NEW INSIGHTS IN DIAGNOSING AND TREATMENT OF GLUCOSE DISORDERS AND OBESITY IN CHILDREN AND ADOLESCENTS

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PUBLISHED IN: Frontiers in Endocrinology and Frontiers in Pediatrics

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88974-020-8
DOI 10.3389/978-2-88974-020-8

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NEW INSIGHTS IN DIAGNOSING AND TREATMENT OF GLUCOSE DISORDERS AND OBESITY IN CHILDREN AND ADOLESCENTS

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Citation: Mozzillo, E., Marigliano, M., Dovc, K., Maltoni, G., Chiavaroli, V., eds. (2021). New Insights in Diagnosing and Treatment of Glucose Disorders and Obesity in Children and Adolescents. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88974-020-8

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frontiers in Pediatrics

EDITORIAL
published: 18 November 2021
doi: 10.3389/fped.2021.786055

Editorial: New Insights in Diagnosing and Treatment of Glucose Disorders and Obesity in Children and Adolescents

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Keywords: obesity, diabetes, childhood, technology, adolescence

Editorial on the Research Topic

New Insights in Diagnosing and Treatment of Glucose Disorders and Obesity in Children and Adolescents

OPEN ACCESS

Edited and reviewed by:
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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *29 September 2021*
Accepted: *20 October 2021*
Published: *18 November 2021*

Citation:
Mozzillo E, Maltoni G, Chiavaroli V, Dovc K and Marigliano M (2021) Editorial: New Insights in Diagnosing and Treatment of Glucose Disorders and Obesity in Children and Adolescents. Front. Pediatr. 9:786055. doi: 10.3389/fped.2021.786055

INTRODUCTION

The diagnosis and treatment of disorders of glucose metabolism and obesity in childhood have significantly changed over the last decades. The impact of new technologies for the management of type 1 diabetes (T1D) (1), the progress in genetic testing for rare forms of diabetes, hyperinsulinism, and obesity (2), and the relevance of secondary diabetes (such as in Cystic Fibrosis) (3) have a growing importance in the diagnostic and therapeutic approach of pediatric endocrinologists and diabetologists.

This Research Topic includes a collection of papers on relevant topics in Pediatric Endocrinology that could help clinicians in better understanding new findings in the endocrine and diabetes fields.

Disorders in glucose metabolism range from hypoglycemia to diabetes mellitus. In fact, in some rare diseases, a mutation in the same gene can cause both hypoglycemia and hyperglycemia, as highlighted in the systematic review by Casertano et al., who propose a novel comprehensive diagnostic flow chart. Diabetes mellitus ranges from T1D, an autoimmune disease requiring insulin, to monogenic diabetes, rare and not necessarily needing insulin, and to secondary diabetes, usually treated with insulin at the onset.

A proper and early diagnosis of diabetes in the childhood and an appropriate therapeutic approach can have a relevant impact on the clinical and psychological outcomes. Peng et al. show that diabetic ketoacidosis (DKA) prevalence was unchanged in a large regional center of China. The authors underline that the increasing awareness of this condition in the community and among primary care physicians could lead to earlier diagnosis, and therefore reduce the rates of DKA at presentation.

Chiesa and Marcovecchio report that vascular complications and the associated mortality remain a major issue for youth with T1D. Thus, it is essential the awareness about their prevalence and the importance of their early prediction and prevention in order to improve the long-term prognosis of youth with T1D. However, cardiometabolic diseases and excess adiposity are strong predictors of morbidity and mortality in children across the spectrum of glucose disorders. Chung et al. show that certain Nuclear Magnetic Resonance derived biomarkers are useful to predict the cardiometabolic risk in youth with dysglycemia.

In order to prevent the cardiovascular complications of T1D, literature has given growing attention to the efficacy of technologies that allow to reach treatment targets for glucose control. Fuchs and Hovorka underline that new technologies, such as *closed-loop* insulin delivery systems, are transforming diabetes management by improving glycaemic control and quality of life in children and young people. Franceschi et al. show in a systematic review of literature that, even though the new technologies have demonstrated to improve metabolic control, it is fundamental to point out to the families the peculiarities of the different devices. On one side Continuous Glucose Monitoring (CGM) allows a tighter metabolic control with less hypoglycemic events compared to intermittent scanning (isCGM); on the other side, isCGM seems to have greater benefits on psychological outcomes. Troncone et al. contribute to the scientific debate on the psychological benefits for patients and their caregivers after participating in a diabetes camp, by examining potential changes in psychological measures of youths' psychosocial adjustment and perception of diabetes, and self-efficacy in disease management.

Over the last decades the diagnosis and management of pediatric monogenic diabetes (4, 5) has substantially improved, thanks to the advanced molecular biology techniques. In their papers Ngoc et al. and Di Iorio et al. underline that the correct genetic diagnosis improves treatment outcomes and prognosis of neonatal diabetes and Wolfram syndrome, respectively. In the latter it is shown that the clinical phenotype of Wolfram syndrome may include optic neuropathy and not only optic atrophy. Often, there is no specific relationship between the mutation and the clinical symptoms in some forms of monogenic diabetes, as stressed by Zhao et al. The authors firstly report EIF2AK3 mutations causing Wolcott-Rallison syndrome, in which diabetes is present.

Whether early detection and treatment of pre-diabetes may contribute to improve the clinical course of secondary diabetes, such as Cystic Fibrosis Related Diabetes, is debated in the systematic review of Mozzillo et al.. The authors underline that early diagnosis and prompt initiation of insulin therapy could have beneficial effects on clinical outcomes of patients with Cystic Fibrosis and pre-diabetes.

Obesity is a major concern worldwide as it can dramatically affect patient's quality of life, particularly among children and adolescents. Its etiology varies from idiopathic to syndromic, up to the rarest monogenic form. Alterations in the mechanism of polyphagia prevail in the forms of obesity secondary to gene mutations, as evidenced by Gregoric et al., who describe two cases of pro-opiomelanocortin (POMC) deficiency. Focusing on idiopathic obesity, the most common type in the pediatric population, special attention deserves the association with metabolic syndrome (MS). Individuals living with MS may show hypertension, diabetes, dyslipidemia, and are at risk of cardiovascular complications. Thus, it is very important to identify those at higher risk already in the pediatric age group. Zhang et al. demonstrate that relative children's lipid accumulation product is an effective indicator for predicting MS, while Yang et al. highlight that C1q is positively associated with obesity and components of MS. In addition, Rahman et al. show an association between insulin-like growth factor binding proteins (IGFBPs) with metabolic homeostasis in adolescents, and suggest its potential use as a biomarker for obesity.

Treating pediatric obesity is not always successful, and which is the most efficient approach is still discussed. Of note, severe obesity is associated not only with physical but also psychological complications, particularly in adolescence. Klemenčič et al. suggest that a reversible bariatric surgery approach led to improvements of psychological factors in adolescents with severe obesity.

We hope that this Research Topic will provide a valuable resource for the diagnosis and therapy of glucose metabolism disorders and the different etiologic types of obesity in pediatric age.

AUTHOR CONTRIBUTIONS

EM, GM, VC, KD, and MM wrote manuscript and made a substantial, direct and intellectual contribution to the work. All authors have approved the submission of the final version of this manuscript for publication, and have agreed to be accountable for all aspects for the work.

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frontiers in Endocrinology

ORIGINAL RESEARCH
published: 30 November 2020
doi: 10.3389/fendo.2020.586440

Complement C1q as a Potential Biomarker for Obesity and Metabolic Syndrome in Chinese Adolescents

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Background: Complement C1q (C1q) has been confirmed to be related to obesity, metabolic syndrome (MetS), and its components. However, human data regarding the associations are relatively scarce. This study aimed to investigate associations of C1q with obesity as well as MetS in Chinese adolescents.

Methods: A total of 1,191 Chinese adolescents aged 13–18 years were enrolled in this study. The biochemical and anthropometric variables of all the subjects were evaluated using standardized procedures. C1q was measured using the immunoturbidometric assay. The relationship between C1q and obesity or MetS was analyzed using multiple regression analyses.

Results: Obesity was more prevalent among participants in the highest tertile than in the lowest tertile of C1q levels. The highest tertile of C1q was related to a greater effect on the risk of MetS, and its trend test was statistically significant. Except for hyperglycemia, the prevalence of other components of MetS significantly increased relative to an increase in C1q tertile. Receiver operating characteristic (ROC) curve analysis of C1q for predicting adolescents with MetS illustrated that the area under the curve (AUC) was 0.82 [95% confidence interval (CI): 0.76, 0.88; $P<0.001$] in the total population after adjusting for confounders.

Conclusions: This study observed a significantly higher prevalence of obesity and MetS features in adolescents with high C1q. The findings of the current study also reported a significant relationship between C1q levels and MetS components [except for fasting plasma glucose (FPG)] in Chinese adolescents. C1q may represent a biomarker for predicting obesity or MetS in adolescents.

Keywords: C1q, biomarker, adolescent, obesity, metabolic syndrome

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *23 July 2020*
Accepted: *20 October 2020*
Published: *30 November 2020*

Citation:
Yang X, Ma Y, Zhao Z, Zhen S and Wen D (2020) Complement C1q as a Potential Biomarker for Obesity and Metabolic Syndrome in Chinese Adolescents. Front. Endocrinol. 11:586440. doi: 10.3389/fendo.2020.586440

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; AST, Aspartate aminotransferase; AUC, Area under the curve; BMI, Body mass index; C1q, Complement C1q; CI, Confidence interval; FPG, Fasting plasma glucose; GGT, Gamma glutamyl transpeptidase; HDL-C, High-density lipoprotein cholesterol; IDF, International Diabetes Federation; LDL-C, Low-density lipoprotein cholesterol; MetS, Metabolic syndrome; OR, Odds ratio; ROC, Receiver operating characteristic; sdLDL-C, Small dense low-density lipoprotein cholesterol; TG, Triglyceride; WHO, World Health Organization; WC, Waist circumference; NW, normal weight; OW, overweight; OB, obesity.

INTRODUCTION

As a major challenge for public health (1), the upward trend of childhood overweight and obesity is generating direct and indirect costs, including lifetime healthcare and productivity costs (2). Childhood obesity has also been associated with the risk of metabolic syndrome (MetS) (3). As previously reported, MetS is a cluster of cardiovascular risk factors, including central obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low levels of high-density lipoprotein cholesterol (HDL-C) (4–6). In addition, MetS was found to influence the risk of cardiovascular diseases, type II diabetes mellitus, chronic kidney disease, non-alcoholic fatty liver disease, and all-cause mortality (7–11). Nowadays, for children and adolescents with MetS, prevalence rates in both developed and developing countries are increasing (6, 12, 13). Considering the scale and problems of obesity and MetS among adolescents, as well as significant and sustained adverse effects on health, it would be critical to identify novel biomarkers for predicting obesity and MetS which would then play a significant role in prevention.

As an important part of the innate immune system (14), the complement system was associated with several components of MetS, including obesity and insulin resistance (15, 16). In adipose tissues of people with obesity, there have been increased expressions of specific complement components (17). Complement C1q (C1q), widely produced by macrophages, immature dendritic, and mast cells (18–23), was referred to as a pattern recognition receptor of the innate immune complement system (24). It is a protein consisting of 18 polypeptides chains of three different types named A, B, and C (25). Recently, these three C1q sub-chains were demonstrated to be upregulated among people with obesity aged 22–36 years (26). Accumulating evidence has indicated that C1q was significantly associated with both cardiovascular disorders, including arterial stiffness, and metabolic health outcomes, such as diabetes mellitus (27–29). In alcoholic liver disease models, C1q was considered to be a key mediator of adipose inflammation caused by alcohol exposure (30–32). According to studies on the complement system and MetS, focus has mostly been on serum complement C3 and its effects on the risk of MetS in both Chinese and Caucasian subjects (33, 34); few studies have addressed the relationship of C1q and MetS. Based on a cross-sectional research among 127 Japanese individuals, serum C1q level was confirmed to be positively associated with blood pressure (27), which has been referred to as a component of MetS (5).

As it stands, most available studies on the relationship between C1q and obesity have mainly been conducted in adults, whereas data on adolescents is largely lacking. With respect to C1q and MetS, previous studies were usually concerned with single or partial components of MetS. Moreover, whether serum C1q level could be beneficial to human subjects with MetS is not yet well-understood, especially in adolescents. In the present study, our objectives were to determine the significance of C1q in adolescents with obesity and to investigate the association of C1q with MetS and its components.

MATERIALS AND METHODS

Study Population

Data of the current study were extracted from the 2017 to 2018 Huanggu District Middle and Primary School Student Physical Fitness Monitoring (HMPSPM) database. The study was a cross-sectional observational study of adolescents enrolled in five middle schools during the 2017–2018 school year in Huanggu District, Shenyang, China. Individuals with missing information, older than 18 years, and with psychiatric disease or severe systemic disease were excluded from this study. In total, 1,191 adolescents aged 13–18 years were included in the analysis. The study was approved by the China Medical University Health Science Ethics Committee, and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent of all the participants and/or their parents were obtained before entering the study.

Anthropometric and Biochemical Measurements

As previously reported (35), all subjects were measured between 8 am and 10 am, and the anthropometric measurements were conducted by a trained research assistant who followed reference protocols recommended by the World Health Organization (WHO) (36). The researcher used standardized equipment to measure the weight and height of each participant to the nearest 0.1 kg and 0.1 cm, respectively. Participants were instructed to wear light clothing and were measured barefoot. Weight was divided by height squared to obtain body mass index (BMI) (kg/m^2). In light of Chinese reference values, age- and sex-specific Z-scores of BMI were calculated for all the participants. Waist circumference (WC) was taken for all participants using established techniques (36). A standardized Omron i-C10 blood pressure monitor (Omron Healthcare Co., Ltd, Kyoto, Japan) was used to measure sitting blood pressure.

Venous blood samples were collected by nurses after participants had fasted overnight for about 12 h. All the samples were immediately transported to the Department of Clinical Laboratory, Shengjing Hospital of China Medical University, where the following parameters were measured by use of standard operating procedures. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transpeptidase (GGT) were determined with an International Federation of Clinical Chemistry method. HDL-C and low-density lipoprotein cholesterol (LDL-C) were assayed directly using the selective solubilization method, and a novel homogeneous enzymatic assay was used to determine small dense low-density lipoprotein cholesterol (sdLDL-C) levels. Concentrations of total triglyceride (TG) were measured by the standard enzymatic method. For fasting plasma glucose (FPG), a modified hexokinase enzymatic method was used, and apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), and C1q were measured by immunoturbidometric assay.

Determination of Overweight and Obesity

To define overweight and obesity in adolescents, categorization was based on criteria set by the WHO. Overweight was defined as

BMI-for-age greater than one standard deviation above the WHO Growth Reference median, and obesity was defined as greater than two standard deviations (1).

Definition of Metabolic Syndrome

MetS and its components in the present study were defined by the criteria of MetS developed by the International Diabetes Federation (IDF) (37). MetS was identified when abdominal obesity (defined as WC≥ 90th percentile for age and gender for individuals between 10 to 16 ages and WC≥90 cm for males or ≥80 cm for females in adolescents over 16 years old) and the following two or more criteria existed simultaneously: 1) high blood pressure (systolic blood pressure≥130mmHg or diastolic blood pressure≥85mmHg); 2) elevated plasma glucose (FPG≥5.6mmol/L); 3) elevated TG (TG≥1.7mmol/L); 4) low HDL-C (HDL-C<1.03mmol/L in both sexes aged 10 to 16 years and <1.03mmol/L for males or <1.29mmol/L for females in adolescents over 16 years old).

Statistical Analysis

Skewness and kurtosis tests were performed for the normal distribution of the data. Descriptive information was presented as means and standard deviations for Gaussian distributions and as medians, together with the upper and lower quartiles, for non-Gaussian distributions. For categorical variables, numbers and percentages were reported. Concentrations of C1q were divided into tertiles. Characteristics of the participants were presented according to the C1q tertiles. Youden's index, a measure of overall diagnostic effectiveness, was used to investigate a C1q cut-off in predicting MetS. Based on the optimal cut-off value of C1q, subjects were divided into "lower" and "upper" groups. Multiple linear regression was performed to identify the association between C1q and BMI Z-scores. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using multiple logistic regression analysis to assess the relationships between C1q and overweight/obesity, obesity, MetS, and MetS components according to C1q tertile. C1q tertile1 was set as the reference. The first multiple regression analysis models adjusted only for age, whereas subsequent models adjusted for age in addition to other potential confounding factors. The receiver operating characteristic (ROC) curve analysis was used to explore sensitivity and specificity. The area under the curve (AUC), together with 95% CI, was used to determine whether C1q could be a biomarker for predicting adolescents with MetS. Data were analyzed using Stata (Version 15.1; StataCorp, College Station, TX, USA), and *P* values were reported as two-tailed with $P < 0.05$ indicating statistical significance.

RESULTS

Characteristics of the Study Population

The basic anthropometric and clinical information of the 1,191 subjects aged 13–18 years, stratified by C1q tertiles, are shown in **Table 1**. In the third C1q tertile, the proportion of overweight,

TABLE 1 | Characteristics of the study population according to tertiles of C1q.

Characteristic	Tertile1 (n=397)	Tertile2 (n=397)	Tertile3 (n=397)
Age, mean (SD), years	16.25 (0.98)	16.25 (0.99)	16.16 (1.02)
Boys, No. (%)	295 (74.31)	194(48.87)	89 (22.42)
Anthropometry			
BMI z-score, mean (SD)	-0.25 (0.75)	0.09 (1.03)	0.24 (1.09)
Weight status, No. (%)			
NW	323 (81.36)	268 (67.51)	241 (60.71)
OW	49 (12.34)	75 (18.89)	85 (21.41)
OB	25 (6.30)	54 (13.60)	71 (17.88)
Waist circumference, mean (SD), cm	72.39 (8.56)	74.88 (11.49)	75.27 (12.04)
Metabolic syndrome outcomes, No. (%)			
Metabolic syndrome	6 (1.51)	25 (6.30)	29 (7.30)
Central obesity	40 (10.08)	81 (20.40)	115 (28.97)
Hypertension	79(19.90)	98 (24.69)	93 (23.43)
Hyperglycemia	2 (0.50)	4 (1.01)	8 (2.02)
High TG	13 (3.27)	17 (4.28)	29 (7.30)
Low HDL-C	71 (17.88)	106 (26.70)	130 (32.75)
Laboratory examinations, median (Q1, Q3),			
ALT, (U/L)	26 (11, 64)	30 (11, 62)	29 (12, 62)
AST, (U/L)	15 (13, 18)	16 (13, 18)	15 (13, 18)
ALP, (U/L)	103 (79, 137)	96 (75, 121)	87 (75, 109)
GGT, (U/L)	16 (13, 19)	16 (12, 22)	16 (13, 22)
FPG, (mmol/L)	4.22 (3.94, 4.52)	4.25 (3.98, 4.53)	4.38 (4.09, 4.67)
HDL-C, (mmol/L)	1.31 (1.13, 1.52)	1.27 (1.14, 1.49)	1.29 (1.11, 1.51)
LDL-C, (mmol/L)	1.93 (1.60, 2.24)	2.16 (1.80, 2.59)	2.29 (1.91, 2.64)
ApoA1, (g/L)	1.30 (1.20, 1.41)	1.31 (1.19, 1.42)	1.32 (1.21, 1.44)
ApoB, (g/L)	0.57 (0.05, 0.66)	0.65 (0.54, 0.74)	0.68 (0.58, 0.79)
sdLDLC-C, (mmol/L)	0.38 (0.31, 0.46)	0.43 (0.34, 0.53)	0.45 (0.36, 0.57)

NW, normal weight; OW, overweight; OB, obesity.

obesity, MetS, and MetS components (including central obesity, hyperglycemia, high TG, and low HDL-C) were highest. The optimal cut-off value for C1q was 184.7mg/L and Youden's index was 0.30. Results after classifying C1q according to the cut-off value are shown in **Supplementary Table 1**.

Multivariable Adjusted β and 95% Confidence Interval for Body Mass Index z-Scores Across C1q Tertiles

Table 2 shows the adjusted β and 95% CIs for predicting BMI z-scores across C1q tertiles. In comparison with the lowest tertile group, BMI z-scores were significantly higher in the third C1q tertile (β=0.49; 95% CI: 0.35, 0.62; *P* for trend<0.001) after adjustment for age. Adjustment for all of age, gender, ALT, AST, ALP and GGT did not show a change in the correlation between BMI z-scores and C1q (β=0.36; 95% CI: 0.23, 0.50; *P* for trend<0.001). There was a statistically significant association between BMI z-scores and C1q in the stratified analysis by the cut-off value of C1q (**Supplementary Table 2**).

Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Overweight, Obesity, and Metabolic Syndrome Across C1q Tertiles

The associations between C1q and overweight, obesity, and MetS are presented in **Table 3**. It was observed that participants in the highest tertile had a 2.26-fold (95% CI: 1.50, 3.40) higher risk of overweight and obesity than those in the lowest tertile after adjusting for age, gender, ALT, AST, ALP, and GGT. The OR (95% CI) for obesity of the highest tertile was 3.76 (2.08, 6.78) when compared with the lowest tertile after adjustment for the same confounding variables. Participants in the highest tertile were more likely to also have MetS compared to those in the lowest tertile, after adjusting for the same variables (OR =5.43; 95% CI: 2.02, 14.60). Similarly, the prevalence of overweight and obesity, obesity, as well as MetS significantly increased across increasing tertiles of C1q (*P* for trend<0.001; *P* for trend<0.001; *P* for trend=0.001). After classifying C1q according to the cut-off value, C1q was significantly related to obesity and MetS (**Supplementary Table 3**).

Multivariable Adjusted Odds Ratios and 95% Confidence Intervals for Metabolic Syndrome Components Across C1q Tertiles

The adjusted ORs and 95% CIs for components of MetS across C1q tertiles are reported in **Table 4**. In comparison to subjects in the lowest tertile, those in the highest tertile had a significantly increased risk of central obesity (OR =3.60; 95% CI: 2.42, 5.34; *P* for trend<0.001), high TG (OR =2.33; 95% CI: 1.19, 4.55; *P* for trend=0.010), and low HDL-C (OR =2.31; 95% CI: 1.65, 3.22; *P* for trend<0.001) after adjusting for age, and even after adjustment for relevant confounders, including age, gender, ALT, AST, ALP, and GGT. Although no association between C1q and hypertension was observed after adjusting for age, participants in the highest tertile had a 1.99-fold (95% CI: 1.30, 3.05) higher risk of hypertension than those in the lowest tertile after adjusting for the additional variables described above. No

TABLE 2 | Multivariable adjusted β and 95% confidence interval (CI) for body mass index (BMI) z-scores across C1q tertiles.

	Tertiles, β (95% CI)			
	Tertile1 (n=397)	**Tertile2 (n=397)**	**Tertile3 (n=397)**	***P* for trend**
Age-adjusted model	0 (Reference)	**0.33 (0.20, 0.47)**	**0.49 (0.35, 0.62)**	**<0.001**
Multiple-adjusted model	0 (Reference)	**0.24 (0.12, 0.37)**	**0.36 (0.23, 0.50)**	**<0.001**

β, regression coefficient; CI, confidence interval; multiple-adjusted model: adjusted for age (in years), sex (boys vs. girls), ALT (U/L), AST (U/L), ALP (U/L), GGT (U/L). P-value for trend was obtained by adjusting tertiles of C1q level as a continuous variable. P-values< 0.05 are in bold.

TABLE 3 | Multivariable adjusted odds ratios and 95% confidence interval (CI) for overweight, obesity, and metabolic syndrome (MetS) across C1q tertiles.

	Tertiles, OR (95% CI)			
	Tertile1 (n=397)	**Tertile2 (n=397)**	**Tertile3 (n=397)**	***P* for trend**
Overweight+obesity				
Age-adjusted model	1 (Reference)	**2.12 (1.53, 2.96)**	**2.80 (2.02, 3.88)**	**<0.001**
Multiple-adjusted model	1 (Reference)	**1.85 (1.27, 2.68)**	**2.26 (1.50, 3.40)**	**<0.001**
Obesity				
Age-adjusted model	1 (Reference)	**2.35 (1.43, 3.87)**	**3.18 (1.97, 5.15)**	**<0.001**
Multiple-adjusted model	1 (Reference)	**2.35 (1.35, 4.08)**	**3.76 (2.08, 6.78)**	**<0.001**
MetS				
Age-adjusted model	1 (Reference)	**4.38 (1.78, 10.80)**	**5.10 (2.09, 12.43)**	**<0.001**
Multiple-adjusted model	1 (Reference)	**4.38 (1.68, 11.40)**	**5.43 (2.02, 14.60)**	**0.001**

OR, odds ratio; CI, confidence interval; Multiple-adjusted model: adjusted for age (in years), sex (boys vs. girls), ALT (U/L), AST (U/L), ALP (U/L), GGT (U/L). P-value for trend was obtained by adjusting tertiles of C1q level as a continuous variable. P-values< 0.05 are in bold.

TABLE 4 | Multivariable adjusted odds ratios and 95% confidence interval (CI) for metabolic syndrome (MetS) components across C1q tertiles.

	Tertiles, OR (95% CI)			
	Tertile1 (n=397)	**Tertile2 (n=397)**	**Tertile3 (n=397)**	***P* for trend**
Central obesity				
Age-adjusted model	1 (Reference)	**2.31 (1.53, 3.49)**	**3.60 (2.42, 5.34)**	**<0.001**
Multiple-adjusted model	1 (Reference)	**1.88 (1.20, 2.95)**	**2.57 (1.60, 4.13)**	**<0.001**
Hypertension				
Age-adjusted model	1 (Reference)	1.32 (0.94, 1.85)	1.22 (0.87, 1.72)	0.251
Multiple-adjusted model	1 (Reference)	**1.56 (1.08, 2.26)**	**1.99 (1.30, 3.05)**	**0.001**
Hyperglycemia				
Age-adjusted model	1 (Reference)	2.01 (0.37, 11.04)	3.96 (0.83, 18.78)	0.063
Multiple-adjusted model	1 (Reference)	1.45 (0.25, 8.49)	2.19 (0.40, 11.93)	0.328
High TG				
Age-adjusted model	1 (Reference)	1.32 (0.63, 2.76)	**2.33 (1.19, 4.55)**	**0.010**
Multiple-adjusted model	1 (Reference)	1.34 (0.62, 2.89)	**2.87 (1.34, 6.16)**	**0.005**
Low HDL-C				
Age-adjusted model	1 (Reference)	**1.68 (1.20, 2.37)**	**2.31 (1.65, 3.22)**	**<0.001**
Multiple-adjusted model	1 (Reference)	1.42 (0.99, 2.02)	**1.70 (1.17, 2.48)**	**0.006**

OR, odds ratio; CI, confidence interval; multiple-adjusted model: adjusted for age (in years), sex (boys vs. girls), ALT (U/L), AST (U/L), ALP (U/L), GGT (U/L). P-value for trend was obtained by adjusting tertiles of C1q level as a continuous variable. P-values< 0.05 are in bold.

association between C1q and hyperglycemia was reported. After classifying C1q according to the cut-off value, the C1q level was significantly related to components of MetS (except for FPG and high TG) (**Supplementary Table 4**).

Receiver Operating Characteristic for Predictive Values of C1q Levels in Detecting Metabolic Syndrome

The ROC curve for tertiles of C1q in predicting MetS is shown in **Figure 1**. As can be observed, the AUC was 0.82 (95% CI: 0.76, 0.88; *P*<0.001) in the total population after adjusting for age, gender, ALT, AST, ALP, and GGT. According to the cut-off value of C1q, the AUC was 0.83 (95% CI: 0.78, 0.88; *P*<0.001) (**Supplementary Figure 1**).

DISCUSSION

This was the first study investigating the association between C1q levels and metabolic parameters among adolescents aged 13 to 18 years. In this cross-sectional observational study, we reported a significantly higher prevalence of obesity and MetS features

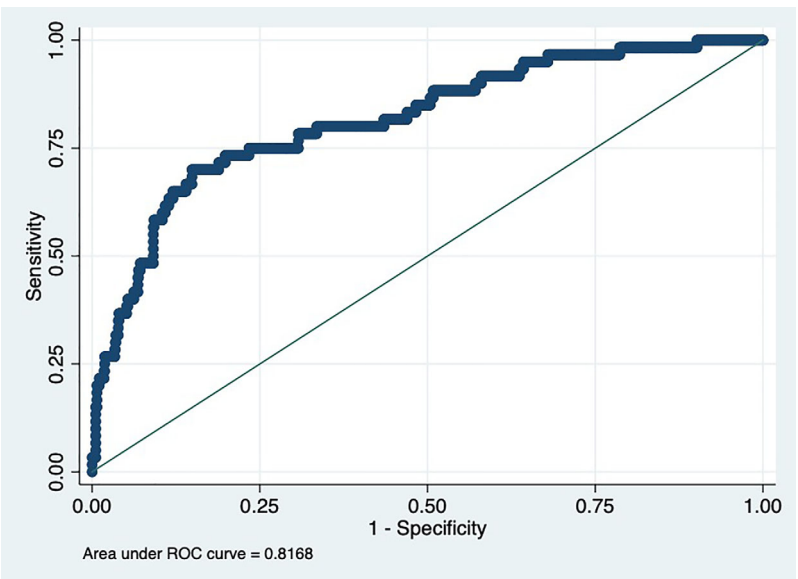

FIGURE 1 | Receiver operating characteristic (ROC) for predictive values of C1q levels in detecting metabolic syndrome (MetS).

among adolescents with elevated serum C1q levels. The adjustment of potential confounding variables described in the study did not have a substantial impact on the above results. We also found that serum C1q levels were significantly related to components of MetS (except for FPG). Our findings raised the possibility that C1q level may be related to the prediction and prevention of obesity or MetS in adolescents.

As a major part of the innate immune system (38), the complement system was considered to have a critical role in obesity (26). Several studies demonstrated that the complement system is widely expressed and regulates inflammation in adipose tissues (14, 17). Moreover, genes of the classical pathway—the specific complement activation pathway—are widely expressed in human adipose tissue (39). By observing the upregulation of complement genes in adipose tissue of heavier co-twins, C1q A-C genes were upregulated in obesity, and C1q stain was more extensive in obese twins (26). Furthermore, a previous study reported that the expression of C1q increased in epididymal adipose tissue among several models of mice, including genetic mice, high-fat diet-induced obese mice, and Zucker obese rats (40). An observational epidemiological survey in 239 Japanese male subjects reported that serum C1q was positively related to adiposity, such as BMI, WC, visceral fat area, and subcutaneous fat area (41). However, there have been very few studies that have examined the relationship between C1q and obesity in human adolescents. In fact, this study suggested that a positive association exists between serum C1q level and BMI, and there was evidence that higher C1q was significantly related to the prevalence of MetS among adolescents.

Our findings also revealed that serum C1q was positively related to MetS and suggested significant associations of serum C1q with a few components of MetS, including central obesity, hypertension, high TG, and low HDL-C. Nevertheless, there was no significant association between C1q and hyperglycemia in the present study. This might be attributed to a relatively small population of hyperglycemia in comparison to other components of MetS among the study subjects. Previous studies on C1q and MetS as well as its components were in line with our results. In a study of subjects aged 30–74 years, there were clear positive associations between C1q and several factors related to MetS, such as systolic blood pressure, diastolic blood pressure, and log TG; the inverse association between C1q and HDL-C was also reported (41). A study conducted among people of Caucasian descent above 40 years showed significant associations of C1q with TG, HDL-C, and FPG. As reported in that study, the relationship between C1q and MetS was modest (42), which was inconsistent with our results. This difference may be attributed to the subjects belonging to a specific population of individuals who already exhibit moderately increased risk of cardiometabolic diseases. Studies have demonstrated that MetS was associated with chronic inflammatory responses (43, 44). Meanwhile, complement activation by C1q could exacerbate many chronic inflammatory diseases (45). Although the pathophysiology of MetS has yet to be fully explained, insulin resistance has proven to be a critical contributor to MetS (46, 47). The complement system is involved in several aspects of the histopathophysiology that lead to insulin resistance. In the C1q-knocked mouse model, the study demonstrated that C1q had a protective role in insulin resistance induced by a high-fat diet (48).

The strengths of this study were the relatively large sample size of the Chinese adolescent population, the appropriate study design, and the standardized information collection procedures. Moreover, measurement biases were largely avoided since blood samples were analyzed by clinical laboratory standards, which improved the reliability of our study. Despite the noteworthy findings revealed in the present study, it has several limitations. First, the cross-sectional design of the current study might limit the strength of these findings as it is not possible to investigate the longitudinal association between C1q and MetS. Further prospective studies are warranted to establish a cause-effect relationship. Second, subjects with inflammations or infections may have altered C1q levels. However, inflammatory markers such as C-reactive protein were not measured in the current study. Inflammatory markers should be included in future studies. Also, not all potential factors to obesity or MetS were included in the present study. To minimize the effect of potential covariates, we have considered a variety of covariates, such as ALT, AST, ALP, and GGT, but we understand that there are many other possible factors to consider. Finally, the present study was limited to Chinese adolescents, which may not be completely representative of other populations.

CONCLUSIONS

This study demonstrated a significant association between C1q and the prevalence of obesity in a large sample of Chinese adolescents. There were also significant associations between C1q level and metabolic parameters (except for FPG) in Chinese adolescents aged 13 to 18 years. Based on these findings, we suggest that C1q may present as a novel biomarker of obesity and MetS in adolescents. The molecular mechanism of complement C1q activation about MetS needs further investigation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the China Medical University Health Science Ethics Committee, and was conducted in accordance with the principles of the Declaration of Helsinki. The written informed consent of all the participants and/or their parents has been obtained before entering the study.

AUTHOR CONTRIBUTIONS

DW, ZZ, and SZ participated in the study design and organized the data collection. XY, YM, and SZ analyzed and interpreted the data. XY wrote the manuscript. All authors have read and agreed to the final manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the National Natural Science Foundation of China [71774173], CMB Open Competition (CMB-OC), Research Program Health Policy and System Sciences [18-291], and the Liaoning province key research and development plan guidance plan resource platform construction project [2017225004].

ACKNOWLEDGMENTS

We thank all the participants for their support in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.586440/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 10 March 2021
doi: 10.3389/fped.2021.650201

Psychological Outcomes in Children and Early Adolescents With Type 1 Diabetes Following Pediatric Diabetes Summer Camp: A 3-Month Follow-Up Study

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *06 January 2021*
Accepted: *05 February 2021*
Published: *10 March 2021*

Citation:
Troncone A, Chianese A, Cascella C, Zanfardino A and Iafusco D (2021) Psychological Outcomes in Children and Early Adolescents With Type 1 Diabetes Following Pediatric Diabetes Summer Camp: A 3-Month Follow-Up Study. Front. Pediatr. 9:650201. doi: 10.3389/fped.2021.650201

Objective: The aim of this study was to assess general psychosocial adjustment to diabetes and perceived disease management among patients with type 1 diabetes (T1D) and their parents before and after patients' participation in a diabetes summer camp.

Methods: In this follow-up study, 20 children and adolescents with T1D (eight boys; mean age = 11.01 ± 0.94 years; mean diabetes duration = 3.02 ± 2.27) attending a southern Italian diabetic center, along with their parents, were assessed prior to and 3 months after the youths participated in a 1 week camp-based intervention involving didactic and interactive child-centered education and recreational activities. Patients and their parents completed measures assessing patients' quality of life and strategies employed by patients to cope with pain. Patients also completed measures evaluating their diabetes psychosocial adjustment, diabetes self-efficacy management, and illness perception; also, their parents completed measures of caregivers' perceived diabetes burden and treatment satisfaction. Youths' glycated hemoglobin (HbA1c) and standardized body mass index (z-BMI) values were also assessed. Within-subjects repeated-measures analyses of variance evaluated pre- and post-camp changes.

Results: Camp attendance showed no beneficial effects on glycemic control, as indicated by HbA1c values both before (7.02%) and after (7.28%) camp being lower than 7.5%. HbA1c values were found to have increased after camp (pre-camp = 7.02%, post-camp = 7.28%; $p = 0.010$), but since they still fell within an acceptable range, they did not reveal clinically relevant changes in glycemic control. No substantial significant improvement in psychosocial measures was observed in children or parents (all $p > 0.05$). According to the parents' evaluation, social support-seeking as a patient pain-coping strategy was slightly increased ($p = 0.044$) after attending the camp.

Conclusions: This study does not provide empirical evidence of benefits of participating in a diabetes camp for either patients or their parents. These findings suggest that healthcare providers rethink such camps as an experience for youths with T1D that actively involves parents and that includes both youth- and parent-focused psychological interventions.

Keywords: type 1 diabetes, adolescence, children, summer camp, psychological adjustment, illness perception, diabetes burden, treatment satisfaction

INTRODUCTION

The psychological burden imposed by type 1 diabetes (T1D) is taxing. Due to the characteristics of the disease, children and adolescents with T1D must monitor and fulfill complex health needs, such as blood glucose monitoring, insulin therapy, and dietary restrictions and planning. Therefore, they are required to change their everyday life in different ways, and all these behavioral challenges place serious demands upon the youth and their family, negatively impacting individual psychological functioning. Unsurprisingly, youths with T1D have frequently been described as being at high risk for psychological symptoms (1). In particular, numerous studies have indicated that individuals with T1D have a greater incidence of depression and anxiety (2, 3), diabetes distress (4), body image concerns (5, 6), and disordered eating behaviors (7) than their peers without diabetes.

Diabetes camp has been deemed worldwide as a part of diabetes care, an opportunity to offer diabetes education to children and adolescents in a group in a safe environment. As underscored in ADA recommendations (8), the mission of specialized camps for children and adolescents with diabetes is to enable youth with diabetes to learn to be more responsible for their condition within a context where they can meet and share their experiences with others and where they can have a safe, integrated educational experience. Camp may be an opportunity for children with T1D to gain or improve the ability to actively manage their illness by enhancing self-management skills (8, 9). In addition, by providing opportunities for children to participate in typical childhood and adolescent activities in a safe and inclusive space, the summer camp may meet children's psychological needs, thereby helping to develop self-confidence and supporting their overall development.

Several studies have examined the psychological effects of participation in a diabetes camp on youth with T1D. In particular, as highlighted by Anarte et al. (10), in terms of the psychological outcomes, the majority of studies has investigated children's quality of life, attitude toward illness, self-efficacy related to disease management, and concepts such as anxiety, affectivity, knowledge about disease management, adaptation, self-esteem, self-reported adherence, and so on.

While general agreement exists on the effectiveness of camp experiences in improving diabetes knowledge and management (11, 12), contradictory results are reported on psychological outcomes. While literature reviews have indicated a general improvement in psychological variables after attending a diabetes camp (13–15), especially in short-term benefits (16), other studies have described no relevant variations in anxiety and psychological adaptation after diabetes camp (17, 18). In the same way, in research investigating children's quality of life after the camp, some studies have found improvement in this dimension (10, 19) while other studies have not (14, 20–22).

Similarly, improvements in attitude toward illness, self-efficacy, competence in diabetes management, adherence, and self-care after the camp experience have been described (12, 17, 22–25), along with other evidence, all indicating mixed results (26).

In addition, to date, some psychological aspects seem to be overlooked in this research area. It is well-known that multiple-injection therapy can induce discomfort and distress to such an extent that fear of injections and finger pricks appear to be fairly common in children and adolescents with T1D (27–30) and significantly correlated with higher injection pain levels (31, 32), regardless of needle diameter (33). Pain associated with insulin injection was found in turn to severely impact the ability to self-manage diabetes, inducing patients to avoid or reduce blood glucose monitoring and insulin injections and thus worsening adherence to insulin therapy (31, 34). Despite how pain affects diabetes care, to our knowledge, no studies have been carried out researching camp's effect on youths' strategies to cope with pain as a dimension of diabetes management.

Furthermore, it should be noted that within the literature evaluating psychological outcomes of diabetes camp experiences, some studies have focused on the effects of participation on campers' parents. In particular, the majority of this research has focused on parents' satisfaction with camp experience (24, 26), on what was changed in their child (in terms of patients' adherence, self-care skills, diabetes knowledge, and management) according to the parents' point of view (12, 21, 25), and on parents' reports of what their child needed to learn (23). However, despite the positive association between parents' well-being and children's metabolic control (35), little research has investigated changes in parents' diabetes treatment-related burden or in parents' feeling and stress around managing a chronic illness after their children attended the camp (10, 22, 36). No evidence has been provided on changes in parents' treatment satisfaction.

In light of the conflicting results in this research area and of the overlooking of certain psychological aspects by previous studies, the present study sought to further investigate the psychological outcomes of patients and their caregivers after the youths participated in a 1-week diabetes camp.

In particular, the psychological benefits evaluated in youths were quality of life, strategies employed by youths to cope with pain, youths' adjustment to and perception of diabetes, and youths' confidence in self-care management of their diabetes.

Some evidence suggests that children and adolescents with T1D may wish to avoid revealing their problems (37), and some evidence indicates that they sometimes report fewer behavioral problems or diverge from their parents' reports regarding their diabetes (38, 39) or aspects of health-related quality of life (13). Thus, where measures were available (i.e., quality of life, strategies to cope with pain), parent-report evaluation was also carried out as an appropriate strategy to enable more accurately analyzing psychological variables under examination.

Additionally, in light of conflicting results on glycemic control changes after camp experiences—such that participation in diabetes camp has been described as either having a positive effect (12, 36, 40) or having no effect (21, 41) on glycemic control—changes in diabetes control, as indicated by glycated hemoglobin (HbA1c) values, were also investigated. Finally, since body mass index (BMI) is commonly considered as a nutritional status and a general health indicator of overall health (42, 43), BMI changes after camp were also evaluated.

In sum, the specific aims of this study were to assess:

(1) Campers' psychological benefits after attending the diabetes camp;
(2) Parents' changes in burden perception and treatment satisfaction following their children's camp experience.

We hypothesized that after the camp experience, changes in general psychological adjustment to diabetes and in related management would be observed in both youths and their caregivers.

MATERIALS AND METHODS

Participants

During the period January–February 2018, youths aged 10–12 years (and their parents) attending a southern Italian diabetic center, who had never gone to a summer camp and who were using multiple daily injections (MDI), were approached during a routine clinic visit and offered to participate in a week-long overnight summer camp for children with T1D. Camp was exclusively proposed to youths treated with MDI because, as is highlighted by results from systematic reviews (44, 45), this treatment is more frequently associated with poorer glycemic control in comparison to continuous subcutaneous insulin infusion. The first 20 parents/youths who agreed and were registered to attend the summer camp were eligible for participation. Parent–youth dyads who were accepted to attend the camp were offered enrollment in the study.

Inclusion criteria for the study included (a) child camper with a diagnosis of T1D for at least 1 year (to avoid the "honeymoon period" and to allow families time to adjust to the diagnosis) and (b) child and primary caregiver present for questionnaire completion, who were able to read and understand the questionnaires. No prespecified HbA1c requirement or insulin administration mode method was set for eligibility. Exclusion criteria included having other illnesses (severe disability due to disease, significant comorbidity, other diagnosed diseases) and presence of recent life stressors. A systematic examination of participants' clinical records was conducted to ascertain that the inclusion/exclusion criteria were met. Demographic and clinical data of participants are shown in **Table 1**.

TABLE 1 | Demographic and clinical data of patients with type 1 diabetes.

	Type 1 diabetes
Sample size (*N*)	20
Gender (*N*) (male/female)	8/12
Age (years)	11.01 (0.94)
HbA1c (%)	7.02 (0.77)
Diabetes duration (years)	3.02 (2.27)
z-BMI	0.15 (1.08)

Data are presented as mean values and standard deviations unless otherwise stated. N, number of subjects; HbA1c, glycated hemoglobin; z-BMI, standardized body mass index.

Measures

Sociodemographic and Clinical Data

A brief interview schedule was specifically designed and completed by the clinicians to record the demographic and clinical data, including the youth's date of diagnosis, age, sex, height, weight, current HbA1c values, and other medical conditions. Possible missing data were obtained by reviewing their medical chart.

Psychosocial/Psychological Measures

Youths

Quality of Life. Youths' quality of life was assessed with the Pediatric Quality of Life Inventory 3.0 Type 1 Diabetes (PedsQL 3.0 DM) self- and parent-report modules (46). The PedsQL 3.0 DM is composed of 28 items across five scales: diabetes symptoms (e.g., I have to go to the bathroom too often, I feel tired or fatigued); treatment barriers (e.g., I am embarrassed about having diabetes, It is hard for me to stick to my diabetes care plan); treatment adherence (e.g., It is hard for me to take blood glucose tests, It is hard to me to exercise); worry (e.g., I worry about "going low," I worry about long-term complication from diabetes); and communication (e.g., It is hard form me to tell the doctors and nurses how I feel, It is hard for me to ask the doctors and nurses questions). Higher scores indicate fewer problems and better quality of life. Validity and reliability studies of the PedsQL 3.0 DM have been conducted in many countries and have indicated satisfactory psychometric properties (47–49). The present study adopted the Italian translation of the PedsQL (8–12 years version), which has demonstrated good validity and reliability (50) and has been used in previous studies (51, 52).

Coping Strategies for Physical Pain. The Waldron/Varni Pediatric Pain Coping Inventory (PPCI) (53) is a self-report questionnaire designed to measure children's strategies of coping with physical pain. It includes both patient and parent-proxy reports and contains items asking to rate how frequently the child uses each

coping skill. The PPCI is composed of five scales: cognitive self-instruction (e.g., pretend I do not have any pain or hurt), seeks social support (e.g., tell my mother or father), problem solving (e.g., ask for medicine), distraction (e.g., try not to think about the pain or hurt or ignore the pain or hurt), and catastrophizing/helplessness (e.g., yell or cry). Higher scores on all subscales indicate more adaptive coping with pain. Both versions of the questionnaire (for children and for parents) have shown good validity and reliability (54–56). A validated 24-item Italian version of the PPCI was used in this study (57).

Psychological Adjustment to Diabetes. The Diabetes Attitude Questionnaire (ATT19) (58) is a 19-item self-report questionnaire designed to assess the emotional adjustment to diabetes and to evaluate the extent to which diabetes is integrated into the patient's lifestyle and personality (e.g., I dislike to be referred as a diabetic; Most people would find it difficult to have diabetes). Higher scores indicate that patients are more likely to be well-adjusted to their chronic illness. A number of studies have examined the ATT's psychometric characteristics and have shown sound reliability and validity (59–61), including an Italian validation study (62).

Perception of Illness. The Brief Illness Perception Questionnaire (Brief IPQ) (63) is a self-report questionnaire composed of eight items aimed at assessing children's cognitive representation (e.g., How long do you think your illness will continue?) and emotional representation (e.g., How concerned are you about your illness?) of their illness, as well as their illness comprehensibility (e.g., How well do you feel you understand your illness?). Higher scores suggest stronger perceptions along that dimension. Several studies have demonstrated that the Brief IPQ has good reliability (64) and overall good psychometric properties (65, 66). A validated Italian version of the Brief IPQ was used in this study (67).

Self-Efficacy in Diabetes Management. The Diabetes Management Self-Efficacy Scale (DMSES) (68) is a 15-item self-report measure that assesses the individual's confidence in self-management of diabetes activities (e.g., to what extent do you feel able to "keep my weight under control;" "adjust my diet when increasing exercise"). Higher scores indicate higher levels of perceived self-efficacy. The DMSES has been validated in several languages and countries, demonstrating acceptable reliability and validity (69–71). A validated Italian version of the DMSES was used in the present study (72).

Parents

Diabetes Treatment Satisfaction. The Diabetes Treatment Satisfaction Questionnaire for parents (DTSQ-parent) (73) is a parent-report measure designed to assess parents' satisfaction with the current treatment of their children. It consists of 14 items concerning general diabetes treatment satisfaction (e.g., How satisfied are you with your child's current treatment?, How easy or difficult is your child's diabetes treatment?), the perceived frequencies of hypoglycemia (e.g., How often have you felt that your child's blood sugars have been too low lately?), and perceived diabetes control and effects on parents' lives (e.g., How well-controlled do you feel your child's diabetes has been lately?). A higher score indicates greater satisfaction. The DTSQ is used internationally to measure treatment satisfaction and has been proven to have good psychometric properties, including parent version (73–75).

Perceived Burden. The Problem Areas in Diabetes parent revised version questionnaire (PAID-PR) (76) is an 18-item measure of the perceived parental burden associated with caring for a child with diabetes (e.g., I feel "burned out" by the constant effort to manage diabetes). Higher scores indicate greater perceived distress and more parental burden. Studies on the psychometric properties of PAID-PR have shown good internal consistency, test–retest reliability, and concurrent validity (76–78). The present study adopted a validated Italian version of the PAID-PR that has also been used in previous Italian studies (52, 79).

Camp Setting

The summer camp was located in a seaside resort on the Cilento coast in southern Italy. It lasted 7 days and was supported by a contribution from a public fund. The facility was adequately equipped for camp purposes, with large rooms for educational activities to be held in groups and suitable space for sports and recreational activities (e.g., a beach).

In line with ADA recommendations (8), the medical staff was composed of one medical director (a physician with expertise in managing type 1 diabetes), one physician with an interest in diabetes, one medical resident, one dietitian with expertise in diabetes, and one psychologist. All staff were previously appropriately trained about routine diabetes management, issues related to lifestyle modification for T1D, signs and symptoms of hypo-/hyperglycemia, and the treatment of diabetes-related emergencies.

The day was organized as follows. About 1 h a day was planned after breakfast for specific educational activities and practical training in groups, designed to extend previous diabetes knowledge and to reinforce self control and self-management skills. Education sessions were directed by a physician (assisted by the medical resident) and by the psychologist and were held in traditional method (e.g., using slides, short films, illustrated handbooks, etc.). These activities consisted of interactive lectures and subsequent group discussion seminars about disease etiology and symptoms; insulin therapy and blood glucose monitoring; diet (carbohydrate measurements); recognition and management of hypoglycemia/hyperglycemia; the relationship connecting exercise, food intake, and insulin doses; the importance of diabetes control; disease evolution control; daily problems related to T1D management; difficulties in living with T1D; and stress management. All content was appropriately adjusted to the age of participants. The remaining hours of the morning and at least 2 h in the afternoon were devoted to recreational activities like going to the beach and sports (volleyball, soccer, etc.). After dinner, all youths participated in leisure activities planned by the facility staff (exhibitions, dances, music, etc.).

Before each meal (breakfast, lunch, dinner, pre-evening snack, and midnight), blood glucose levels were analyzed, and insulin doses were calculated by a member of the medical staff in

collaboration with the child and adapted to that day's meals on the basis of the previous day's values. Values were achieved by finger-prick blood sample tests. Additional blood glucose measurements were made if the youth reported symptoms ascribable to hypoglycemia. The nutritionist planned the diet for all participants according to their physical requirements, and it could be modified on the basis of their caloric intake daily needs.

Campers attended the camp program free of charge.

Study Design and Procedure

This study was a follow-up investigation. All participants signed an informed consent form before participating in any study-related activities. The study was approved by the local Ethics Committee. Participation was voluntary, and no incentives were offered.

The youth and their primary caregiver were seen at baseline (T0) and at 3 months (T1) after the camp. The primary caregiver was identified as the person who is most responsible for the daily care of the youth with T1D. Two weeks prior to the camp session, all registered campers were met at the clinic in order to inform them about the camp and to have the informed consent form signed by the parents (children provided assent to participate in the study) (baseline, T0). After clinicians gathered demographic and medical data, interviews and test administrations were conducted by a psychologist with a bachelor's degree in Psychology who was adequately trained in the techniques and who had prior experience with the instruments. Evaluations were made individually and anonymously in a quiet, comfortable room made available by the clinic. The order of questionnaire administration was randomly assigned. At T1, which was planned during a routine clinic visit, HbA1c measurements and questionnaire completion were conducted following the same procedure.

Statistical Analysis

Cronbach's alpha (α) was used to assess the homogeneity of the scales. Comparisons of means at two different time points (baseline–T1) were conducted separately for patients and parents using repeated-measures analyses of variance (ANOVA). Scores of changes were computed for HbA1c, BMI, and psychosocial measures. Results were considered significant at alpha = 0.05 for a two-sided test. Effect size was reported as partial Eta square. The statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) version 21.0 for Macintosh.

RESULTS

Sample Characteristics

Of the 20 camp attendees, 20 parent–youth dyads agreed to participate in the study. Twenty youths (eight boys) and their caregivers (one father) consented to the study and completed the 3-month post-camp follow-up study. One parent (mother) was excluded after failing to complete the full evaluation (post-test).

Table 1 presents the gender, age, duration of illness, HbA1c, BMI, and standardized BMI (z-BMI) of the participants.

Glycemic Control and z-BMI

As shown in **Table 2**, no significant clinical improvements were found in HbA1c values from before camp to after camp; HbA1c values were even found to increase after camp ($p = 0.010$). No significant differences were found in z-BMI values ($p = 0.085$).

Psychological Outcomes

Cronbach's alpha for all adopted measures (total score) demonstrated adequate internal consistency (PedsQL child self-report $\alpha = 0.873$; ATT19 $\alpha = 0.886$; PPCI–patient proxy report $\alpha = 0.852$; DMSES $\alpha = 0.899$; PedsQL parent proxy report $\alpha = 0.848$; PAID-PR $\alpha = 0.779$; PPCI–parent proxy report $\alpha = 0.738$; DTSQ $\alpha = 0.829$) except for Brief IPQ ($\alpha = 0.338$).

Youths

The first 3 months after camp, the youths' quality of life (according to youths' and parents' opinions) was not found to be significantly changed, as measured by the PedsQL 3.0 DM's subscales scores (Diabetes symptoms, Treatment barriers, Treatment adherence, Worry, Communication, all $p > 0.05$). From T0 to T1, strategies employed by youths to cope with pain (according to youths' and parents' perspectives) were found not substantially changed, as measured by PPCI's subscales (Cognitive self-instruction, Problem solving, Distraction, Social support seeking, Catastrophizing/helplessness, all $p > 0.05$). According to parents' evaluations, social support seeking as a pain coping strategy was slightly increased ($p = 0.044$) after their child participated in the camp.

Similarly, patients did not report significant improvements in their adjustment to diabetes (ATT19 scores $p > 0.05$), confidence in self-care management of their diabetes (Disease management and Lifestyle management DMSES subscales, $p > 0.05$), or perception of disease (as Illness Cognitive/Emotional Representations and Comprehensibility IPQ scores subscales, $p > 0.05$).

In **Table 2**, the mean values and effect sizes for HbA1c, z-BMI, and psychological measures for participants at each time point are shown.

Parents

From T0 to T1, all parents' psychological scores remained stable. In particular, after their child participated in the camp, parents did not report significant improvement in diabetes burden perception or in treatment satisfaction, according to PAID-PR scores and DTSQ subscales scores, respectively (general Treatment satisfaction, Perceived diabetes control, Perceived hypoglycemia, all $p > 0.05$).

In **Table 3**, the mean values and effect sizes of the psychological measures for participants' parents at each time point are shown.

DISCUSSION

This study aimed to investigate the psychological outcomes for patients and their caregivers after the youths participated in a 1-week diabetes camp. It seeks to contribute to the scientific

TABLE 2 | Means (*SD*) for Hb1Ac, z-BMI, PedsQL, PPCI, ATT19, DMSES, and B-IPQ for participants ($N = 20$) at each time point.

Outcomes	Baseline (T0) *M* (*SD*)	3 months (T1) *M* (*SD*)	Overall changes Repeated measures ANOVA *F*	*p*	n^2
HbA1c	7.02 (0.77)	7.28 (0.84)	8.172	0.010	0.301
z-BMI	0.15 (1.08)	0.27 (1.05)	3.291	0.085	–
PedsQL self-report					
Diabetes symptoms	68.83 (14.11)	62.73 (12.84)	3.700	0.070	–
Treatment barriers	82.18 (16.75)	79.06 (20.41)	0.455	0.508	–
Treatment adherence	84.82 (15.75)	83.57 (17.93)	0.082	0.777	–
Worry	60.83 (24.65)	58.75 (24.55)	0.126	0.726	–
Communication	77.92 (24.82)	79.58 (20.32)	0.065	0.801	–
PPCI					
Cognitive self-instruction	3.8 (2.17)	3.65 (2.3)	0.051	0.823	.
Problem-solving	4.7 (2.62)	3.8 (2.55)	1.856	0.189	–
Distraction	6.9 (3.51)	7.4 (3.22)	0.492	0.491	–
Seek social support	5.7 (2.83)	5.45 (2.84)	0.216	0.647	–
Catastrophizing/helplessness	4.3 (1.69)	3.6 (1.31)	2.160	0.158	.
ATT19	61.2 (19.96)	66.2 (12.5)	2.408	0.137	–
DMSES					
Disease management	7.54 (1.63)	7.64 (1.23)	0.039	0.845	–
Lifestyle management	6.98 (2.1)	7.22 (1.58)	0.257	0.618	
B-IPQ					
Cognitive representations	37.21 (3.71)	37.63 (4.36)	0.302	0.589	
Emotional representations	12.26 (5.24)	12.89 (3.62)	0.540	0.472	
Comprehensibility	8.00 (2.75)	7.74 (1.69)	0.195	0.664	

Data are presented as mean values and standard deviations unless otherwise stated.
HbA1c, glycated hemoglobin; z-BMI, standardized body mass index; PedsQL, Pediatric Quality of Life Inventory; PPCI, Pediatric Pain Coping Inventory; ATT19, Diabetes Attitude Questionnaire; DMSES, Diabetes Management Self-Efficacy Scale; B-IPQ, Brief Illness Perception Questionnaire.

debate about the utility of diabetes camp in improving youths' adaptation to their illness.

Contrary to our hypothesis, the present findings showed that the camp experience was not associated with significant changes in youths' general psychological adjustment to diabetes or their perceived disease management, or in parents' overall distress regarding their child's diabetes management. In particular, as already found in previous research (14, 20–22), after youths attended summer camp, they did not report an improvement in quality of life nor were parents found to perceive their child's quality of life differently. Similarly, contrary to some previous studies (12, 17, 22–25) but consistent with other evidence indicating mixed results (26), youths did not report improvements in their ability to manage their diabetes, in their adjustment to illness, or in their diabetes perception after the camp experience.

In a comparison of pre-camp and post-camp values, a significant change following camp attendance was only found in youths' strategies to cope with pain according to the parents' perception. Specifically, after the camp, parents reported that their child had an increased tendency to cope with pain by seeking social support. Nevertheless, it should be noted that this finding was at borderline significance; therefore, this improvement cannot be considered a relevant change in general coping pain strategies adopted by youths after diabetes camp.

In terms of parents' perceived burden and treatment satisfaction, no significant changes were detected. It should be noted that few studies have explored and supported the beneficial effect of diabetes camp on parents' burden (22, 36), and no publications exist on longitudinal changes in parents' treatment satisfaction as an outcome of camp experience. As such, this is the first study evaluating this psychological dimension of parents, and it is potentially relevant to stimulating further research in this area.

In addition, HbA1c values were found to have actually increased after diabetes camp—albeit in an acceptable range, analogous to population data in similar age groups (80, 81)—supporting previous evidence that did not reveal any improvement in glycemic control after camp experience (21, 41).

Overall, this study does not provide empirical evidence of the benefits of participation in a diabetes camp, either in campers or in their parents, and it could be included among the studies in this research area reporting conflicting results about psychological positive outcomes of camp. In this regard, it should be noted that some authors have highlighted that an overall conclusion on the psychological outcomes of this experience is difficult to reach, due to huge variations in the methodological approach, the characteristics of study populations, and the definition of camp programs (15). Several methodological limitations (e.g., the lack of a control group or longer-term follow-up measures in most

TABLE 3 | Means (*SD*) for PedsQL, PPCI, PAID-PR, DTSQ for participants' parents ($N = 20$) at each time point.

Outcomes	Baseline (T0) *M* (*SD*)	3 months (T1) *M* (*SD*)	Overall changes Repeated measures ANOVA *F*	*p*	n^2
PedsQL parents' version					
Diabetes symptoms	63.16 (15.07)	65.6 (12.99)	0.822	0.377	–
Treatment barriers	68.42 (18.57)	64.8 (19.23)	0.397	0.536	–
Treatment adherence	74.47 (16.64)	77.76 (18.09)	0.573	0.459	–
Worry	44.74 (24.72)	37.72 (20.29)	1.209	0.286	–
Communication	79.38 (20.85)	73.25 (20.52)	0.939	0.345	–
PPCI					
Cognitive self-instruction	2.55 (1.54)	3.2 (1.15)	2.097	0.164	.
Problem-solving	4.7 (2.54)	5.65 (2.18)	3.537	0.075	–
Distraction	7.4 (3.86)	7.6 (3.59)	0.025	0.877	–
Seek social support	5.8 (2.14)	6.8 (1.64)	4.634	0.044	0.196
Catastrophizing/helplessness	3.95 (1.85)	4.4 (1.31)	1.222	0.283	–
PAID-PR	33.00 (14.49)	35.53 (12.46)	0.350	0.562	.
DTSQ					
Treatment satisfaction	42.58 (12.87)	42.11 (9.68)	0.023	0.880	.
Perceived diabetes control	6.95 (2.32)	6.63 (1.86)	0.178	0.678	
Perceived hypoglycemia	2.89 (1.61)	3.11 (1.23)	0.186	0.671	

Data are presented as mean values and standard deviations unless otherwise stated.
PedsQL, Pediatric Quality of Life Inventory; PPCI, Pediatric Pain Coping Inventory; PAID-PR, Problem Areas in Diabetes parent revised; DTSQ, Diabetes Treatment Satisfaction Questionnaire.

studies) prevent drawing robust conclusions about the positive impact on youths' psychosocial functioning and health (16).

However, the lack of improvement in children's general diabetes adjustment and perceived management as well as in parents' perceived burden and treatment satisfaction, as found in the present study, lead us to consider the extent to which these results may be related to the specific camp setting.

First, it could be supposed that the lack of any notable psychological benefit following camp participation may be associated with the absence of specific, structured psychological interventions in the camp activities. Given that the camp was not designed to offer psychological treatment to individuals, it is possible that the brief camp experience is not strong enough to be associated with changes in the psychosocial aspects that were examined here. Although diabetes camps may provide an opportunity for recreation and education that can help youths with diabetes better cope with the stresses related to diabetes management, only a structured psychological intervention specifically focused on improving psychosocial functioning may ensure effectiveness. As clearly highlighted in the International Society for Pediatric and Adolescent Diabetes' (ISPAD's) guidelines for psychological care of children and adolescents with T1D (1), an integrated approach to T1D management addressing psychological and physical management of diabetes, both for the youths and their parents, is recommended. Dedicated meetings lead by a psychologist should be included in camp activities and can be held every day, with the aim of discussing various topics, some suggested by the youths, concerning their main issues (e.g., recognizing emotions, daily problems related to T1D management, fears and difficulties of living with T1D); promoting emotional support among campers with similar experiences; and encouraging participants to share their experiences, to give and receive help, and to learn from others' experiences. This intervention could provide the opportunity to bond with others and to share the feelings (such as fear, shame, anger, etc.) that one can experience as a result of having T1D; it could also reinforce the opportunity to form relationships with peers. In addition, to improve long-term camp effects, it could be useful to provide continuous education and psychological intervention through periodical group psychoeducational sessions during clinic visits. Thanks to the support of the multidisciplinary team (diabetologist, psychologist, dietician, and nurse), these group sessions might allow clinicians to monitor and possibly reinforce the skills and knowledge acquired during camp. At the same time, they might also help children and parents to identify attitudes and behaviors that potentially affect good metabolic control, providing further insights for use in planning and organizing diabetes camps.

Second, the lack of camp activities focused on parents' needs may have played a role in the present findings.

It should be noted that other evidence in studies examining parents' diabetes-specific emotional distress after camp (22) and parents' general perceptions of the camp experience (21, 25, 26) overwhelmingly (except 35) came from studies on camps that only included youths as campers. Given how seriously diabetes management impacts parents' lives—as demonstrated by the stress and burden frequently reported in studies on caregivers, who are described as overwhelmed by the demands of their children's T1D (82, 83)—and given the positive association connecting parents' well-being, family dynamics, and children's

metabolic control (35), it is difficult to image significant changes in parents' diabetes perceptions after a camp experience without their direct involvement. Parent-oriented activities should be planned alongside camp activities. If the camp experience is conceived to also promote self-care skills and independence in management (especially from parental monitoring), it is possible to promote parent-oriented activities that do not necessarily require the parents to take part in group activities. In order to avoid negatively affecting the self-management experience provided by the camp, these activities should be appropriately scheduled and possibly carried out in a dedicated location.

Finally, an explanation of these results should also consider that the duration of the camp was only 1 week long and that data were collected at only one time point (after 3 months). We do not know whether the length of the camp was too brief to observe significant changes following the experience and/or whether possible changes in diabetes improvement might be identifiable over a longer time period. Collecting data not only before and 3 months after camp but also at several months to years after camp ended might have allowed us to identify potential changes. Additionally, no activities after the camp were organized to continue to reinforce the skills learned during camp. It could be hypothesized that youths, lacking an opportunity to strengthen their new learning, may have lost the skills as soon as they returned home.

Generally, the present findings must be interpreted with caution, due to some methodological limitations of the study. First, the self-selection aspect of the participants' recruitment may have introduced sample selection bias. Additionally, the final sample size was small, and all participants attended the same pediatric diabetology service; thus, it is not representative of the entire population. This limitation affects the generalizability of the findings and the external validity of the study. Furthermore, neither the time of diagnosis nor the age at diagnosis were considered nor a comparison or control group of youths who did not attend camp. Moreover, even though only those who attended the camp for the first time were included in this study, so that their responses could not biased by previous experiences, this study relied on self-report data; therefore, subjective perceptions of behaviors, thoughts, and feelings might not have been sincerely, accurately, or fully revealed.

Further research is necessary to address these limitations and to expand knowledge on the psychological experience of patients and families associated with youths' camp experiences.

Despite these limitations, the present results have important theoretical and practical implications. In its attempt to update and expand the existing literature on the benefits of summer camp experiences for youths with T1D, this study sheds light on the need to further analyze themes for how to make camp an experience that enhances the youth's attitude toward their illness and their confidence in diabetes management. Conflicting literature results on camp efficacy fail to answer the question of which aspects of programs should be altered and which elements make programs successful or unsuccessful. Even though much evidence supports the positive effects of camp experiences, the elements responsible for that success have largely remained unclear, leaving this issue as an unresolved problem. From a practical point of view, the present findings clearly indicate that, in addition to didactic and interactive child-centered education, it is also important to involve a psychologist in order to better structure possible activities. Clinical psychologists should play a role in designing camp curricula and in determining, together with medical team, how the entire camp program/organization might be adapted to meet the needs of campers, potentially according to age groups. Psychologists should also play a role in evaluating camping programs and making the information obtained from such program evaluations available to camp staff and other members of the care team (26).

An important next step in this line of research is longitudinal research assessing whether camp participation is related to long-term improvement outcomes in self-management and in psychosocial functioning, as this will provide valuable information for designing diabetes camp programs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Commitee of University of Campania "Luigi Vanvitelli". Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AT designed the study, analyzed the data, and wrote the manuscript. DI supervised this work, designed the study, and contributed to the manuscript. CC, AC, and AZ collected data and contributed to the data analyses and to the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The research leading to these results received funding from the project, DiabEaT1, which received funding from University of Campania "Luigi Vanvitelli" through the program V:ALERE 2019, funded with D.R. 906 del 4/10/2019, prot. n. 157264, October 17, 2019.

ACKNOWLEDGMENTS

The authors thank the staff of the outpatient clinic at the pediatric diabetes unit G. Stoppoloni at the University of Campania "Luigi Vanvitelli" for their help in data collection, in particular Dr. Marianna Capasso, Dr. Serena Rollato, Dr. Stefano Curto, Dr. Vittorio Picone, Dr. Veronica Testa, and all the patients, parents, and children who agreed to take part in the investigation.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past collaboration with the authors DI and AZ.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 27 April 2021
doi: 10.3389/fendo.2021.653519

10-Year Incidence of Diabetic Ketoacidosis at Type 1 Diabetes Diagnosis in Children Aged Less Than 16 Years From a Large Regional Center (Hangzhou, China)

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OPEN ACCESS

Edited by:
Valentino Cherubini,
Azienda Ospedaliero Universitaria Ospedali Riuniti, Italy

Reviewed by:
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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 14 January 2021
Accepted: 23 March 2021
Published: 27 April 2021

Citation:
Peng W, Yuan J, Chiavaroli V, Dong G, Huang K, Wu W, Ullah R, Jin B, Lin H, Derraik JGB and Fu J (2021) 10-Year Incidence of Diabetic Ketoacidosis at Type 1 Diabetes Diagnosis in Children Aged Less Than 16 Years From a Large Regional Center (Hangzhou, China). Front. Endocrinol. 12:653519. doi: 10.3389/fendo.2021.653519

Background: Diabetic ketoacidosis (DKA) is a potentially life-threatening complication of type 1 diabetes (T1D), and a leading cause of death in children aged <15 years with new-onset T1D.

Aims: i) to assess the incidence of DKA in children and adolescents newly diagnosed with T1D over a 10-year period at a large regional center in China; and ii) to examine the clinical symptoms and demographic factors associated with DKA and its severity at diagnosis.

Methods: We carried out a retrospective audit of a regional center, encompassing all youth aged <16 years diagnosed with T1D in 2009–2018 at the Children's Hospital, Zhejiang University School of Medicine (Hangzhou, China). DKA and its severity were classified according to ISPAD 2018 guidelines.

Results: 681 children were diagnosed with T1D, 50.1% having DKA at presentation (36.0% mild, 30.0% moderate, and 33.9% severe DKA). The number of patients diagnosed with T1D progressively rose from approximately 39 cases/year in 2009–2010 to 95 cases/year in 2017–2018 (≈2.5-fold increase), rising primarily among children aged 5–9 years. DKA incidence was unchanged but variable (44.8% to 56.8%). At T1D diagnosis, 89% of patients reported polyuria and 91% polydipsia. Children presenting with DKA were more likely to report vomiting, abdominal pain, and particularly fatigue. DKA was most common among the youngest children, affecting 4 in 5 children aged <2 years (81.4%), in comparison to 53.3%, 42.7%, and 49.3% of patients aged 2–4, 5–9, and ≥10 years, respectively. Children with severe DKA were more likely to report vomiting, fatigue, and abdominal pain, but less likely to report polyuria, polydipsia,

and polyphagia than those with mild/moderate DKA. Rates of severe DKA were highest in children aged <2 years (51.1%).

Conclusions: The number of children diagnosed with T1D at our regional center increased over the study period, but DKA rates were unchanged. With 9 of 10 children reporting polyuria and polydipsia prior to T1D diagnosis, increasing awareness of this condition in the community and among primary care physicians could lead to earlier diagnosis, and thus potentially reduce rates of DKA at presentation.

Keywords: DKA, complications, symptoms, children, adolescents

INTRODUCTION

The incidence of type 1 diabetes (T1D) has been increasing worldwide (1–3). Diabetic ketoacidosis (DKA) is a potentially life-threatening complication of T1D that occurs with severe insulin deficiency, consisting of hyperglycemia, ketosis, and metabolic acidosis (4). This acute condition is responsible for most of the diabetes-related morbidity and mortality in affected children (5, 6). Indeed, recent data showed that DKA remains the leading cause of death in individuals aged <15 years newly diagnosed with T1D (7).

Worldwide, rates of DKA at T1D presentation vary markedly, ranging from 13% in Sweden to 80% in the United Arab Emirates (8). DKA is mainly a result of the delay in diagnosing T1D, and the concurrent failure to start appropriate insulin replacement. Therefore, DKA is a metabolic complication that is relatively easily avoidable, since it primarily reflects lack of awareness of T1D symptoms (4). Other risk factors for DKA at diabetes presentation include young age, minority ethnic groups, lower socioeconomic status, limited access to medical services, lack of medical insurance, and absence of first-degree relatives with T1D (4, 9–11). Note that, in contrast to what occurs at diagnosis, insulin omission (either inadvertently or deliberately) is the main cause of recurrent DKA (7).

DKA is associated with a large number of clinical symptoms, which include dehydration, nausea and/or vomiting, abdominal pain that may mimic an acute abdominal condition, thrombotic events, drowsiness, brain swelling, and coma (7). Although uncommon, severe brain swelling is associated with 20-30% mortality (12–14). Children with DKA may need prolonged hospital stay, with severe DKA often requiring intensive care admission. Thus, it is important to assess the epidemiology of DKA and identify the associated risk factors.

A recent study examined the incidence of T1D in 13 different regions across China (15). The authors reported a DKA rate of approximately 51.4% within 6 months of diagnosis among children aged ≤14 years, but no data from Zhejiang province were included in that study (15). Further, there are still relatively few studies comparing the clinical characteristics of patients newly diagnosed with T1D in relation to DKA severity. Therefore, we assessed the incidence of DKA among children and adolescents newly diagnosed with T1D at a regional centre in Zhejiang province over a 10-year period. In addition, we examined the clinical symptoms and demographic factors associated with the likelihood of DKA and its severity at diagnosis.

METHODS

Ethics Approval

This study was approved by the Medical Ethics Committee of the Children's Hospital, Zhejiang University School of Medicine. Written or verbal informed consent from individual patients was not required, as this study involved an audit of data from routine clinical practice based on de-identified data.

Participants

Participants were all children aged <16 years diagnosed with T1D over a 10-year period (between 1 January 2009 and 31 December 2018) at the Children's Hospital, Zhejiang University School of Medicine. The hospital is located in Hangzhou (the capital of Zhejiang province), a large city whose population increased from 6.89 million in 2010 to 7.74 million in 2018; during the same period the population of children and adolescents aged ≤17 years increase from 1.05 million to 1.33 million (+27%) (16, 17). The Children's Hospital is one of only two National Clinical Research Centres for Child Health in China, recording 81,000 inpatient and 3.5 million outpatient visits per year. It provides specialized care for children with diabetes in Zhejiang Province.

Study Parameters

T1D was diagnosed based on clinical and biochemical features: all patients had elevated blood glucose at presentation (a random measurement >11.1 mmol/l and/or fasting blood glucose >7.1 mmol/l), and with classical symptoms of diabetes. Further, all patients met at least one of the following criteria: 1) DKA; 2) presence of T1D-associated antibodies (glutamic acid decarboxylase, islet antigen 2, islet cell, or insulin autoantibodies); and/or 3) on-going requirement for insulin therapy.

Family and personal medical history prior to diagnosis was recorded for all children. A range of demographic information and data on clinical symptoms were collected at diagnosis from interviews with the parent(s) and patient. Clinical symptoms recorded included polyuria (excessive urination), polydipsia (excessive thirst), polyphagia (excessive eating), anepithymia (loss of appetite), weight loss, vomiting, fatigue, and abdominal

pain. All patients were weighed at presentation, but height at diagnosis was not consistently measured over the study period, therefore body mass index (BMI) could not be calculated. Nonetheless, weight data were converted into standard deviation scores (SDS) as per World Health Organization standards (18, 19).

Participants underwent blood tests, and recorded parameters of interest were pH, bicarbonate, and glycated hemoglobin (HbA1c). DKA at diagnosis was defined according to ISPAD 2018 guidelines as the combination of ketosis, hyperglycemia, and acidosis (venous pH <7.3 or bicarbonate <15 mmol/L) (20). DKA was further classified as mild (venous pH <7.3 or bicarbonate <15 mmol/L), moderate (pH <7.2 or bicarbonate <10 mmol/L), or severe (pH <7.1 or bicarbonate <5 mmol/L). Antibody positivity was based on the presence of islet antigen 2 and/or islet cell autoantibodies.

Subsequently, hospitalization data (such as length of stay in hospital and the estimated cost of treatment) were obtained from hospital records.

It should be noted that all patients with DKA in our study were treated following the latest protocol as per ISPAD Clinical Practice Consensus Guidelines (20–22).

Statistical Analyses

Data on demographic characteristics and clinical symptoms were compared between participants with and without DKA at T1D diagnosis using one-way ANOVA, Fisher's exact tests, or non-parametric Kruskal-Wallis tests, as appropriate. Similar analyses were run comparing the three groups with DKA according to severity (i.e. mild vs moderate vs severe DKA), while differences in DKA rates among age groups (<2, 2–4.99, 5–9.99, and ≥10 years) were assessed using Fisher's exact tests.

A generalized linear regression model was run to examine the associations between key demographic factors and the likelihood of having DKA at T1D diagnosis. The model included the following predictors: family history of T1D (yes vs no) and sex (male vs female) as categorical variables; and age at diagnosis and year of diagnosis as covariates. Results are reported as the adjusted relative risks (aRR) and respective 95% confidence intervals.

Analyses were performed using SPSS v25 (IBM Corp, Armonk, NY, USA), SAS v9.4 (SAS Institute, Cary, NC, USA), and Minitab v16 (Pennsylvania State University, State College, Pennsylvania, USA). All statistical tests were two-tailed with the significance level maintained at $p<0.05$. Figures were created using GraphPad Prism v8.2.1 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

A total of 681 children and adolescents aged between one month and 15.8 years (314 boys and 367 girls) were diagnosed with new-onset T1D over the 10-year period, all of whom were Han Chinese. There was a progressive increase in the number of patients aged <16 years diagnosed with T1D, rising from approximately 39 cases per annum in 2009–2010 to 95 cases per annum in 2017–2018, i.e. a near 2.5-fold increase (**Figure 1A**). Notably, the increase in the number of new T1D cases occurred mostly among children aged 5–9 years (**Figure 2**).

DKA

Half of all new cases of T1D diagnosed had DKA (n=341; 50.1%) (**Table 1**). Across the 10-year period, there was no change in the incidence of DKA (p=0.31), with variable yearly rates ranging from 44.8% to 56.8% (**Figure 1A**). Rates of DKA were also highly variable across all age groups, with no evidence of a change in incidence within these groups (**Figure 2**).

A slightly higher proportion of children without DKA had a family history of diabetes (27.1% vs 30.6%; **Table 1**). The likelihood of DKA was not associated with the sex of the child (p=0.77; **Table 1**), but increasing age was associated with lower risk of having DKA at diagnosis, which decreased by 4% per additional year of age [aRR 0.96 (95% CI 0.94, 0.98); p=0.0003]. Thus, on average, children with DKA were slightly younger

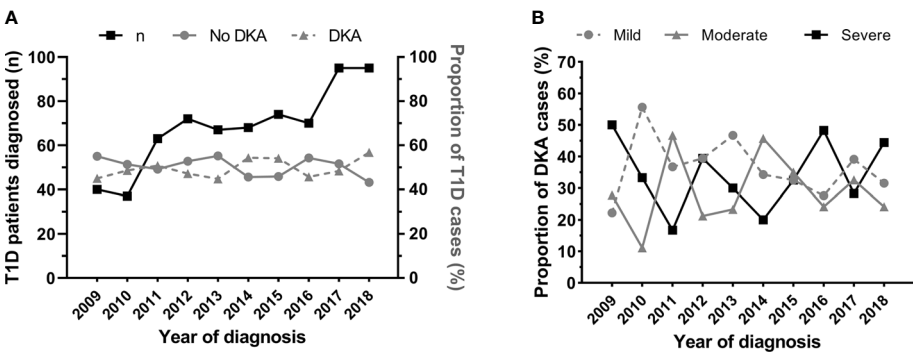

FIGURE 1 | Number of patients aged <16 years newly diagnosed with type 1 diabetes (T1D) at the Children's Hospital of Zhejiang University School of Medicine (Hangzhou, China) in 2009-2018, and rates of diabetic ketoacidosis (DKA) at diagnosis. **(A)** Number of patients newly diagnosed with T1D (black line, scale on left *y* axis) and the respective rate of DKA (solid grey line, scale on right *y* axis) and no-DKA (dashed grey line, scale on right *y* axis) at diagnosis. **(B)** Proportion of patients with mild (dashed grey line), moderate (solid grey line), and severe (black line) DKA at T1D diagnosis.

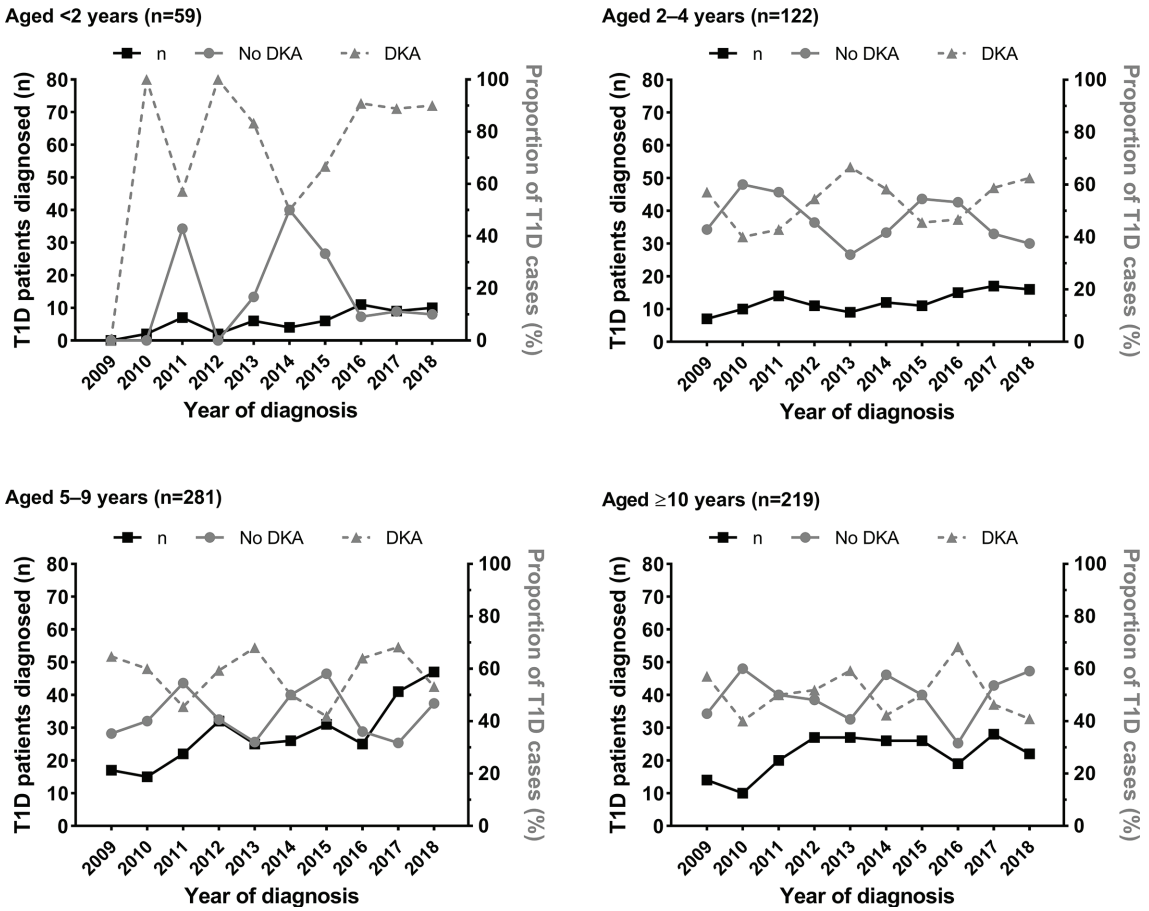

FIGURE 2 | Number of patients aged <16 years newly diagnosed with type 1 diabetes (T1D) at the Children's Hospital of Zhejiang University School of Medicine (Hangzhou, China) in 2009-2018 according to age group, and rates of diabetic ketoacidosis (DKA) at diagnosis. Data are the number of patients newly diagnosed with T1D (black line, left y axis), and the respective rates of DKA (solid grey line, right y axis) and no-DKA (dashed grey line, right y axis) at T1D diagnosis.

(-0.8 years; p=0.001), and were also 0.34 SDS lighter (p=0.0001) (**Table 1**). DKA was particularly common among the youngest group of children, present in more than 4 in 5 children aged <2 years (81.4%; 48/59) at T1D diagnosis, compared to 53.3%, 42.7%, and 49.3% of patients aged 2–4, 5–9, and ≥10 years, respectively (p<0.0001; **Figure 3A**).

Overall, 89% of patients experienced polyuria and 91% polydipsia prior to T1D diagnosis. Children who presented with DKA were markedly more likely to report vomiting, abdominal pain, and in particular fatigue that was reported by ≈40% of them (**Table 1**). Conversely, reported rates of polyuria, polydipsia, polyphagia, and weight loss were similar in patients with or without DKA (**Table 1**). Children with DKA had a median hospital stay that was 1 day longer, and incurred median hospitalization costs that were 24% higher (**Table 1**).

DKA Severity

A total of 120 children had mild DKA (36.0%), 100 children had moderate DKA (30.0%), and 113 children had severe DKA (33.9%) (**Table 2**). The proportion of children with severe DKA did not change over the study period, with a highly marked (and apparently random) variation observed from year to year (**Figure 1B**).

Children with severe DKA were more likely to report vomiting, fatigue, and abdominal pain than those with mild or moderate DKA (**Table 2**). Conversely, children with severe DKA were less likely to report the classical symptom of T1D at diagnosis than children with mild or moderate DKA, such as polyuria, polydipsia, polyphagia, and weight loss (**Table 2**).

The proportion of cases according to DKA severity varied among age groups, with rates of severe DKA highest in children aged <2 years (n=23/45; 51.1%) (**Figure 3B**).

Note that there were no deaths recorded in association with DKA at presentation.

DISCUSSION

Over the 10-year study period (2009–2018), the number of children aged <16 years newly diagnosed with T1D increased

TABLE 1 | Demographic and clinical data at type 1 diabetes (T1D) diagnosis in children and adolescents at the Children's Hospital of Zhejiang University School of Medicine (Hangzhou, China), according to their diabetic ketoacidosis (DKA) status.

		All patients	No DKA	DKA	*P*-value
n (%)		681	340 (49.9%)	341 (50.1%)	
Demography	**Age (years)**	7.9 [4.7, 10.8]	8.2 [5.8, 10.9]	7.4 [3.6, 10.7]	**0.001**
	Sex (females)	367 (53.9%)	183 (53.8%)	184 (54%)	>0.99
	Family history of T1D	184 (27.1%)	104 (30.6%)	80 (23.5%)	**0.047**
Anthropometry	**Weight SDS**	-0.50 ± 1.15	-0.33 ± 1.09	-0.67 ± 1.18	**0.0001**
Symptoms at diagnosis	**Polyuria**	603 (88.5%)	307 (90.3%)	296 (86.80%)	0.19
	Polydipsia	620 (91.0%)	311 (91.5%)	309 (90.6%)	0.79
	Polyphagia	193 (28.3%)	103 (30.3%)	90 (26.4%)	0.27
	Weight loss	345 (50.7%)	172 (50.6%)	173 (50.7%)	>0.99
	Vomiting	50 (7.3%)	5 (1.5%)	45 (13.2%)	**<0.0001**
	Anepithymia	14 (2.1%)	3 (0.9%)	11 (3.2%)	0.055
	Fatigue	153 (22.5%)	19 (5.6%)	134 (39.3%)	**<0.0001**
	Abdominal pain	35 (5.1%)	4 (1.2%)	31 (9.1%)	**<0.0001**
Biochemical parameters	**pH**	7.31 ± 0.13	7.39 ± 0.04	7.22 ± 0.14	**<0.0001**
	Bicarbonate (mmol/l)	16.0 ± 7.0	21.1 ± 3.1	10.9 ± 6.1	**<0.0001**
	HbA1c (%)	12.47 ± 2.06	12.37 ± 2.14	12.57 ± 1.98	0.21
	HbA1c (mmol/mol)	113 ± 23	112 ± 23	114 ± 22	0.21
Hospitalization	**Length of stay (days)**	8 [7, 10]	8 [6, 10]	9 [7, 11]	**<0.0001**
	Cost of treatment (RMB)[†]	4938 [4134, 5991]	4436 [3782, 5219]	5524 [4532, 6664]	**<0.0001**
	Cost of treatment (USD)[‡]	765 [640, 928]	687 [586, 808]	856 [702, 1032]	**<0.0001**

Data are n (%), mean ± standard deviation, or median [quartile 1, quartile 3], as appropriate.

P-values refer to comparisons between children with and without DKA at T1D diagnosis, with p-values for statistically significant differences at p<0.05 shown in bold.

HbA1c, glycated hemoglobin; RMB, Chinese Yuan; SDS, standard deviation score; USD, United States dollars.

[†]Original recorded costs, unadjusted for inflation.

[‡]Conversion to USD accurate as of 6 January 2021.

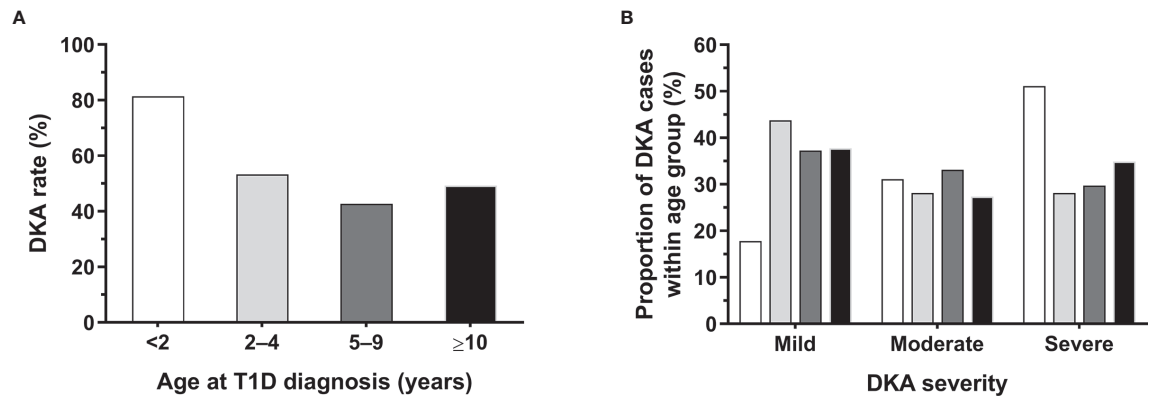

FIGURE 3 | Rates and severity of diabetic ketoacidosis (DKA) among patients aged <16 years newly diagnosed with type 1 diabetes (T1D) at the Children's Hospital of Zhejiang University School of Medicine (Hangzhou, China), according to age group. **(A)** Percentage of patients with DKA according to age at diagnosis. **(B)** Proportion of cases according to DKA severity within age group, whose corresponding bars are color coded as per panel **(A)**; the number of patients who had DKA at T1D diagnosis and data on its severity in each age group was: <2 years, n=45; 2–4 years, n=64; 5–9 years, n=118; and ≥10 years, n=106.

markedly at our large clinical centre in Hangzhou (China), with the observed increase recorded mostly among children <10 years of age. However, DKA rates were unchanged (although variable). Of note, half of all new cases of T1D presented with DKA, of which two-thirds were moderate or severe, and markedly more common in younger children (aged <2 years).

Stable rates of DKA (without a decline) have been reported in several countries (10, 23–25). For instance, despite an increase in the incidence of children newly diagnosed with T1D in Austria in 1989-2008, DKA rates at diabetes onset were unchanged (24). Similarly, the incidence of DKA was unchanged among children with new-onset T1D in Auckland (New Zealand) over a 15-year period (1999–2013) (10). Consistently with these findings, DKA rates at our regional centre were largely unchanged, despite the increasing number of youth diagnosed with T1D over the study period. Conversely, in a retrospective study of youth newly diagnosed with T1D in New York (USA) in 2010–2013, there was a modest decline in the rate of DKA compared to a similar study 15 years earlier (from 38% to 29%) (26). More recently, a comprehensive nationwide study in Italy also reported a slight reduction in average DKA rates at T1D diagnosis from 40.3% in 2004–2013 to 36.9% in 2014–2016,

TABLE 2 | Demographic and clinical data at type 1 diabetes (T1D) diagnosis in children and adolescents at the Children's Hospital of Zhejiang University School of Medicine (Hangzhou, China), according to diabetic ketoacidosis (DKA) severity.

		Mild DKA	Moderate DKA	Severe DKA	*P*-value
n		120 (36.0%)	100 (30.0%)	113 (33.9%)	
Demography	**Age (years)**	7.3 [4, 10]	7.7 [4, 11]	7.4 [3, 11]	0.67
	Sex (females)	57 (47.5%)	61 (61.0%)	61 (54.0%)	0.14
	Family history of T1D	29 (24.4%)	29 (29.0%)	21 (18.6%)	0.20
Anthropometry	**Weight SDS**	-0.57 ± 1.21	-0.74 ± 1.07	-0.78 ± 1.24	0.36
Symptoms at diagnosis	**Polyuria**	113 (94.2%)	92 (92%)	86 (76.1%)	**<0.0001**
	Polydipsia	115 (95.8%)	97 (97%)	92 (81.4%)	**<0.0001**
	Polyphagia	42 (35.0%)	27 (27.0%)	19 (16.8%)	**0.007**
	Weight loss	75 (62.5%)	52 (52.0%)	44 (38.9%)	**0.001**
	Vomiting	8 (6.7%)	9 (9.0%)	27 (23.9%)	**0.0003**
	Anepithymia	2 (1.7%)	3 (3.0%)	5 (4.4%)	0.48
	Fatigue	21 (17.5%)	42 (42.0%)	68 (60.2%)	**<0.0001**
	Abdominal pain	8 (6.7%)	3 (3.0%)	19 (16.8%)	**0.002**
Biochemical parameters	**pH**	7.31 ± 0.06	7.23 ± 0.09	7.12 ± 0.17	**<0.0001**
	Bicarbonate (mmol/l)	14.3 ± 3.6	9.8 ± 5.2	7.7 ± 6.7	**<0.0001**
	HbA1c (%)	12.74 ± 2.14	12.32 ± 1.72	12.73 ± 2.01	0.23
	HbA1c (mmol/mol)	116 ± 23	111 ± 19	116 ± 22	0.23
Hospitalization	**Length of stay (days)**	8 [7, 10]	9 [8, 11]	9 [8, 11]	**0.008**
	Cost of treatment (RMB)†	4915 [4191, 5986]	5612 [4545, 6766]	6302 [5230, 8232]	**<0.0001**
	Cost of treatment (USD)‡	761 [649, 927]	869 [704, 1048]	976 [810, 1275]	**<0.0001**

Data are n (%), mean ± standard deviation, or median [quartile 1, quartile 3], as appropriate.

P-values for statistically significant differences overall at p<0.05 are shown in bold.

HbA1c, glycated hemoglobin; RMB, Chinese Yuan; SDS, standard deviation score; USD, United States dollars.

Information on DKA severity was missing for 8 of the 341 cases of new-onset T1D who had DKA at presentation.

†Original recorded costs, unadjusted for inflation.

‡Conversion to USD accurate as of 6 January 2021.

with a more marked reduction observed among children aged <5 years (27). A greater decline was reported in Saudi Arabia, from 55.1% to 32.5% between 2005 and 2015 (28). In contrast, in Colorado (USA), DKA rates increased from 41% to 58% during 2010-2017 (29), while in Malaysian children DKA rates increased from 54.5% to 66.7% in 2000–2009 (30). The reasons for these contrasting changes in DKA rates in different countries are unclear, but higher rates are generally associated with reduced community awareness of diabetes symptoms and decreased access to health care services (8, 31). Of note, our observed DKA rate (50.1% overall) was nearly identical to that reported from 13 areas across China (51.4%), although the latter figure referred to DKA up to 6 months since diagnosis (15).

In children with new-onset T1D, young age has been identified often as an important risk factor for DKA at diabetes presentation (4, 10, 27). Studies in Canada, Italy, and the UK have reported higher rates of DKA at T1D diagnosis in young children aged 0–4 years (25, 27) and <2 years (28), respectively. A meta-analysis involving 32 studies found that children aged <2 years of age had 3 times the risk of presenting with DKA compared to older children (32). In line with these studies, we observed that DKA was more common among the youngest children (<2 years of age), who also had the highest rates of severe DKA. The latter observation is in agreement with the published evidence, as DKA at diagnosis in younger children (especially <2 years of age) is usually more severe, and it is often a consequence of delayed treatment or diagnostic error (1–3, 27, 33).

In our study, children with severe DKA were less likely to report the classical symptom of T1D at diagnosis than children with mild or moderate DKA (i.e. polyuria, polydipsia, polyphagia, and weight loss). As the youngest group of children were over-represented amongst those with DKA, the reported differences might have resulted from an increased difficulty to recognize polydipsia or polyuria in very young children (10). Conversely, vomiting, anepithymia, fatigue, and abdominal pain were common symptoms among children with severe DKA. In this context, it is important to be aware of both typical and less typical symptoms of T1D in children, as early recognition of this condition allows for a timely diagnosis, which in turn minimizes the risk of DKA.

To this regard, community educational campaigns to prevent DKA have been proposed as a way of increasing awareness about T1D symptoms and related acute complications, in both parents/caregivers and health care practitioners (34–39). For instance, the 'Parma campaign' (Italy), which delivered posters promoting the link between enuresis, polyuria, vomiting, abdominal pain, and diabetes to schools, parents, and pediatric practices, was associated with a marked reduction in DKA incidence at diagnosis (from 78% to 12.5% over two years) (39). Recently, the Stuttgart Ketoacidosis Awareness Campaign (Germany) also focused on the typical clinical symptoms of T1D and reduced the incidence of DKA (from 28% to 16% over three years) (35). However, not all awareness campaigns have been successful in reducing DKA rates at T1D diagnosis, achieving limited or no impact (34, 36). In addition, mixed findings have also been observed. In Italy, Rabbone et al. reported 2-year nationwide data

on DKA incidence among children and adolescents aged 0–18 years, soon after the initiation of a national awareness campaign (40). Surprisingly, while the overall DKA rate increased (from 38.5% in 2012–2014 to 47.6% in 2016-2017), it decreased markedly in children aged <6 years (73.8% vs 52.5%); further, in contrast to the latter observation, the rate of severe DKA actually increased among these preschoolers (from 16.6% to 21.7%) (40). Nonetheless, the key strategy for campaign success seems to be close cooperation among families, school teachers, and health care practitioners, in particular primary health care providers, such as family pediatricians as in the Italian campaign (40). In any case, our study corroborates the relevance of focusing on the classical symptoms of T1D in such campaigns (34, 40), as 89% of our patients experienced polyuria and 91% polydipsia prior to T1D diagnosis.

Apart from potential adverse neurocognitive outcomes (41, 42), DKA can also lead to death, being associated with a mortality rate <1% (8, 43). The recent study reporting on DKA at T1D diagnosis in 13 different regions across China recorded two deaths as a result of DKA among 5018 patients (15), i.e. a mortality rate of 0.04%. In our study, there were no recorded fatalities among our 681 patients.

The main limitation of the present study was the lack of key demographic data, such as access to medical services and medical insurance, and in particular socioeconomic status, as numerous studies have reported that greater socioeconomic deprivation is associated with an increased risk of DKA at T1D diagnosis (9, 11, 27, 32, 44–51). Of note, a number of studies have shown an increased risk of DKA among ethnic minorities (11, 23, 45, 50, 52), and since ethnicity and socioeconomic status are strongly intertwined (53), our homogeneous cohort consisting solely of Han Chinese patients likely mitigated some of the potential effects of socioeconomic status. Further, a key strength of our study was the relatively large number of patients examined from a large city in China over a 10-year period. In addition, all our patients presented to a single large center, so that they were all attended to according to the same treatment protocol.

In conclusion, we observed an increasing number of children being diagnosed with T1D over the study period at our large regional centre. While the rates of DKA were unchanged overall, they remained relatively high. With approximately 9 of 10 children reporting the two main clinical symptoms of T1D at diagnosis (i.e. polyuria and polydipsia), educational campaigns to increase awareness of this condition in the community and among primary care physicians could lead to earlier diagnosis, and thus potentially reduce the high rates of DKA at presentation in the region.

DATA AVAILABILITY STATEMENT

The clinical data supporting this article are not readily available because of the conditions of the ethics approval. The anonymized data on which this article was based could be made available to other investigators upon bona fide request, and following all the necessary approvals (including ethics) of the detailed study proposal and statistical analyses plan. Requests to access the datasets should be directed to Professor Junfen Fu (fjf68@zju.edu.cn).

ETHICS STATEMENT

This study was approved by the Medical Ethics Committee of the Children's Hospital, Zhejiang University School of Medicine. Written or verbal informed consent from individual patients was not required, as this study involved an audit of data from routine clinical practice based on de-identified data.

AUTHOR CONTRIBUTIONS

JF, JD, JY, HL, and BJ conceived and designed the study. JF, JY, GD, HL, KH, and WW carried out clinical assessments and collected the data. JY, HL, BJ, RU, JD, WP, GD, HL, KH, and WW contributed to data curation. JD analyzed the data. WP, VC, and JD wrote the manuscript with critical input from all other authors. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the National Key Research and Development Program of China (No. 2016YFC1305301); Fundamental Research Funds for the Central Universities (2020XZZX002-22); the Research Fund of Zhejiang Major Medical and Health Science and Technology & National Ministry of Health (WKJ-ZJ-1804), and Zhejiang Provincial Key Science and Technology Project (LGF21H070004).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

CASE REPORT
published: 29 April 2021
doi: 10.3389/fped.2021.661434

Case Report: Ophthalmologic Evaluation Over a Long Follow-Up Time in a Patient With Wolfram Syndrome Type 2: Slowly Progressive Optic Neuropathy as a Possible Clinical Finding

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Wolfram syndrome (WFS) is a rare autosomal recessive neurodegenerative disease whose diagnosis requires diabetes mellitus and optic atrophy (OA). WFS includes a wide spectrum of other possible complications such as diabetes insipidus, sensorineural deafness, urinary tract problems, neurological and psychiatric disorders. Most WFS patients show type 1 syndrome (WFS1) caused by mutations in the WFS1 gene, encoding Wolframin protein, while few patients are affected by WFS type 2 (WFS2) due to a pathogenetic variants in the CISD2 gene encoding an endoplasmic reticulum intermembrane small protein. WFS2 is considered a phenotypic and genotypic variant of WFS, from which differs only for the increased risk of bleeding and presence of peptic ulcers. OA and diabetes are considered cardinal features of WFS. We hereby report the ophthalmologic evaluation in a patient, previously described, with WFS2 after 8 years of follow-up. A 20-year-old white woman was referred to our retinal center for the first time in 2012 following a diagnosis of a novel intragenic exon 2 CISD2 homozygous deletion, for the suspicion of an associated bilateral OA. Fundus examination, spectral-domain optical coherence tomography, visual field, visual evoked potentials were performed and confirmed the presence of an optic neuropathy that remained stable over 8 years follow up. A slowly progressive optic neuropathy, rather than OA can characterize patients with WFS2 and CISD2 intragenic deletion.

Keywords: CISD2 gene, optic atrophy, optic neuropathy, neurodegeneration, non-autoimmune diabetes, Wolfram syndrome

OPEN ACCESS

Edited by:
Siri Atma W. Greeley, University of Chicago, United States

Reviewed by:
David Zangen, Hadassah Medical Center, Israel
Sulev Kõks, University of Tartu, Estonia
Lisbeth Tranebjærg, University of Copenhagen, Denmark

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *30 January 2021*
Accepted: *07 April 2021*
Published: *29 April 2021*

Citation:
Di Iorio V, Mozzillo E, Rosanio FM, Di Candia F, Genesio R, Testa F, Iovino C, Franzese A and Simonelli F (2021) Case Report: Ophthalmologic Evaluation Over a Long Follow-Up Time in a Patient With Wolfram Syndrome Type 2: Slowly Progressive Optic Neuropathy as a Possible Clinical Finding. Front. Pediatr. 9:661434. doi: 10.3389/fped.2021.661434

INTRODUCTION

Wolfram syndrome (WFS) is a rare autosomal recessive neurodegenerative disease characterized by a wide phenotypic spectrum including non-autoimmune diabetes mellitus (DM) (1), and optic atrophy (OA), often associated with diabetes insipidus (DI) and deafness, hence the historical acronym: DIDMOAD (2). Other common clinical signs include urinary tract problems and renal dysfunction, related to neurogenic bladder, endocrine disorders, severe gastrointestinal ulcers, psychiatric symptoms, and progressive neurological degeneration (2). The most frequent form of WFS type 1 (WFS1) includes mutations in the WFS1 gene, whereas the rare form of WFS type 2 (WFS2) involves the CISD2 gene. There is no clear genotype-phenotype correlation in this complex syndrome (3). WFS2 is distinguishable from WFS1 by the increased risk of bleeding, presence of peptic ulcers and the absence of DI, albeit a case of a patient with WFS2 and DI has been reported (4). WFS2 (MIM #604928) was firstly described in the year 2000 in a large consanguineous Jordanian family (5). Only in 2007 it was reported to be linked to a missense mutation in a novel gene, CISD2, mapping in 4q24 (6). The CISD2-encoded endoplasmic reticulum intermembrane small protein, does not interact directly with Wolframin, the WFS1 gene-encoded protein (6).

Our group reported the first Italian Caucasian girl with WFS2 (7), carrying a novel intragenic exon 2 CISD2 homozygous deletion from C-4OKFJ (102,885,416 bp) to C-6MGSK (102,886,154 bp), mapping on chromosome 4q24, showing optic neuropathy rather than OA, and impaired platelet aggregation to adenosine diphosphate and not to collagen, as described in the Jordanian family (5). Since this mutation had never been reported, consanguinity was hypothesized. Although patient's parents reported to be not consanguineous a microsatellite analysis confirmed the hypothesis of a common ancestor (7).

Later on, some authors reported other two Italian siblings with a novel homozygous CISD2 mutation mapping within the donor splice site of intron 1. Authors hypothesized that the alteration presumably would have caused a skipping of exon 1 with subsequent disrupting of the mRNA splicing by eliminating exon 2 (4). Among the clinical peculiar features of WFS, the two sisters presented both DM, deafness and peptic ulcers. The platelet aggregation defect was present only in the youngest sister, while the eldest showed DI, not diagnosed with the water deprivation test. OA was described in both patients (4). OA is considered one of the cardinal features of WFS and generally occurs after onset of DM at an average age of 11 years with a progressive loss of visual acuity and color vision (2). In fact, clinical suspicion of WFS is mainly based on the observation of OA after the diagnosis of diabetes in patients. Although there are currently no effective therapies for OA, annual eye examination is highly recommended. Retinal thinning is also considered a reliable marker of disease progression (8).

We hereby report the evolution of optic neuropathy over 8 years of follow-up of our patient with WFS2 and CISD2 intragenic deletion.

CASE PRESENTATION

The previous clinical history of our patient, who currently is a young woman of 28 years, has been described in the previous report (7). She came to our attention when she was 18 years old presenting non-autoimmune insulin-dependent diabetes mellitus, sensorineural hearing loss, intestinal ulcers, optic neuropathy, and defective platelet aggregation to ADP.

Actually, her diabetes is moderately-controlled (latest glycated hemoglobin values 7%) with a very low total daily dose of insulin requirement (<0.5 IU/kg).

The annual audiological evaluation showed a stable mild bilateral sensorineural hearing loss on high frequencies, without the need for hearing aids throughout the follow-up period.

She is still on pump inhibitor therapy and no longer presented peptic ulcers at endoscopic controls.

From July 2014, medical history was characterized by the presence of recurrent febrile urinary tract infections. Renal ultrasonography was performed showing grade IV vesicoureteral reflux (VUR), for which prophylactic antibiotic therapy was started, and bilateral pyelectasis with thickened distended bladder. Voiding cystourethrography and urodynamic test confirmed severe VUR and showed a low-capacity corrugated-walled bladder with sphincteric dyssynergia and pathological post-voiding residue. Diagnosis of neurogenic bladder was made, and the attempt of oxybutynin therapy failed due to drug side effects (blurred and flashing vision). Intermittent bladder catheterization was started. Patient underwent annual endoscopic examinations for peptic ulcers, which did not recur, and had no further bleeding episodes. Apart from the neurogenic bladder, our patient did not develop any other neurological disorders. Currently, she has a successful career as a lawyer.

In March 2020, she performed the last brain MRI that showed a slight increase in the antero-posterior diameter of both eyes with mild prominence of the CSF sheaths of both optic nerves, mild lower worm hypoplasia and multiple T2/FLAIR hyperintense areas in the cerebral bi-hemispheric white matter.

From 2012 to date, she has been regularly followed up at the Referral Center for Inherited Retinal Dystrophies of the Eye Clinic, University of Campania "Luigi Vanvitelli." Periodic ophthalmological examinations were performed, including best corrected visual acuity (BCVA), measurements with the Snellen visual chart, slit lamp anterior segment examination, measurements of intraocular pressure, fundus examination, Octopus visual fields assessment (Octopus 900 Haag Streit), electroretinography (ERG) and visual evoked potentials (VEP) (LKC UTAS E3000 LKC Technologies, Inc., United States). Spectral-domain optical coherence tomography (SD-OCT, Cirrus 4000 HD, Carl Zeiss Meditec, Inc., Dublin, CA, United States) was performed to evaluate retinal nerve fiber layer (RNFL) thickness and monitoring disease progression.

At the last ophthalmic examination in September 2020, patient's BCVA was 20/40 in right eye (RE) and 20/50 in left eye (LE), the same visual acuity presented at the first visit in 2012. Anterior segment slit lamp examination and intraocular pressure (<20 mmHg) were normal bilaterally

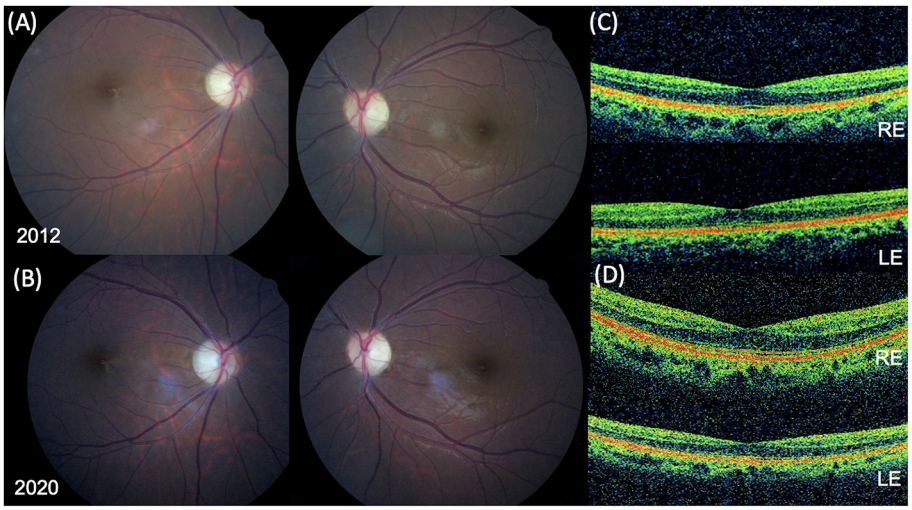

FIGURE 1 | Fundus photographs and spectral-domain optical coherence tomography (SD-OCT) findings. Fundus photograph in 2012 **(A)** and in 2020 **(B)** showing bilateral optic disc temporal pallor with normal macula in both eyes. **(C)** SD-OCT foveal B-scan in 2012 revealing a normal ellipsoid zone (EZ) and a central foveal thickness (CFT) of 246 μm in the right eye (RE) and 239 in left eye (LE). **(D)** SD-OCT foveal B-scan in 2020 confirming a normal EZ and a CFT of 229 μm in the RE and 239 in the LE.

2012	OD	OS
Average RNFL Thickness	62 μm	57 μm
RNFL Symmetry	47%	

2020	OD	OS
Average RNFL Thickness	64 μm	58 μm
RNFL Symmetry	48%	

FIGURE 2 | Retinal nerve fiber layer (RNFL) thickness evaluated with spectral domain-optical coherence tomography. Average RNFL thickness and RNFL symmetry did not change significantly throughout the follow-up period.

at each control. Pupils were isochoric, normoreactive to light and no relative afferent pupillary defect was detected. Patient presented no signs of nystagmus, strabismus, and dyschromatopsia on color test evaluation. Fundus examination was stable over time showing bilateral optic disk temporal pallor with normal macula in both eyes and no signs of diabetic retinopathy (**Figures 1A,B**). Macular and optic nerve SD-OCT measurements were stable after 8 years of follow-up (**Figures 1C,D**, **2**). Central foveal thickness, in 2012 was 246 and 239 μm in RE and LE, respectively. In 2020 it was 229 μm in the RE and 239 μm in LE, with a preservation of the inner and outer retinal layers (**Figures 1C,D**). Regarding optic nerve analysis, in 2012 SD-OCT showed a reduction of the peripapillary RNFL, a suffering of superior and inferior axonal fibers of optic nerve in both eyes and of nasal axonal fibers in LE. As shown in **Figure 2** all these findings were stable during the follow-up. The visual field tests were also stable over the time. **Figure 3** shows Octopus visual field with values of a mean deviation of 6.5 dB in RE and 8.9 dB in LE in 2015, and a mean deviation of 6.2 dB in RE and 8.6 dB in LE in 2020. ERG tests were normal in all visits, whereas VEP showed latency increase and amplitude reduction in P100 waves stable throughout the follow-up.

DISCUSSION

To the best of our knowledge, this is the longest follow-up in the literature in a patient with WFS2 and CISD2 intragenic deletion.

In this report we show that WFS2 can be characterized by an optic neuropathy rather than an OA, with a very slow progression over the time.

To date, few cases of WFS2 with mutations in CISD2 have been reported in addition to our case: 3 consanguineous families of Jordanian descent (6), two Italian siblings (4), a Moroccan patient (9), and a Chinese patient (10). All the aforementioned papers report patients with a severe vision loss developing OA and, in some cases, neurological impairment with progressive cognitive disturbances (11). This variability could be partially due to the different mutations in the CIDS2 gene and to different degrees of penetration. The described phenotype of all cases suggests a heterogeneity of the clinical spectrum, that is: all cases described (with

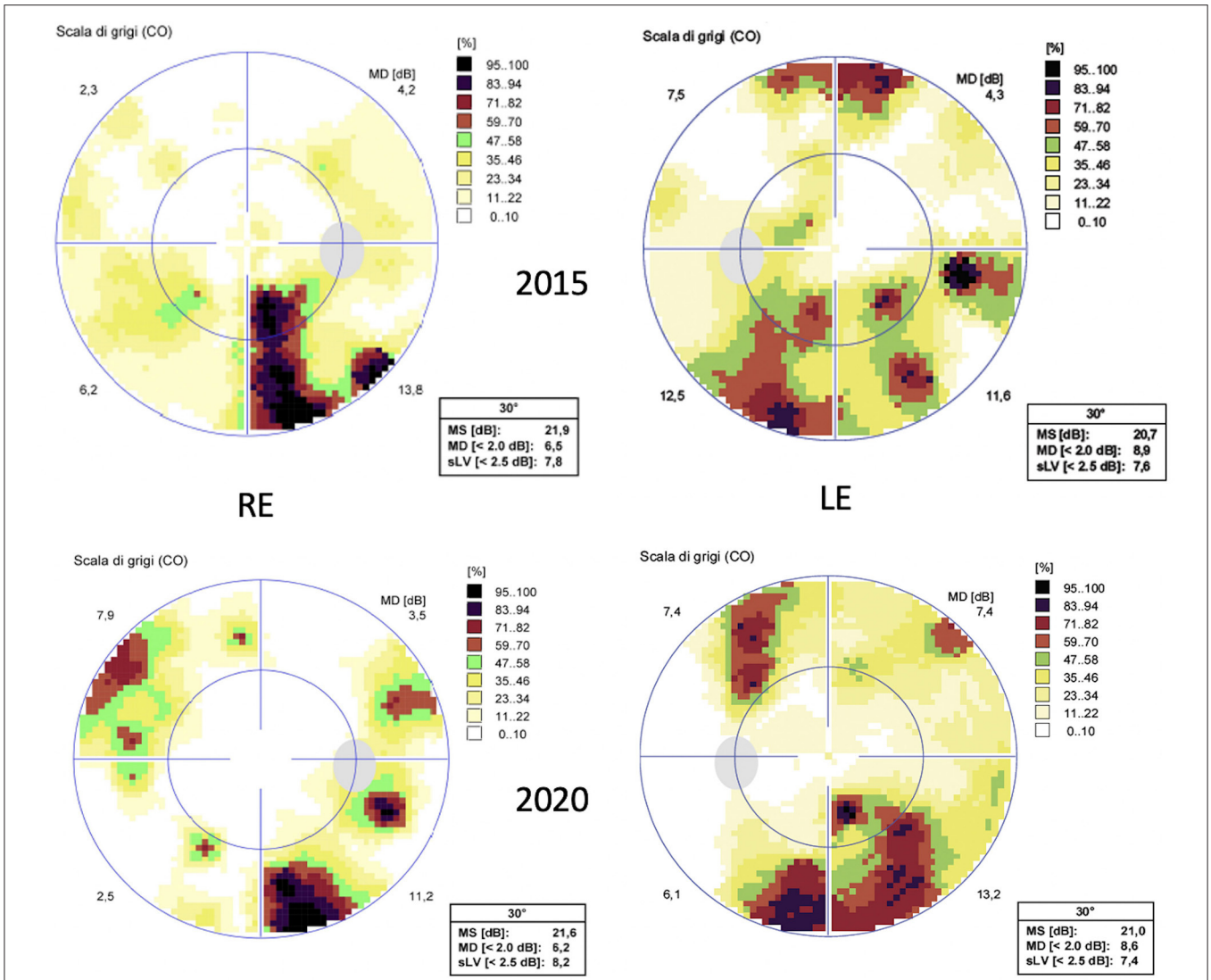

FIGURE 3 | Octopus visual field testing (32 standard). Above the exam performed in 2015 and below the exam performed in 2020, showing stable perimeter indices over the years.

different exon mutations of the CISD2 gene) reported non-autoimmune diabetes, deafness and OA as prevalent clinical features. Among these, OA was absent only in our Caucasian case (7), even after a long follow up; while bleeding intestinal ulcers and defective platelet aggregation were absent in a Moroccan case (9).

Our patient was followed up yearly and the clinical picture including all macular and optic nerve examinations along with the BCVA remained stable over time. In our patient the mutation detected probably led to encode a protein that doesn't cause OA but optic neuropathy.

Our findings further confirm that the involvement of the optic nerve in patients with WFS2 does not always manifest with OA. Additionally, the optic neuropathy progression over the years can be very slow allowing for a stable visual acuity.

Ophthalmological evaluations in WFS2 patients with CISD2 intragenic deletion should be performed longitudinally. All retinal examinations may be helpful in distinguish the patients presenting with OA from those presenting with optic neuropathy, considering the differences in term of prognosis. Indeed, WFS symptoms have a negative impact on individuals' daily function and quality of life. Apart from the finding of a neurogenic bladder, the clinical picture of our patient has been stable over the years, especially as regards the optic neuropathy which allowed her to maintain a good visual acuity and not go blind. She also managed to graduate, due to the stability of the clinical picture over time. The long follow-up of our patient allows us to hypothesize that not all WFS2 cases evolve to blindness. Further studies with comprehensive evaluation of the visual function of

patients with WFS2 are needed to clarify the results of our study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

VD and EM were responsible for the initial plan and study design. VD, EM, FR, and FD collected and extracted data. VD and EM interpreted data and drafted the manuscript. VD, EM, FR, FD, RG, FT, CI, AF, and FS did a critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors are grateful to the European Reference Network dedicated to Rare Eye Diseases (ERN-EYE) and to Rare Endocrine Conditions (Endo-ERN) for useful discussions.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 29 April 2021
doi: 10.3389/fendo.2021.673539

Diabetes and Prediabetes in Children With Cystic Fibrosis: A Systematic Review of the Literature and Recommendations of the Italian Society for Pediatric Endocrinology and Diabetes (ISPED)

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Cystic fibrosis related diabetes (CFRD) is a comorbidity of cystic fibrosis (CF) that negatively impacts on its clinical course. Prediabetes is an important predictor of either CFRD development and unfavorable prognosis of CF in both pediatric and adult patients. International guidelines recommend insulin only in case of CFRD diagnosis. Whether early detection and treatment of prediabetes may contribute to improve the clinical course of CF is still debated. A subgroup of pediatric diabetologists of the Italian Society for Pediatric Endocrinology and Diabetology (ISPED) performed a systematic review of the literature based on predefined outcomes: impact of pre-diabetes on clinical outcomes and on the risk of developing CFRD; diagnosis of diabetes and pre-diabetes under 10 years of age; effectiveness of therapy on glycemic control, impact of therapy on pulmonary function and nutritional status. Thirty-one papers were selected for the analysis data presented in these papers were reported in tables sorted by outcomes, including comprehensive evidence grading according to the GRADE approach. Following the grading of the quality of the evidence, the entire ISPED diabetes study group achieved consensus for the Italian recommendations based on both evidence and clinical experience. We concluded that in patients with CF, prediabetes should be carefully considered as it can evolve into CFRD.

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 27 February 2021
Accepted: 29 March 2021
Published: 29 April 2021

Citation:
Mozzillo E, Franceschi R, Piona C, Passanisi S, Casertano A, Pjetraj D, Maltoni G, Calcaterra V, Cauvin V, Cherubini V, D'Annunzio G, Franzese A, Frongia AP, Lombardo F, Lo Presti D, Matteoli MC, Piccinno E, Predieri B, Rabbone I, Scaramuzza AE, Toni S, Zucchini S, Maffeis C and Schiaffini R (2021) Diabetes and Prediabetes in Children With Cystic Fibrosis: A Systematic Review of the Literature and Recommendations of the Italian Society for Pediatric Endocrinology and Diabetes (ISPED). Front. Endocrinol. 12:673539. doi: 10.3389/fendo.2021.673539

In patients with CF and prediabetic conditions, after complete evaluation of the OGTT trend, glucometrics, glycemic values measured during pulmonary exacerbations and/or steroid therapy, early initiation of insulin therapy could have beneficial effects on clinical outcomes of patients with CF and prediabetes.

Keywords: cystic fibrosis-related diabetes, prediabetes, oral glucose tolerance test (OGTT), continuous glucose monitoring, abnormal glucose tolerance, systematic review, recommendations, glargine insulin

INTRODUCTION

Improving survival in cystic fibrosis (CF) has caused an increase of the prevalence of comorbidities, with cystic fibrosis-related diabetes (CFRD) being the most common and affecting at least half of the adult CF population (1). CFRD has a negative impact on the clinical course of the disease increasing its mortality (2). In recent years, several lines of evidence have demonstrated that pulmonary function, microbiological colonization and nutritional status start to worsen several years prior to the diagnosis of CFRD (3). Early detection of pre-diabetes, defined in CF patients by the presence of abnormal glucose tolerance (AGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG) or indeterminate glycaemia (INDET) is therefore essential, although to date few studies have focused on pre-diabetes and its negative significant impact on the course of CF

The main cause of CFRD is insulin insufficiency with insulin secretion being increasingly impaired in correlation with exacerbation of pre-diabetes (4). According to this pathophysiological evidence, current guidelines of the International Society for Pediatric and Adolescent Diabetes (5) recommend insulin therapy initiation for the treatment of CFRD. However, no specific insulin therapies appear to have significantly distinct advantages both for an effective treatment of hyperglycemia and for their possible positive impact on the clinical course of CF (6). In addition, whether CF patients diagnosed with pre-diabetes could benefit from 'early' initiation of insulin therapy it is still debated (7).

The screening and diagnosis of CFRD and pre-diabetic conditions, as long as the effectiveness of the therapy for the treatment of CFRD and pre-diabetes represent two topics of major interest in the field of diabetes and CF. For this reason, a subgroup of pediatric diabetologists of Italian Society for Pediatric Endocrinology and Diabetology (ISPED) performed a systematic review of the literature and Grading of Recommendations, Assessment, Development and Evaluation (GRADE) profiles focusing on the above mentioned debated topics.

METHODS

A systematic review based on pre-defined outcomes for each question has been performed. The entire diabetes study group of ISPED compiled evidence profiles and achieved consensus for the final recommendations.

In regards to screening and diagnosis of diabetes and pre-diabetes in CF patients, the outcomes were: 1) impact of pre-diabetes on clinical outcomes (pulmonary function, number of pulmonary exacerbations and nutritional status) and 2) on the risk of developing CFRD; 3) diagnosis of diabetes and pre-diabetes in children under 10 years of age.

In regards to effectiveness of therapy in CFRD and pre-diabetes the outcomes were: 1) effectiveness of therapy on biochemical measures of glycemic control, as glycosylated hemoglobin (HbA1c), fasting and 2 h post-meal serum blood glucose values, and data derived from CGM download; 2) effectiveness of therapy in improving pulmonary function, 3) effectiveness of therapy in improving nutritional status.

Pulmonary function was analyzed in terms of forced expiratory volume in the 1st second (FEV1) and forced vital capacity (FVC). Pulmonary exacerbations were analyzed considering the following criteria: increased sputum volume, more frequent coughing, increased dyspnea, weight loss, change in the chest physical examination, absence from school or work because of illness, requiring hospitalization and antibiotic therapy. Pathogens colonization was considered on the results of microbiological investigations on deep pharyngeal aspirates. Pathogens colonization on deep pharyngeal aspirates was considered on the results of microbiological investigations. Nutritional status was evaluated as body mass index (BMI) and standard deviation scores (SDS) of weight and height.

The method used to perform this systematic review was based on the PICOS model (Population, Intervention, Comparison, Results, Study design). Inclusion criteria of studies are listed in **Table 1**. Exclusion criteria were studies not meeting the established outcomes and studies with animals. No restrictions were applied regarding the published paper's language and patients' age. The articles selected for this literature review include all those published from 1/01/2006 to 30/10/2020. The keywords used, also called "mesh" (MEdical Subject Headings) on PubMed, are the following: "diabetes diagnostic test AND cystic fibrosis," "cystic fibrosis AND diabetes management," "cystic fibrosis AND AGT," "cystic fibrosis AND IFG," "cystic fibrosis AND IGT," "cystic fibrosis AND INDET," "cystic fibrosis AND diabetes." Systematic searches, using relevant keywords and search strings, were conducted on electronic databases (PubMed, Scopus, Google Scholar, CINAHL, Nursing reference center, Up to date and PsycINFO, Embase, CENTRAL) and clinical trial registers (http://clinicaltrials.gov; www.controlled-trials.com).

In order to derive recommendations, a GRADE approach to rank the quality of a body of evidence was applied as reported in **Table 2** (8, 9). The final assessment of the quality of evidence was discussed and established by the entire subgroup.

For each risk of bias (i.e. imprecision, inconsistency, indirectness, and publication bias), the authors had the option

TABLE 1 | PICOS model (population, intervention, comparison, results, study design) adopted in the systematic review.

Population	Patients with CF
Intervention	Diagnostic test and treatment of pre-diabetes and/or diabetes in CF
Comparison	Patients with CF not screened or not treated for pre-diabetes or diabetes
Results	Screening and diagnosis of diabetes and pre-diabetes -Impact of pre-diabetes on the clinical course of CF - Pre-diabetes and risk to develop CFRD -Diagnosis of diabetes and pre-diabetes in patients with CF under 10 years of age Effectiveness of therapy in CFRD and pre-diabetes -Biochemical measures of glycemic control -Pulmonary function and number of pulmonary exacerbations -Assessment of nutritional status
Study design	RCTs, observational studies, prospective studies, cross-sectional studies, exploratory studies, case series, case reports, mix of qualitative and quantitative studies

TABLE 2 | GRADE approach to ranking the quality of a body of evidence.

High	=	Further research is very unlikely to change confidence in the estimate of effect.
Moderate	=	Further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate.
Low	=	Further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate.
Very low	=	Any estimate of effect is very uncertain.

of decreasing their level of certainty one or two levels (e.g., from high to moderate). Since GRADE cannot be implemented mechanically, there is by necessity a considerable amount of subjectivity in each decision.

Following the assessment of the quality of the evidence, an assessment of the strength of the recommendation was made. A consensus for the final recommendations was achieved from the entire diabetes study group of the ISPED, and summarized in **Table 3**. According to the GRADE method, the strength of each recommendation is classified in four mutually exclusive categories: "strong" and "weak or conditional" in favor (positive) or against (negative) the use of a specific intervention, as reported in **Table 4** (10).

TABLE 3 | Factors than can reduce or increase the quality of evidence.

Factors that can reduce the quality of the evidence	
Factor	**Consequence**
Limitations in study design or execution (risk of bias)	↓ 1 or 2 levels
Inconsistency of results	↓ 1 or 2 levels
Indirectness of evidence	↓ 1 or 2 levels
Imprecision	↓ 1 or 2 levels
Publication bias	↓ 1 or 2 levels
Factors that can increase the quality of the evidence	
Factor	Consequence
Large magnitude of effect	↑ 1 or 2 levels
All plausible confounding would reduce the demonstrated effect or increase the effect if no effect was observed	↑ 1 level
Dose-response gradient	↑ 1 level

TABLE 4 | Assessment of the strength of a recommendation.

Strength of recommendation	Rationale
Strong	The panel is confident that the desirable effects of adherence to the recommendation outweigh the undesirable effects
Weak or • **conditional** (depending on patient values, resources available or setting) • **discretionary** (based on opinion of patient or practitioner) • **qualified** (by an explanation regarding the issues which would lead to different decisions).	The panel concludes that the desirable effects of adherence to a recommendation probably outweigh the undesirable effects. However: - the recommendation is only applicable to a specific group, population or setting or - new evidence may result in changing the balance of risk to benefit or - the benefits may not warrant the cost or resource requirements in all settings
No recommendation possible	Further research is required before any recommendation can be made

We used these standard expressions and if sufficient evidence was not available recommendations were based on the panel opinion according to the current daily clinical practice.

RESULTS

After a careful evaluation of all databases available, 592 papers were found, 317 of whom were removed because they were duplicates (**Figure 1**). Among the 105 papers left, after reading the full text of each of them, only 34 papers were selected for the analysis because all the inclusion criteria were met.

Data from the 34 studies selected, including comprehensive evidence grading, are presented in **Tables 5**–**10**, sorted by outcomes.

According to the ISPAD guidelines (5), the following two diagnostic categories of CFRD have been established for patients screened with oral glucose tolerance test (OGTT) during periods of stable CF clinical conditions, based on fasting and 2-h glucose values: CFRD without fasting hyperglycemia (CFRD-FH$^-$) and CFRD with fasting hyperglycemia (CFRD-FH$^+$) (**Table 11**). In addition, in symptomatic patients, the CFRD can be diagnosed if random blood glucose level is ≥200 mg/dL (≥11.1 mmol/L) on 2 or more occasions, and if HbA1c is ≥ 48 mmol/mol (6.5%) (48 mmol/mol), even though diagnosis of diabetes can also be made in CF patients that show HbA1c value below this range (45). During flare-up phases of the disease, when intravenous antibiotic therapy and/or systemic corticosteroid therapy is required, the diagnosis of CFRD can be made if a fasting glycemia ≥126 mg/dL (≥7 mmol/L) or a post-prandial blood glucose ≥200 mg/dL (≥11.1 mmol/L) is present for 48 h.

Due to the insidious onset of CFRD, once a year OGTT in all patients aged 10 and above is crucial for the diagnosis of CFRD and for the identification of high-risk subjects (5).

OGTT identifies patients with CFRD and with pre-diabetes using the following diagnostic categories: normal glucose tolerance (NGT); INDET; IGT (**Table 11**). Two more categories named AGT (4, 37) and impaired fasting glucose (IFG) can also to be considered (46) (**Table 11**).

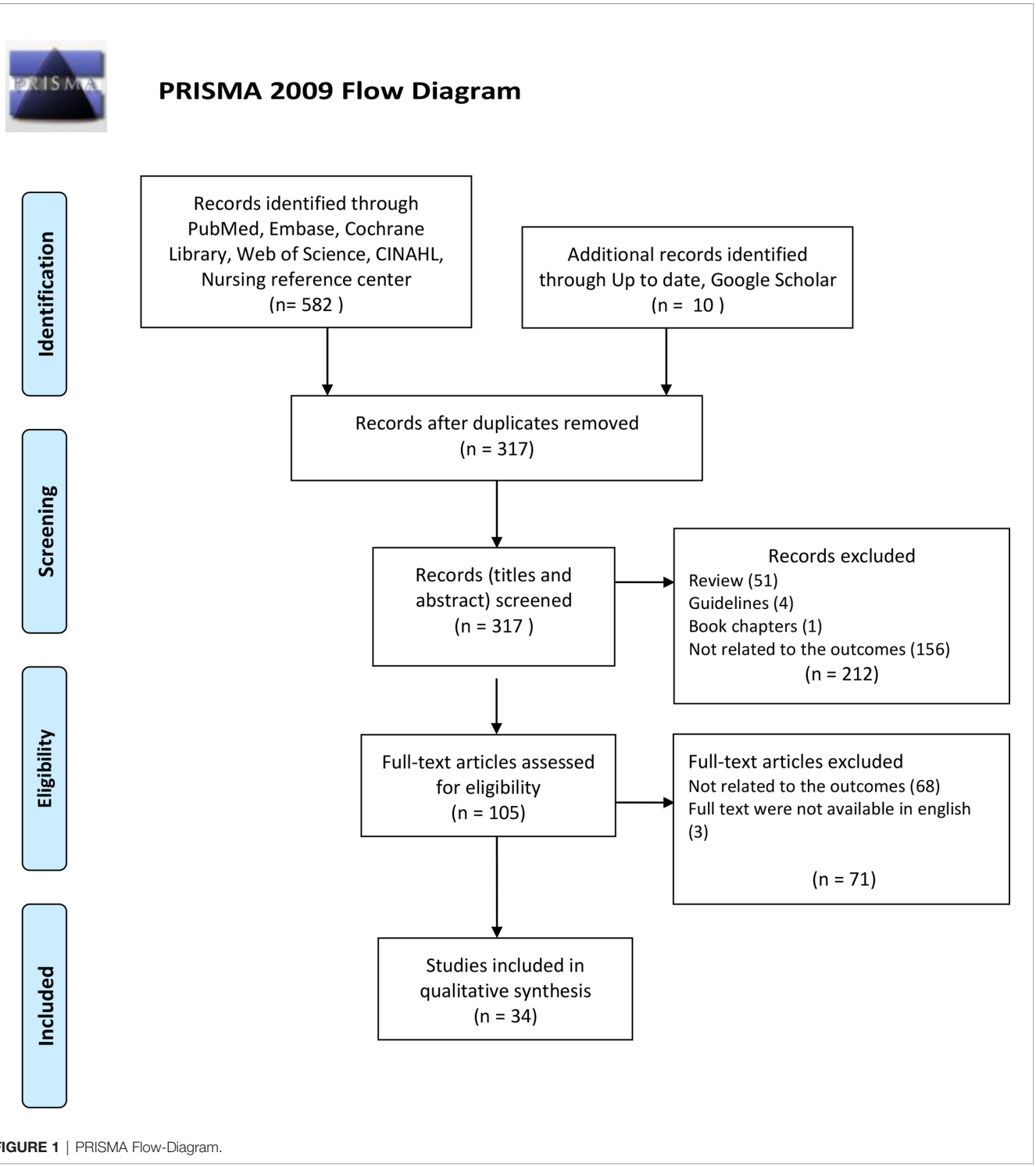

FIGURE 1 | PRISMA Flow-Diagram.

Impact of CFRD and Pre-Diabetes on CF Outcomes

Regarding the outcome "Impact of pre-diabetes on the clinical outcomes of CF", 13 paper were included in this systematic review, 2 are multi-center and 11 are single-center studies; 8 were prospective observational studies while 5 were retrospective-observational studies. The number of enrolled patients ranged from 16 to 4,293 including both children and adults (**Table 5**).

Impact on Pulmonary Function

CF patients diagnosed with IGT or INDET had lower FEV1 and FVC levels compared to CF patients without glucose abnormalities

TABLE 5 | Impact of prediabetes on the clinical outcomes in CF patients.

Reference/ Study	Study design Follow up period	Participants and Diagnostic Test	Pulmonary Function	Growth and Nutritional status	Pulmonary exacerbations and microbiological colonizations	Rating upgrade/ downgrade	Evidence level
Banavath et al. (11)	Single-center observational case-control study Period of data collection: 2006 – 2016.	25 children tested with OGTT and divided in two groups: - Group A: 16 children with AGT (including IFG, INDET, IGT, and CFRD) - Group B: 9 children with NGT	Significant lower FEV1/FVC in children with AGT (p< 0.0001)	Significant lower BMI Z-score in children with AGT (p< 0.0001)	Significant higher rate of colonization by *Pseudomonas aeruginosa* and number of hospitalization in children with AGT (p<0.0001)	Downgrade (imprecision)	Moderate
Lavie et al. (12)	Single-center observational case-control study Period of data collection: not declared.	51 adolescents and adults tested with OGTT and divided in two groups: - Group A: 38 subjects with NGT - Group B: 13 subjects with IGT	Significant lower FEV1 in subjects with IGT (p= 0.014); significant negative correlation between FEV1 and fasting, 30 min, 1-h, 2-h and peak glucose measured during OGTT.	Significant lower BMI-SDS in subjects with IGT (p< 0.001)	Not evaluated	Downgrade (imprecision)	Moderate
Coriati et al., (13)	Single-center observational case-control study. Period of data collection: 2014-2016.	252 adults tested with OGTT categorized according their glucose tolerance status: - 99 subjects with NGT - 66 subjects with IGT - 45 subjects with INDET - 42 subjects with *De novo* CFRD	Significant trend of worsening of FEV1% across glucose tolerance categories (p= 0.038); CFRD and INDET subjects have comparable decreased FEV1% (p=0.996)	No significant trend of weight and BMI across glucose tolerance categories (p=0.723 and p=0.813 respectively)	Not evaluated	No	Moderate
Terliesner et al. (14)	Single-center retrospective observational case control study. Period of data collection: 1990 – 2014; mean follow-up duration of 13 years	16 adolescents who developed CFRD during the follow-up period compared to 16 age and gender-matched subjects who not developed CFRD.	Significant lower FEV1% and FVC% both at baseline and during Follow-up in subjects with CFRD	Significant lower Height and weight SDS% both at baseline and during Follow-up in subjects with CFRD, no significant differences in BMI and BMI-SDS.	Not evaluated	No	Moderate
Tommerdahl et al. (15)	Single-center observational study. Period of data collection: not declared. Follow-up duration: unavailable	52 CF patients (11 with NGT, 33 with AGT, and 8 with CFRD) aged 10-18 year were tested with OGTT. Three alternative criteria were considered: curve shape (biphasic vs. monophasic), time to glucose peak (≤30 min vs. > 30 min), 3. 1-h glucose (< 155 mg/dl vs. ≥155 mg/dl).	There were no differences between groups (biphasic vs. monophasic, ≤30 min vs. > 30 min, < 155 mg/dl vs. ≥155 mg/dl) in FEV %1 or FVC%	BMI z-score was significantly higher in the peak glucose at > 30 min group vs. the peak glucose at ≤30 min group. No other differences were found.	Not evaluated	Downgrade (risk of bias)	Low
Leclercq et al. (16)	Single-center observational case-control study. Period of data collection: 2009 – 2012.	38 children aged >10 years with NGT at OGTT tested with 3-day CGM: - Group A: 26 subjects with BG max <200 mg/dl (11 mmol/l); - Group B: 12 subjects with BG max > 200 mg/dl (11 mmo/l)	Significant lower FEV1% and FVC% in Group B (p=0.01 and p= 0.021, respectively)	No significant difference in BMI SDS between the 2 groups (p = 0.079)	Significant higher rate of colonization by *Pseudomonas Aeruginosa* in Group B (p=0.024)	Downgrade (imprecision)	Moderate
Olszowiec-Chlebna et al. (17)	Single-center retrospective observational	61 children tested with OGTT	Impaired glucose tolerance status was a significant	Not evaluated	Not evaluated	Upgrade (Large	Moderate

(Continued)

TABLE 5 | Continued

Reference/ Study	Study design Follow up period	Participants and Diagnostic Test	Pulmonary Function	Growth and Nutritional status	Pulmonary exacerbations and microbiological colonizations	Rating upgrade/ downgrade	Evidence level
	study Period of data collection: 1996 -2009; mean follow-up duration of 8 years.		risk factor for decline in FEV1% in children older than 10 years of age (p= 0.027)			magnitude of effect)	
Milla et al. (18)	Single-center prospective longitudinal observational study. Period of data collection: not declared; mean follow-up duration of 4 years.	187 children and 65 adults tested with OGTT divided according to their glucose tolerance status in three groups: - 69 subjects with NGT - 59 subjects with IGT - 24 subjects with CFRD	A significant decline in FEV1% and FVC% during the follow up period was observed in CFRD and IGT subjects, and not in NGT subjects	No significant differences in BMI trends across the glucose tolerance groups.	Not evaluated	No	Moderate
Bizzarri et al. (19)	Single-center prospective longitudinal observational study. Period of data collection: not declared; mean follow-up duration of 3 years.	Children tested with OGTT during puberty divided according to their glucose tolerance status in three groups: - Group A: 52 subjects with NGT - Group B: 17 subjects with CFRD	Significant lower FEV1% and FVC% in Group B A both at baseline and during follow-up period (p = 0.01 and p = 0.02, respectively)	Significant lower BMI-SDS in Group B both at baseline and during follow-up period (p = 0.01 and p = 0.04, respectively).	No significant differences in the rate of colonization by *Pseudomonas Aeruginosa* in Group B both at baseline and during follow-up period (p= 0.78 and p=0.38, respectively); Significant higher rate of hospitalizations and outpatient clinic visits during the follow-up in Group B (p= 0.003 and p=0.01, respectively).	No	Moderate
Van Sambeek et al. (20)	Single- center retrospective case-control study. Period of data collection: 2012 to 2013.	88 children and adults tested with OGTT and categorized according their glucose tolerance status in three groups: NGT, CFRD and IGT.	Significant lower FEV1% and FVC% in CFRD subjects with HbA1c level > 6.5% compared to CFRD subjects with HbA1c level < 6.5%	Not evaluated	Significant higher rate of pulmonary exacerbation in CFRD subjects	No	Moderate
Limoli et al. (21)	Single center observational case-control study. Period of data collection: 2012 or 2013.	91 children and 134 adults tested with OGTT categorized according to their glucose tolerance status in two groups: - 136 subjects with NGT - 89 subjects with CFRD	Significant lower FEV1% in CFRD subjects	Not evaluated	Significant higher rate of *P. aeruginosa* and *S. aureus* confections and pulmonary exacerbations requiring IV antibiotics in CFRD subjects.	No	Moderate
Reece et al. (22)	Multicenter retrospective observational case control study. Period of data collection: 2013.	749 children and adults categorized in 6 groups depending on their colonization status for *Pseudomonas aeruginosa* and *Aspergillus fumigatus*	Not evaluated	Not evaluated	Patients persistently colonized with PA had a higher prevalence of CFRD diagnosis (p = 0.012).	No	Low
Merlo et al. (23)	Multicenter longitudinal observational study Period of data collection: 1998 – 2002, follow-up duration of 5 years.	4293 children and adults in which data about demographics, anthropometrics, spirometry, respiratory culture results, comorbidities, antibiotic usage, and hospitalizations were collected.	Not evaluated	Not evaluated	CFRD was a significant risk factor for the acquisition of multiple antibiotic resistant P. aeruginosa infection (hazard ratio [HR], 1.64; 95% confidence interval [CI], 1.11 to 2.43).	No	Elevated

TABLE 6 | Impact of prediabetes on the risk to develop CFRD.

References	Study design Follow up duration	Participants	Main outcomes	Rating upgrade/ downgrade	Evidence level
Larson Ode K et al., 2010	Single-center retrospective study. Follow-up: 5 years.	62 CF children aged 6-9 years tested with OGTT. Glucose tolerance categories: AGT (50%) and NGT (50%)	Odds of developing diabetes were 11 times greater AGT patients (p<0.001)	No	Moderate
Schmid et al. ((24)	Multicenter longitudinal study. Mean follow-up: 3.6 ± 2.1 years.	1093 CF patients aged 10 years or older with at least two valid OGTTs each and no CFRD at their first OGTT. Glucose tolerance categories groups: NGT (76.7%), IFG (6.4%), IGT without IFG (14.2%), IGT with IFG (2.7%). INDET (269 patients) and no-INDET (269 patients) only for a subgroup of patients)	Incidence of CFRD was significantly higher in the IFG (p = 0.0005), IGT without IFG (p = 0.0007), and IGT with IFG (p = 0.00009) groups compared with the NGT group	No	High
Sheikh et al. (25)	Single-center retrospective study. Follow-up: 5 years.	80 CF children aged 12 years (IQR 9.0-14.5). Glucose tolerance categories at baseline: INDET (8.7%), 1-h plasma glucose > 160 mg/dl (35%), NGT (56.3%)	INDET was associated with a significantly increased risk for future CFRD compared with no-INDET (OR 2.81, 1.43–5.51)	No	High
Schiaffini et al. (26)	Single-center prospective study. Follow-up: 2-5 years.	17 CF patients + 14 healthy controls tested with OGTT and CGM. Glucose tolerance categories at baseline: NGT (58%), IFG (5.9%), IGT (17.6%), IGT + INDET (11.8%), CFRD (5.9%)	Patients with PG1>160 mg/dl at baseline had 4 times more risk of developing CFRD; patients with PG1>200 mg/dl at baseline had 10 times more risk of developing CFRD	No	Moderate

(11–14) (evidence Moderate); glucose peaks >200 mg/dl (11.1 mmol/l) during a continuous glucose monitoring were associated with worse spirometry pulmonary function parameters (16) (evidence Low). Pulmonary function is not associated with alternative OGTT criteria (i.e. monophasic curve, glucose peak > 30 min, and/or 1 h ≥155 mg/dl) (15) (evidence Low).

Prediabetes was one of the most relevant predictors of deterioration of lung function defined as a significant decrease in FEV1 predicted value, during a 5 year-follow-up (17, 18) (evidence Moderate).

Impact on Growth and Nutritional Status

Auxological parameters, height-SDS and BMI-SDS, may be negatively influenced by prediabetes status in CF patients (11, 12, 14) (evidence Moderate).

Pediatric CF patients experienced a deterioration of their nutritional status and a negative impact on their final height due to prediabetes (14, 19) (evidence Moderate).

Impact on Pulmonary Exacerbations and Microbiological/Pathogens Colonization

A higher rate of pulmonary exacerbations, hospital admissions and outpatient clinic visits was observed in CF patients with CFRD and early glucose tolerance abnormalities compared to patients with NGT (11, 12, 19–21) (evidence Moderate).

CFRD and prediabetes diagnosis was recognized as independent risk factors for colonization by common CF pathogens, in particular for the acquisition of *Pseudomonas Aeruginosa*, its multiple antibiotic-resistant infection and its co-infection with other pathogens (11, 12, 19, 21–23) (evidence Moderate).

Regarding the outcome "Impact of prediabetes on the risk to develop CFRD", 4 papers were included, 1 is a multi-center prospective study, 1 is a single-center prospective study and 2 are single-center retrospective studies. The sample size of the study populations ranged from 17 to 1,093 patients, especially children and adolescents (**Table 6**).

The results of these studies strongly support the evidence for an early detection of prediabetic conditions in CF patients, because IGT, IFG or INDET presented a five-years CFRD risk at least 10 times higher compared to CF patients with NGT (3, 24, 25). (evidence High). In addition, early glucose tolerance alterations defined by INDET during continuous glucose monitoring were also related to a higher risk of developing CFRD (25, 26) (evidence Moderate).

Regarding the outcome "Diagnosis of diabetes and prediabetes in patients with CF under 10 years of age" we included in this systematic review 7 papers. Among these, one is a multi-center prospective study, 4 are single-center prospective studies, one is a single-center retrospective study, and one is a case report. The number of enrolled patients ranged from 11 to 152 and age ranged from a few months-old to less than 10 years of age (**Table 7**). The analysis of these studies showed that in infants and young children with CF glucose derangements detected by OGTT are often diagnosed (3, 27–31) (evidence Moderate) and annual diabetes screening program in patients <10 years of age increased the early detection of CFRD (27, 28, 32) (evidence Low), leading to a more prompt and appropriate therapeutic approach (32) (evidence Very Low). Continuous glucose monitoring may be an alternative method for detecting glucose derangements in very young children with CF (29, 30) (evidence Moderate), particularly those with *Pseudomonas Aeruginosa* colonization (29) (evidence Moderate). Current evidence has also demonstrated that early diagnosis of prediabetes may be related to early clinical deterioration, particularly lung function and auxological parameters (29, 30) (evidence Moderate).

TABLE 7 | Diagnosis of diabetes and prediabetes in children with Cystic Fibrosis under 10 years of age.

References	Study design Follow up duration	Participants	Main outcomes	Rating upgrade/ downgrade	Evidence level
Yi et al. (27)	Multicenter prospective study. Follow-up: unavailable	23 CF children aged 3 months to 5 years and 11 healthy control subjects tested with OGTT	All control subjects were NGT, while 39% of CF children had AGT status (2 CFRD, 2 INDET and 6 IGT). AUC of glucose was significantly higher in subjects with CF than control subjects (p=0.02)	No	Moderate
Fattorusso et al. (28)	Case report. Follow-up: 16 years	A CF patient diagnosed with GMD at 1 year with a very long-term follow-up.	This case report confirms the importance of paying attention to early GMDs in very young CF patients and seems to suggest that earlier therapy could ameliorate CF natural history	No	Low
Larson Ode K et al., 2010	Single-center retrospective study. Follow-up: 5 years.	62 CF children aged 6-9 years tested with OGTT. Glucose tolerance categories: AGT (50%) and NGT (50%)	10 years after study onset, 42% of AGT patients developed diabetes vs 3% of NGT patients. Age of CFRD onset was 12 ± 1 years in boys and 11 ± 1 years in girls	No	Moderate
Prentice et al. (29)	Single-center, observational, case-control study. Follow-up: unavailable	18 CF patients aged 0·9–5.5 years and 4 control subjects tested with CGM for 3 days	Peak SG was >11.1 mmol/L in 39% of CF patients. SG levels before age 6 years are associated with increased pulmonary inflammation and Pseudomonas aeruginosa infection	Downgrade (imprecision)	Moderate
Prentice et al. (30, 31)	Single-center observational study. Follow-up: unavailable	27 CF patients aged <10 years tested with OGTT. OGTT results were performed with results from CGM performed in 11 participants	There was a significant inverse correlation between weight and height z-scores with BG max (both p=0.02). AUC total was inversely correlated with weight, height and BMI z-score (p=0.01, p=0.009, p=0.02 respectively). A significant inverse correlation was also identified between fasting insulin level and elevated glucose on CGM, defined as AUC >7.8 mmol/L (p=0.027) or as % time > 7.8 (p=0.011)	No	High
Mozzillo et al. (32)	Single-center observational study. Follow-up: unavailable	152 CF children aged 2.4-18 years tested with OGTT Age groups: <6 years (n° 24), 6-10 years (n° 42), and >10 years (n° 86)	Prevalence of GMDs among three age groups were: between 2.4 and 5.9 years (n° 24), between 6 and 9.9 years (n° 42), and >10 years (n° 86)	No	High
Prentice et al. (30, 31)	Single-center observational study. Follow-up: 24 months	11 CF children (mean age 3.8 ± 2.5 years) tested with 3-day CGM at baseline, 12 months, and 24 months.	Three of the participants (27%) had normal CGM at all time-points. Seven children (64%) had a peak SG ≥11.1 mmol/L. None of the children had a peak SG ≥11.1 at every time point. Only four of the subjects (36%) did not have a peak SG ≥11.1 mmol/L at any time-point. Eight children (73%) spent more than 4.5% of their total time in the impaired range (> 7.8 mmol/L) at any time-point, and 5 (63%) had elevated percent time on more than one test	Downgrade (imprecision)	Moderate

Effectiveness of Therapy in CFRD

Regarding the outcome "Effectiveness on glycemic control", we included in this systematic review 10 studies, 5 are randomized controlled trials (RCT), 3 are prospective cohort studies, one is a prospective case-control study, and one is a retrospective case-control study. The number of enrolled patients ranged from 9 to 100, from infancy to adulthood. Glycemic control has been analyzed in terms of glycated hemoglobin (HbA1c), fasting and 2 h post-meal blood glucose (**Table 8**). Different insulin and other drugs used are reported in **Table 8**.

Among the 3 studies on NPH insulin, in a retrospective - prospective case-control study, 10 pediatric patients with CFRD were treated with a mixture of short and intermediate acting insulin over a 10-year period, with no evidence of HbA1c improvement when compared to 14 non-diabetic matched controls (33) (evidence Low). In 28 adult patients, who were insulinopenic, diagnosed with IGT or CFRD- FH^-, NPH insulin treatment (0.12–0.25 IU/kg/day) has been able to improve HbA1c during the first 2 years, but the effect vanished during the third year of treatment. NPH insulin did not increase the risk of hypoglycemia (34) (evidence Moderate*)*. In an RCT, 19 adults with CFRD-FH^+ were randomly assigned to treatment with NPH or with glargine while continuing pre-meal insulin aspart (at least 3 administration *per* day). After 12-week treatment, NPH and glargine had similar efficacy on metabolic control in terms of HbA1c and postprandial glycaemia, whereas in the group treated with glargine, FPG was significantly reduced. No

TABLE 8 | Effectiveness of therapy on glycemic control (HbA1c, fasting and 2 h post-meal serum blood sugar values).

References	Study design Follow-up duration	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
Rolon et al. (33)	Retrospective-prospective case-control F/up: 5 years retrospective plus 5 years prospectively	14 CFRD patients age at T0: 15.3 y (range: 9 y 10 months to 21 y) compared with 14 non-diabetic CF patients	10 patients on two daily injections of a mixture of short- and intermediate-acting insulin 1 on basal-bolus regimen with short-acting insulin 1 on pre meal bolus-only 2 died shortly after insulin start	**HbA1c mean in cases** T0: no differences between cases and controls. T1 (n = 12) = 6.59 T2 (n = 8) = 7.37 T3 (n = 7) = 8.08 T4 (n = 7) = 7.51 T5 (n = 7) = 7.84	Downgrade (imprecision)	Low
Kolouskova et al. (34)	Prospective case–control F/up: 3 years	28 CF Insulinopenic patients 17 CFRD FH- and 11 IGT compared with 28 OGTT normal patients matched by sex, age and DOB	NPH insulin, once a day, 0.12 IU/kg (mean; range 0.09 – 0.25) before breakfast	**HbA1c %** T1 year: 5.5 vs 4.7 (p < 0,01) T2 Years: 5.3 vs 5.2 (N.S.) T3 Years: 6.4 vs 5.2 (p < 0,01)	Downgrade (imprecision, risk of bias)	Moderate
Grover et al. (35)	Randomized, cross-over F/up 12 weeks	19 CFRD with FH adults	12 week therapy with bedtime NPH vs 12 weeks of bedtime Glargine Boluses (Aspart) at least 3 times/day according to I:CHO of each patient	**HbA1c % (delta)** Glargine 6.4 ± 0.2 (-0.2 ± 0.2 from baseline) vs NPH 6.6 ± 0.2 (-0.2 ± 0.2 from baseline) (p 0,96) **Fasting Blood glucose (mg/dl)** Glargine 123 ± 4 (-8 ± 2, compared to baseline) vs NPH 125 ± 5 (0 ± 2, compared to baseline) (p 0,03) **2 H Post prandial glucose (mg/dl)** Glargine 150 ± 4 (+1.2± 0.5, compared to baseline) vs NPH 155 ± 9 (0.2 ± 0.5 compared to baseline) (p 0,85)	No	High
Frost et al. (36)	Prospective cohort study 2016-17 F/up: 12 months	52 Adults with CFRD diagnosed by CGM	15 patients: dietary modification 35 patients: on Detemir once daily (average initial dose 4.9 U) 2 patients on Lispro 2 U	**HbA1c** At baseline 39.4 mmol/mol At 3 months 31/37 HbA1c had <48 mmol/mol 20/32 had HbA1<40 mmol/mol	Downgrade (imprecision, risk of bias)	Low
Mozzillo et al. (37)	Prospective cohort study F/up: 12 months	22 CF patients: 4 AGT-CGMS 9 IGT 7 DM FH- 2 DM FH+ Mean age 12.4 ± 4.2 yr (range 2.6–19)	Glargine 1 daily before breakfast initial dose: 0.20 U/kg adjusted to obtain glycemia 70 - 140 mg/dl	**HbA1c** (average on 4 values in the last year before starting therapy vs 1 year on therapy) baseline 6.1 ± 0.1 (min 5.2 max 8.4) vs 6.3 ± 0.3 (min 5.5 max 11.6)	No	High
Minicucci et al. (38)	Multicenter, controlled, two-arm, randomized clinical study F/up: 18 months	34 CF patients with IGT (18 in the Glargine arm and 16 in the control arm). Median age 20 (range 11- 53)	Once daily Glargine up to a dosage of 0.15 U/kg/day, vs ordinary therapy (no hypoglycemic pharmacological treatment)	**HbA1c % difference** 0 - 18 months: –0.11 (–0.80; 0.30) vs 0.26 (–0.66; 0.95) p 0.04	No	Moderate
Moran et al. (39)	Multicenter, double blinded, comparative, randomized trial F/up: 12 months prospectively, plus 12 months	74 adults with CFRD FH- and 26 with severe IGT at OGTT	Three arms of randomization: - Insulin Aspart (0.5 unit/15 g of CHO) - Repaglinide 2.0 mg orally - Oral placebo three times a day	+12 months **Fasting glucose**: no differences between groups **90-min postprandial glucose** CFRD FH-: significantly lower in those treated with Aspart vs placebo (p 0.06)	Downgrade (imprecision)	High

(Continued)

TABLE 8 | Continued

References	Study design Follow-up duration	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
	retrospectively. (Dec 2007-2009)			116 ± 4 mg/dl in the insulin group 138 ± 12 mg/dl in the placebo group 131 ± 7 mg/dl in the repaglinide group IGT: significantly lower in those treated with Aspart vs repaglinide (p 0.03) 114 ± 3 mg/dl in the insulin group 122 ± 4 mg/dl in the placebo group and 131± 9 mg/dl in the repaglinide group		
Ballmann et al. (40)	Multicenter, open-label, comparative, randomized trial Dec 2009-2011 F/up: 24 months	75 patients >10 years, diagnosis of CFRD based on two consecutive OGTT in 6 months	34 patients on Repaglinide starting dose 0.5 mg ×3/die 41 patients on Insulin Regular (Actrapid) starting dose 0,05 UI/Kg/dose	**Fasting Glucose** No differences between the two groups **HbA1c** No difference between the two groups at baseline or after 12 or 24 months with repaglinide: 0.2% ± 0.7 with insulin vs –0.2% ± 1.3 (p 0.15)	Downgrade (risk of bias)	High
Hardin et al. (41)	Prospective cohort study F/up: 6 months	9 adults CFRD FH+	5 pts on D-Tron Plus pump 4 pts on Medronic paradigm 520 pump Therapy adjusted to maintain the following target -first morning: 95–120 mg/dl -pre-meal 75–110 mg/dl -postprandial: < 150 mg/dl.	**HbA1c** baseline 8.2% ± 1.9 vs 6 months after CSII 7.1%± 1.5 (p 0.05) **Fasting Blood glucose** (mg/dl) baseline 141 ± 41 vs 6 months after CSII 111± 27 (p 0.04) **2 H Post prandial glucose** (mg/dl) baseline 184± 44 vs 6 months after CSII 158± 32 (p 0.04)	Downgrade (imprecision)	Moderate
Geyer et al. (42)	Randomized, double-blind, crossover design Exenatide vs Placebo	Six patients, 10-25 years, 3 M 3 F, with CF and IGT	8.00-9.00 pm after at least 10 h long fasting. 48 h interval between the two drugs (exenatide/Placebo), ensuring complete clearance of exenatide. Intervention (Exenatide) in a dose of 2.5 micrograms 15 min prior to commencement of the test meal (standard meal)	**Postprandial blood glucose** Area under the curve over 240 min (AUC240) Exenatide 1431 ± 54 vs Placebo 1814 ± 109 mmol/L/min (p<0.0001) **Glucose peak postprandial value** Exenatide 7.65 ± 0.34 vs Placebo 9.53 ± 0.63 mmol/L (p<0.0001) Significant reduction of the glycemic response	Downgrade (imprecision)	Low
Gnanapragasam et al. (43)	Case report F/up: 3 months	A 21 year old CFRD patient	Semaglutide 0.13-0.16 mg weekly replaced prandial insulin Lispro in combination with Glargine 15 U	-Reduction in HbA1c from 9.1% to 6.7% -CGM: stable euglycemic pattern on CGM (TIR 68-77%; mean glucose, 142-163 mg/dl; SD, 51-65) during f/up -patient lost 2 Kg over the treatment period	No	Low

TABLE 9 | Effectiveness of therapy on pulmonary function (eg forced expiratory volume (FEV1) and forced vital capacity (FVC).

References	Study design Follow-up period	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
Rolon et al., (33)	Retrospective-prospective case-control F/up: 5 years retrospective plus 5 years prospectively	14 CFRD patients age at T0: 15.3 y (range: 9 y 10 mo to 21 y) compared with 14 non-diabetic CF patients	10 on two daily injections of a mixture of short- and intermediate-acting insulin 1 on basal-bolus regimen with short-acting insulin 1 on pre meal bolus-only 2 died shortly after insulin start	**FVC and FEV1** T-5 to T-1: lower in the cases (ns) -6 months: FVC 52 ± 20% vs 79 ± 20% (p= 0.01) FEV1 37 ± 19% vs 72 ± 23% (p = 0.01) FVC and FEV1 improved in the cases after the start of insulin therapy. Rate of FVC decline demonstrated in 5 of 7 patients after 5 y of insulin therapy (p = 0.1) Symptomatic cases seemed to benefit more than the screened cases	Downgrade (imprecision)	Low
Kolouskova et al., (34)	Prospective case–control F/up: 3 years	28 CF Insulinopenic patients, 17 DM FH - and 11 IGT) compared with 28 OGTT normal patients matched by sex, age and DOB	NPH insulin, once a day, 0.12 IU/kg (mean; range 0.09 – 0.25) before breakfast	**FEV1%** After 3 years FEV1 was lower in the untreated group compared to insulin treated patients who showed stable FEV 1 during insulin administration (61.0 ± 4.0 vs 73.5 ± 4.4; p 0.03)	Downgrade (imprecision, risk of bias)	Moderate
Frost et al. (36)	Prospective cohort study 2016-17 F/up: 12 months	52 Adult with CFRD diagnosed by CGM	15 patients on dietary modification 35 on Detemir once daily (average initial dose 4.9 U) 2 pz on Lispro 2 U	**FEV1** at 3 months +4.27% (1.1–7.48) p 0.01 worsened at 12 months (+1.07% from baseline, p 0.27)	Downgrade (imprecision, risk of bias)	Moderate
Hameed et al. (44)	Prospective cohort study F/up: median treatment of 0.8 years (range 1.3–2.2 years)	18 pts (7.2–18.1 yrs): 6 patients with CFRD 12 patients with early insulin deficiency (CFID1 and CFID2)	Detemir x 1/day starting from 0.1 unit/kg then adjusted for a blood glucose target of 4–8 mmol/l (72 -144 mg/dl) Median dose 0.13 units/kg/day median treatment duration 0.8 years (IQR 1.03)	**Delta %FEV 1** Insulin deficiency: -9.8% ± 9.3 vs +5.3 ± 11.5%, p=0.004 CFRD: +0.3 ± 8.3% vs –4.3 ± 18.6%, p=0.56 Whole Group: -7.9% ± 2.8 vs + 5.8 ± 13.4%, p=0.024 **Delta %FVC** Insulin deficiency: -6.8% ± 10.3 vs +5.8 ± 13.4%, p=0.024 CFRD: +4.0 ± 12.5% vs –3.8 ± 21.2%, p=0.34 Whole Group: -5.8% ± 14.3 vs +5.2 ± 12.7%, p=0.013	Downgrade (imprecision, risk of bias)	Moderate
Minicucci et al. (38)	Multicenter, controlled, two-arm, randomized clinical study F/up: 18 months	34 IGT adults with at least one of: 1. BMI <10th pc 2. loss of 1 pc class of BMI 3. FEV1 ≤80% than predicted; 4. FEV1 decrease ≥10%	Once daily Glargine up to a dosage of 0.15 U/kg/day, vs ordinary therapy (no hypoglycemic pharmacological treatment)	**FEV1%** There were no significant differences in FEV1 values between the two groups nor within groups.	No	Moderate
Mozzillo et al. (37)	Prospective cohort study F/up: 12 months	22 CF patients: 4 AGT-CGMS 9 IGT 7 CFRD FH- 2 CFRD FH+ Mean age 12.4 ± 4.2 yr (range 2.6–19)	Glargine x 1/day before breakfast initial dose: 0.20 U/kg adjusted to obtain glycemia 70 -140 mg/dl	**FEV1** (% of predicted for age sex race weight height), after 12 month therapy vs baseline 68.2 ± 6.2 (24.0/117.0) vs 77.1 ± 6.4 (37.0/118.0) p 0.01.	No	High

(Continued)

TABLE 9 | Continued

References	Study design Follow-up period	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
Fattorusso et al. (28)	Case report F/up: 16 years	Female CF patient. Intermittent diabetes during early childhood, IGT at 10 years, CFRD FH+ at 13 years.	0–9 y CFRD, intermittent requirement of rapid insulin 10 y IGT: Glargine 1/day (0.35 U/kg/day) 13 y CFRD-FH+ Rapid insulin + glargine (0.9 U/kg/day)	**FEV1%** At the age of 10 years: 97 13y: 97 16 yrs: 70.5 Earlier Glargine administration could have reduced the worsening of pulmonary function	No	Low
Moran et al. (39)	Multicenter, double blinded, comparative, randomized trial F/up: 12 months prospectively, plus 12 months retrospectively. (Dec 2007-2009)	74 adults with CFRD FH- and 26 with severe IGT at OGTT	Three arms of randomization: -Insulin Aspart (0.5 unit/15 g CHO) -Repaglinide 2.0 mg orally -Oral placebo three times a day	**FVC** CFRD FH group Insulin:-0.5 ± 2.0 (p 0.21) Repaglinide: -2.1 ± 2.2 (p 0.25) Placebo: -1.1 ± 2.5 (p 0.37) IGT group Insulin: -10.3± 4.2 (p 0.05) Repaglinide: -3.1± 5.6 (p 0.96) Placebo: -5.1± 3.7 (p 0.6) **FEV1 + 12 months** less decline in FEV1 in the insulin and repaglinide arms, but ns CFRD FH group Insulin:-1.8 ± 2.2 (p 0.21) Repaglinide: -1.3 ± 2.2 (p 0.1) Placebo: -3 ± 2.7 (p 0.5) IGT group Insulin: 12.1± 5.6 (p 0.12) Repaglinide: -4.9± 7.4 (p 0.82) Placebo: -11.5 ± 4.9 (p 0.05)	Downgrade (imprecision)	High
Ballmann et al. (40)	Multicenter, open-label, comparative, randomized trial F/up: 24 months (Dec 2009-2011)	75 patients >10 yrs. Diagnosis CFRD based on two consecutive OGTT in 6 months	34 patients on Repaglinide starting dose 0,5 mg x 3/day 41 on Regular Insulin (Actrapid) starting dose 0.05 UI/Kg/Dose	**FVC percentage of predicted** Change from baseline to 12 and 24 months: N.S. **FEV1** Change from baseline to 12 and 24 months: N.S.	Downgrade (risk of bias)	High

severe hypoglycemia event occurred and the frequency of minor hypoglycemic episodes was not significantly different in the two groups (35) (evidence High).

Only one prospective cohort study used insulin detemir, showing that this insulin administered once a day has been able to maintain HbA1c value below 6.5% (48 mmol/mol) in adult patients with CFRD after 3 months of treatment. Moreover, 62.5% of patients had HbA1c values below 5.8% (40 mmol/mol), with an 8% decrease in time spent above 140 mg/dl (7.8 mmol/l) (36) (evidence Low).

Three studies evaluated the efficacy of basal insulin therapy using glargine, without any apparent effect of this insulin on HbA1c (**Table 8**). A significant decrease in HbA1c has been observed only after 18-month therapy in one study (38) (evidence Moderate).

Pre-meal insulin (aspart or regular Actrapid) was compared to repaglinide in two studies, with conflicting results (**Table 8**). A study in 81 adults with CF and FPG or IGT showed repaglinide more effective after 6-month follow-up but not after 12-month follow-up (39) (evidence High). In pediatric patients Actrapid insulin and repaglinide showed similar efficacy, with no severe hypoglycemia episodes in the 2 groups (40) (evidence High).

One study in 9 patients with CFRD-FH+ used insulin pump therapy for 6 months achieving a significant reduction of HbA1c, fasting and postprandial blood glucose, without severe hypoglycemia events (41) (evidence Moderate).

Two studies have been published on the use of GLP-1 receptor agonists (GLP-1 RA) in the treatment of CFRD (42, 43). In a double blind RCT study conducted on 6 adolescents and young adults with IGT, exenatide was administered for 48 h showing a significant reduction in postprandial blood glucose (42) (evidence Low); as well as Semaglutide administered weekly at a low dose, was able to replace prandial Lispro and control glycemia in combination with Glargine (43).

In regard to the outcome "Effectiveness on pulmonary function", 9 studies were included in this systematic review, 3 are RCT, 3 are prospective cohort studies, and 3 are case-control studies, either prospective or retrospective. The number of

TABLE 10 | Effectiveness of therapy on nutritional status [eg body mass index (BMI)].

References	Study design Follow-up duration	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
Rolon et al. (33)	Retrospective-prospective case-control F/up: 5 years retrospective plus 5 years prospectively	14 CFRD patients age at T0: 15.3 y (range: 9 y 10 mo to 21 y) compared with 14 non-diabetic CF patients	10 on two daily injections of a mixture of short- and intermediate-acting insulin 1 on basal-bolus regimen with short-acting insulin 1 on pre meal bolus-only 2 died shortly after insulin start	**BMI z-score** Symptomatic cases had a decrease in their BMI z-score in the year preceding the onset of the insulin therapy ($p = 0.03$). BMI values increased significantly after the start of insulin therapy ($p < 0.05$).	Downgrade (imprecision)	Low
Kolouskova et al. (34)	Prospective case–control F/up: 3 years	28 CF 17 DM FH – and 11 IGT compared with 28 OGTT normal patients matched by sex, age and DOB Age at the diagnosis of CF: 0.1 – 13.3 years (median 3.6) Age at onset of the study: 11.2 – 21.6 years (median 15.4)	NPH insulin, once a day, 0.12 IU/kg (mean; range 0.09 – 0.25) before breakfast	**Weight z-score** NPH vs Controls T-1 Years: – 1.01 ± 0.13 vs – 1.03 ± 0.14 N.S. T0 – 1.08 ± 0.14 vs – 1.01 ± 0.13 N.S. T1 Years – 0.96 ± 0.14 vs – 0.98 ± 0.14 N.S. but significant increase from baseline in treated ($p < 0.001$) T3 Years – 0.83 ± 0.14 vs – 0.77 ± 0.16 N.S. but significant increase from baseline in treated ($p < 0.001$) **BMI z-score** NPH vs Controls T-1 Years – 0.78 ± 0.13 vs – 0.84 ± 0.13 N.S. T0 – 0.88 ± 0.14 vs – 0.77 ± 0.11 N.S. T1 Years – 0.75 ± 0.16 vs – 0.79 ± 0.13 N.S. but significant increase from baseline in treated ($p < 0.05$) T3 Years – 0.50 ± 0.20 vs – 0.62 ± 0.14 N.S. but significant increase from baseline in treated ($p < 0.05$) Weight and BMI significantly improved in the insulinopenic group following insulin administration	Downgrade (imprecision, risk of bias)	Moderate
Grover et al. (35)	Randomized, cross-over F/up 12 weeks	19 CFRD adults with FH	12 week therapy with bedtime NPH vs 12 weeks of bedtime Glargine Boluses (Aspart) at least 3 times/day according to I:CHO of each patient	**Weight (Kg)** Glargine 64.3 ± 2.4 (+1.2 ± 0.5 from baseline) vs NPH 65.7 ± 2.5 (+0.2 ± 0.5 from baseline) p 0.07 **Lean Body Mass (by DEXA) in Kg** Glargine 45.7 ± 1,9 (+0.3 ± 0.2 from baseline) vs NPH 45.7 ± 2 (+0.1 ± 0.2 from baseline) p 0.5 **Fat Body Mass (by DEXA) in Kg** Glargine 16.1 ± 1.4 (+0.7 ± 0.4 from baseline) vs NPH 16.7 ± 1.5 (+0.4 ± 0.4 from baseline) p 0.09	No	High
Hameed et al. (44)	Prospective cohort study F/up: median treatment of 0.8 years (range 1.3–2.2 years)	18 subjects (7.2–18.1 yrs): 6 patients with CFRD 12 patients with early insulin deficiency (CFID1 and CFID2)	Detemir x 1/day starting from 0.1 unit/kg then adjusted for a blood glucose target of 4–8 mmol/l (72 -144 mg/dl) Median dose 0.13 units/kg/day median treatment duration 0.8 years (IQR 1.03)	**Delta Weight z-score**: change pre insulin detemir vs post insulin detemir treatment Period of comparison pre therapy: 1 year in those who received insulin treatment for ≤1 year (n=10). Equivalent to duration of treatment in those who received insulin treatment for >1 year (range 1.3–2.2 years, n=8), Insulin deficiency (n = 12): -0.41 ± 0.43 vs + 0.22 ± 0.31 $p = 0.003$ CFRD (n = 6): –0.52 ± 0.25 vs +0.11 ± 0.24 $p = 0.014$	Downgrade (imprecision, risk of bias)	Moderate

(Continued)

TABLE 10 | Continued

References	Study design Follow-up duration	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
				Whole Group: -0.45 vs ± 0.38 vs + 0.18 ± 0.29 p=0.0001 ΔWtSDS improved in 16 patients		
Frost et al. (36)	Prospective cohort study F/up: 12 months (2016-17)	52 Adult with CFRD diagnosed by CGM	15 pt dietary modification 35 pt on Detemir once daily (average initial dose 4.9 U) 2 pt on Lispro 2 U	**Weight (Kg)** In the insulin group at 3 months there was gain of weight (+ 1.23 kg, p 0.01); the improvement was not confirmed at 12 months. In the dietary modification group, no significant differences in weight after 3 and 12 months.	Downgrade (imprecision, risk of bias)	Moderate
Mozzillo et al. (37)	Prospective cohort study F/up: 12 months	22 CF patients: 4 AGT-CGMS 9 IGT 7 CFRD FH- 2 CFRD FH+ Mean age 12.4 ± 4.2 yr (range 2.6–19)	Glargine x 1/day before breakfast initial dose: 0.20 U/kg adjusted to obtain glycemia 70 -140 mg/dl	**BMI z score** (after 12 months of therapy vs baseline) Whole population: baseline 20.56 ± 0.26 (min -2.8 max 2.2) vs -0.37 ± 0.25 (min -2.9 max 2.2) N.S. After stratification for BMI z score ≤ -1 or > -1 The difference was significant (p 0.017) only in patients with BMI z-score ≤ -1.	No	High
Minicucci et al. (38)	Multicenter, controlled, two-arm, randomized clinical study F/up: 18 months	34 IGT adults with at least one of: 1. BMI <10th pc 2. loss of 1 pc class of BMI 3. FEV1 ≤80% than predicted; 4. FEV1 decrease ≥10%	Once daily Glargine up to a dosage of 0.15 U/kg/day, vs ordinary therapy (no hypoglycemic pharmacological treatment)	**BMI z-score** Glargine vs Controls At baseline: –0.45 (–3.06; 1.34) vs 0.05 (–1.46; 2.20) p 0.12 0 - 3 months: 0.00 (–0.72; 0.98) vs 0.05 (–0.34; 0.41) p 0.73 0 - 6 months: 0.00 (–1.40; 0.79) vs –0.11 (–1.67; 0.48) p 0.98 0 - 9 months: – 0.09 (–0.91; 0.59) vs –0.13 (–1.57; 0.54) p 1.00 0 - 12 months: –0.10 (–1.15; 1.19) vs –0.11 (–1.89; 1.27) p 0.98 0 - 15 months: –0.14 (–1.77; 1.50) vs –0.04 (–2.06; 0.61) p 0.87 0 – 18 months: –0.13 (–1.92; 1.44) vs 0.00 (–1.94; 0.78) p 0.97	No	Moderate
Fattorusso et al. (28)	Case report F/up: 16 years	Female CF patient. Intermittent diabetes during early childhood, IGT at 10 years, CFRD FH+ at 13 years.	0–9 y CFRD, intermittent requirement of rapid insulin 10y IGT: Glargine 1/day (0.35 U/kg/day) 13y CFRD-FH + Rapid insulin + glargine (0.9 U/kg/day)	**BMI z-score** At the age of 10 years: -0.40 13y: - 0.18 16 yrs: - 1.52 Earlier Glargine administration could have reduced the worsening of nutritional status	No	Low
Moran et al. (39)	Multicenter, double blinded, comparative, randomized trial F/up: 12 months prospectively, plus 12 months retrospectively. (Dec 2007-2009)	74 adults with CFRD FH- and 26 with severe IGT at OGTT	Three arms of randomization: -Insulin Aspart (0.5 unit/15 g CHO) -Repaglinide 2.0 mg orally -Oral placebo three times a day	**BMI (kg/m^2)** -12 - 0 months: BMI decline in all groups 0 + 12 months CFRD FH- group Insulin: 0.39 ± 0.21 (p 0.02) Repaglinide: 0.15 ± 0.21 (p 0.33) Placebo: -0.02 ± 0.25 (p 0.45) Patients who received repaglinide had an initial significant gain of 0.53 ± 0.19 BMI units within the first 6 months of therapy (p 0.01); not sustained at 12 months (p 0.33) IGT group Insulin: -0.42 ± 0.30 (p 0.45) Repaglinide: -0.71 ± 0.28 (p 0.45) Placebo: 0.24 ± 0.27 (p 0.02)	Downgrade (imprecision)	High
Ballmann et al. (40)	Multicenter, open-label, comparative, randomized trial	75 patients >10 yrs. Diagnosis CFRD based on two consecutive OGTT in 6 months	34 patients on Repaglinide starting dose 0.5 mg x 3/day 41 on Regular insulin (Actrapid) starting dose 0,05 UI/Kg/dose	**BMI z-score** A significant change in BMI z-score was seen after 12 months Repaglinide –0.1 ± 0.4	Downgrade (imprecision)	High

(Continued)

TABLE 10 | Continued

References	Study design Follow-up duration	Participants	Treatment	Main outcomes	Rating upgrade/ downgrade	Evidence level
Hardin et al. (41)	F/up: 24 months (Dec 2009-2011) Prospective cohort study F/up: 6 months	9 adults CFRD FH+	5 pts on D-Tron Plus pump 4 pts on Medronic paradigm 520 pump Therapy adjusted to maintain the following target -first morning: 95–120 mg/dl -pre-meal 75–110 mg/dl -postprandial: < 150 mg/dl.	Insulin +0,1 ± 0.4 p 0.02 but not after 24 months (N.S.) **Weight (Kg)** baseline 55.63 ± 3.5 vs 6 months after CSII 59.2 ± 3.3 p 0,01 **Lean Body Mass (Kg) by DEXA** baseline 48.2 ± 1.5 vs 6 months after CSII 50.6 ± 1.6 p 0.03	Downgrade (imprecision, publication bias)	Moderate

TABLE 11 | Glucose Tolerance Categories in Cystic Fibrosis.

	Fasting Plasma Glucose (FPG)		2 h Plasma glucose value		Notes
	mg/dl	*mmol/l*	*mg/dl*	*mmol/l*	
Normal glucose tolerance (NGT)	< 100	< 5.6	< 140	< 7.8	All glucose levels < 140 mg/dl (7.8 mmol/L)
Impaired fasting glucose (IFG)	100 - 126	5.6 - 7	< 140	< 7.8	All glucose levels ≤ 140 mg/dl (7.8 mmol/L)
Abnormal glucose tolerance 140 (AGT 140)	< 100	< 5.6	< 140	< 7.8	Mid-OGTT glucose ≥ 140 mg/dl (7.8 mmol/L)
Indeterminate glycemia (INDET)	< 100	< 5.6	< 140	< 7.8	Mid-OGTT glucose ≥ 200 mg/dl (11.1 mmol/L)
Impaired glucose tolerance (IGT)	< 100	< 5.6	140 - 199	7.8 – 11	
CFRD with fasting hyperglycemia	≥ 126	≥ 7	≥ 200	≥ 11.1	
CFRD without fasting hyperglycemia	< 126	< 7	≥ 200	≥ 11.1	

enrolled patients ranged from 9 to 100, aged from less than 1 year to adulthood. The following therapies were tested in these studies, intermediate/NPH insulin (2 studies), insulin detemir (2 studies), insulin glargine (3 studies), rapid analogue insulin (2 studies), rapid insulin (1 study), repaglinide (2 studies), with conflicting results as shown in **Table 9**.

In a few studies, especially in pediatrics, it seems that insulin therapy has beneficial effects either on FVC or FEV1 (33) (evidence Low), while in adult patients the efficacy of insulin therapy seems to disappear after some time (34) (evidence Moderate). Similar data have been observed in other studies using insulin analogs either in adults (37) (evidence High) (36, 38) (evidence Moderate), or in pediatrics (44) (evidence Moderate) (28) (evidence Low).

Insulin aspart treatment compared to repaglinide or placebo, gave significant changes in the annual rate of decline in FVC, but not in FEV1 and in the number of acute exacerbations in subjects with Fc and IGT (39) (evidence High), while others observed that insulin Actrapid did not have any impact (40) (evidence High), or just limited one (28) (evidence Low) on pulmonary function decline.

Regarding the outcome "Effectiveness on nutritional status", 11 papers were included in this systematic review, four are RCT, four are prospective cohort studies, one is a prospective case-control, and one is a case-control study. The number of enrolled patients ranged from 9 to 100, aged from a few months to adult age (**Table 10**).

Details about the relationship between the therapies used and the nutritional status of the patients with CF are given in **Table 10**. Summarizing, there is Low evidence that NPH insulin allows a significant improvement in BMI in children (33), but not in adults (34) (evidence Moderate), even if data are conflicting (35) (evidence Low). Better results have been found using insulin analogues, such as detemir either in pediatric patients (44) (evidence Moderate), or in adults (36) (evidence Moderate).

No significant differences in BMI-SDS were observed either in adolescents treated with Repaglinide compared to those treated with insulin Actrapid (40) (evidence High), or in adults (39) (evidence Moderate). Insulin pump therapy seemed to be effective in increasing weight and lean mass (41) (evidence Moderate).

DISCUSSION

This systematic review of the literature and the GRADE approach (8, 9) were able to demonstrate how much CFRD impacts on the clinical course of CF in terms of pulmonary function, pulmonary exacerbations, pulmonary microbiological colonization and auxological parameters.

Moreover, prediabetes emerged as an important predictor factor of either CFRD or worse prognosis of CF outcomes in both pediatric and adult patients. Evidence of prediabetes in children under 10 years of age is not unusual and is once again associated with a significant risk of progression to CFRD and worse clinical course of CF. A significant decline in pulmonary function was in fact observed in pediatric patients with prediabetes (14), as well as, in adult patients with prediabetes compared to patients with NGT (11–14).

Among prediabetes, IGT represents one of the most relevant predictors of the decline in pulmonary function (17) (18) and both CFRD and IGT have been recognized as independent risk factors for pulmonary exacerbations and colonization by

common CF pathogens, in particular by *Pseudomonas Aeruginosa*, and its form of infection characterized by multi-resistance to antibiotic therapy and co-infection with other pathogens (11, 16, 19, 21–23, 25).

Glycemic peaks higher than 200 mg/dl (11.1 mmol/l) were recorded with continuous glucose monitors in patients screened with OGTT, with normal fasting and 2-h blood glucose, and these glycemic peak were related to a worsening of spirometry parameters (16). A very recent study, not found by the search for the systematic review that was updated till October 2020, analyzed the correlation between intermittent scan CGM (isCGM) and OGTT data in a cohort of 32 children with CF. The isCGM percent of measurements >140 mg/dL (7.8 mmol/L) and the number of peaks per day >200 mg/dL (11 mmol/L) have correlations with intermediate OGTT glucose time points, but not the 2-h glucose value. Moreover patients with abnormal glucose tolerance (AGT) had lower lung function than those with normal glucose tolerance demonstrated by both FEV1% predicted and lung clearance index (47).

Among patients with CF, auxological parameters are significantly impaired in patients with CFRD than patients with NGT, also before diabetes diagnosis (14) and can be negatively influenced by prediabetes (11, 12, 44).

Evaluating the impact of different therapeutic strategies on glycemic control, pulmonary function and auxological parameters in patients with CFRD, glargine seems to be the most studied insulin. Data showed with moderate to high level of evidence that glargine therapy led to a significant improvement in HbA1c, the gold standard metric for long-term glycemic control, only in adult IGT patients (38), but not in children (37). In addition, in CFRD-FH^+ adult patients glargine had the same efficacy than NPH insulin (35). Data about detemir are more confusing (36, 44).

A significant improvement in HbA1c value was demonstrated in CFRD patients treated with insulin pump therapy (41).

The analysis of the efficacy of pre-meal insulin or oral antidiabetic agents in controlling postprandial hyperglycemia in CFRD showed that aspart insulin is more effective than repaglinide (39), and regular insulin is as effective as repaglinide in patients with HbA1c <7% (40).

Glargine insulin in CFRD patients did not increase the risk of hypoglycemia when compared to NPH (35), and repaglinide does not increase the number of hypoglycemic events compared to regular insulin (40).

In both, adult and pediatric patients with CFRD or prediabetes, insulin therapy with NPH (32), as well as with Detemir (44) and glargine (37, 38) preserved pulmonary function and reduced the number of respiratory exacerbations, whereas therapy with regular human insulin or rapid analogue insulin or repaglinide had no impact on pulmonary function (28, 39, 40).

Regarding auxological and nutritional status parameters, in adults with diabetes or prediabetes, NPH insulin preserved the decay of BMI-SDS (34). Insulin detemir in pediatric patients with CFRD improved BMI-SDS (44), while this effect was not sustained in adult patients 37. In pediatric subjects diagnosed with AGT, IGT or CFRD, glargine insulin improved the BMI-SDS only in patients with poor nutritional status (BMI SDS <−1) (37, 38). In adults, insulin therapy with rapid analogue increased BMI in those diagnosed with IGT, but not in those with CFRD FH^+ (39), while insulin pump therapy produces an increase in weight and lean mass measured with DEXA after 6 months of therapy (41). Repaglinide and regular insulin in CFRD patients have no impact on BMI-SDS (40).

LIMITATIONS

To obtain the best evidence to answer our research questions, we have made a great effort to collect studies by establishing strict inclusion criteria. Even if low-quality studies have been excluded, a few limitations still exist, that should be acknowledged for the evaluation and interpretation of the results, and consequently, the recommendation summarized in this review: 1) few RCT studies have been found, particularly for the outcome related to "effectiveness of treatment"; 2) only a few studies had sample sizes larger than 100 patients, and 3) continuous glucose monitoring was used in a limited number of studies.

CONCLUSIONS AND RECOMMENDATIONS FOR THE CLINICAL PRACTICE

CF is a complex multi-organ disease requiring a comprehensive multidisciplinary treatment program. CFRD deserves special knowledge and expertise to be adequately diagnosed and treated. For these reasons, after a systematic review of the literature, the Diabetes Study Group of the ISPED has agreed to provide practical recommendations based on both evidence from current literature and clinical experience.

1. In patients with CF, OGTT is the gold-standard method for the screening of glucose abnormalities during periods of clinical stability (absence of respiratory exacerbation and/or use of antibiotic and/or steroid therapy, and in case of organ transplantation). Patients should be tested once a year -**Strong in favor-.**
2. Random BG and fasting BG measurement are recommended during periods of pulmonary exacerbations and/or use of glucocorticoid therapy, enteral nutrition and in case of diabetes symptoms -**Strong in favor**-.
3. Current evidence did not support the use of continuous glucose monitoring as a diagnostic and/or screening tool; however, it is considered very useful for monitoring subjects with IFG, IGT or INDET and during high risk conditions (i.e.: pulmonary exacerbations and/or use of steroids) - **Strong in favor-.**
4. Subjects with prediabetes are at risk of developing CFRD, thus close monitoring of glucose metabolism with OGTT and 2-week CGM at 6-month intervals is recommended **-Conditional in favor-.**

5. In children <10 years of age, starting from at least 6 years of age or even earlier if possible, it is advisable to screen for alterations of glucose metabolism at least once a year, whenever possible, and if IGT or INDET conditions are diagnosed a close monitoring of BG levels is recommended (OGTT at 6-month intervals; continuous glucose monitoring as a possible adjunctive tool for glucose monitoring during respiratory exacerbations and/or use of steroids), **-Conditional in Favor-.**
6. Treatment with a basal analogue (glargine) should be started in patients affected by CFRD FH^+ at a dosage of 0.2 IU/kg/day **-Conditional in Favor-.**
7. Postprandial hyperglycemia should be treated with rapid analogue at initial dosage of 0.05 to 0.1 UI/kg before meal or an insulin/carbohydrate ratio starting with 1UI: 15 g and 1UI: 30 g ratio and subsequently modified on the basis of different intakes of the day and of the degree of insulin resistance at each moment. **-Conditional in favor-.**
8. The risk of hypoglycemia due to insulin therapy is low**, -Strong in favor-.** Treatment of CFRD with insulin could result in an improvement in fasting and postprandial blood sugar levels more than in the HbA1c value, **-Strong in favor-.**
9. Insulin pump therapy, although rarely used in these patients, is a valid and effective alternative to multiple daily injections **-Strong in favor-.**
10. In patients diagnosed with IGT, if clinically compromised, treatment with basal insulin analogue is recommended starting with 0.1 to 0.2 UI/kg/day with subsequent adjustments based on the glycemic trend, -**Conditional in Favor**-.
11. In patients diagnosed with other prediabetic conditions, in particular INDET and AGT, early initiation of insulin therapy could be beneficial and this possibility should be taken into account after a complete evaluation of the patient on the basis of the annual trend of the OGTT results, glucometrics, glycemic values measured during pulmonary exacerbations and/or steroid therapy, -**Conditional in Favor**-.
12. In all CF patients with diabetes and prediabetes treated with insulin analysis of glucose metrics with CGM may be useful for monitoring insulin treatment, -**Conditional in Favor**-.

COLLABORATORS

Collaborators of the Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology (ISPED). A complete list of the members of the Diabetes Study Group of the ISPED can be found in the supplementary material online.

AUTHOR CONTRIBUTIONS

EM and RF engaged in literature retrieval of the articles, have analyzed the results, and wrote the manuscript. CP, SP, AC, and DP performed records screening and assessed eligibility, compilation of evidence and of evidence tables. AES reviewed the records screening and contributed to wrote the manuscript. GM, VCal, VCau, VCh, GD, AF, APF, FL, DP, MCM, EP, BP, IR, ST, and SZ discussed and commented on literature analysis. RS and CM critically revised the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.673539/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

MINI REVIEW
published: 30 April 2021
doi: 10.3389/fped.2021.679484

Benefits and Challenges of Current Closed-Loop Technologies in Children and Young People With Type 1 Diabetes

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Recent advances in diabetes technology have led to the development of closed-loop insulin delivery systems for the management of type 1 diabetes. Several such systems are now commercially available for children and young people. While all available systems have been shown to improve glycaemic control and quality of life in this population, qualitative data also highlights the challenges in using closed-loop systems, which vary among different pediatric age-groups. Very young children require systems that are able to cope with low insulin doses and significant glycaemic variability due to their high insulin sensitivity and unpredictable eating and exercise patterns. Adolescents' compliance is often related to size and number of devices, usability of the systems, need for calibrations, and their ability to interact with the system. Given the speed of innovations, understanding the capabilities and key similarities and differences of current systems can be challenging for healthcare professionals, caregivers and young people with type 1 diabetes alike. The aim of this review is to summarize the key evidence on currently available closed-loop systems for children and young people with type 1 diabetes, as well as commenting on user experience, where real-world data are available. We present findings on a system-basis, as well as identifying specific challenges in different pediatric age-groups and commenting on how current systems might address these. Finally, we identify areas for future research with regards to closed-loop technology tailored for pediatric use and how these might inform reimbursement and alleviate disease burden.

Keywords: diabetes technology, young people, type 1 diabetes (or diabetes), closed-loop insulin delivery, artificial pancreas (AP), children

OPEN ACCESS

Edited by:
Marco Marigliano, Integrated University Hospital Verona, Italy

Reviewed by:
Valentino Cherubini, Azienda Ospedaliero Universitaria Ospedali Riuniti, Italy
Bonfanti Riccardo, San Raffaele Hospital (IRCCS), Italy

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *11 March 2021*
Accepted: *01 April 2021*
Published: *30 April 2021*

Citation:
Fuchs J and Hovorka R (2021) Benefits and Challenges of Current Closed-Loop Technologies in Children and Young People With Type 1 Diabetes. Front. Pediatr. 9:679484. doi: 10.3389/fped.2021.679484

Type 1 diabetes is a lifelong, incurable condition characterized by a deficiency of insulin caused by immune-mediated destruction of pancreatic beta-cells in genetically predisposed individuals (1). Its incidence in the pediatric population is increasing by around 3% per year (2), and more than one million children and young people under the age of 20 years are living with the condition worldwide (3). Tight glycaemic control is challenging to achieve, and the majority of children and young people with type 1 diabetes do not meet treatment guidelines for target glycated hemoglobin (HbA1c) (4–7), or only achieve good glycaemic control at the expense of high management burden (8–10). Meeting glycaemic targets is vital, as higher HbA1c levels are associated with an increased risk of premature morbidity and mortality (11–13).

Over the past decades, several new technologies have been developed to improve management of type 1 diabetes, including insulin pumps and real-time continuous glucose monitoring (CGM) devices (14). However, rather than improving, HbA1c levels have worsened in the pediatric age group over the last 10 years (5). While insulin pumps and CGM devices have been shown to lower HbA1c levels and reduce the risk of diabetic ketoacidosis (15–17), they require significant user-input and frequent insulin dosing adjustments to achieve good glycaemic control (10). In recent years, the development of closed-loop systems, which link insulin delivery to sensor glucose levels, have started to transform management of type 1 diabetes (18, 19). These closed-loop systems utilize an algorithm that automatically adjusts insulin delivery via an insulin pump based on real-time sensor glucose levels. This glucose-responsive automated insulin delivery more closely replicates normal physiology. Current hybrid closed-loop systems continue to require user-initiated prandial insulin boluses.

This review summarizes key evidence on current closed-loop systems for children and young people with type 1 diabetes, as well as commenting on user experience and specific challenges in different pediatric age-groups.

CLOSED-LOOP SYSTEMS

Four hybrid closed-loop systems are currently commercially available and licensed for use in children and young people, with varying minimum age for use. These systems are: 670G hybrid closed-loop (HCL) system and 780G advanced HCL (AHCL) (Medtronic, Northridge, California); CamAPS FX interoperable app (CamDiab, Cambridge, UK); and the Control IQ system (Tandem Inc., San Diego, California). Further hybrid closed-loop systems are in development, with pivotal trials under way (20). **Table 1** summarizes the key clinical trial evidence of commercialized systems in children and young people.

The closed-loop algorithm is embedded in the software of the tethered insulin pump and communicates wirelessly with the compatible glucose sensor for the Medtronic and Tandem systems (14). For CamAPS FX, the algorithm is embedded in an app, classed as a medical device, residing on an unlocked smartphone that communicates wirelessly with a compatible insulin pump and glucose sensor (14). As more hybrid closed-loop systems become commercially available, it is becoming increasingly complex for people with type 1 diabetes, their families and healthcare professionals to navigate different technologies. While similar in basic principle, there are important differences between each hybrid closed-loop system, and clinicians need to understand key device characteristics in order to provide appropriate clinical support and guidance to children, young people and their families. Several available closed-loop systems now provide online training and education modules, both for healthcare professionals and users, and increasingly module completion is a requirement to allow initiation of closed-loop. **Table 2** outlines the capabilities and key similarities and differences of current systems using the CARES paradigm, an educational tool developed by Messer et al. (32), that provides a practical framework to identify key concepts for each closed-loop system. Understanding these key concepts will allow healthcare professionals to set appropriate expectations of hybrid closed-loop system capabilities, and to adjust settings for treatment optimisation to maximize the benefits of this novel therapy.

Clinical trials show that hybrid closed-loop insulin delivery is safe and improves glycaemic outcomes in children of all ages (10, 33), but high quality real-world data remains scarce, particularly for newer systems licensed in the last 12–18 months. Considering the evidence available in terms of system capabilities, qualitative research and observational real-world data, we discuss age-specific challenges in children and young people and how closed-loop systems might address these.

TODDLERS AND YOUNG CHILDREN

Type 1 diabetes is challenging to manage at any age, but management is further complicated in young children under the age of 7 by a variety of unique physiological, behavioral and developmental factors. Young children have higher variability in insulin requirements and higher insulin sensitivity than older children and adults (34), as well as more unpredictable eating and activity patterns. Furthermore, hypoglycaemia is frequently asymptomatic and can be prolonged, particularly at night-time (35, 36). These factors and resulting parental worry lead to high management burden for parents and caregivers with negative impact on family quality of life (9).

Closed-loop studies in this age-group have been of short duration in small cohorts (**Table 1**). One of the main benefits of hybrid closed-loop insulin delivery in young children is improved glycaemic control at night-time. In a 3-week closed-loop study in 24 very young children, time in the target range 3.9–10.0 mmol/L was highest overnight with reduction in hypoglycaemia compared to daytime (31) (**Table 1**). Variability in insulin requirements in young children is highest overnight (34), and closed-loop systems are uniquely positioned to address this by delivering insulin in a glucose-responsive manner. Qualitative data shows that parents noted improvements in quality of sleep with closed-loop, both for themselves and their child (37, 38).

Improvements in glycaemic control are less marked in the daytime, which is likely related to unpredictable eating and activity patterns. Bolus timing is challenging in this age group, as children frequently graze or do not complete meals. Many parents choose to bolus with or shortly after the start of the meal, leading to post-prandial hyperglycaemia (39, 40). The pharmacokinetics and pharmacodynamics of current rapid-acting insulins limit the closed-loop system's ability to mitigate immediate post-prandial hyperglycaemia (41), and the resultant increase in algorithm-driven insulin delivery increases the risk of delayed hypoglycaemia. New ultra-rapid acting insulins, which have faster onset and offset of action, have the potential to address this issue, but

TABLE 1 | Key clinical trials of closed-loop insulin delivery in children and young people using commercialized systems.

Age group (no. of participants)	Duration of closed-loop treatment	Type of study	Comparator	Baseline HbA1c	Glycaemic outcomes	Year and Key reference
Medtronic 670G HCL / 780G AHCL						
14-21 years ($n = 30$)	3 months (670G)	Single-arm, non-randomized	None	61mmol/mol (7.7%)	TIR 67%, no control arm.	2017 (21, 22)
7-13 years ($n = 105$)	3 months (670G)	Single-arm, non-randomized	None	63mmol/mol (7.9.%)	TIR 65%, no control arm.	2019 (23)
14-29 years ($n = 113$)	3 months (AHCL)	Randomized, crossover	670G HCL	63mmol/mol (7.9%)	TIR 67% with 780G and 63% with 670G ($p<0.0001$).	2021 (24)
7-80 years ($n = 59$) [7-13 years ($n = 19$); 14–21 years ($n = 14$)]	4 weeks (AHCL)	Randomized crossover	670G PLGM	60mmol/mol (7.6%)	TIR 70% overall, increased 12 percentage points compared to control ($p<0.001$); increased 12 percentage points in 7-13yr olds and 14 percentage points in 14-21yr olds compared to control.	2021 (25)
Tandem Control IQ						
6-13 years ($n = 101$)	16 weeks	Randomized, parallel	Sensor-augmented pump	61mmol/mol (7.7%)	TIR 67%, increased 11 percentage points compared to control ($p<0.001$).	2020 (26)
14-24 years ($n = 63$)	6 months	Randomized, parallel	Sensor-augmented pump	65mmol/mol (8.1%)	TIR 64%, increased 13 percentage points compared to control ($p<0.001$).	2020 (27)
2-5 years ($n = 12$)	3 days	Single-arm, non-randomized	None	56mmol/mol (7.3%)	TIR 71%, no control arm.	2020 (28)
CamAPS FX (CamDiab)						
10-18 years ($n = 12$)	3 weeks	Randomized, crossover	Sensor-augmented pump	69mmol/mol (8.5%)	TIR 67%, increased 19 percentage points compared to control ($p<0.001$).	2016 (29)
6-65 years ($n = 86$) [6-12 years ($n = 33$); 13-21 years ($n = 19$)]	12 weeks	Randomized, parallel	Sensor-augmented pump	68mmol/mol (8.3%)	TIR 65% overall, increased 11 percentage points compared to control ($p<0.0001$); increased 15 percentage points in 6-12yr olds and 14 percentage points in 13-21yr olds compared to baseline.	2018 (30)
1-7 years ($n = 24$)	3 weeks	Randomized, crossover	Closed-loop with diluted insulin U20.	57mmol/mol (7.4%)	TIR 70% closed-loop with U100 and 72% closed-loop with U20, no difference ($p=0.16$).	2018 (31)

HCL, hybrid closed-loop; AHCL, advanced hybrid closed-loop; PLGM, predictive low glucose management; TIR, time in range; HbA1c, glycated hemoglobin.

these have not been trialed in closed-loop systems in the pediatric age-group.

Another limitation of current closed-loop systems is the minimum total daily insulin dose required for optimal system performance. While this does not preclude use in those young children with a very low total daily dose, it can limit the benefit of closed-loop therapy due to the high variability of absorption with such small volumes (42). A randomized trial comparing closed-loop insulin delivery using diluted and standard strength insulin showed no difference in glycaemic outcomes between the two groups (31). However, only a small number of participants had a total daily insulin dose of <10 units in this cohort. Previous shorter closed-loop studies using diluted insulin in this age group showed reduced inter-individual variability in time to peak insulin action with diluted insulin (42), suggesting that insulin dilution may be beneficial on a case-by-case basis in those with a very low total daily insulin dose.

In spite of these limitations, qualitative studies reported parents spending less time performing diabetes-related activities and feeling less stressed when their child was using closed-loop, resulting in reduced management burden overall (37, 38).

There is limited real-world closed-loop data available for very young children. This is in part due to the fact that closed-loop insulin delivery is only licensed for one commercialized system (CamAPS FX) in this age group, with other systems being used off-license in some centers. A retrospective case series of the 670G HCL system in 16 children under the age of 7 years showed improvements in glycaemic control compared to baseline (43). There was an increase in time in hypoglycaemia, however this was still low at 2.4% (43). Importantly, the results of further clinical closed-loop trials in very young children are expected to be reported in the near future, and should result in licensing of a wider variety of systems in this age group.

TABLE 2 | Comparison of commercially available closed-loop systems using the CARES paradigm (32).

CARES		670G/780G	Control IQ	CamAPS FX
	Licensing	7 years+	6 years+	1 year+
	Availability	USA and Europe	USA & Europe	Europe
	Pump	Medtronic 670G / 780G	Tandem t:slim X2	Dana RS, Dana-i
	Insulin	Rapid-acting	Rapid-acting	Ultra-rapid and rapid-acting
	Closed-loop term	Auto Mode	Control IQ	Auto mode
Calculate	Algorithm	Treat-to-target proportional integral derivative with insulin feedback (670G); added fuzzy logic component (780G)	Treat-to-range predictive control	Treat-to-target MPC
	Set-up	TDD, weight, basal rates, ICR, ISF, active insulin time. 7 days of manual mode	TDD, weight, basal rates, ICR, ISF	TDD and weight
	Adaptive learning	Overall	Not specified by manufacturer	Overall, diurnal, meals
	Automated insulin delivery	Based on total daily insulin dose last 2-6 days	Based on pre-programmed basal rates	Based on adaptive learning
	Automated corrections used to supplement basal delivery	670G: No 780G: Yes	Yes	No
Adjust	Glucose target	670G: Target 6.7mmol/L non-customisable. 780G: Customisable target of 5.5, 6.1, or 6.7mmol/L.	Target range 6.2 – 8.9mmol/L. Sleep range 6.2 – 6.7mmol/L. Non-customisable.	Target 5.8mmol/L. Customisable between 4.4-11mmol/L, adjustable in 0.1mmol/L increments.
	Adjustable settings in CL	ICR, active insulin time, glucose target	Basal rates, ICR, ISF, target range	ICR, glucose target
	Non-adjustable in CL	Basal rates, ISF (automatically calculated and adapted)	Active insulin time (set at 5 hours)	Basal rates, active insulin time, ISF (all automatically calculated and adapted)
	Exercise mode	670G: No 780G: Yes	Yes	Yes
	Boost mode	No	No	Yes
Revert	Sick day rules	——————————————Recommended to revert to open loop for illness and/or ketones—————————————-		
	Automatically reverts to open loop if…	Prolonged hyperglycemia (670G only), max/min insulin delivery, loss of CGM data, sensor integrity concerns, lack of calibrations.	Loss of CGM data	Loss of CGM data or loss of pump connectivity
Educate	Meal bolus	——————————————-Pre-meal bolusing recommended for optimal outcomes—————————————		
		Late bolusing can lead to insulin stacking and hypoglycaemia as CL insulin delivery increases in response to rising glucose		
	Hypo treatment	———————Consider treating hypoglycaemia with fewer carbohydrates depending on recent insulin delivery———————-		
	System optimisation	• System requires finger pricks for HCL functioning • Use of temp target will turn off automated corrections (780G only) • Extended bolus / combo bolus function not available in CL	• Set sleep activity schedule overnight for tighter target • Adjust doses for individuals with shorter active insulin time • Extended bolus possible in CL, max 2 hours	• Use exercise mode "Ease-Off" following hypoglycaemia • Use "Boost" mode during periods of high glucose • Extended bolus / combo bolus function not available in CL
	Online training	No	Yes	Yes
Sensor	Type of sensor	Guardian 3	Dexcom G6	Dexcom G6
	Calibrations	670G: 4–6 per day 780G: 1–2 per day	Rarely required (factory calibrated)	Rarely required (factory calibrated)
	Sensor life	7 days	10 days	10 days
Share	Remote monitoring	No (App in development for 780G)	Yes – Dexcom follow	Yes – Text Alert (Dexcom Follow planned for 2021)
	Upload/Data sharing	Manual downloading	Automated cloud storage for Dexcom data; manual downloading for pump	Automated cloud storage to Diasend (Clarity in 2021)
	Remote bolusing	No	No	Yes

MPC, model predictive control; CGM, continuous glucose monitor; TDD, total daily insulin dose; ICR, insulin-to-carbohydrate ratio; ISF, insulin-sensitivity factor; CL, closed-loop.

CHILDREN

There is significantly more evidence of closed-loop safety and efficacy in school-aged children, compared to those below the age of 7 years. Studies of longer duration in larger cohorts show significant improvements in glycaemic control (26, 30), with no difference in time in hypoglycaemia (**Table 1**).

Despite their young age, school-aged children often independently manage their diabetes to a significant extent (10). This is in part due to a high turnover of caregiving adults, whose diabetes management knowledge is often minimal (44). This leads parents and children to tolerate higher glucose levels to avoid hypoglycaemia (10, 45), and may limit children's ability to participate in certain activities or events without parental supervision.

Closed-loop systems address this issue in two ways. The automation of insulin delivery in response to real-time sensor glucose levels reduces the need for user input, and events such as post-prandial hyperglycaemia or exercise-induced hypoglycaemia may be prevented or attenuated by the closed-loop system itself. This system-innate responsiveness has the potential to give children more freedom in their activities by increasing parents' confidence in the child's safety. In a qualitative study interviewing parents of children using a closed-loop system, they reported being more willing to allow their child to participate in activities such as school trips or sleepovers than before (21). Secondly, the remote monitoring capabilities of some closed-loop systems give reassurance to parents and children, by allowing parents to adopt a watchful waiting approach, and to intervene and support their child's decision making if required (46). Both parents and children reported closed-loop insulin delivery improving their quality of life and reducing diabetes management burden (21, 38).

While clinical trial evidence shows significant benefits with closed-loop insulin delivery in terms of glycaemic control, parents noted the importance of trusting the closed-loop system for optimum benefit (21). They noted that an initial adjustment period was required, during which they realized that taking action to address low or high glucose levels could be counter-productive to the system's ability to manage glucose levels (21). Additional education around minimizing interventions when using closed-loop insulin delivery could be beneficial when commencing this therapy.

Similar to younger children, real-world closed-loop data is limited. All commercially available closed-loop systems, apart from the 670G HCL system, were only licensed for children in the last 12–18 months. A prospective observational study of people aged 9–61 years using the 670G HCL system for 1 year found that closed-loop use declined significantly over time with a high proportion of closed-loop discontinuation (47). Children and adolescents were more likely to discontinue closed-loop. The main reasons for discontinuation were frequent sensor calibration requirements and a high number of closed-loop exits (47). Another prospective observational study of youth aged 2–25 years using the 670G HCL system showed similar results, with a steady decline of closed-loop use over time (48). Newer generation systems, such as Control IQ and CamAPS FX, using a factory-calibrated sensor alleviate a key reason for closed-loop discontinuation. While the 780G AHCL system still requires sensor calibration, clinical trials show a significant reduction in closed-loop exits (24), suggesting improved usability in this newer iteration.

YOUNG PEOPLE

HbA1c levels are highest in young people aged 13–17 years (5). Diabetes self-management is particularly challenging in this age group due to a variety of factors, including peer group influences, importance of body image, less parental oversight, greater risk-taking, and fear of hypoglycaemia, leading to higher levels of diabetes distress (49, 50). Closed-loop insulin delivery offers a novel way to address these issues, although important considerations remain with regards to choice of system for individual users.

Clinical studies have shown that closed-loop insulin delivery significantly improves glycaemic control in this age-group (25, 51), including in those with sub-optimal glycaemic control (30), and that improvements are sustained over time (27, 52). Importantly, qualitative studies of young people using closed-loop and their parents have reported significant improvements in quality of life and reduced diabetes management burden (21, 46, 49, 50).

Fitting in with peers and taking part in normal activities is very important to young people (50), which can lead them to neglect diabetes self-management tasks such as finger prick blood glucose checks and pre-meal bolusing (53). Glucose sensors reduce burden and allow young people to discreetly check glucose levels, as well as allowing glucose-responsive insulin delivery in closed-loop. While sensors requiring calibration can be a significant factor in low closed-loop usage (38, 48, 54), several systems now use factory-calibrated glucose sensors, with high sensor wear reported in clinical trials (52) and reduced device-burden reported in qualitative studies (49). In a qualitative study of young people using closed-loop from onset of diabetes, participants reported that the closed-loop system had helped them continue to lead normal lives despite having diabetes (49) by alleviating the need for disruptive finger pricking and automatically adjusting insulin delivery in response to high or low glucose levels (50, 55).

Current closed-loop insulin delivery systems are all hybrid systems, which require user-initiated prandial boluses for optimal efficacy. However, studies have shown that systems have the ability to cope with missed boluses, while still providing improvements in short-term glycaemic control without an increase in hypoglycaemia (53). Data from a recent 6-month closed-loop study using Control IQ in young people aged 14–24 years showed sustained glycaemic improvements (27), suggesting that closed-loop remains efficacious in

a group where there is higher likelihood of sub-optimal compliance (53).

Common barriers to closed-loop insulin delivery in this age group are device burden and alarm frequency (49). Young people preferentially wear devices in non-visible locations (21) and at times avoid activities where devices may be visible to others, such as swimming (49). A system with remote data viewing and bolusing capability via a mobile phone was positively received by young people, as this offered maximum discretion in peer environments (50) while enabling them to make management decisions (21, 49). Audible alarms can negatively impact quality of life, and in a qualitative study of closed-loop in this age group parents reported some young people opting to disconnect from the system when socializing with peers to limit alarms sounding in public (50). Most systems now feature personalisable alarm settings and healthcare providers should support young people in choosing settings that minimize interruptions while providing an adequate safety net.

Real-world data of the first commercially available hybrid closed-loop system, the 670G HCL system, showed high rates of closed-loop discontinuation, due to frequent sensor calibration requirements and a high number of closed-loop exits (47, 48). As described above, newer generation systems have shown much higher closed-loop use and fewer closed-loop exits in clinical trials (24, 27). A recently published real-world study assessed glycaemic control and quality of life by administering questionnaires to more than 1,000 Control-IQ users aged 14 years and over (21). Users reported a positive impact on their quality of life and sleep quality over a 2-month period after starting closed-loop therapy. Minimizing device and alarm burden and enabling easy and discreet user interaction, while maintaining glycaemic benefits should be the main goals of further closed-loop system developments in this age group.

FUTURE RESEARCH

Across all pediatric age groups device burden and connectivity problems remain an issue with regards to closed-loop insulin delivery (49, 50, 55). System-integration with factory-calibrated sensors is paramount to ensure high and sustained closed-loop usage. Connectivity issues resulting in closed-loop exits need to be improved, for example by increasing allowable distance between devices or integrating the algorithm with smart devices, such as watches. Remote data viewing and bolusing capabilities are highly valued by parents of young children, as this minimizes disturbance during sleep or play, and also by young people, where it allows discreet interaction with the closed-loop system, and this should become standard to all commercial closed-loop systems (21). Furthermore, automatic cloud storage and data sharing facilitates interaction with healthcare providers and improves remote consultations, reducing burden (56). Currently, most systems are limited to one pump and CGM model, with specific devices having user-dependent pros and cons. Inter-operable systems, where users can mix and match devices that suit their individual needs, should be the focus of future developments.

The majority of systems are not licensed for very young children, and there is lack of clinical trial evidence with regards to efficacy and safety over longer periods. Clinical guidance is required for those whose total daily insulin dose is below the required threshold for closed-loop operation. Particularly for young people, new faster insulins could provide an increasingly realistic pathway to a more fully closed-loop system, where accurate carbohydrate counting and prandial bolusing is no longer required. Closed-loop studies in the pediatric age-group with ultra-rapid acting insulins are required to assess feasibility and safety.

Due to the novelty of closed-loop insulin delivery technology there is little real-world evidence to guide clinicians and users. Longer-term real-world studies are required to assess whether glycaemic and quality of life benefits are sustained long-term and what features are most desired by users to improve ease-of-use. This will inform system reimbursement, facilitating wider access across the diabetes population. Furthermore, several systems now incorporate personalisable glucose targets, as well as user-initiated modes that reduce or intensify insulin delivery. While users have expressed a wish for more collaboration with closed-loop systems in qualitative studies (49), safety and efficacy of these features needs to be evaluated to help optimize their use.

One of the most important issues facing healthcare providers and the diabetes community is access to closed-loop therapy. Insurance coverage of closed-loop therapy is currently poorly established, and the high cost of CGM and insulin pumps is a significant barrier to uptake for those who cannot afford to self-fund the technology (57). This may lead to growing disparities in those from lower socio-economic backgrounds (57). Future research needs to incorporate robust health economic analysis and should aim to show long-term cost-effectiveness to aid reimbursement for closed-loop therapy.

CONCLUSION

Closed-loop insulin delivery improves glycaemic control in all pediatric age groups, while crucially reducing the high management burden associated with this chronic disease, thus improving quality of life for the whole family. Children, young people, and their families now have a variety of commercially available closed-loop systems to choose from, with further systems in development. Future research should focus on improving systems to further reduce diabetes management burden and optimize efficacy, ultimately informing system reimbursement and facilitating wider access across the diabetes population.

AUTHOR CONTRIBUTIONS

JF wrote the manuscript. RH edited, critically reviewed, and approved the final submitted version of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Supported by the Efficacy and Mechanism Evaluation National Institute for Health Research (14/23/09); National Institute of Diabetes and Digestive and Kidney Diseases (UC4DK108520); JDRF; The Leona M. and Harry B. Helmsley Trust (#2016PG-T1D046); National Institute for Health Research Cambridge Biomedical Research Centre; European Union Horizon 2020 research and innovation programme (grant agreement no. 731560); and Wellcome Trust Strategic Award (100574/Z/12/Z).

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Disclaimer: The views expressed are those of the authors and not necessarily those of the funders. No sponsor had any role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript.

Conflict of Interest: RH reports having received speaker honoraria from Eli Lilly and Novo Nordisk, serving on advisory panel for Eli Lilly and Novo Nordisk; receiving personal fees from BBraun, Medtronic and Abbott Diabetes Care; patents related to closed-loop insulin delivery, shareholder and director at CamDiab Ltd.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

SYSTEMATIC REVIEW
published: 05 May 2021
doi: 10.3389/fped.2021.660173

Intermittently Scanned and Continuous Glucose Monitor Systems: A Systematic Review on Psychological Outcomes in Pediatric Patients

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: 28 January 2021
Accepted: 06 April 2021
Published: 05 May 2021

Citation:
Franceschi R, Micheli F, Mozzillo E, Cauvin V, Liguori A, Soffiati M and Giani E (2021) Intermittently Scanned and Continuous Glucose Monitor Systems: A Systematic Review on Psychological Outcomes in Pediatric Patients. Front. Pediatr. 9:660173. doi: 10.3389/fped.2021.660173

Aim: To explore the impact of real-time continuous glucose monitoring (rtCGMs) or intermittently scanned/viewed CGM (isCGM) on psychological outcomes in children and caregivers, and to grade the level of evidence.

Method: Systematic review of the literature from PubMed, Embase, Cochrane Library, Web of Science, CINAHL, Nursing reference center, Up to date, Google Scholar, and PsycINFO databases. The studies selected used validated questionnaires for investigating the psychological outcomes. We applied GRADE (Grading of Recommendations Assessment, Development and Evaluation) to rank the quality of a body of evidence.

Results: A total of 192 studies were identified in the initial search and after the process of evaluation 25 studies were selected as appropriate to be included in this systematic review. We found in moderate quality studies that isCGM in adolescents can improve diabetes related distress, family conflicts, fear of hypoglycemia, and quality of life, while depression, anxiety, and quality of sleep have not yet been evaluated by validated questionnaires. In moderate—high quality studies, rtCGM technology does not impact on diabetes burden, diabetes specific family conflict, and depressive symptoms. The effect on fear of hypoglycemia, sleep quality, and anxiety is still debated and RCT studies powered to find significant results in psychological outcomes are lacking. RtCGM increases satisfaction and quality of life in parents and patients wearing rtCGM.

Conclusion: these data present an interesting point to consider when families are deciding whether or not to start CGM use, choosing between rtCGM to reach a tighter metabolic control, or isCGM which allows greater benefits on psychological outcomes.

Keywords: psychological outcomes, isCGM, CGM, type 1 diabetes, child

INTRODUCTION

The advent of real-time continuous glucose monitoring systems (rtCGMs) or intermittently scanned/viewed CGM (isCGM) is one of the major technological innovation for the treatment of Type I Diabetes (T1D). Real-time CGM allows individuals with diabetes to follow their glucose concentration simultaneously, and to obtain information on glucose trends and trajectories. Moreover, the systems can provide warnings on upcoming hypoglycemia or hyperglycemia as well as alarms for rapid glycemic excursions (1). Meta-analyses provided evidence for real-time CGM to lower hemoglobin A1c (HbA1C) levels without increasing hypoglycemic events (1).

Importantly, recent studies confirmed that the use of isCGM has a positive impact on glucose control, by limiting glucose variability, reducing hypoglycemia, and improving long-term glucose control (2).

In addition to the stand-alone rtCGM systems, the integrated combination of pump therapy with rtCGMs allows to automatically suspend insulin delivery in the case of upcoming hypoglycemia, thus reducing or avoiding nocturnal hypoglycemia (3).

Although a clear evidence that the benefits associated with the use of rtCGMs are strictly related to a near daily use (1, 4, 5), a constant rtCGM use remains problematic for many patients in the pediatric age group (6, 7). Indeed, a better glycemic control is achieved by patients who use rtCGM for the majority of time, generally considered to be 70% or more (1, 8). Nevertheless, recent data from the Type 1 Diabetes Exchange Clinic Registry still reports that only one third of T1D-affected youth regularly wears rtCGM, although there has been an increase of use from 2013 (4% of T1D youth) to 2015 (14%) and 2017 (31%) (9). Furthermore, rtCGM wearing declines significantly over-time among T1D users (10). Barriers to a regular rtCGM use in pediatrics are reported in the following Table:

Barrier	Description
Physical barriers	Pain due to sensor insertion, skin reactions to sensor, adhesive and lack of skin areas for sensor placement in young children (11, 12)
Clinical barriers	Multiple alerts and alarms can lead to alarm fatigue
Education barriers	A well-experienced diabetes team has to ensure a proper training for patients and families and a continuous support in problem solving on ways to break down barriers;
Financial barriers	Lack of insurance coverage and high costs for rtCGM supplies (13)
Psychological barriers related to rtCGM	Diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality, and quality of life (QoL).

A deeper understanding of the factors related to technologies uptake and adherence remains a crucial topic of investigation. In particular, studies on psychological factors that may predict sensor success or interruption are still limited. On the contrary, identifying psychological issues related to the sensor use would support both diabetologists in tailoring the best treatment for each patient, and youth and families in setting realistic expectations. The impact of rtCGM and isCGM on psychological outcomes in children and caregivers remains controversial (6, 14, 15). This may be due to the fact that psychological measures are usually considered as secondary outcomes in trials involving CGMs (Laffel LM 2020 JAMA, Massa GG 2019, JDRF-CGM Study Group, Diabetes Care 2010), compared to the metabolic control (HbA1c, hypoglycemia, CGM glucose metrics). Moreover, different questionnaires are used to assess the outcomes in the published studies. Also, each area of investigation (depression, fear of hypoglycemia, QoL) could be explored by different validated measures, self-reported or administered by health care providers, as summarized in **Table 1** (16–42).

AIM

The aim of this systematic literature review is to explore the impact of rtCGM or isCGM on psychological outcomes (diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality, quality of life, and satisfaction with the CGM system) in children and caregivers and to grade the level of evidence.

METHODS

Criteria for Study Selection

Types of Studies

We included RCTs, observational studies, prospective studies, cross-sectional studies, exploratory studies, mix of qualitative, and quantitative studies. We included only published studies.

Types of Participants

We included patients with T1D aged between 0 and 18 years and their caregivers.

Types of Interventions

We included the following comparisons:

Comparison 1: rtCGM on psychological outcomes (diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality and quality of life, satisfaction) vs. capillary glucose testing for glycemic assessment in children and caregivers;

Comparison 2: isCGM on psychological outcomes (diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired

Abbreviations: T1D, type 1 diabetes; DRD, diabetes related distress; FOH, fear of hypoglycemia; QoL, quality of Life; HRQoL, health related quality of life; BG, blood glucose; BGM, blood glucose monitoring; isCGM, intermittently scanned/viewed CGM; CGM, continuous glucose monitoring; FGM, flash glucose monitoring; CSII, Continuous subcutaneous insulin infusion; PLGM, predictive low glucose management.

TABLE 1 | Review of psychological measures in children used in the studies, sorted by outcome.

Construct	Measure	Self-report or proxy-report	Number of items	Score range	Interpretation: ↑ score indicates
Diabetes Burden	Problem Areas in Diabetes survey-Pediatric (PAID-Peds) (16); Problem Areas in Diabetes survey-Parent Revised (PAID-PR) (17)	Youth self-report Parent self-report	20 (PAID-Peds), 18 (PAID-PR)	0–100	↑ burden
	The Diabetes Distress Scale (T1-DDS) (18)	Parent self-report	28	Average of all 28 items, each rated on a 1–6 scale	
Diabetes-Specific Family Conflict	Diabetes Family Conflict Scale (DFCS) (19)	Youth self-report Parent self-report	19	0–100	↑ diabetes-specific family conflict
Parent Involvement	Diabetes Family Responsibility Questionnaire (DFRQ) (20)	Youth self-report Parent self-report	19	0–100	↑ parent involvement
Depressive Symptoms	Center for Epidemiologic Studies Depression Scale for Children (CES-DC) (21); Center for Epidemiologic Studies Depression Scale (CES-D) (22)	Youth self-report Parent self-report	20	0–60	↑ depressive symptoms
	The Children's Depression Inventory (CDI) (23)	Youth self-report	27	0–54	
	The Depression Anxiety Stress Scale (DASS) (24)	Parent self-report	42	0–126	
	Patient Health Questionnaire depressive scale (PHQ-8) (25)	Youth self-report	8	0–24	
State Anxiety, Trait Anxiety	Spielberger State-Trait Anxiety Inventory (STAI) (26, 27)	Youth self-report Parent self-report	20 (state), 20 (trait)	20–60	↑ anxiety
	The Diabetes Worry Scale (DWS) (28)	Youth self-report Parent self-report	50	50–250	
Fear of Hypoglycemia	Hypoglycemia Fear Survey—Worry scale (HFS) (29, 30)	Youth self-report Parent self-report	15	0–100	↑ fear of hypoglycemia
	The Hypoglycemia Confidence Scale (HCS) (31)	Parent self-report	9	0–36	
Sleep quality	The Pittsburgh Sleep Quality Index (PSQI) (32)	Parent self-report	19	0–21	↑ poor sleep quality
Youth QoL	Pediatric Quality of Life Inventory (PedsQL)—Generic and Diabetes-specific (33, 34)	Youth self-report Parent proxy-report	23 (generic), 28 (diabetes)	0–100	↑ quality of life
	Social Functioning Health Survey (SF-12) (35)	Parent proxy-report	12	0–100	
	The WHO Five Well-Being Index (WHO-5) (36)	Youth self-report Parent proxy-report	5	0–25	
	The Diabetes-Specific Quality of Life Scale (DSQOLS) (37)	Parent proxy-report	64	0–320	
	The Diabetes Quality of Life Clinical Trial Questionnaire—Revised (DQLCTQ-R) (38)	Parent proxy-report	57	0–100	
	The Appraisal of Diabetes Scale (ADS) (39)	Parent proxy-report	7	7–35	
Satisfaction with the CGM system	The CGM Satisfaction Scale (CGM-SAT) (40)	Youth self-report Parent self-report	44	44–220	↑ satisfaction with CGM use
	The Glucose Monitoring Survey (GMS) (40)	Youth self-report Parent self-report	22	44–154	
	The Blood Glucose Monitoring Communication Questionnaire (BGMC) (41)	Youth self-report Parent self-report	8	8–24	
	The Diabetes Treatment Satisfaction Questionnaire status (DTSQs) (42)	Youth self-report Parent self-report	8	0–48	

↑*increased.*

sleep quality and quality of life, satisfaction) vs. capillary glucose testing for glycemic assessment in children and caregivers.

Comparison 3: rtCGM vs. isCGM on psychological outcomes (diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality and quality of life, satisfaction) in children and caregivers.

Outcomes

Psychological outcomes in children and caregivers included: diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality, quality of life, satisfaction.

A detailed description of outcomes and related measures is reported in **Table 1** (16–42).

Search Methods

We conducted a systematic search of the literature according to the PICOS model (Population, Intervention, Comparison, Results, Study design).

Population	Pediatric patients (0–18 years old) with Type I diabetes and theirs caregivers
Intervention	Use of Intermittently Scanned Continuous Glucose Monitoring (isCGM) or Real-Time Continuous Glucose Monitoring (rtCGM) Systems
Comparison	Capillary blood glucose monitoring or isCGM
Results	Variations in diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality, and QoL
Study design	RCTs, observational studies, prospective studies, cross-sectional studies, exploratory studies, mix of qualitative, and quantitative studies

The study exclusion criteria were:

- patients >18 years; patients with Type II Diabetes;
- studies not meeting the established primary and secondary outcomes;
- animal research studies;
- devices: use of closed loop systems;
- reviews, conference abstracts, full texts not available.

We did not apply language restrictions.

Sources used for literature review included: PubMed, Embase, Cochrane Library, Web of Science, CINAHL, Nursing reference center, Up to date, Google Scholar, and PsycINFO.

Articles published from 1/01/2006 to 31/12/2020 were considered for the current review. Search terms, or "mesh" (MEdical Subject Headings) for this systematic review included: *"CGM AND distress," "CGM AND sleep quality," "CGM AND psychological variables," "Glucose monitoring AND distress," "Glucose monitoring AND sleep quality," "Glucose monitoring AND psychological variables," "Flash glucose monitoring AND distress," "Flash glucose monitoring AND sleep quality," "Flash glucose monitoring AND psychological variables."*

According to the PICOS detailed above, filters for participants' age (0–18 years), and study characteristics were activated.

Data Extraction and Management

Two review authors independently extracted data by using the forms integrated in the sources' systems.

The following characteristics were reviewed for each included study:

- reference aspects: authorship(s); published or unpublished; year of publication; year in which study was conducted; other relevant papers cited;
- study characteristics: study design; type, duration; informed consent; ethics approval;
- population characteristics: age, number of participants;
- intervention characteristics: type, duration, mode of use of rtCGM and isCGM;
- evaluation of the outcomes as reported in **Table 1** (16–42).

Disagreements were solved by discussion.

Assessment of the Certainty of the Evidence

We used the GRADE approach (Grading of Recommendations Assessment, Development and Evaluation) to rank the quality of a body of evidence (www.gradeworkinggroup.org) for the following outcomes: diabetes distress/burden, diabetes-specific family conflicts, depressive symptoms, anxiety, fear of hypoglycemia, alarm fatigue, impaired sleep quality, quality of life, and satisfaction with the rtCGM and the isCGM systems.

Two review authors independently assessed the certainty of the evidence for each of the outcomes above. In the case of risk of bias in the study design, imprecision of estimates, inconsistency across studies, indirectness of the evidence, and publication bias, we had the option of decreasing the level of certainty by one or two levels according the GRADE guidelines (43).

The GRADE approach results in an assessment of the certainty of a body of evidence and allocation to one of four grades:

High	=	Further research is very unlikely to change confidence in the estimate of effect.
Moderate	=	Further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate.
Low	=	Further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate.
Very low	=	Any estimate of effect is very uncertain.

RESULTS

A total of 192 studies were identified following the literature review. After screening, we excluded 20 records as they were duplicates. When we reviewed titles and abstracts we excluded 112 records: 9 studies were published only in abstract form, 100

studies did not investigate the outcomes of interest (**Table 1**), 3 studies were not available in the full text form.

A total of 60 full-text manuscripts were assessed for eligibility: 27 studies were excluded as no data were available for the analysis, besides the ones reported in the abstracts; 4 studies were excluded as they reported data from the same cohort of patients; 4 studies were excluded as they resulted to be literature reviews when the full-texts were analyzed. A final number of 25 studies, 6 on isCGM, 19 on rtCGM, were included in this systematic review.

The PRISMA flow diagram in **Figure 1** shows the process of study evaluation.

A summary of results from the studies included in this systematic review is reported in **Tables 2**, **3**.

DISTRESS/DIABETES BURDEN

This outcome is analyzed in 3 studies on isCGM use and in 12 studies on rtCGM use in youth and their caregivers.

In pediatric patients **isCGM** reduced psychological distress for all the domains analyzed during a 12-weeks prospective study in children/adolescents [(44), Moderate] and in a 4-weeks qualitative study in adolescents/young adults [(45), Low]. This effect was reported also in parents of children and adolescents in a qualitative study [(46), Low].

RtCGM reduced diabetes burden in adolescent patients according to a cross-sectional study [(47), Moderate]. A similar effect was described for caregivers in five studies [(48–50), Low, (47, 51), Moderate]. In two studies no variation in diabetes burden was found both in children and caregivers [(13, 14), High-Moderate]. Broad effects were highlighted in three studies [(52–54), Moderate-Low].

FAMILY CONFLICT IN THE MANAGEMENT OF DIABETES

This outcome is measured in 2 studies on isCGM use and in 5 studies on rtCGM use in youth and their caregivers.

IsCGM use was associated with a reduction in diabetes specific parent-child conflict and parental conflict in patients aged 13–20 years in 2 qualitative studies [(45, 46), Low].

RtCGM use was associated with both a reduction in family conflicts and an improvement in rtCGMs related family functioning in 2 studies included in the review [(51, 55), Moderate]. These benefits were related to a decrease in the workload associated to blood glucose monitoring (BGM) and to an increased sense of safety [(51), Moderate]. In a RCT very similar levels of family conflict between the intervention group (rtCGM) and the control group (BGM) were found [(52), Moderate]. In other two studies no differences in family conflict were reported after the initiation of rtCGM use [(13, 53), Moderate]. The perception of a high number of obstacles and barriers related to the use of rtCGM sensors is related to a greater number of family conflicts and difficulties in managing the disease [(53), Moderate].

DEPRESSION

Depression in youth using **rtCGM** is evaluated in two studies. In a cross-sectional study on rtCGM use in adolescents, more depressive symptoms were reported by those who faced more barriers [(53), Moderate]. In a RCT in children 8–17 years old, rtCGM parent-proxy report of depression was significantly higher than that reported by BGM parents [(52), Moderate]. Data on depression in youths using **isCGM** are lacking.

ANXIETY

This outcome is measured in 3 studies on rtCGM use in youth. In a RCT evaluating children in the age 2–12 years and their parents, parental stress level was lower in the arm using **rtCGM** compared to the control group (51, Moderate). In another study including 16 children aged 2–17 years, rtCGM use was associated with an improvement in children and parents' anxieties [(56), Low].

In a RCT study, the group of youth with rtCGM reported more trait anxiety than BGM youth, whereas rtCGM adults reported less state and trait anxiety than BGM adults [(52), Moderate].

Data on anxiety in youths using **isCGM** are lacking.

FEAR/WORRY OF HYPOGLYCEMIA

This outcome is measured in 1 study on isCGM use and in 14 studies on rtCGM use in youth.

Fear of hypoglycemia (FOH) was reduced by **isCGM** use in adolescents older than 12 years in a 3-month prospective study [(59), Moderate]. Similarly, **rtCGM** use reduced FOH in 16 children aged 2–12 years in a 12-month cohort study [(56), Low]. Likewise, fear associated with hypoglycemic events resulted significantly lower in parents of youth using rtCGM in several studies [(51, 57, 58), Moderate, (56), Low]. RtCGM reduced the fear of nocturnal hypoglycemia in youth when integrated with a pump that automatically suspend insulin delivery in case of hypoglycemia [(54), Low].

On the contrary, in several studies no differences were found in FOH in both youth using rtCGM/isCGM [(13, 14, 52, 57), Moderate-High] and their caregivers [(6, 13, 14, 52, 60), Moderate-High]. The fear of hypoglycemic events resulted higher in parents than in children [(52), Moderate] although the sensor use. This is probably related to the fact that not all parents have full confidence in rtCGM systems: some parents are worried that the sensor may not work properly and it does not intercept hypoglycemic events [(53), Moderate].

SLEEP QUALITY

This outcome is measured in 7 studies on **rtCGM** use in youth. In an observational study, overall 67% of children with T1D met the criteria for poor sleep quality; a worse child sleep quality was associated with worse metabolic control and poorer parental sleep quality. Child sleep was not related to the use of diabetes-related technology (rtCGM, insulin pump) [(58), Moderate]. About caregivers, most experimented better sleep patterns with rtCGM [(51, 54), Low-Moderate], while others

TABLE 2 | Analysis of the 25 papers included in the systematic review.

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Al Hayek et al. (44)	Effect of isCGM on DRD	12-week prospective study 187 children and adolescents (13–19 years) with T1D, using the conventional fingerprick method. 31% were on CSII Region: Saudi Arabia	At baseline sensors were fixed. T1-DDS (diabetes distress) questionnaire was administered at T0 and + 12 weeks	T +12 weeks, in comparison to the baseline (fingerprick) showed significant decrease in all the seven the subdomains and in total T1-DDS (diabetes distress score). Increased frequency of glucose monitoring with isCGM. Substantial drop in HbA1c and in the frequency of hypoglycemia was observed.	Lack of a control group; limited number of risk factors assessed. **- Moderate -**
Boucher et al. (45)	Early experiences with isCGM	4 week qualitative study 15 participants with T1D (age 13–20 years) Device: isCGM Region: New Zeeland	Interviews 1-month from starting the isCGM. The interview analyzed: -Impacts of isCGM -Facilitators and challenges of using isCGM -Supporting patients in using isCGM	Participants perceived isCGM to be easy to use and discrete. All participants reported that isCGM alleviated burden of managing diabetes. Most (n = 12/15) participants perceived an improvement in their diabetes self-management. Other benefits: Facilitate to do insulin all the time Improved concentration Increased physical activity Improved sleep: reduced nocturnal hyperglycemia and helps to identify how to prevent reoccurring nocturnal hypoglycemia Less parental conflict Reduces worry about glucose level Improved social life Barriers: the most common challenges of isCGM use were: premature sensor loss, forgetting to scan, skin irritation, technical problems. All participants anticipated continuing to use isCGM	This finding may not be generalizable to longer periods of use. The sample may not be representative of the general population **- Low -**
Boucher et al. (46)	Parental perspectives after isCGM start.	Qualitative study 12 parents (age of children and adolescents with T1D: 13–20 years) Device: isCGM 11% of children used CSII Region: New Zeeland	A interview explored: -Impacts of isCGM -Facilitators/challenges of using isCGM -Supporting patients in using isCGM	The following themes were identified: (1) improved parental *well-being:* "peace of mind" while their adolescent slept; reduced diabetes-specific worry and improvement in sleep quality (2) reduced diabetes-specific parent–child conflict (3) facilitated parental role in management: easier to perform glucose checks; helped guide treatment decision isCGM has the potential to reduce diabetes management burden for both adolescents and parents. Barriers: premature sensor loss and sensor malfunction, isCGM costs.	Limitation were the small sample size. The parents included in this study were predominantly of European ethnicity and the findings may not apply to minority populations. **- Low -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Vesco et al. (47)	Diabetes technology use on adolescent and DRD	Cross-sectional study. Adolescents with T1D (12–18 years) and parents ($N = 1,040$; primarily mothers) 64% were on CSII, 11% rtCGM+CSII Region: USA	Adolescents were categorized by technology use: rtCGM Alone, CSII Alone, rtCGM+CSII, or No Technology Adolescents (PAID-T) and parents (P-PAID-T) completed an online questionnaire	Adolescents: rtCGM use was associated with less DRD compared to No Technology, rtCGM+CSII and CSII Alone Parents: results were similar but with smaller effect size for parent-reported distress rtCGM Alone was associated with lower HbA1c compared to No Technology CSII alone and CSII+rtCGM Alone was associated with lower HbA1c compared to No Technology. rtCGM+CSII gave advantage over CSII Alone.	The sample was composed of mostly Caucasian participants from higher income families which is not representative of all youth with T1D. Small number of participants in the rtCGM Alone technology use group. **- Moderate -**
Erie et al. (48)	rtCGM practices in homes and schools, attitudes and expectations of parents and caregivers	Cross-sectional, using quantitative and qualitative methods Parents and daytime caregivers (school nurse, daycare teacher, nanny). Age of the children cared for by the respondents was 2–17 years 32 patients wore Dexcom® G4 or G5 sensors and 1 patient wore a Medtronic Enlite® Sensor Region: USA	Anonymous survey assessing characteristics of rtCGM use 57 survey pairs were distributed. 33 parents and 17 daytime caregivers responded	All parents and most caregivers (78%) reported decreased overall worry/stress. Parents felt positive about rtCGM use, it brought them peace of mind and a sense of security. Daytime caregivers felt comfortable with rtCGM and many of them felt that use of these systems allowed to work in a collaborative manner with parents to provide intensive diabetes management Frequency of sensor use was very high with 94% of respondents stating their child used the sensor 7 days a week	Relatively small sample size and response rate of 58% amongst parents and 1/3 of daytime caregivers Respondents were extremely adherent to sensor technology **- Low -**
Barnard et al. (49)	Impact of diabetes-related technology in spouses and caregivers of people with T1D	Survey, quantitative, and qualitative mix 100 parents/caregivers and 74 partners 83% of children and 72% of adults were on CSII	Participants were recruited *via* the Glu online community website. Online questions (PAID-5, WHO-5) and specific questions exploring the impact of technology	High use of rtCGM in both groups-partners and parents/caregivers. Parents/caregivers reported more negative emotions and decreased *well-being* related to their family members T1D, compared to partners, DRD was common, as was sleep disturbance associated with device alarms and fear of hypoglycemia. 87% of partners and 66% of parents/caregivers rated their own QoL as good Disrupted sleep was commonly reported with 73% of parents/caregivers and 59% of partners reporting waking because of diabetes technology. Of these, 54% of parents/caregivers and 12% of partners reported waking at least 4 times a week. The main reasons reported were rtCGM alarms and fear of hypoglycemia. False alarms were uncommon with 26 and 23%, respectively.	This study reaches only participants who are members of the Glu community (membership may be more tech savvy) as an online community **- Low -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Kashmer et al. (50)	Characteristics of patients most willing to use rtCGM	Exploratory study Parents of children (0–18 years) with T1D responded to the online survey (no. 457) 70% used CSII Region: USA	Online survey software was utilized to administer a 50-item questionnaire to parents of children with T1D. Primary outcomes were parental interest, attitudes and concerns	Only 12% of parents whose child had previously used a rtCGM Over 90% of the parents indicated a high level of interest in having their child use a rtCGM. Primary variables related to interest in rtCGM, were use of CSII, checking BG more than six times daily and parental worry about high or low BG. Age of the child and HbA1c were not related to parental interest in a rtCGM. Only a very few parents (6%) believed that using a rtCGM would increase their diabetes-related stress. Less than 2% of parents indicated believing that they would be overwhelmed Some (7%) were concerned that they would give too much or too little insulin if they saw glucose readings continuously.	The survey instrument was not formally validated. **- Low -**
Burckhardt et al. (51)	Effect of rtCGM with remote monitoring on psychosocial outcomes in parents of children with T1D	RCT, two 3-month periods (participants spended 3 months in each of the two study arms) 49 children with T1D, 2–12 years, along with their parents	Participants "naïve" for rtCGM At the first visit and after each 3-month period, parents and children (aged 8–12 years) completed: HFS, PedsQL, DASS, STAI, PSQI, RTCGM-SAT The primary outcome was parental HFS	Parental Hypoglycemia fear scores (HFS) were lower while the child was using rtCGM with remote monitoring. Parental health-related QoL and family functioning, stress, anxiety, and sleep measures also improved significantly after intervention	Relatively small sample size **- Moderate -**
Beck et al. (14)	Impact of rtCGM on QoL among individuals with T1D	Multicenter trial RCT, 26 weeks f/up 206 children and 228 adults with T1D 110 Children on rtCGM, 106 on capillary BG. Most on CSII	HFS, PAID, SF-12 questionnaires were completed at baseline and 26 weeks by all participants and by parents (<18 years old). The rtCGM-SAT was completed by the rtCGM group (participants and parents) at 26 weeks.	Survey completion was high (rtCGM group: adults 98%, youth 93%, parents 97%; control group: 94–100%). There was substantial satisfaction with rtCGM technology after 26 weeks among participants and parents. QoL scores remained largely unchanged for both the treatment and the control group, although there was a slight difference favoring the adult rtCGM group on several subscales High baseline levels of QoL were found in this population No variation in parental burden associated with diabetes	High baseline levels of QoL in the participants who were predominantly non-Hispanic white, well-educated, privately insured, and most commonly treated with insulin pumps at enrollment **- High -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Giani et al. (13)	Biomedical and psychosocial factors associated with rtCGM use	6 months observational study 61 T1D (8–17 years) and their parents 80% were treated with CSII Region: USA	At the first visit and after 6 months period, patients and their parents completed: HFS, DFRQ, DFCS, CES_D, STAI-CP, PAID, P-PAID, PedsQL	There was no decline in any of the psychosocial factors At baseline parents of youth using rtCGM consistently reported higher QoL for their children than the parents of youth using rtCGM less often. Youth scores were lower than parent scores for parent fear of hypoglycemia, state anxiety, traitanxiety, and diabetes burden; were higher for youth generic QoL and youth diabetes-specific QoL Youth and parent scores were significantly positively correlated for parent involvement, diabetes-specific family conflict, diabetes burden, youth generic QoL and youth diabetes-specific QoL rtCGM use declined over the 6 months	Modest sample size; the study sample presented a large proportion of participants treated with CSII and high frequency of BG monitoring at baseline, relatively low HbA1c. Therefore, the results may not be generalizable to the general population of youth with T1D. **- Moderate -**
Markowitz et al. (52)	Impact of rtCGM on psychological variables that may influence diabetes treatment adherence	RCT Children (8–17 years old) and adults, randomized to the rtCGM or BGM group for 6 months. 86% were on CSII Region: USA	49 participants were enrolled and completed at 0 and 6 months: HFS, PedsQL, SF-12, CDI, CES-D, STAI, BGM, DFCS, PAID	There were no differences in reported fear of hypoglycemia between rtCGM and BGM groups Parents in both groups reported significantly more FOH than youth. rtCGM youth and their parents and rtCGM adults reported more negative affect around BGM than the BGM group. rtCGM youth reported more trait anxiety than BGM youth, whereas rtCGM adults reported less state and trait anxiety than BGM adults. rtCGM parent-proxy report of depression was significantly higher than that reported by BGM parents. Reported levels of diabetes-specific family conflict were similar between groups.	This study was not powered to find significant result **Moderate**
Messer et al. (53)	Adolescent reported barriers to diabetes device use and to determine targets for clinician intervention	Cross-sectional study Survey on 411 adolescents (12–19 years) with T1D. 75% were on CSII Region: USA	411 adolescents completed the survey. 225 (55%) were on rtCGM Online survey with PHQ-8, PAID-Peds, SEDM, and General Technology Attitudes Survey, the Diabetes Technology Attitudes Survey	Barriers: cost/insurance related concerns; wear related issues: hassle of wearing the device, dislike of device on body Adolescents who endorsed more barriers also reported more diabetes distress, family conflict and depressive symptoms Pump and rtCGM discontinuers both endorsed more barriers and more negative perceptions of technology than current users, but reported no difference from device users in diabetes distress, family conflict, or depression.	Potential underrepresentation of adolescents not using any diabetes technology or using intermittently scanned rtCGM **- Moderate -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Pickup et al. (54)	To analyze narratives about experiences of real-time rtCGM in people with T1D	Qualitative study 50 children with T1D (3–17 years) using rtCGM and 50 caregivers Most participants (87%) used rtCGM+CSII Region: UK	Online survey on rtCGM duration, frequency of sensor wear, funding and a free narrative about experiences or views about rtCGM. Qualitative framework analysis to analyze 100 responses was analyzed 71% used sensors ≥75% of the time	Experiences were overwhelmingly positive, with reported improved -sleep: most participants who mentioned sleep (81%) wrote that they were able to sleep more easily, with less disturbance, FOH, and a feeling of safety, with rtCGM -QoL, and physical and psychological *well-being* (reduced stress for patient and caregiver, reassurance and security, more confidence and independence, improved energy, mood, and QoL) -reduced frequency of SMBG Barriers: sensor inaccuracy and unreliability, and "alarm fatigue." The advantages of rtCGM used with CSII with PLGM were recorded by several participants, noting reduced hypoglycemia frequency and fear of nocturnal hypoglycemia.	Responses were based on perception Participants who were funded might tend to be biased toward the positive features of rtCGM to justify the funding. **- Low -**
Telo et al. (55)	Patient and family behavioral and clinical characteristics associated with rtCGM	Cross-sectional study 358 children with T1D (age 8–18 years) Device: rtCGM 70% of patients with rtCGM used CSII, and 84% of controls Region: USA	Youth and their parents completed: DMQ, DFCS, DFRQ, PedsQL.	rtCGM group performed more frequent BGM; reported greater adherence to diabetes care; higher youth QoL; less diabetes-specific family conflict. No differences with respect to parent involvement in diabetes management. Patients who are already wearing CSII may be less reluctant	Only 28% of eligible youth who were approached for the rtCGM study agreed to wear a rtCGM device compared with 66% of the eligible general pediatric population who were approached. This probably because they recognized potential burdens related to current rtCGM technology. **- Moderate -**
Ng et al. (56)	Effects of rtCGM on patient and caregiver well-being, worry, fear of hypoglycemia and glycemic control.	12 months cohort study 16 children with T1D (age 2–17 years) Device: rtCGM (Dexcom G4®) All the patients were on pump therapy Region: United Kingdom	Children aged >12 years completed the HFS Parents completed a modified version of the HFS-P12	Improvement in fear of hypoglycemia (FOH), for both parents and children, were observed. rtCGM gave to parents and children the confidence to modify treatment regimen and rtCGM improved their anxieties, fear, and worry. rtCGM improved the children's and their parents' *well-being*. After 8 months follow up, 5 patients used rtCGM intermittently and up to 58% were not using their rtCGM routinely.	The small sample size limits transferability of the findings to the whole clinic population. **- Low -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Burckhardt et al. (57)	rtCGM and psychosocial outcomes	2 months prospective cohort study 65 parents and 46 children with T1D (age 15 ± 1.81 years) Some patients were treated with CSII. Device: Dexcom® G5 and Medtronic Guardian Connect. Approximately 70% of the participants were using systems with remote monitoring. Region: Western Australia	To children over 12 years of age and their parents: HFS, PSQI, DTSQs, Gold Hypoglycemia awareness questionnaire after starting rtCGM	Total parental Hypoglycemia Fear and worry decreased, no difference in children were observed. Satisfaction regarding diabetes treatment improved both in parents and children Frequency of overnight BG testing decreased significantly. The percentage of children with reduced awareness of hypoglycemia decreased. Reported parental sleep quality improved Parents reported to miss fewer work days 11 children stopped rtCGM because of: sensor connection issues, general dislike, sensor falling off during exercise and problems with sensor change.	The small sample size limits transferability of the findings to the whole clinic population. Moreover, rtCGM was discontinued due to technical issues and dislike of the system. **- Moderate -**
Jaser et al. (58)	Associations between rtCGM and child sleep, glycemic control and adherence, parent sleep and well-being, parental fear of hypoglycemia, and nocturnal caregiving behavior	Descriptive observational study 515 parents of 2–12-year-old participants in the T1D Exchange clinic registry. Device: rtCGM 80% used insulin pump	Surveys were emailed to parents: CSHQ, PSQI, HFS, WHO-5 questionnaires	67% of children met criteria for poor sleep quality. Child sleep was not related to the use of diabetes-related technology (rtCGM, insulin pump) Child sleep quality and duration was related to HbA1c but not to mean frequency of BG monitoring. Children with poor sleep quality were more likely to experience severe hypoglycemia and DKA. Poorer child sleep quality was associated with poorer parental sleep quality, parental *well-being*, and fear of hypoglycemia.	Use of parent-report measures of child sleep **- Moderate -**
Al Hayek et al. (59)	Effect of isCGM on glycemic control, hypoglycemia, HTQoL, and FOH	3 months prospective study 47 youth with T1D (age 13–19 years) Device: isCGM 38% of children used CSII Region: Saudi Arabia	At the baseline and after 3 months validated questionnaires were administered: HFS-C, PedsQL 3.0 DM.	isCGM scanning can effectively reduce fear of hypoglycemia (FOH), worry and HbA1c level. It also improves QoL. The frequency of self-testing by isCGM is 8 times greater than in BGM by finger pricking. A higher frequency of isCGM scan positively correlates with behavior and QoL Significant improvement in behavior, worry, and hypoglycemia among the CSII patients.	Small sample size and inclusion of only one center for study. **- Moderate -**
Mauras et al. (6)	rtCGM benefit in young children aged 4–9 years with T1D	RCT, 26 weeks 146 children with T1D, 4–9 years 64% were on pumps Region: USA	Participants were "naïve" for rtCGM Parents completed at baseline and at 26 weeks: GMS, PAID, HFS, CGM-SAT The primary outcome was HbA1c	rtCGM wear was well-tolerated, and parental satisfaction with rtCGM was high. However, parental fear of hypoglycemia was not reduced. rtCGM wear decreased over time	**- High -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Laffel et al. (60)	Effect of rtCGM on glycemic control and 20 secondary outcomes	RCT 153 youth with T1D (age 14–24 years), HbA1c 7.5–10.9% Device: rtCGM (Dexcom G5®) 70% of youth used CSII Region: USA	Youth completed at the baseline and after 26 weeks: PAID, HCS, PSQI	rtCGM use gave reduction in the time spent in hyperglycemia and hypoglycemia; difference in the glucose monitoring satisfaction. No difference in diabetes problem areas, hypoglycemia confidence and sleep quality were reported. The use of rtCGM device does not increased burden.	rtCGM used in the trial required twice-daily calibrations with BGM. **- High -**
Lawton et al. (61)	Participants' experiences using rtCGM.	Qualitative study 15 children aged <12; 13–15; >16 years HbA1c 7.5–10% 9 parents Device: Guardian™ Sensor 3, Medtronic 640G (100%) Region: United Kingdom	Interview, after ≥4 weeks of rtCGM use, analyzed: Previous experience of using rtCGM and SMBG; understandings, expectations and impact on diabetes self-management; likes and dislikes of the technology; views about information and training needed to support effective use of rtCGM.	Benefits deriving from the use of rtCGM: -increased awareness about glycemic values -instant and effortless access to data -prevents hypoglycemia and hyperglycemia events -short-term lifestyle changes (diet, physical activity) -better understanding of how insulin, food and physical activity impact on BG levels. -promote diabetes self-management -high treatment satisfaction Sleep quality: in some cases offered peace of mind that in target and stable BG control was being achieved and a better quality of sleep. Alarms have been identified as a factor causing decreased sleep quality and interrupted sleep. Alarm fatigue: in general individuals reported clear clinical and psychological benefits to alarms alerting. Others noted how alarms could result in distractions in the workplace or at school. Barriers: difficulty inserting and/or removing the device, finding a discreet place on the body to place it on, occasional signal loss and difficulties resulting from the need to regularly calibrate their devices (12 every hour). However, all emphasized that the clinical and psychological benefits of rtCGM outweighed any challenges encountered.	Limited observation time; CSII population, the results may not be generalizable to those using insulin injection regimens. **- Low -**
Sinisterra et al. (62)	Sleep characteristics and nocturnal BGM (NBGM) Pediatric and parental HRQOL Relationship with RTCGM use.	Prospective study, only baseline data are presented 46 parent-child dyads (age 2–5 years). Device: rtCGM Region: USA	Participants complete PedsQL Sleep quality was assessed with specific questions listed accelerometry devices were used to objectively measure child sleep for a subset of participants.	rtCGM use: -may be helpful for improving child sleep and QoL -may assist child sleep duration by minimizing their wake periods throughout the night, given that parents are less likely to wake their child up for NBGM. Parents of children on rtCGM reported a higher frequency of NBGM which may contribute to greater sleep disturbances.	This study does not include a validated parent-report sleep measure. Small sample size **- Moderate -**

(Continued)

TABLE 2 | Continued

References	Main objective	Characteristics	Methodology	Main results—outcomes	Limits of the study and evidence level
Diabetes Research in Children Network (DirecNet) Study Group (63)	Psychological impact of clinical use of a rtCGM	RCT, 6 months A multi-center sample of 200 youths, aged 7–17 years, with T1D and their parents 46% were on CSII Use of the GlucoWatch G2® Biographer (GW2B) as rtCGM Region: USA	DSMP, DWS, PedsQL, CGM-SAT were administered at 0 and 6 months The DSMP was completed by telephone interview, the other on a tablet or personal computer Satisfaction with use of the GW2B was measured at end of study	Little evidence that GW2B use resulted in either beneficial or adverse psychological effects on either parents or older youths. GW2B use declined steadily during the study. Better treatment adherence (DSMP) and quality of life (PedsQL) as reported by parents at baseline was associated with more frequent GW2B use during the study.	The study was designed with the assumption that GW2B use would be relatively stable over the 6-mo study period. This was not the case as GW2B use declined steadily during the study The present study did not systematically assess how patients and parents used and responded to GW2B data **- Moderate -**
Al Hayek et al. (64)	Treatment satisfaction and sense of well-being with isCGM	12 weeks prospective cohort study 33 patients with T1D (age 14–21 years) 30% of children used CSII Device: isCGM Region: Saudi Arabia	At baseline and after 12 weeks: DTSQ and WHO-5 questionnaire	At 12 weeks: improvements in treatment satisfaction and mental *well-being* scores were detected. Improvements in the overall Diabetes treatment satisfaction questionnaire (DTSQ) score from baseline to 12 weeks. The well-being percentage score showed a statistically significant difference in *well-being* (WHO-5).	Small sample size **- Moderate -**
Pintus et al. (65)	Metabolic outcomes and QoL in children that used isCGM.	12 months prospective observational study 52 children with T1D (age 5–18 years) Device: isCGM Region: United Kingdom	The Peds QL 3.2 questionnaire was used to assess QoL before and 3 months after the use of the system. PedsQL parent report was used for parents.	The results demonstrated significant improvement in patient QoL, reduction of diabetes symptoms and treatment barriers. The use of isCGM associated with structured education improves QoL and glycemic control of children and their family.	The small sample size, limited time in observing QoL (3 months), 31–42% of patients stopped using isCGM at 6 and 12 months. **- Moderate -**

For each study, the analyzed "Psychological Outcome" is underlined.

TABLE 3 | Summary of the evidence: rtCGM and isCGM impact on psychological outcomes in children (and parents/caregivers where specified).

References	Device	Distress diabetes burden	Diabetes family conflicts	Depression	Anxiety	Fear/worry of hypo	Sleep quality	Alarm fatigue	QoL/well-being	Satisfaction
Al Hayek et al. (44)	isCGM	↓								
Boucher et al. (45)	isCGM	↓	↓							
Boucher et al. (46)	isCGM	↓ ↓parents	↓						↑parents	
Vesco et al. (47)	rtCGM	↓ ↓parents								
Erie et al. (48)	rtCGM	↓caregivers								↑caregivers
Barnard et al. (49)	rtCGM	↓ parents/ caregivers					↓ parents/caregivers		↑partners more than parents/caregivers	
Kashmer et al. (50)	rtCGM	↓parents								
Burckhardt et al. (51)	rtCGM	↓parents	↓		↓parents	↓parents	↑parents		↑parents	
Beck et al. (14)	rtCGM	– –caregivers				– – caregivers			–caregivers	↑ ↑parents
Giani et al. (13)	rtCGM	– –caregivers	Device: Guardian™ Sensor 3, Medtronic 640G (100%)			– – caregivers			–caregivers	
Markowitz et al. (52)	rtCGM	↓↑	–	↑adults	↑ ↓adults	– –adults				
Messer et al. (53)	rtCGM	↓↑	–	–				↑		
Pickup et al. (54)	rtCGM	↓↑				↓	↑parents	↓↑ parents	↑	
Telo et al. (55)	rtCGM		↓						↑	
Ng et al. (56)	rtCGM				↓	↓ ↓parents			↑ ↑parents	

(Continued)

TABLE 3 | Continued

References	Device	Distress diabetes burden	Diabetes family conflicts	Depression	Anxiety	Fear/worry of hypo	Sleep quality	Alarm fatigue	QoL/well-being	Satisfaction
Burckhardt et al. (57)	rtCGM					– ↓parents	↑parents			↑ ↑parents
Jaser et al. (58)	rtCGM					↓parents	–			
Al Hayek et al. (59)	isCGM					↓			↑	
Mauras et al. (6)	rtCGM					–parents				↑parents
Laffel et al. (60)	rtCGM	–				–	–			↑
Lawton et al. (61)	rtCGM						↑ ↑parents	↑↓		↑
Sinisterra et al. (62)	rtCGM						↑ ↓parents		↑	
Diabetes Research in Children Network (DirecNet) Study Group (63)	rtCGM	–			–			↑	–	–
Al Hayek et al. (64)	isCGM								↑	
Pintus et al. (65)	isCGM								↑ ↑parents	

↓, *reduced;* ↑, *increased; –, no variation;* ↑↓, *heterogeneity in the response*

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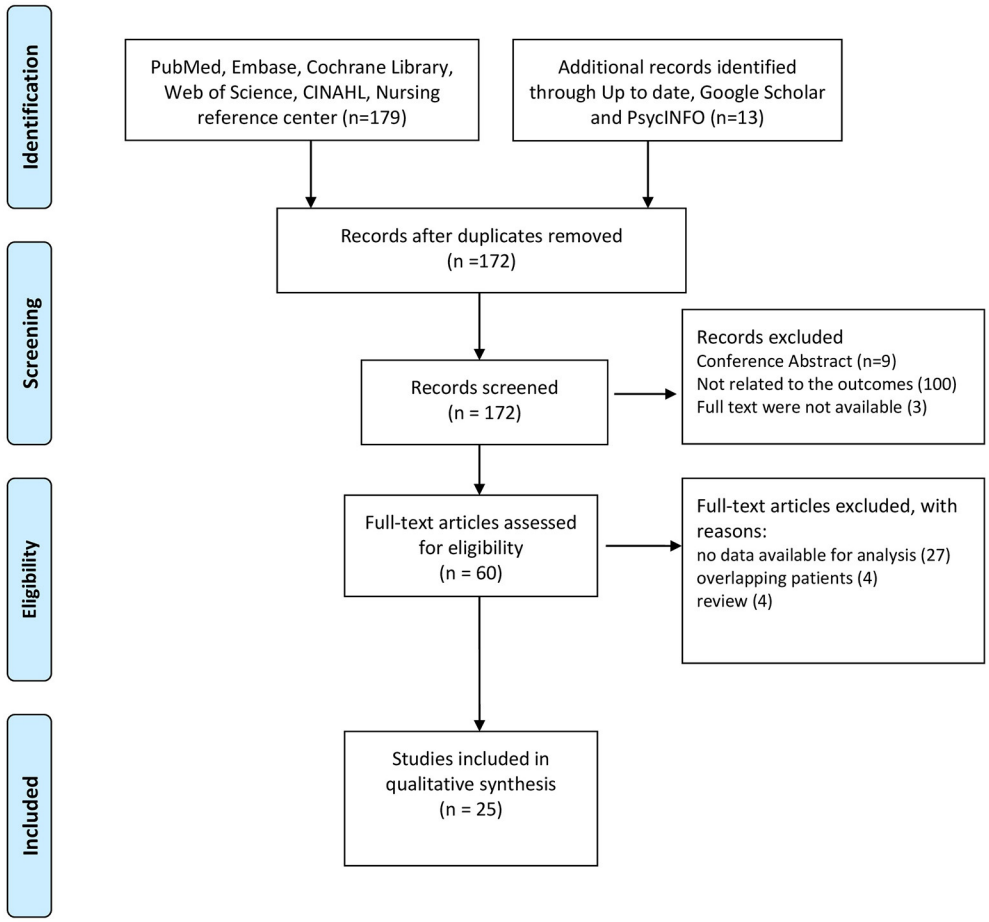

FIGURE 1 | Preferred Reporting Items for Systematic Reviews (PRISMA) flow diagram showing the progress of studies through the review.

reported disturbed sleep due to the presence of alarms and to the fear of hypoglycemia [(49), Low].

In a qualitative study, 9 pairs of children and parents reported improved sleep quality with the sensor use [(61), Low]. A prospective study on 46 children and their parents found that kids who used rtCGM experienced fewer sleep disturbances than those who did not, but their parents had greater sleep disturbances related to a higher frequency of nocturnal blood glucose monitoring (NBGM) [(62), Moderate]. A RCT on youth aged 14–24 years using rtCGM, reported there were no differences in sleep quality between sensors users and non-users [(60), High]. Data on sleep quality in youths using **isCGM** are lacking.

ALARM FATIGUE

This outcome is measured in 5 studies on rtCGM use in youth. Parents of children aged 3–17 years using **rtCGM** reported both positive and negative responses for alarms: helpful when signaling hypoglycemia but annoying when repeatedly sounding during the night; thus, most parents reported they would like to louder alarms [(54), Low]. In a qualitative study, most parents reported clear clinical and psychological benefits associated with alarms alerting, but others noted that alarms could interfer with daily activities in the workplace or at school [(61), Low]. While alarms could reinforce a sense of hypoglycemic safety, some individuals expressed ambivalent views, especially those who perceived alarms as signaling personal failure to achieve optimal glycemic control [(61), Low]. Two additional studies included in the review highlighted that alarms can often cause annoyance and discomfort [(53, 63), Moderate].

Day caregivers, teachers or school nurses, generally appreciate alarm systems and these are not perceived as a source of distraction or disturbance but as a tool that simplifies the management of the disease [(48), Low].

QUALITY OF LIFE/WELL-BEING

Four studies reported on this outcome in patients with isCGM, as well as 9 studies in patients with rtCGM. The use of **isCGM** has been reported to improve QoL in children

and adolescents [(59, 64), Moderate] as well as in their parents [(46, 65), Moderate-Low].

RtCGM systems has been reported to improve QoL in children, for easier management of insulin dosages, diet, physical activity and in school and extra-home management [(54, 55, 62), Moderate-Low]. Similarly, in parents of youths, rtCGM has been reported to improve QoL and well-being [(51, 56), Moderate-Low].

In 3 studies included in this review no variations in QoL were found after rtCGM intervention [(13, 14, 63), Moderate-High] in youths and their parents.

Parents scores regarding the QoL are significantly higher (indicative of a less favorable QoL) than the youth's one, confirming that the perception of parents regarding the QoL of their children is less favorable than the prospects of youth regarding their QoL [(63), Moderate]. Moreover, parents/caregivers compared to partners, reported more negative emotions and decreased well-being related to their family members with T1D [(49), Low].

SATISFACTION

This outcome is measured in 7 studies on **rtCGM** use in youth. Most patients using rtCGM and their parents reported high treatment-related satisfaction [(49, 57, 61), Low-Moderate].

Three RCTs of high quality confirmed the satisfaction with rtCGM use (6, 14, 60). In the first RCT, 90% of parents of 4–9 years old children, reported a high degree of satisfaction with rtCGM: the use of rtCGM makes adjusting insulin easier, shows patterns in blood glucose not seen before, and makes them feel safer knowing that they will be warned about low blood glucose before it happens [(6), High]. In the second RCT, patients aged 14–24 years using rtCGM, reported higher glucose monitoring satisfaction compared to the BGM group over a 26-weeks study period [(60), High]. In the third RCT, in patients aged 7–17 years, satisfaction scores at 26 weeks were higher for both, youths and parents, with higher scores associated with a more frequent use of rtCGM [(14), High].

In a cross-sectional study using qualitative and quantitative methods, parents and caregivers of children aged 2–17 years, felt positive about rtCGM use [(48), Low].

Data on satisfaction in youths using **isCGM** are lacking.

DISCUSSION AND CONCLUSIONS

A large percentage of pediatric patients with T1D experiences negative emotions, including state of anxiety, fear, discouragement, and frustration for the burden of the disease management. The use of CGM systems improves glycemic control (60) but demands for extra efforts from patients and their parents. Therefore, it is important to assess if the use of rtCGM and isCGM systems is related to psychological issues (52).

Studies on how isCGM and rtCGM impact the psychological outcomes in children and their caregivers were evaluated in this systematic review. Some limitations of the revised studies need to be addressed (**Table 2**):

(i) the sample size resulted small or not representative of the general population is some studies; (ii) psychological measures were included as secondary outcomes in most of the studies; thus, in some cases, the study design was not adequate to support significant results; (iii) some of the questionnaires used to measure the psychological outcomes were not previously validated. Also, questionnaires varied from one study to another.

Data on psychological outcomes in the pediatric population using isCGM systems are still limited, probably due to their recent availability on the market. The use of isCGM in adolescents can reduce psychological distress, family conflicts and fear of hypoglycemia (44, 59) and improves QoL (59, 65) as reported by a Saudi Arabia group (44) in moderate quality studies. Currently, there is no evidence of a negative impact of the isCGM system on the psychological outcomes evaluated in this review. However, results from our literature review highlighted the lack of data on depression, anxiety, and quality of sleep in pediatric patients using isCGM.

Most of the studies reported that the use of rtCGM did not increase diabetes burden in adolescents and their parents/caregivers with a moderate-high quality of evidence and using the PAID-T and P-PAID-T questionnaires (6, 13, 14, 52, 60). Likewise, rtCGM did not impact the diabetes specific family conflict, as measured by DFRQ and DFCS questionnaires in a moderate quality study (13, 52). Furthermore, rtCGM did not change depressive symptoms assessed with CDI, CES-D (13), and PHQ8 questionnaires (53).

On the other hand, rtCGM resulted improving parental anxiety in a moderate quality RCT using the STAI questionnaire by Burckhardt et al. (51). However, these results were not confirmed in a moderate quality observational study using the same questionnaire, by Giani et al. (13).

Fear of hypoglycemia remains the most common diabetes-related issue among T1D, both for youth and their parents/caregivers. In a RCT (51), parental fear of hypoglycemia (FOH) evaluated by the HFS score resulted lower in the group using rtCGM. However, other moderate-high quality studies using the HFS and HCS questionnaire did not confirm this outcome (6, 13, 14, 60).

In a RCT, adolescents' sleep quality measured with the PSQI questionnaire was not different in youth using rtCGM (60). On the contrary, parental sleep quality improved with the use of rtCGM, both when measured with the PSQI questionnaire as well by accelerometry devices in parents of adolescents and of young children, respectively (62).

Alarm fatigue was broadly evaluated in patients using rtCGM by non-validated interviews. In most cases, individuals reported clear clinical and psychological benefits to alarms setting (61), but in some contexts alarms resulted annoying and intrusive (53).

In most of the studies the perceived QoL assessed by the PedsQL in patients and caregivers, resulted improved by the use of rtCGM (55, 62). In some other studies no variations in the PedsQL were reported (13, 14), probably due to the number of variables that may influence the perceived QoL in diabetes or due to the short-term follow-up. An increased satisfaction related with the rtCGM use was assessed in both parents and

youth with the DTSQ, CGM-SAT, and GMS questionnaires in moderate-high quality studies (6, 14, 51, 60).

In conclusion, the benefits of isCGM and rtCGM use on glycemic control have been previously demonstrated (1, 2, 66, 67). Findings from the studies included in this systematic review suggest that: (i) the use of isCGM in adolescents can improve diabetes related distress, family conflicts, FOH and perceived QoL; depression, anxiety, and quality of sleep have not yet been evaluated with validated questionnaires; (ii) the use of rtCGM does not impact diabetes burden, diabetes specific family conflict and depressive symptoms. The effect of rtCGM use on the fear of hypoglycemia, the sleep quality and the anxiety is still debated. Further RCT studies specifically powered to investigate psychological outcomes are needed. The use of rtCGM increases both satisfaction and perceived QoL in youth and their parents, although alarm fatigue need to be prevented with alarm targeting.

Altogether, these findings represent an interesting overview to consider when families are in the process of deciding whether or not to start CGM use.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RF and FM made a substantial contribution to the design of this literature review, in the acquisition of data, and their interpretation and analysis as well as in the writing of the manuscript. FM and RF selected the articles of this literary review. VC, MS, EM, and EG contributed to the critical revision of the manuscript for intellectual reasons and performed a thorough proofreading of the manuscript. All the authors have definitely approved the version to publish.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 18 May 2021
doi: 10.3389/fendo.2021.665292

Nuclear Magnetic Resonance Derived Biomarkers for Evaluating Cardiometabolic Risk in Youth and Young Adults Across the Spectrum of Glucose Tolerance

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Youth with obesity have an increased risk for cardiometabolic disease, but identifying those at highest risk remains a challenge. Four biomarkers that might serve this purpose are "by products" of clinical NMR LipoProfile® lipid testing: LPIR (Lipoprotein Insulin Resistance Index), GlycA (inflammation marker), BCAA (total branched-chain amino acids), and glycine. All are strongly related to insulin resistance and type 2 diabetes (T2DM) in adults (glycine inversely) and are independent of biological and methodological variations in insulin assays. However, their clinical utility in youth is unclear. We compared fasting levels of these biomarkers in 186 youth (42 lean normal glucose tolerant (NGT), 88 obese NGT, 23 with prediabetes (PreDM), and 33 with T2DM. All four biomarkers were associated with obesity and glycemia in youth. LPIR and GlycA were highest in youth with PreDM and T2DM, whereas glycine was lowest in youth with T2DM. While all four were correlated with HOMA-IR (Homeostatic Model Assessment for Insulin Resistance), LPIR had the strongest correlation (LPIR: r = 0.6; GlycA: r = 0.4, glycine: r = –0.4, BCAA: r = 0.2, all $P < 0.01$). All four markers correlated with HbA1c (LPIR, GlycA, BCAA: r ≥ 0.3 and glycine: r = –0.3, all $P < 0.001$). In multi-variable regression models, LPIR, GlycA, and glycine were independently associated with HOMA-IR (Adjusted $R^2 = 0.473$, $P < 0.001$) and LPIR, glycine, and BCAA were independently associated with HbA1c (Adjusted $R^2 = 0.33$, $P < 0.001$). An LPIR index of >44 was associated with elevated blood pressure, BMI, and dyslipidemia. Plasma NMR-derived markers were related to adverse markers of

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Edited by:
Klemen Dovc, University Medical Centre Ljubljana, Slovenia

Reviewed by:
Urh Groselj, University of Ljubljana, Slovenia
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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *07 February 2021*
Accepted: *08 April 2021*
Published: *18 May 2021*

Citation:
Chung ST, Matta ST, Meyers AG, Cravalho CK, Villalobos-Perez A, Dawson JM, Sharma VR, Sampson ML, Otvos JD and Magge SN (2021) Nuclear Magnetic Resonance Derived Biomarkers for Evaluating Cardiometabolic Risk in Youth and Young Adults Across the Spectrum of Glucose Tolerance. Front. Endocrinol. 12:665292. doi: 10.3389/fendo.2021.665292

cardiometabolic risk in youth. LPIR, either alone or in combination with GlycA, should be explored as a non-insulin dependent predictive tool for development of insulin resistance and diabetes in youth.

Clinical Trial Registration: Clinicaltrials.gov, identifier NCT:02960659

Keywords: NMR, youth, insulin resistance, cardiometabolic risk, lipoprotein insulin resistance index, GlycA, glucose tolerance

INTRODUCTION

Cardiometabolic diseases are a major cause of morbidity and mortality worldwide. Excess adiposity in childhood is an important modifiable risk factor and one of the strongest predictors of future disease in adults (1, 2). Yet global rates of childhood obesity continue to rise unabatedly despite numerous primary prevention campaigns (3). To tackle this growing public health problem, prevention and intervention strategies should be coupled and targeted to youth and young adults at highest risk (4). Current approaches in pediatrics rely heavily on clinical and single laboratory parameters, such as BMI percentile and screening tests for hyperglycemia, to help clinicians diagnose and assess risk for cardiometabolic diseases. Although these tools are useful for the diagnosis of obesity and diabetes among growing children, they are relatively insensitive to risk stratification of insulin resistance, a primary pathophysiologic factor in cardiometabolic diseases (5).

Quantitatively assessing insulin resistance in the clinical arena has been challenging as it requires a test that is simultaneously effective and suitable for widespread clinical use. Although multiple invasive provocative methods are available to measure insulin resistance, none are suitable for routine clinical application. Alternatively, simple fasting blood tests to measure glucose and insulin are more economical and less labor intensive. For example, the homeostasis model of insulin resistance (HOMA-IR) is frequently used in research and epidemiological analyses for metabolic risk stratification (6, 7). However, outside of the research arena, its clinical application is limited because of lack of standardization of insulin assays resulting in poor reproducibility across clinical laboratory platforms (8, 9).

Four potential nuclear magnetic resonance (NMR)-derived markers—lipoprotein insulin resistance index (LPIR), GlycA (a composite marker of inflammation), total branched chain amino acids (BCAA), and glycine—have emerged as useful tools in adults with the prospect that they could augment existing clinical phenotypes to improve cardiometabolic risk stratification (10, 11). These surrogate markers of insulin resistance, derived from NMR analyzers, are attractive for routine clinical assessments because they provide reliable quantification without the need for specialized assays or time-consuming resources. These biomarkers are efficiently and inexpensively obtained from automated NMR analyzers that are already routinely used for clinical lipid and lipoprotein analysis (12).

Notably, despite the potential for improving care, there is a paucity of data on the use of these NMR biomarkers across the spectrum of insulin resistance and glucose tolerance in the pediatric population, especially in minority youth at risk for T2DM. NMR biomarkers of insulin resistance are important to explore in youth because they would facilitate estimation of an insulin independent marker of insulin resistance. Dyslipidemia of insulin resistance is a key risk factor for cardiovascular disease, precedes the onset of dysglycemia, and reflects adipose tissue and hepatic responses to insulin signaling defects (13). Since adolescence is associated with physiologic changes in lipid profiles, it is important to investigate the association of these markers with glycemia and insulin resistance in youth. We hypothesized that LPIR, GlycA, and total BCAA would be higher in youth with abnormal glucose tolerance and would positively correlate, while glycine would be negatively correlated with insulin resistance. Our primary objectives were to compare these four NMR biomarkers (LPIR, GlycA, BCAA, and glycine) in youth with and without obesity across the spectrum of glucose tolerance and determine the association of these four biomarkers with insulin resistance and glycemia.

MATERIALS AND METHODS

This was a secondary analysis of participants enrolled in two observational cohort cross-sectional studies that were designed to evaluate the pathophysiology of T2DM in youth: The MIGHTY study (Metformin Influences Gut Hormones in Youth) cohort was recruited from two clinical sites (Baylor College of Medicine, Houston TX and National Institutes of Health (NIH), Bethesda, MD) and The Dyslipidemia and Cardiovascular (CV) Risk Factors in Pediatric Obesity and Type 2 Diabetes study cohort was recruited from the Children's Hospital of Philadelphia (CHOP). Data on primary analyses for the cohorts have been previously published (14, 15). The studies were approved by the Institutional Review Boards at Baylor College of Medicine, NIH, and CHOP. All enrollees 18 years or older and parents of participants <18 years gave written informed consent prior to participation. Participants <18 years gave verbal consent and signed a written assent.

All youth who had a baseline evaluation for fasting lipoprotein profile were included in this analysis (**Figure 1**). Youth were pubertal (Tanner 2–5), 78% African American (self-reported), age 10–25 years, had no known major chronic illnesses (except T2DM and/or obesity), were not pregnant, and were not on medications known to affect insulin sensitivity (*e.g.* statins, vitamin A, or oral steroids). Tanner stage was determined based on breast examination in girls and

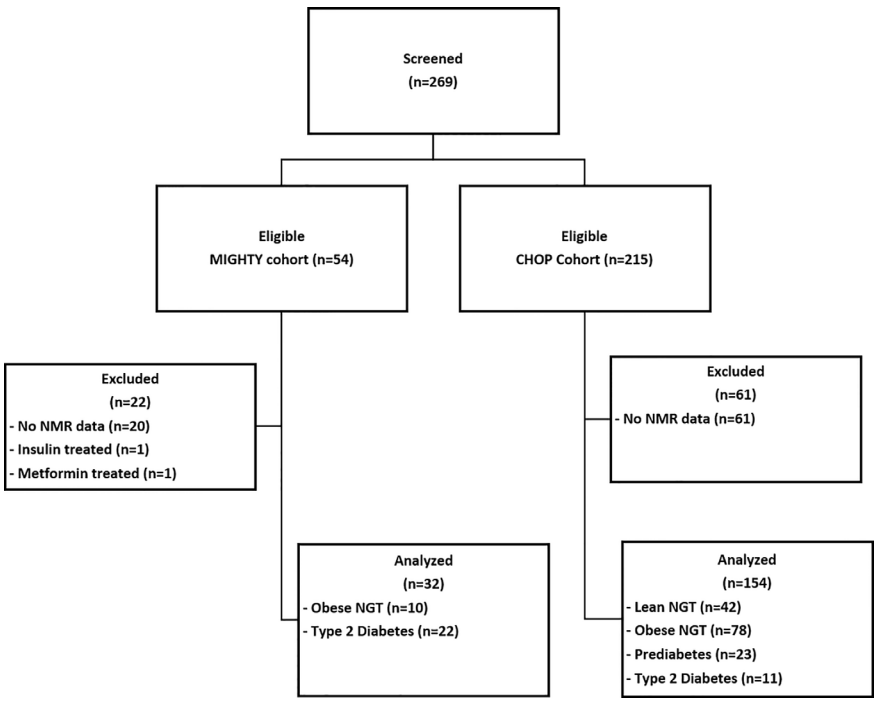

FIGURE 1 | Participant Flow Diagram. MIGHTY, Metformin Influences Gut Hormone Study in Type 2 Diabetes Youth; CHOP, Children's Hospital of Philadelphia; NMR, nuclear magnetic resonance; NGT, normal glucose tolerant; PreDM, prediabetes; T2DM, type 2 diabetes.

testicular examination in boys. The following conversion of testicular volume to Tanner stage was used: <4 cc: Stage 1; 4–6 cc: Stage 2; 7–10 cc: Stage 3; 11–15 cc: Stage 4; >15 cc: Stage 5. Pubic hair and bone age were not used to characterize pubertal stage. Blood pressure was measured in the seated patient's right arm after 10–15 minutes at rest. Blood pressure percentiles for age, sex, and height were calculated based on American Academy of Pediatrics 2017 guidelines (16, 17). BMI was calculated as weight in kilograms divided by height in meters squared, and BMI percentiles were assessed using age and sex specific BMI reference data (18). Height measurements were repeated three times, and the average value was utilized for BMI calculations. Obesity was defined as BMI ≥95%ile and lean as BMI: 5–85%ile for age and using CDC growth charts. Youth with T2DM and prediabetes (PreDM) were previously diagnosed by their physician or by a 2-h standard oral glucose tolerance test (OGTT) and American Diabetes Association criteria; the same criteria were used to classify youth as having NGT (19). Briefly, the ADA defines prediabetes as fasting plasma glucose levels of 100 mg/dl to 125 mg/dl or 2-h plasma glucose during a 75-g oral glucose tolerance test of 140 mg/dl to 199 mg/dl or an HbA1c of 5.7–6.4%. Diabetes is defined as a fasting glucose of ≥126 mg/dl or a 2-h plasma glucose ≥200 mg/dl during OGTT or an HbA1c ≥6.5%, or in patients with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl.

Figure 1 details the patient flow diagram: 186 youth were eligible to participate in this secondary analysis: 42 lean control, 88 obese NGT, 23 obese PreDM, 33 obese T2DM. In the youth with T2DM, nine were drug-naïve and 24 were treated with metformin which was discontinued 4–7 days prior to the assessment. Six participants from the CHOP cohort were regularly treated with insulin but were withdrawn from their insulin treatment prior to fasting blood draw as follows: 36 h for insulin glargine, 24 h for non-protamine Hagedorn (NPH) insulin, and 12 h for short-acting insulin prior to baseline evaluation. All participants had blood samples for plasma glucose, insulin, lipid panel, and lipoprotein profile obtained after a 10–12 h overnight fast.

Biochemical Analyses and Calculations

Lipid and Lipoprotein Analyses

All plasma samples were obtained at the baseline visit after participants fasted overnight. Samples were processed and stored at −80°C prior to analysis. Lipoprotein particle size and subclass concentrations were measured by the amplitudes of the lipid-methyl group NMR signals and reported in particle concentration units (nmol/L). Analysis was conducted with a 400 Mhz proton NMR Profiler and Vantera Clinical Analyzer platforms (20, 21). The lipid methyl signal envelope algorithm analysis (LP4.17) was used to characterize particle size and concentration. Because of the combination of two different study cohorts using different lipid measurement methodologies, we did not feel it scientifically sound to combine the original lipid values. Therefore, for this analysis we estimated the lipid values from partial least square regression models using a validated methodology (12).

The lipid panel (total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations), apolipoprotein B, and apolipoprotein A concentrations were estimated from partial least square regression models of lipid methyl and methylene region (0.494–1.592 ppm; 1,600 data points) of serum NMR LipoProfile spectra (12). Estimated low-density lipoprotein cholesterol values were calculated as follows (22):

$$LDL\text{-}C\ (mg/dl) = (TC/0.948) - (HDL\text{-}C/0.971) - (TG/8{:}56 + TG \times Non\text{-}HDL\text{-}C/2140 - TG^2/16{,}100 - 9{:}44).$$

The lipoprotein insulin resistance index (LPIR) was calculated from a composite score of six NMR lipoprotein (LP) parameters scored 0–100, with higher score indicating higher insulin resistance (23–25). BCAA, glycine, and GlycA were quantified from spectral deconvolution of signal amplitudes using *NMR LipoProfile®*. The three BCAAs (valine, leucine, isoleucine) were quantified from NMR signals. Since levels of valine, leucine, and isoleucine are highly correlated, their sum ("BCAA") is reported for ease of epidemiologic investigation. Thus, BCAA concentrations were calculated as the sum of valine, leucine, and isoleucine concentrations in each participant.

Insulin and Metabolites

Serum insulin concentrations were measured with standardized assays at each institution (reported coefficient variations ≤10%); NIH: Roche Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN), CHOP: ELISA, ALPCO Diagnostics (Salem, NH), and Baylor: Elecsys 1010 Analyzer (Roche Diagnostics, Indianapolis, IN). HbA1c was measured with high-performance liquid chromatography. Data for HbA1c (n = 7), glucose (n = 1), and insulin (n = 2) in some participants were not available due to technical difficulties.

Calculations

The equation used to calculate HOMA-IR was

$$\frac{fasting\ \ blood\ \ glucose\ \ (mg/dl) * fasting\ \ insulin\ \ (mIU/L)}{405}$$

HOMA-IR strongly correlates with insulin resistance measured by the gold-standard hyperinsulinemic clamp and has been evaluated in individuals with and without diabetes ($r > 0.6$, $P < 0.001$) (26, 27). The advantage of HOMA-IR in this research protocol was the ability to evaluate a large number of youths without invasive blood sampling required for clamp measurements and rigor and reliability of insulin measurements using standardized research insulin assays. To account for calibrations to the contemporary insulin assays and the insulin-glucose dynamics with hyperglycemia, we also used a corrected nonlinear model (the HOMA2 Calculator from ©The University of Oxford 2004–2021) to estimate HOMA-2 (28).

Statistical Analyses

Data are presented as mean ± SD, except where otherwise indicated. Insulin, triglycerides, BCAA, and HOMA-IR were natural log transformed prior to analysis. Continuous variables were compared across groups with one-way analysis of variance with Bonferroni corrections and categorical variables using Chi-squared tests. Spearman correlations (r) were used to determine the association of biomarkers with HbA1c and HOMA-IR. Multi-linear regression models were created to determine the relationship between HOMA-IR or HbA1c (outcome variables) and biomarkers (predictor variables), adjusted for Tanner stage, age, race/ethnicity, and sex. These covariates were chosen because they are known mediators of insulin resistance. To test for multi-collinearity among independent variables in the regression models we used variance inflation factors. The variance inflation factors were <2 indicating weak correlation among variables. *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using STATA (version 16.1; Stata Corp, College Station, TX).

RESULTS

Participant Demographic and Metabolic Characteristics

Youth were 14.7 ± 1.5years, 49.5% female, 89.7% Tanner 4–5, and 78.0% African American (**Table 1**). By design, BMI was higher and comparable between obese NGT, obese PreDM, and obese T2DM groups *vs.* lean NGT. There was no significant age difference among the four groups. Youth with T2DM had the highest HbA1c, fasting glucose, and fasting insulin. HOMA-IR and HOMA2-IR were similar in obese PreDM and obese T2DM and were higher compared to obese NGT and lean NGT. Similarly, the dyslipidemic pattern (elevated triglycerides, higher apolipoprotein B, higher LDL cholesterol, and lower HDL) was similar in obese PreDM and T2DM and higher compared to lean and obese NGT.

NMR Biomarkers

The median LPIR was 26 (range 0–91) and was higher in youth with obesity compared to lean (**Figure 2A**). Among youth with obesity, there was also variation in LPIR scores: LPIR was lower in obese NGT compared to obese PreDM and T2DM ($P < 0.01$), but there was no difference in LPIR between obese PreDM and T2DM ($P = 0.21$). Median GlycA was 376 μmol/L (range 239–574) and highest in obese T2DM compared to the other three groups (**Figure 2B**). Total BCAA was comparable across groups (median 392 μmol/L, range 296–595), although total BCAA was higher in obese T2DM compared to lean NGT, $P = 0.003$ (**Figure 2C**). As expected, glycine was inversely related to glucose tolerance status in a stepwise fashion (median 240 μmol/L, range 123–388, **Figure 2D**). The biomarkers did not differ by age (**Supplemental Table 1**), African ancestry (**Supplemental Table 2**), sex, or Tanner stage (data not shown).

LPIR, GlycA, and total BCAA were positively correlated, and glycine was negatively correlated with HOMA-IR (LPIR: $r = 0.6$; GlycA: $r = 0.4$, glycine: $r = -0.4$, BCAA: $r = 0.2$, all $P < 0.01$). All four markers correlated with HbA1c (LPIR, GlycA, BCAA: $r \geq 0.3$ and glycine: $r = -0.3$, all $P < 0.001$) (**Supplemental Table 3**). Among the four biomarkers, LPIR had the strongest correlation with both HbA1c and HOMA-IR/HOMA2-IR ($r = 0.48$ and $r \geq 0.60$, respectively). In a combined model using all four

TABLE 1 | Participant Demographic and Metabolic Characteristics.

Variable	Overall (n = 186)	Lean NGT (n = 42)	Obese NGT (n = 88)	Obese-PreDM (n = 23)	Obese T2DM (n = 33)	Overall p-value
Demographic						
Age (years)	14.65 ± 1.53	14.7 ± 1.31	14.5 ± 1.46	14.4 ± 1.41	15.0 ± 1.98	0.34
Female	92 (49.5)	21 (50)	49 (55.7)	9 (39.1)	13 (39.4)	0.30
Race/Ethnicity						
African American	145 (78)	34 (81.0)	65 (73.9)	21 (91.3)	25 (75.8)	
White	14 (7.5)	2 (4.76)	8 (9.1)	1 (4.4)	3 (9.1)	0.13
Hispanic/Latino	10 (5.4)	0 (0.00)	6 (6.8)	0 (0.00)	4 (12.1)	
Mixed	14 (7.5)	6 (14.3)	6 (6.8)	1 (4.4)	1 (3.0)	
Unknown	3 (1.6)	0 (0.00)	3 (3.4)	0 (0.00)	0 (0.00)	
Tanner Stage						
2 and 3	19 (10.3)	2 (4.8)	10 (11.4)	6 (27.3)	1 (3.0)	
4 and 5	166 (89.7)	50 (95.2)	78 (88.6)	16 (72.7)	32 (97.0)	**0.02**
BMI (kg/m^2)	32.2 ± 8.8	20.0 ± 1.85^{a,b}	34.9 ± 5.77 ^{a}	36.1 ± 7.4 ^{b}	38.2 ± 7.14 ^{a}	**<0.0001**
Systolic BP (mmHg)	113.7 ± 12.0	109.5 ± 12.2^{a}	112.0 ± 10.0^{b}	115.8 ± 13.8	122.2 ± 11.7 a^{,b}	**<0.0001**
Diastolic BP (mmHg)	63.2 ± 7.6	64.0 ± 8.06	61.6 ± 6.37	61.7 ± 6.04	67.6 ± 9.59	**0.0010**
Systolic BP >90th percentile	32 (17)	4 (10)	10 (11)	5 (22)	13 (40)	**0.002**
Diastolic BP >90th percentile	5 (3)	1 (2)	0 (0)	0 (0)	4 (14)	**0.001**
Metabolic (Fasting)						
Glucose (mg/dl)	95.4 ± 22.1	86.0 ± 5.8^{a}	88.4 ± 5.82^{b}	96.3 ± 6.89^{c}	124.9 ± 38.5 a^{,b,c}	**<0.0001**
Insulin (μU/L)	22 ± 19.8	8.28 ± 4.13^{a,b}	21.3 ± 12.5^{a}	29.7 ± 15.4 ^{b}	39.9 ± 32.1 ^{a}	**<0.0001**
HOMA-IR	3.8 (2.1, 6.8)	1.7 (1.1, 2.0)^{a,b}	4.0 (2.8, 5.8) ^{a,b}	7.5 (3.5, 9.7)^{a}	10.2 (4.7, 16.7) ^{b}	**<0.0001**
HOMA2-IR	1.9 (1.1, 3.3)	0.91 (0.58, 1)^{a,b}	2.0 (1.5, 2.8) ^{a,c}	3.5 (1.5, 4.9) ^{b}	4.1 (1.9, 5.7) ^{a,c}	**<0.0001**
Hemoglobin A1c (%)	5.60 ± 0.74	5.27 ± 0.30 a	5.35 ± 0.30^{b}	5.47 ± 0.43 ^{c}	6.77 ± 0.90 a^{,b,c}	**<0.0001**
Total Cholesterol (mg/dl)	135 ± 26	131 ± 23	134 ± 25	135 ± 28	143 ± 31	0.223
Triglycerides (mg/dL)	97 (75, 133)	85 (67, 100)^{a,b}	95.5 (71, 130)^{c}	121 (86, 155)^{a}	122 (95, 179) ^{b,c}	**0.0001**
HDL Cholesterol (mg/dl)	47 ± 11	58 ± 10^{a,b}	46 ± 10^{a}	42 ± 7^{b}	41 ± 8^{a}	**<0.0001**
LDL Cholesterol (mg/dl)	64 ± 20	54 ± 17^{a,b}	65 ± 17^{a}	66 ± 22	73 ± 24^{b}	**0.0003**
Apolipoprotein A1 (mg/dl)	112.73 ± 17.2	128.3 ± 15.7^{a,b,c}	110.2 ± 14.7^{a}	107.0 ± 12.3^{b}	103.6 ± 15.9^{c}	**<0.0001**
Apolipoprotein B (mg/dl)	57 ± 17	46 ± 12^{a,b}	57 ± 14^{a,c}	61 ± 19^{b}	67 ± 19^{a,c}	**<0.0001**

Data are n (%), mean ± SD or median (25th, 75th percentiles) Groups with the same letter are significantly different (Bonferroni post hoc analysis, p<0.05). BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ln, natural logarithm; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA2-IR, revised homeostatic model of insulin resistance. Continuous variables compared with one-way analysis of variance and Bonferroni correction. Insulin, HOMA-IR, HOMA2-IR, and triglycerides natural log-transformed prior to analysis. Categorical variables compared with Chi-squared tests.
Bolded values indicate significant p-values < 0.05.

biomarkers, LPIR, GlycA, and glycine were independently associated with HOMA-IR (Adjusted R^2 = 0.473, P < 0.001, **Table 2**). In a separate model, LPIR, glycine, and BCAA were independently associated with HbA1c (Adjusted R^2 = 0.33, P < 0.001, **Table 2**). To explore whether LPIR could discriminate individuals with highest risk, we divided LPIR into quartiles and compared patient metabolic and demographic variables (**Table 3**). Participants in quartile 4 with an LPIR score of >44 had significantly higher BMI, blood pressure, and metabolic markers of dyslipidemia and dysglycemia ($P < 0.001$).

DISCUSSION

This analysis confirms the association of NMR biomarkers (LPIR, GlycA, BCAA, and glycine) with insulin resistance and glycemia and provides new information on the distribution of these variables in a predominantly African American youth and young adult cohort who were at risk for or who had T2DM. Although each of the four biomarkers was associated with insulin resistance and HbA1c, the strengths of the association differed by BMI and glucose tolerance category. Youth with preDM and T2DM had the highest levels of LPIR and GlycA and the lowest values for glycine—a biochemical profile consistent with high cardiovascular risk in adults. Moreover, LPIR had the strongest and most robust associations with insulin resistance and glycemia (HbA1c) compared to the other three biomarkers. In contrast, BCAA was only weakly correlated with glycemia and insulin resistance. Although higher total BCAA concentrations were observed in youth with obesity, BCAA levels did not differ by glucose tolerance category. Therefore, of the four biomarkers evaluated, LPIR and GlycA would be ideal candidates for long term evaluation as predictor(s) of cardiometabolic diseases in youth and young adults.

Identifying biomarkers of insulin resistance that can be universally applied across race/ethnic populations, regardless of age, is of high clinical significance and urgently needed to simplify and refine primary prevention of cardiovascular diseases (29). Current invasive methodologies for measuring insulin resistance reliably assess hepatic and peripheral tissue responsiveness to insulin, but the extensive time and resources required to perform these metabolic assessments relegate these techniques to the research arena (8). In contrast, each of the four biomarkers evaluated in this study could be efficiently and inexpensively obtained from automated NMR analyzers that

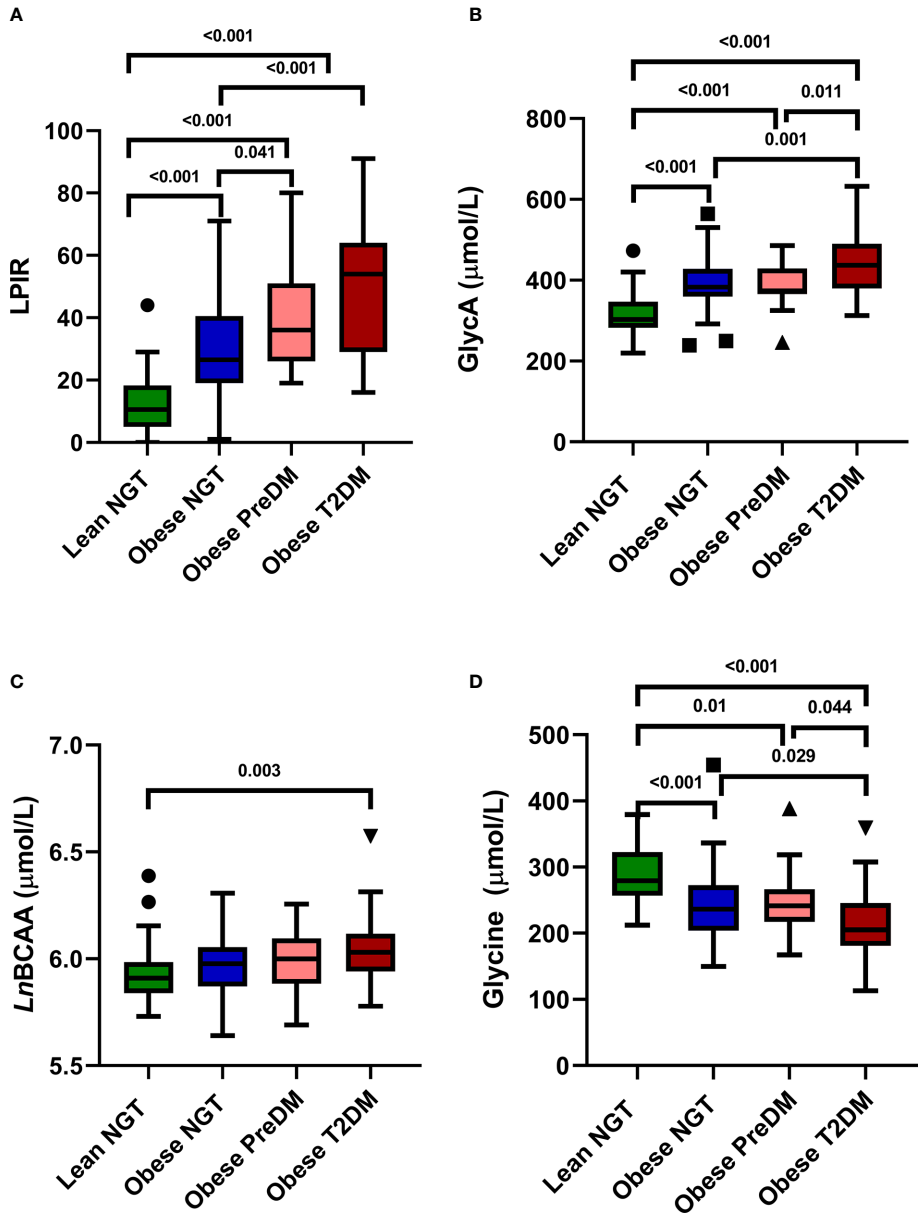

FIGURE 2 | NMR Biomarkers in Youth. Tukey Box and Whisker plots of LPIR score **(A)** GlycA **(B)** Total *Ln*BCAA **(C)** and Glycine concentrations **(D)** in lean NGT (green), obese NGT (blue), obese PreDM (pink) and obese T2DM (red). Groups were compared with one-way ANOVA with Bonferroni corrections. LPIR, lipoprotein insulin resistance score; *Ln*BCAA, natural log transformed branched chain amino acids; NGT, normal glucose tolerance; PreDM, prediabetes; T2DM, type 2 diabetes.

TABLE 2 | Association of NMR-derived biomarkers with HOMA-IR and HbA1c.

	***Ln* HOMA-IR (n = 181)**			**HbA1c (n = 176)**		
	Adjusted R^2 = 0.4733, P < 0.001			**Adjusted R^2 = 0.293, P < 0.001**		
	β	**SE**	**95% CI**	**β**	**SE**	**95% CI**
LPIR	0.202	0.003	0.014, 0.026	0.012	0.003	0.007, 0.018
GlycA	0.002	0.001	0.0008, 0.004	0.001	0.001	–0.001, 0.002
***Ln* BCAA**	0.537	0.34	–0.133, 1.207	1.016	0.355	0.316, 1.717
Glycine	–0.002	0.001	–0.004, –0.0006	–0.003	0.001	–0.005, –0.001

Multi-linear regression models were adjusted for age, Tanner stage, self-reported race/ethnicity, and sex. NMR, nuclear magnetic resonance; HOMA-IR, homeostatic model of insulin resistance; HbA1c, hemoglobin A1c; Ln, natural logarithm.

TABLE 3 | Participant demographic and metabolic characteristics and Lipoprotein Insulin Resistance Index Score.

Variable	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	P-value
	0–16	17–26	28–44	>44	
	n = 47	n = 49	n = 44	n = 46	
Demographic					
Age (years)	14.5 ± 1.5	15 ± 1.4	14.4 ± 1.2	14.7 ± 1.9	0.2881
Female	27 (57.5)	27 (55.1)	18 (40.9)	20 (43.5)	0.288
Race	40 (85.1)	38 (77.6)	35 (79.6)	32 (69.6)	**0.018**
African American White	1 (2.13)	8 (16.3)	3 (6.8)	2 (4.35)	
Hispanic/Latino	2 (4.26)	0 (0.00)	1 (2.27)	7 (15.2)	
Mixed	4 (8.51)	3 (6.12)	3 (6.82)	4 (8.7)	
Unknown	0 (0.00)	0 (0.00)	2 (4.55)	1 (2.17)	
Tanner Stage					
2 and 3	1 (2.13)	3 (6.25)	8 (18.2)	7 (15.2)	
4 and 5	46 (97.9)	45 (93.8)	36 (81.8)	9 (84.8)	**0.038**
Prediabetes	0 (0)	6 (12.2)	10 (22.7)	7 (15.2)	**<0.001**
Type 2 diabetes	2 (4.3)	5 (10.2)	5 (11.4)	21 (45.7)	**<0.001**
BMI (kg/m^2)	24.5 ± 7.18	31.8 ± 7.19	35.0 ± 7.70	38.0 ± 7.04	**<0.001**
Systolic BP (mmHg)	111.0 ± 12.7	111.0 ± 10.7	115.9 ± 12.0	117.2 ± 11.8	**0.0165**
Diastolic BP (mmHg)	64.1 ± 8.8	61.8 ± 6.5	62.4 ± 6.6	65 ± 8.7	0.15
Systolic BP > 90th percentile	6 (12.8)	5 (10.4)	10 (22.7)	11 (24)	0.205
Diastolic BP > 90th percentile	1 (2)	0 (0)	0 (0)	4 (9)	**0.024**
Metabolic (Fasting)					
Glucose (mg/dl)	88.5 ± 9.8	90.2 ± 9.1	92.9 ± 12.4	110.2 ± 36.7	**<0.00001**
Insulin (μU/L)	9.2 (5.9, 15.1)	14.5 (10.2, 17.5)	23.6 (12.3, 32.3)	35.5 (19.6, 46.7)	**<0.00001**
HOMA-IR	2.0 (1.1, 3.2)	3.5 (2.1, 4.0)	5.4 (2.9, 7.3)	8.2 (4.6, 15.2)	**<0.0001**
HOMA2-IR	1.0 (0.6, 1.7)	1.6 (1.1, 1.9)	2.7 (1.4, 3.5)	3.9 (2.4, 5.4)	**<0.0001**
Hemoglobin A1c (%)	5.32 ± 0.32	5.45 ± 0.50	5.47 ± 0.53	6.18 ± 1.05	**<0.001**
Total Cholesterol (mg/dl)	145.4 ± 23.7	122.3 ± 23.8	127.5 ± 21.4	145.2 ± 27.3	**<0.001**
Triglycerides (mg/dl)	88 (70, 109)	80 (60, 93)	104 (84, 130)	159 (122, 196)	**<0.001**
HDL Cholesterol (mg/dl)	60.1 ± 9.70	45.8 ± 7.70	42.9 ± 6.74	39.1 ± 5.9	**<0.001**
LDL Cholesterol (mg/dl)	64.9 ± 19.5	58.1 ± 19.7	59.4 ± 16.9	72.5 ± 20.1	**0.001**
Apolipoprotein A (mg/dl)	129.4 ± 15.2	108.5 ± 14.6	107.8 ± 13.1	104.9 ± 13.9	**<0.0001**
Apolipoprotein B (mg/dl)	53.4 ± 13.9	50.3 ± 15.0	54.5 ± 14.3	68.6 ± 17.2	**<0.001**

Data are n (%), mean ± SD or median (25th, 75th percentiles) BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA2-IR, revised-model Homeostatic Model of Insulin Resistance. Continuous variables compared with one-way analysis of variance and Bonferroni correction. Insulin, HOMA-IR, HOMA2-IR, and triglycerides natural log-transformed prior to analysis. Categorical variables compared with Chi-squared tests. Bolded values indicate significant p-values <0.05.

are already routinely used for clinical lipid and lipoprotein analysis (12). They are indirect measures of insulin resistance and were chosen because of their potential to identify the tissue-specific effects of insulin resistance, independent of the biological and assay variations in insulin concentrations.

LPIR is a non-insulin dependent fasting test that quantifies one of the earliest manifestations of insulin resistance—dyslipidemia characterized by elevations in triglyceride and lower high-density lipoprotein cholesterol concentrations. LPIR is already an emerging diagnostic and risk stratification tool in adults (30, 31) because elevations in LPIR precede the development of abnormal glucose tolerance (23, 32). However, its diagnostic and predictive ability in youth is unknown. In the current study, we show that LPIR progressively increased across glucose tolerance categories and was associated with insulin resistance and glycemia. Furthermore, an LPIR score of >44 identified individuals with the highest biochemical and demographic markers of cardiometabolic risk. A previous analysis in youth demonstrated an association of LPIR with insulin resistance in a healthy population before and after an exercise intervention (33). The current study confirms the positive association of LPIR with insulin resistance and extends those findings to youth at highest risk (youth with preDM and T2DM). Prior data also suggest that LPIR may be more sensitive to underlying lipid changes associated with adiposity; Magge et al., 2019 found that in youth with Down syndrome compared to controls, LPIR was higher despite no differences in fasting insulin concentrations or HOMA-IR, even when adjusted for demographics, pubertal stage and BMI z-score (34).

Similar to our findings in youth, an LPIR of >44 was associated with the highest cardiometabolic risk in adults and could be used to predict 20 year progression to T2DM (24, 31). The advantage of using LPIR resides with its simplicity in use and its potential as a risk stratification and therapeutic monitoring tool. Due to the cross-sectional nature of this analysis, we were unable to evaluate the predictive ability of LPIR for future cardiometabolic disease in youth. However, taken together, our findings and others, support using a universal cut-point of ≥45 to denote increased cardiometabolic risk in children and adults.

A notable strength of our analyses was identifying the strong association of LPIR with obesity and glycemia in youth of

African ancestry. Although simple lipid indices, such as the TG: HDL ratio, have been proposed as clinical biomarkers of insulin resistance in pediatric and adult cohorts (35), these ratios are weak correlates of insulin resistance, especially in African ancestry individuals, who are known to have lower triglyceride concentrations but greater insulin resistance (25, 36). LPIR strongly correlates with the gold-standard hyperinsulinemic euglycemic clamp across a range of ethnicities (23).

An important outstanding question is whether LPIR could be superior to HOMA-IR in risk prediction. HOMA-IR has been used across pediatric populations, but its ability to risk discriminate and predict intervention response in individuals with prediabetes with declining beta-cell function is controversial (26, 29, 37, 38). This study determined the relationship of NMR biomarkers with this existing research and epidemiological tool but was not designed to compare the diagnostic or predictive accuracy of LPIR *vs.* HOMA-IR for future cardiometabolic risk prediction. Prospective analyses are needed to extensively evaluate the predictive potential, sensitivity, and specificity of LPIR cutoffs in children and young adults.

Next, we demonstrated that GlycA correlated with insulin resistance, and there was a stepwise increase in GlycA across categories of obesity and glucose tolerance (**Figure 2B**). Some, but not all studies (39), have identified GlycA as an independent correlate of HOMA-IR, with the added advantage that GlycA had low intra-individual variability in youth and adults compared to other inflammatory markers such as C-reactive protein (33, 40, 41). GlycA is a promising assay because it is an integrated marker of inflammation derived from a composite signal of N-acetyl methyl group resonances from several of the most abundant inflammation response serum proteins (11). In population-based studies, elevated levels were indicators of chronic asymptomatic systemic inflammation from childhood to adulthood (42). However, its value in predicting cardiometabolic diseases in youth is unclear, and in prior analysis, it was not related to exercise-induced improvements in insulin resistance in a cohort of Latino adolescents (39). In agreement with adult studies, our study findings demonstrate a moderately strong correlation of GlycA with insulin resistance and provide new knowledge about the relationship of this inflammatory marker in youth with abnormal glucose tolerance.

Total BCAA is another serum biomarker that has been extensively studied in the last few decades. In this study, we examined the NMR derived total BCAA and showed that BCAA was a weak correlate of insulin resistance across categories of glucose tolerance in our cohort. Our findings agree with previous studies in adults; total BCAA concentrations are elevated in T2DM, but because of its relatively weak correlation with insulin resistance and glycemia, it is not a readily used clinical risk tool (43, 44). Data linking BCAA with insulin resistance is conflicting, especially in cross-sectional studies in which dietary intake is not controlled (43, 45). High BCAA concentrations in individuals with obesity and insulin resistance may be caused by abnormal catabolism. Reduced catabolism, related to impaired branched-chain α-keto-acid dehydrogenase, is linked to insulin resistance in murine models (46). Alternatively, BCAAs are essential amino acids whose concentrations directly correlate with dietary intake, and plasma levels may also be altered by differences in microbiome composition and anti-diabetic medications such as metformin therapy (45, 47, 48). Given the multiple lifestyle and environmental factors that influence BCAA concentrations, our data posit that NMR-derived BCAA concentrations are not robust markers of insulin resistance or worsening glycemia in youth. The weak relationship of BCAA with insulin resistance was observed in our cohort despite strict protocols to minimize any effects of medication.

Lastly, we provide novel information on glycine concentrations in youth. Glycine is a relatively new biomarker that is a non-essential amino acid associated with reduced incidence of coronary heart disease and lower risk of T2DM (49, 50). This is the first study, to our knowledge, that has systematically evaluated the relationship of glycine and demonstrated a weak inverse relationship with insulin resistance and glycemia in youth. Despite a small range, glycine was significantly different between each of the four groups and lowest in youth with T2DM. However, the effect size of the differences between groups was small, and the underlying mechanisms of this inverse relationship are not clear and need further validation. Murine data suggest that glycine is important for waste elimination of excess free fatty acid and BCAA catabolism (51, 52). Alternatively, glycine supplementation has been associated with improved insulin secretion and inhibition of inflammation (50). Therefore, glycine's role as a biomarker and indicator of insulin resistance, especially in youth, remains to be determined.

Some study limitations are noteworthy. This was a secondary cross-sectional analysis in a predominantly African American youth and young adults. Although we acknowledge that the homogeneity of our study participants limits the generalizability of findings, our population demographics reflect the burden of disease in youth at risk for T2DM. Therefore, it was imperative that these biomarkers of interest were evaluated in a population-specific manner that included youth with preDM, and T2DM of African ancestry in whom traditional markers of insulin resistance (*e.g.* TG/HDL ratio) are imprecise. Larger studies will be required to validate NMR biomarker prediction in a multi-ethnic youth population. In addition, nutritional habits were not collected during this study and the influence of diet on glycine, BCAAs, and serum lipid concentrations could not be evaluated. Nevertheless, our study design reflected 'real-world' clinical conditions in which detailed dietary profiles are not available. Another limitation of this analysis was the use of frozen samples that may have underestimated biomarker levels (23), though in a consistent manner across all study samples. To minimize bias related to systematic underestimation, all samples were processed after only one freeze–thaw cycle and batched for analysis. Additionally, we acknowledge that HOMA-IR was not a gold-standard measure of insulin resistance, and its use in individuals with dysglycemia is not without controversy (37, 53). We justified choosing HOMA-IR *a priori* because it allowed us to determine associations with a commonly used fasting research index in large group of at-risk youth, in which invasive measurements of a insulin resistance were not possible.

CONCLUSION

The ability to risk-stratify youth with insulin resistance, especially in the clinical setting, has been challenging. This study demonstrated the utility of NMR-derived biomarkers obtained from the clinical LipoProfile® lipid testing in a high-risk pediatric population across the spectrum of glucose tolerance. LPIR and GlycA were superior biomarkers of glycemia and insulin resistance compared to BCAA and glycine. LPIR was the strongest correlate of insulin resistance and a value of >44 was associated with the highest cardiometabolic risk—findings that strongly support the versatility of using a universal LPIR scoring system across race/ethnicities in both children and adults. Biomarkers of insulin resistance that can be applied across different racial and ethnic groups in both youth and adults, with a single blood draw, could prove extremely useful in a clinical setting. LPIR, alone or in combination with GlycA, is potentially an important addition to the clinician's toolbox and should be explored as predictive tools of cardiometabolic risk in prospective studies of diverse youth populations.

DATA AVAILABILITY STATEMENT

A limited dataset containing the raw data supporting the conclusions of this article will be made available by the authors upon request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board at Baylor College of Medicine, NIH, and CHOP. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

STC conceptualized and designed the study, recruited, and collected the data, conducted the analysis, and wrote the manuscript. STM conceptualized the study, conducted the analysis, and wrote the manuscript. SNM designed the study, recruited and collected the data, and edited and revised the manuscript. AGM, CKC, AV-P, JMD, VRS, MLS, and JDO contributed to study design, and data collection, and revised and edited the manuscript. STC is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

The Division of Intramural Research supports SC (National Institute of Diabetes & Digestive & Kidney Diseases), MS (the NIH Clinical Center), and AM (National Institute of Child Health and Development). SM received NIH funding (K23 PA05143). The study was presented as a moderated poster at the 80th Scientific Sessions of the American Diabetes Association, June 2020.

ACKNOWLEDGMENTS

We would like to thank the volunteers (and their families) whose participation made this study possible.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.665292/full#supplementary-material

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Conflict of Interest: JO was employed by the company Laboratory Corporation of America Holdings (LabCorp), Morrisville, NC, USA.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 20 May 2021
doi: 10.3389/fendo.2021.645825

Relative Children's Lipid Accumulation Product Is a Novel Indicator for Metabolic Syndrome

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Background: The children's lipid accumulation product (CLAP) is associated with MS in Chinese children and adolescents. The aim of this study was to develop a more effective indicator, the relative children's lipid accumulation product (RCLAP) was evaluated for correlation with MS and the density of lipid accumulation.

Methods: A stratified cluster sampling method was used to recruit 683 students aged 8–15 years in this study. The presence of MS was defined according to the NCEP-ATP III criteria. The participants' guardians signed informed consent before the medical examination. This study was approved by the Medical Ethics Committee of the Bengbu Medical College [(2015) No.003] and was conducted in accordance with the Declaration of Helsinki.

Results: The overall prevalence of MS was 4.8% (male 6.6%, female 2.8%). After adjusting for sedentary activity time, relative children's lipid accumulation product per height (RCLAP-H) and relative children's lipid accumulation product per sitting height (RCLAP-SH) significantly increased the risk of MS in girls [OR (95% CI): 96.13 (11.11–831.97) and 96.13 (11.11–831.97), respectively]. After adjusting for ages and moderate-to-vigorous physical activity time, RCLAP-H, and RCLAP-SH significantly increased the risk of MS in boys [OR (95% CI): 171.75 (33.60–878.00) and 133.18 (27.65–641.39), respectively]. The AUCs of RCLAP-H and RCLAP-SH for predicting MS were 0.950, 0.948 in girls, and 0.952, 0.952 in boys, which were higher than BMI, WHtR, Tg/HDL-C, CLAP, and CLAP combining height, sitting height.

Conclusions: The RCLAP-H and RCLAP-SH were more effective indicators for predicting MS than BMI, WHtR, Tg/HDL-C, and CLAP in children and adolescents.

Keywords: metabolic syndrome, relative children's lipid accumulation product, obesity, children, adolescents

OPEN ACCESS

Edited by:
Giulio Maltoni, Sant'Orsola-Malpighi Polyclinic, Italy

Reviewed by:
Maurizio Delvecchio, Giovanni XXIII Children's Hospital, Italy
Alberto Casertano, University of Naples Federico II, Italy

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *24 December 2020*
Accepted: *22 February 2021*
Published: *20 May 2021*

Citation:
Zhang L, Zhang Z, Wang B, Yuan Y, Sun L, Gao H and Fu L (2021) Relative Children's Lipid Accumulation Product Is a Novel Indicator for Metabolic Syndrome. Front. Endocrinol. 12:645825. doi: 10.3389/fendo.2021.645825

INTRODUCTION

Over the past two decades, there has been a striking increase in the number of people with metabolic syndrome (MS) (1). In 2009, the overall age-standardized estimated prevalence of MS was 21.3% based on the criteria of the revised National Cholesterol Education Program—Third Adult Treatment Panel (NCEP-ATPIII) (2). A meta-analysis showed that MS increased the risk of type

2 diabetes and cardiovascular diseases (CVD) (3). Concomitantly with the increasing prevalence of childhood obesity, the prevalence of metabolic syndrome is rising among children and adolescents (4). According to the International Diabetes Federation (IDF), NCEP-ATPIII, and Chinese Children Metabolic Syndrome Righteousness and Prevention Advice (CHN2012) criteria, the prevalence of MS among Chinese children was 1.8, 2.6, and 2.0%, respectively. In addition, the MS prevalence in children who were overweight and obese was 4.7 and 17.3% based on IDF criteria in 2004–2014, respectively (5). Childhood MS is associated with hypertension, hyperlipidemia, insulin resistance, and type 2 diabetes, which can also lead to cardiometabolic diseases during adulthood. The definition of MS includes the presence of three or more components: central obesity, hypertriglyceridemia, high fasting glucose, low high-density lipoprotein (HDL), and hypertension (6). Many factors may induce MS, including unhealthy eating habits and lack of exercise (7, 8), the etiology and pathogenesis of MS are very complex (9, 10). Thus, more effective indicators to predict MS are very important in children and adolescents.

Some research has reported that body mass index (BMI), waist circumference (WC), abdominal skinfold thickness (AST), Waist-to-height ratio (WHtR), triglycerides (TG), triglycerides-to-HDL-C ratio (Tg/HDL-C), and wrist circumference (WrC) were effectively related with MS (11–17). However, the above indicator is limited to distinguishing adipose tissue from lean mass and showing circulating lipid accumulation. The lipid excess coincides with expansion of visceral adipocytes and elevated blood concentrations of certain lipids, which is referred to as lipid overaccumulation, which could lead to ectopic deposition of lipids in non-adipose tissues, insulin resistance, and other metabolic dysfunctions (18–20). Kahn et al. (18) proposed a new marker, the lipid accumulation product (LAP), which reflects the total lipid accumulation in the body to predict MS in adults. The LAP is a product of waist circumference (WC) and fasting triglycerides (TG) concentration. Studies have shown LAP is a powerful marker for predicting MS and is better than BMI, WC, and WHtR in adults. However, LAP may not directly reflect lipid accumulation in children and adolescents. Zhang et al. (21) developed a novel indicator, the children's lipid accumulation product (CLAP), associated with MS in Chinese children and adolescents. The CLAP is a product of WC, AST, and TG concentration [CLAP = WC (cm) × AST (mm) × TG (mmol/L) / 100]. They reported that CLAP was an effective indicator associated with MS and was better than BMI and WHtR. Wang et al. (22) showed that CLAP was significantly associated with hypertension in children and adolescents, and can more effectively predict childhood hypertension than WC, WHtR, BMI, AST, and TG. Yuan et al. (23) showed that the CLAP was significantly associated with impaired fasting glucose (IFG) in Chinese boys, and it performed better than WC, WHtR, AST, and TG. From the formula of CLAP, we know that AST refers to the accumulation of skin fat at a point on the abdomen. Multiplying by WC shows the accumulation of whole abdominal fat and then multiplying by TG, to a certain extent, shows the accumulation of body lipid. However, CLAP could not reflect the density of lipid accumulation in body.

It is well known that there are multifarious types of obesity, and different obesity types have different characteristic locations of fat accumulates. Exogenous and endogenous obesity are two types of Childhood obesity (24). The relative children's lipid accumulation product (RCLAP) that was the CLAP at per unit body height, sitting height, and weight, may reflect the density of lipid accumulation among children and adolescents. However, it has been unclear whether the RCLAP were more effective indicators related to MS than CLAP, BMI, and WHtR. The purpose of this study was to develop more effective RCLAP indicators for predicting MS.

MATERIALS AND METHODS

Study Subjects

In this study, a total of 683 students aged 8–15 years were selected from two 9-year schools *via* the stratified cluster sampling methods, including 317 girls (46.4%) aged at (10.98 ± 1.83 years and 366 boys (53.6%) aged at (10.77 ± 1.80 years).

Measurement

The medical staff who received standardized training measured the participants' body weight, height, sitting height (SH), Diastolic blood pressure (DBP), Systolic blood pressure (SBP), WC, and AST (21). Venous blood samples (3 ml) were taken by nurses with standardized training after at least 8 h of overnight fasting by the children and adolescents. The enzyme-linked immunoassay method was used to detect HDL-C. Enzymatic methods were used to detected TG and FBG levels.

Definition of Metabolic Syndrome (MS)

In this study, MS was diagnosed according to the amended NCEP-ATP III criteria (25). High fasting blood glucose (FBG) ≥ 110 mg/dl; abdominal obesity: WC ≥90th age- and sex-specific percentile for Chinese children (26); high blood pressure: SBP and/or DBP ≥90th percentile for gender and age (27); low high-density lipoprotein cholesterol (HDL-C) ≤40 mg/dl; high triglycerides(TG)≥110 mg/dl; when three or more of the five components were present then a diagnosis of MS was made.

Calculation of the Derivative Variables

Children's lipid accumulation product (CLAP) = WC (cm) × AST (mm) × TG (mmol/L) / 100; BMI = weight (kg)/height^2 (m^2); WHtR = waist circumference (cm)/height (cm); Relative children's lipid accumulation product per height (RCLAP-H) = WC (cm) × AST (mm) × TG (mmol/L)/height (cm); Relative children's lipid accumulation product per sitting height (RCLAP-SH) = WC (cm) × AST (mm) × TG (mmol/L)/sitting height (cm); Relative children's lipid accumulation product per weight (RCLAP-W) = WC (cm) × AST (mm) × TG (mmol/L)/ weight (kg).

Surveys of Behavioral Indexes

We investigated dietary behaviors and physical activities. The healthy dietary behaviors (including breakfast, milk, fruits, nuts,

fresh vegetables) and risk dietary behaviors (including fried foods, eating out, carbonated drinks, high-energy snacks, western-style fast food)—each item was assigned 0 points for never, 0.25 points for 1 time per month, 0.5 points for 2 times per month, 2 points for 1–3 times per week, 2 points for 1–3 times per week, 5 points for 4–6 times per week, 7 points for 7 times per week. The total scores of dietary behaviors were defined by the two grades of ≥ the 75th percentile (P75) and <75th percentile (P75) [21–23]. Children's Leisure Activities Study Survey (CLASS) questionnaire was used to investigate physical activities (21, 28, 29).

Statistical Analysis

Data were analyzed using SPSS 23.0 software. The data were described using proportion and mean ± standard deviation. The height, siting height, weight, WC, AST, BMI, WHtR, Tg/HDL-C, logarithmic CLAP, RCLAP-H, RCLAP-SH, RCLAP-W, SBP, and DBP were standardized for genders and ages using the Z-score method (Abbreviations of above standardized indexes: SH, SSH, SW, SWC, SAST, SBMI, SWHtR, STg/HDL-C, SlnCLAP, SRCLAP-H, SRCLAP-SH, SRCLAP-W, SSBP, and SDBP, respectively). The t-test, chi-square test, and logistic regression models were used to analyze the associations of SBMI, SWHtR, STg/HDL-C, SlnCLAP, SRCLAP-H, SRCLAP-SH, and SRCLAP-W with MS, respectively. Receiver Operating Characteristic (ROC) curves were used to evaluate the predictive efficiency of above indexes for predicting MS. $P < 0.05$ was considered statistically significant.

RESULTS

Demographics

A total of 683 children aged 8–15 years (366 boys and 317 girls) were included in this study. The overall prevalence of MS was 4.8% (2.8% for girls and 6.6% for boys), as shown in **Table 1**. The results showed that weight, WC, AST, BMI, WHtR, CLAP, RCLAP-H, RCLAP-SH, RCLAP-W, SBP, DBP, Tg/HDL-C, and TG among girls with MS were significantly higher than those without MS; the boys with MS had higher values of height,

TABLE 1 | The comparison of anthropometric characteristics, dietary behaviors, physical activities, CLAP, and RCLAP among children with Non-MS and MS.

Variables	Girls (n = 317)		t/χ^2	*P*	Boys (n = 366)		t/χ^2	*P*
	Non-MS (97.2%)	MS (2.8%)			Non-MS (93.4%)	MS (6.6%)		
SH	−0.01 ± 0.99	0.32 ± 1.04	−0.98	0.329	−0.03 ± 0.97	0.40 ± 1.17	−2.05	0.041
SSH	−0.01 ± 0.98	0.41 ± 1.19	−1.27	0.204	−0.04 ± 0.96	0.58 ± 1.24	−2.99	0.003
SW	−0.02 ± 0.99	0.83 ± 0.67	−2.59	0.010	−0.10 ± 0.91	1.45 ± 0.91	−8.07	<0.001
SWC	−0.05 ± 0.95	1.74 ± 0.73	−5.61	<0.001	−0.11 ± 0.91	1.58 ± 0.65	−8.90	<0.001
SAST	−0.03 ± 0.98	0.96 ± 0.72	−3.00	0.003	−0.10 ± 0.92	1.48 ± 0.72	−10.22	<0.001
SBMI	−0.02 ± 0.99	0.76 ± 0.52	−2.36	0.019	−0.11 ± 0.91	1.56 ± 0.69	−11.26	<0.001
SWHtR	−0.05 ± 0.95	1.79 ± 0.79	−5.77	<0.001	−0.11 ± 0.92	1.55 ± 0.65	−11.82	<0.001
SlnCLAP	−0.04 ± 0.97	1.43 ± 0.23	−15.42	<0.001	−0.11 ± 0.92	1.60 ± 0.51	−14.91	<0.001
SRCLAP-H	−0.05 ± 0.94	1.85 ± 0.67	−6.01	<0.001	−0.15 ± 0.78	2.20 ± 1.13	−10.03	<0.001
SRCLAP-SH	−0.05 ± 0.94	1.85 ± 0.66	−5.98	<0.001	−0.15 ± 0.79	2.19 ± 1.11	−10.14	<0.001
SRCLAP-W	−0.05 ± 0.96	1.57 ± 0.80	−5.01	<0.001	−0.14 ± 0.80	2.02 ± 1.19	−8.76	<0.001
SSBP	−0.04 ± 0.97	1.32 ± 0.87	−4.18	<0.001	−0.08 ± 0.96	1.09 ± 0.84	−5.84	<0.001
SDBP	−0.04 ± 0.96	1.34 ± 1.02	−4.22	<0.001	−0.06 ± 0.97	0.86 ± 0.91	−4.46	<0.001
HDL-C	1.51 ± 0.29	1.18 ± 0.27	3.32	0.001	1.56 ± 0.30	1.20 ± 0.24	5.81	<0.001
TG	0.94 ± 0.38	1.39 ± 0.27	−3.47	0.001	0.83 ± 0.34	1.50 ± 0.39	−9.24	<0.001
STg/HDL-C	−0.05 ± 0.93	1.86 ± 1.15	−6.02	<0.001	−0.12 ± 0.86	1.71 ± 1.12	−9.83	<0.001
FBG	5.09 ± 0.45	5.26 ± 0.34	−1.08	0.283	5.18 ± 0.42	5.30 ± 0.44	−1.36	0.176
Ages (years)			0.47	0.491			7.06	0.008
8–	187 (96.4)	7 (3.6)			221 (96.1)	9 (3.9)		
12–15	121 (98.4)	2 (1.6)			121 (89.0)	15 (11.0)		
Healthy dietary behaviors			0.13	0.723			0.08	0.769
$<P_{75}$	241 (96.8)	8 (3.2)			247 (93.2)	18 (6.8)		
$\geq P_{75}$	67 (98.5)	1 (1.5)			95 (94.1)	6 (5.9)		
Risk dietary behaviors			0.00	1.00			1.33	0.250
$<P_{75}$	256 (97.3)	7 (2.7)			238 (94.4)	14 (5.6)		
$\geq P_{75}$	52 (96.3)	2 (3.7)			104 (91.2)	10 (8.8)		
Moderate-to-vigorous physical activity time			0.61	0.434			4.46	0.035
<60 min	182 (96.3)	7 (3.7)			166 (90.7)	17 (9.3)		
≥60 min	126 (98.4)	2 (1.6)			176 (96.2)	7 (3.8)		
Sedentary activity time			3.65	0.056			0.38	0.537
<120 min	124 (94.7)	7 (5.3)			179 (94.2)	11 (5.8)		
≥120 min	184 (98.9)	2 (1.1)			163 (92.6)	13 (7.4)		

SH, standardized height; SSH, standardized sitting height; SW, standardized weight; SWC, standardized waist circumference; SAST, standardized abdominal skinfold thickness; SBMI, standardized body mass index; SWHtR, standardized waist-height ratio; SlnCLAP, standardized logarithmic children's lipid accumulation product; SRCLAP-H, standardized relative children's lipid accumulation product per height; SRCLAP-SH, standardized relative children's lipid accumulation product per sitting height; SRCLAP-W, standardized relative children's lipid accumulation product per weight; SSBP, standardized systolic blood pressure; SDBP, standardized diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; TG, triacylglycerol; STg/HDL-C, standardized triglycerides-to-HDL-C ratio; FBG, fasting blood glucose.

siting height, weight, WC, AST, BMI, WHtR, CLAP, RCLAP-H, RCLAP-SH, RCLAP-W, SBP, DBP, Tg/HDL-C, and TG compared with those without MS ($P < 0.05$). In contrast, girls and boys with MS had lower values of HDL-C than those without MS, respectively ($P < 0.05$). The prevalence of MS among boys aged 12–15 years was significantly higher than that aged 8–11 years ($P < 0.05$). The proportion of moderate-to-vigorous physical activity time (≥60 min) among boys with MS was significantly lower than those without MS ($P < 0.05$).

The Factors Associated With MS

The results of chi-square test showed that WHtR, CLAP, RCLAP-H, RCLAP-SH, and RCLAP-W were significantly associated with MS among boys and girls (**Table 2**). In girls, after adjusting for sedentary activity time factor, SWHtR ≥1, SBMI ≥1, STg/HDL-C ≥1, SRCLAP-H ≥1, SRCLAP-SH ≥1, and SRCLAP-W ≥1 significantly increased the risk of MS compared with SWHtR <1, SBMI <1, STg/HDL-C <1, SRCLAP-H <1, SRCLAP-SH <1, and SRCLAP-W <1 (OR were 15.79, 3.73, 32.97, 96.13, 96.13, 18.28, respectively). In boys, after adjusting for ages and moderate-to-vigorous physical activity time factors, SWHtR ≥1, SBMI ≥1, STg/HDL-C ≥1, SlnCLAP ≥1, SRCLAP-H ≥1, SRCLAP-SH ≥1, and SRCLAP-W ≥1 significantly increased the risk of MS compared with SWHtR <1, SBMI <1, STg/HDL-C <1, SlnCLAP <1, SRCLAP-H <1, SRCLAP-SH <1, and SRCLAP-W <1 (OR were 37.43, 68.33, 25.70, 105.86, 171.75, 133.18, 50.13, respectively) (**Table 3**).

The Power for Predicting MS

As shown in **Table 4** and **Figure 1**, the AUCs of SBMI, SWHtR, STg/HDL-C, SlnCLAP, SRCLAP-H, SRCLAP-SH, SRCLAP-W, SlnCLAP and SH, SlnCLAP and SSH, SlnCLAP and SW for predicting MS among girls were 0.828, 0.925, 0.929, 0.946, 0.950, 0.948, 0.920, 0.947, 0.947, 0.949. The AUCs of above indicators for predicting MS among boys were 0.916, 0.916, 0.931, 0.946, 0.952, 0.952, 0.929, 0.946, 0.946, 0.949, respectively.

DISCUSSION

Metabolic syndrome (MS) has become a major public health issue worldwide (30). This study showed that the overall prevalence of MS was 4.8% (males 6.6%, girls 2.8%) in children and adolescents aged 8–15 years. The similar prevalence of MS was reported among Indian studies aged 12–17 years (4.2%) (31) and US adolescents (4.5%) (32). Rodríguezmorán et al. (33) reported that there was higher prevalence of MS among Mexico adolescents (6.5%) and Esmaillzadeh et al. (34) reported that there was 10.1% of Iranian adolescents with MS. The present study showed that prevalence of MS among girls (2.8%) was lower than that in boys (6.6%), which was consistent with the results of most previous studies (25, 35, 36). The reasons might be because the boys had lower levels of moderate-to-vigorous physical activity and higher levels of risk dietary behaviors, so they have a higher prevalence of overweight or obesity among boys than girls. However, other studies had also reported there was no significant difference in the prevalence of MS between sex (9). The current study showed that the prevalence of MS among boys aged 12–15 years was significantly higher than that aged 8–11 years, which was in line with the result of a study by Gooty et al. (37). This may be associated with an increased exposure of risk factors for MS as boys age (38). It was reported

TABLE 2 | The associations between WHtR, BMI, Tg/HDL-C, CLAP, RCLAP, and MS among children.

Variables	Girls (n = 317)		χ^2	*P*	Boys (n = 366)		χ^2	*P*
	Non-MS	MS			Non-MS	MS		
SWHtR			18.48	<0.001			58.11	<0.001
<1	258 (99.2)	2 (0.8)			293 (98.3)	5 (1.7)		
≥1	50 (87.7)	7 (12.3)			49 (72.1)	19 (27.9)		
SBMI			2.48	0.115			72.38	<0.001
<1	275 (97.9)	6 (2.1)			298 (98.7)	8 (1.3)		
≥1	33 (91.7)	3 (8.3)			44 (68.8)	20 (31.3)		
STg/HDL-C			31.86	<0.001			71.73	<0.001
<1	277 (89.9)	2 (22.2)			312 (91.2)	7 (29.2)		
≥1	31 (10.1)	7 (77.8)			30 (8.8)	17 (70.8)		
SlnCLAP			44.22	<0.001			90.72	<0.001
<1	268 (100.0)	0 (0.0)			299 (99.3)	2 (0.7)		
≥1	40 (81.6)	9 (18.4)			43 (66.2)	22 (33.8)		
SRCLAP-H			47.67	<0.001			116.98	<0.001
<1	280 (99.6)	1 (0.4)			311 (99.4)	2 (0.6)		
≥1	28 (77.8)	8 (22.2)			31 (58.5)	22 (41.5)		
SRCLAP-SH			47.67	<0.001			111.81	<0.001
<1	280 (99.6)	1 (0.4)			309 (99.4)	2 (0.6)		
≥1	28 (77.8)	8 (22.2)			33 (60.0)	22 (40.0)		
SRCLAP-W			22.78	<0.001			92.46	<0.001
<1	278 (98.9)	3 (1.1)			309 (98.7)	4 (1.3)		
≥1	30 (83.3)	6 (16.7)			33 (62.3)	20 (36.7)		

SWHtR, standardized waist-height ratio; SBMI, standardized body mass index; STg/HDL-C, standardized triglycerides-to-HDL-C ratio; SlnCLAP, standardized logarithmic children's lipid accumulation product; SRCLAP-H, standardized relative children's lipid accumulation product per height; SRCLAP-SH, standardized relative children's lipid accumulation product per sitting height; SRCLAP-W, standardized relative children's lipid accumulation product per weight.

TABLE 3 | The adjusted associations between WHtR, BMI, Tg/HDL-C, CLAP, and RCLAP on MS using logistic regression models.

Variables	β (S.E.)	Wald	P	OR (95% CI)
Girls				
SWHtR				
<1	0			1
≥1	2.76 (0.82)	11.24	0.001	15.79 (3.15–79.21)
SBMI				
<1	0			1
≥1	1.32 (0.74)	3.14	0.076	3.73 (0.87–15.95)
STg/HDL-C				
<1	0			1
≥1	3.50 (0.84)	17.35	<0.001	32.97 (6.37–170.80)
SlnCLAP				
<1	0			1
≥1	19.74 (2,358.36)	0.00	0.993	–
SCLAP-H				
<1	0			1
≥1	4.57 (1.10)	17.19	<0.001	96.13 (11.11–831.97)
SCLAP-SH				
<1	0			1
≥1	4.57 (1.10)	17.19	<0.001	96.13 (11.11–831.97)
SCLAP-W				
<1	0			1
≥1	2.91 (0.75)	15.18	<0.001	18.28 (4.24–78.87)
Boys				
SWHtR				
<1	0			1
≥1	3.62 (0.60)	37.09	<0.001	37.43 (11.67–120.10)
SBMI				
<1	0			1
≥1	4.22 (0.67)	40.20	<0.001	68.33 (18.51–252.20)
STg/HDL-C				
<1	0			1
≥1	3.25 (0.51)	40.91	<0.001	25.70 (9.50–59.50)
SlnCLAP				
<1	0			1
≥1	4.66 (0.80)	32.80	<0.001	105.86 (21.99–509.68)
SCLAP-H				
<1	0			1
≥1	5.15 (0.83)	38.21	<0.001	171.75 (33.60–878.00)
SCLAP-SH				
<1	0			1
≥1	4.89 (0.80)	37.20	<0.001	133.18 (27.65–641.39)
SCLAP-W				
<1	0			1
≥1	3.92 (0.60)	42.63	<0.001	50.13 (15.48–162.37)

SWHtR, standardized waist-height ratio; SBMI, standardized body mass index; STg/HDL-C, standardized triglycerides-to-HDL-C ratio; SlnCLAP, standardized logarithmic children's lipid accumulation product; SRCLAP-H, standardized relative children's lipid accumulation product per height; SRCLAP-SH, standardized relative children's lipid accumulation product per sitting height; SRCLAP-W, standardized relative children's lipid accumulation product per weight.

TABLE 4 | The areas under ROC curves of SBMI, SWHtR, SlnCLAP, SRCLAP-H, SRCLAP-SH, SRCLAP-W, STg/HDL-C, SlnCLAP combining SH, SlnCLAP combining SSH, SlnCLAP combining SW for predicting MS among children.

Variables	AUC	S.E.	P	95% CI of AUC
Girls				
SBMI	0.828	0.032	0.001	0.782–0.868
SWHtR	0.925	0.029	<0.001	0.890–0.951
SlnCLAP	0.946	0.014	<0.001	0.916–0.968
SRCLAP-H	0.950	0.014	<0.001	0.920–0.971
SRCLAP-SH	0.948	0.014	<0.001	0.918–0.970
SRCLAP-W	0.920	0.027	<0.001	0.884–0.947
STg/HDL-C	0.929	0.025	<0.001	0.895–0.955
SlnCLAP combining SH	0.947	0.014	<0.001	0.916–0.969
SlnCLAP combining SSH	0.947	0.014	<0.001	0.916–0.969
SlnCLAP combining SW	0.949	0.013	<0.001	0.918–0.969
Boys				
SBMI	0.916	0.020	<0.001	0.883–0.942
SWHtR	0.916	0.019	<0.001	0.883–0.942
SlnCLAP	0.946	0.020	<0.001	0.917–0.967
SRCLAP-H	0.952	0.020	<0.001	0.925–0.972
SRCLAP-SH	0.952	0.020	<0.001	0.925–0.971
SRCLAP-W	0.929	0.027	<0.001	0.898–0.953
STg/HDL-C	0.931	0.017	<0.001	0.900–0.955
SlnCLAP combining SH	0.946	0.020	<0.001	0.917–0.967
SlnCLAP combining SSH	0.946	0.020	<0.001	0.918–0.967
SlnCLAP combining SW	0.949	0.019	<0.001	0.922–0.969

SBMI, standardized body mass index; SWHtR, standardized waist-height ratio; SlnCLAP, standardized logarithmic children's lipid accumulation product; SRCLAP-H, standardized relative children's lipid accumulation product per height; SRCLAP-SH, standardized relative children's lipid accumulation product per sitting height; SRCLAP-W, standardized relative children's lipid accumulation product per weight; STg/HDL-C, standardized triglycerides-to-HDL-C ratio.

that the ability to regulate glucose was progressively lost with age (39). Moreover, in the present study we found that moderate-to-vigorous physical activity time of less than 60 min was a risk factor for MS in boys, which was consistent with previous studies (40, 41). Styne et al. (42) also showed that at least 20 min of vigorous short bursts of physical activity a day, for 3 to 5 days per week can improve metabolic measures in children and adolescents. Physical activity is helpful in improving the lipid profile by increasing HDL concentration and decreasing both LDL and triglycerides concentrations (43).

The additional findings of this cross-sectional study were that the children with MS demonstrated higher BMI, WHtR, Tg/HDL-C, and CLAP levels compared to children without MS, which was in line with previous studies (11, 13, 14, 21). However, these indexes were limited to showing the accumulation of lipids. BMI cannot show an indication of body fat distribution, and it is not only related to fat mass but also related to fat-free mass (44), WHtR was limited to showing the accumulation of lipids in blood circulation, Tg/HDL-C was only showing the accumulation of lipids in blood circulation. CLAP is a better marker to predicting MS than BMI and WHtR in Chinese children and adolescents; however, it only reflects the state of lipid accumulation, not the density of lipid accumulation. Now our results suggested SRCLAP-H and SRCLAP-SH were significantly associated with MS [in girls, the OR values (95% CI) were 96.13 (11.11–831.97) and 96.13 (11.11–831.97), respectively; in boys, the OR value (95% CI) were 171.75 (33.60–878.00) and 133.18 (27.65–641.39), respectively] and the abilities of RCLAP-H and RCLAP-SH for predicting MS were all higher than those of BMI, WHtR, Tg/HDL-C, -CLAP, and CLAP combining height, sitting height. RCLAP-H reflected the lipid accumulation at per unit height which could reflect different metabolic risks based on children's height; for example, the children with the same CLAP have a greater risk of MS with shorter heights. RCLAP-SH reflected the lipid accumulation at per upper half of body. There have been studies that showed that an upper body or centralized deposition of excess body fat carries an increased risk for obesity-associated

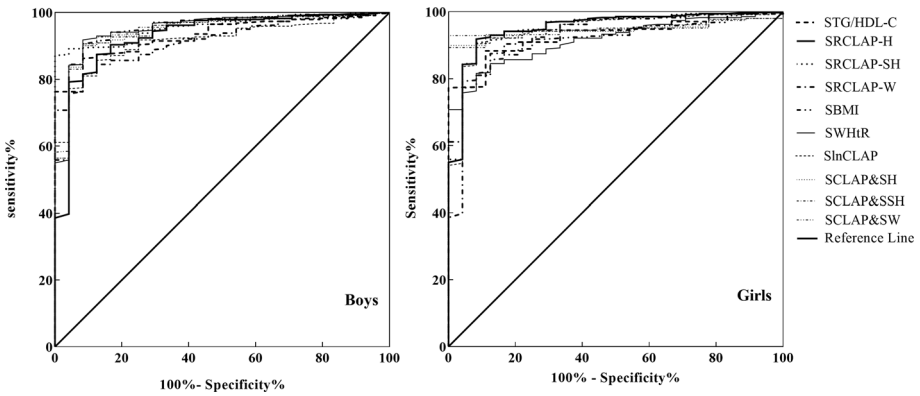

FIGURE 1 | ROC curves of SBMI, SWHtR STg/HDL-C, SlnCLAP SRCLAP-H, SRCLAP-SH, SRCLAP-W, SlnCLAP combining SH, SlnCLAP combining SSH, SlnCLAP combining SW to predict MS among boys and girls. SBMI, standardized body mass index; SWHtR, waist-height ratio; STg/HDL-C, triglycerides-to-HDL-C ratio; SlnCLAP, logarithmic children's lipid accumulation product; SRCLAP-H, relative children's lipid accumulation product per height; SRCLAP-SH, relative children's lipid accumulation product per sitting height; SRCLAP-W, relative children's lipid accumulation product per weight by sex and age using the z-score method.

metabolic complications (45, 46). In our study population, the effect of SRCLAP-W was not obvious, which may be that WC and AST reflect weight to some extent, so the effect of CLAP divided by weight will be weakened. However, the effect of CLAP combining weight for predicting MS was higher than that of SRCLAP-W, which may be that CLAP combining weight reflects the superposition effect of WC and AST.

Excess lipid material will increasingly be deposited in non-adipose tissues (e.g., liver, kidneys, skeletal muscle, heart, blood vessels, and pancreas) where it may adversely modify cellular metabolism and accelerate apoptosis (47, 48). Commonly adopted predictive indicators of abdominal obesity include WC and related indexes such as the waist-to-height and waist-to-hip ratios (49, 50). Ectopic lipid deposition is difficult to quantify directly in children and adolescents, but an increased RCLAP value may indicate that various tissues or organs have become more vulnerable to injury from lipid overaccumulation. The metabolically obese normal-weight (MONW) (51) individuals who having normal body weight but with obesity, are characterized by the presence of a cluster of cardiovascular risk factors. Janssen (52) proposed that those that also fulfill the criteria for the MS should be classified as MONW. Du et al. (53) showed that LAP and visceral adiposity index (VAI) are effective markers for identifying the Chinese adults with MONW phenotype. We speculate that RCLAP-H or RCLAP-SH may be applicable to identifying MONW in children and adolescents.

The present study has several strengths. The RCLAP at per unit body height, sitting height can reflect the density of lipid accumulation in body, the study demonstrated that the RCLAP-H and RCLAP-SH were more effective indicators for predicting MS than BMI, WHtR, Tg/HDL-C, and CLAP in children and adolescents. However, this study also has some limitations. Firstly, it was a cross-sectional study and the causality between RCLAP and MS cannot be inferred. Secondly, we only studied Chinese children and adolescents, thus the generalizability to other ethnic groups is limited. Finally, the sample size in our study is limited, so we cannot provide a representative cut-off value in different ages and gender for the time being. Therefore, the results need to be confirmed by other studies.

The RCLAP was associated with MS and reflect the density of lipid accumulation among children and adolescents. It is an accurate and simple method for predicting the risk of MS in children and adolescents. Furthermore, we reported that the relative children's lipid accumulation product per height (RCLAP-H) and relative children's lipid accumulation product per sitting height (RCLAP-SH) may be more predictive power for MS than BMI, WHtR, Tg/HDL-C, and CLAP.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: This dataset is kept in the School of Public Health, Bengbu Medical College, and can be applied to LF. Requests to access these datasets should be directed to LF, lianguofu@163.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the Bengbu Medical College. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LZ conceptualized and designed the study, analyzed and interpreted the data, drafted the initial manuscript, reviewed and revised the manuscript. ZZ, BW, YY, and LS collected data, analyzed and interpreted the data, and critically reviewed the

manuscript for important intellectual content. HG and LF conceptualized and designed the study, coordinated and supervised data collection, analyzed and interpreted the data, reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported by grants from the National Natural Science Foundation of China (No. 81502823), Outstanding Young Talent Key program of College and University in Anhui Province (No. gxyqZD2017063), 512 Talent Cultivation Plan of Bengbu Medical College (No. by51201204), and Overseas Visiting and Training Project for Outstanding Young Talents in Universities (No. by15200053).

ACKNOWLEDGMENTS

We would like to thank the students and teachers who participated in the current study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 26 May 2021
doi: 10.3389/fped.2021.679646

Identification of Two Novel Compound Heterozygous *EIF2AK3* Mutations Underlying Wolcott–Rallison Syndrome in a Chinese Family

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OPEN ACCESS

Edited by:
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Reviewed by:
Hua Yue, Shanghai Sixth People's Hospital, China
Stefano Zucchini, Sant'Orsola-Malpighi Polyclinic, Italy

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *12 March 2021*
Accepted: *12 April 2021*
Published: *26 May 2021*

Citation:
Zhao N, Yang Y, Li P, Xiong Q, Xiao H and Wu C (2021) Identification of Two Novel Compound Heterozygous EIF2AK3 Mutations Underlying Wolcott–Rallison Syndrome in a Chinese Family. Front. Pediatr. 9:679646. doi: 10.3389/fped.2021.679646

Objective: Wolcott–Rallison syndrome is a rare autosomal recessive inheritance disorder caused by the defectiveness of eukaryotic translation initiation factor 2 alpha kinase 3 (*EIF2AK3*), which encodes the PKR-like endoplasmic reticulum kinase (PERK). Defect in *EIF2AK3* results in a permanent diabetes in early infancy or newborn period, a tendency to develop skeletal fractures and other associated disorders such as severe liver and renal dysfunction, and central hypothyroidism. Two patients with Wolcott–Rallison syndrome-like manifestations in a Chinese family and family members were genetically analyzed to identify if any variations that occurred in *EIF2AK3*, which may cause Wolcott–Rallison syndrome.

Methods: Whole-exome sequencing (WES) was performed to identify genetic variations, and Sanger sequencing was conducted to verify the identified variations in the family members with Wolcott–Rallison syndrome (WRS) clinical manifestations. Several bioinformatics tools were employed to predict the effect of *EIF2AK3* variations on the protein function. The impact on PERK protein was analyzed by sequential analysis and evolution conservation study.

Results: Two novel *EIF2AK3* heterozygous single base variations (c.2818C>T and c.2980G>C) were detected in the proband. PERK has two functional domains: one is regulatory domain (aa 1–576), and the other is catalytic domain (aa 577–1,115). Both variations are missense mutations and locate in catalytic domain of PERK; c.2818C>T resulted in a residue substitution of proline for serine at amino acid site 940 (p.Pro940Ser), and variation c.2980G>C caused an amino acid change at position 994 from glutamic acid to glutamine (p.Glu994Gln). These novel missense variations may affect the physiological functions of PERK protein.

Conclusions: Two novel compound heterozygous *EIF2AK3* variations (c.2818C>T, p.Pro940Ser and c.2980G>C, p.Glu994Gln) were found in a Chinese family. The

identification of the variations and verification of their pathogenicity extended the variation spectrum of *EIF2AK3* variations causing Wolcott–Rallison syndrome and enriched valuable information for precise medical intervention for Wolcott–Rallison syndrome in China.

Keywords: WRS, EIF2AK3, PERK, diabetes, variation

INTRODUCTION

Wolcott–Rallison syndrome (WRS, OMIM: 226980) is caused by an autosomal eukaryotic translation initiation factor 2-a kinase 3 (*EIF2AK3*) deficiency, associated with permanent diabetes and skeletal dysplasia in newborn period or early infancy, hepatic dysfunction, and growth retardation, which was first reported in 1972 by Wolcott and Rallison (1–3). As an autosomal recessive inheritance disorder, WRS is more common in consanguineous families. In 2000, *EIF2AK3* was confirmed to be the disease-causing gene of Wolcott–Rallison syndrome (2). So far, almost 100 *EIF2AK3* variations causing WRS have been reported in the Human Gene Mutation Database (HGMD, http://www.hgmd.org/;2019.9).

The *EIF2AK3* gene (NC_000002, NM_004836.7) is located in chromosome 2p11.2, containing 17 exons, and encodes PKR-like endoplasmic reticulum kinase (PERK) (2). PERK is a transmembrane enzyme, which is highly expressed in both pancreatic beta cells and bone tissue. It is essential for normal fetal and early beta cell proliferation, differentiation, proinsulin processing, and stimulation of bone development (4). PERK acts as a major physiological effector of the unfolded protein response (UPR) following endoplasmic reticulum (ER) stress (5, 6). Once activated by the accumulation of misfolded proteins during ER stress, PERK phosphorylates the alpha subunit of the eukaryotic initiation factor-2 (EIF2A), thereby reducing the synthesis of misfolded proteins and increasing the expression of activating transcription factor 4 (ATF4), which regulates autophagy, amino acid metabolism, oxidative stress, and apoptosis. Loss-of-function mutations in the *EIF2AK3* gene decrease the ability of the ER to cope with stress, which results in loss of functional coordination among PERK-dependent ER chaperones responsible for controlling protein synthesis and proinsulin aggregation. These effects lead to β-cell defects and cell apoptosis, which results in permanent neonatal diabetes and epiphyseal dysplasia. Long-term regular insulin therapy has been demonstrated to improve the survival rates of WRS patients. Organ transplantation is a treatment for WRS; so far, three cases with organ transplantation have been reported (7–9), and these three patients were fully physically and socially rehabilitated.

In our study, we found compound heterozygous variations in *EIF2AK3*, which cause WRS in two children from non-consanguineous parents.

METHODS

Patients' Information

This study was approved by the Ethics Committees of Shanxi University, and written informed consent was obtained from all the members of the family. The patient has been subjected to clinical and physical examinations, and all the medical records were reviewed and evaluated. The proband was diagnosed with diabetes at The Affiliated Hospital of Xuzhou Medical University (Xuzhou, China) in 2009. In 2017, the proband was diagnosed with WRS in The First Hospital of Peking University (Beijing, China).

Molecular Genetic Studies

Whole-exome sequencing and validation by PCR were performed as described in our previous studies (10). The blood samples used for whole-exome sequencing were obtained from patients II-2 and II-3; the blood samples used for PCR and Sanger sequencing were obtained from family members I-1, I-2, and II-2. Variants were functionally annotated and filtered using our cloud-based rare disease NGS analysis platform (https://www.gene.ac/) in which analyses were performed by comparison with public databases [dbSNP, OMIM, ESP, Clivar, 1000 Genomes (11), and ExAc (12)] and HGMD Professional database.

The primer pairs for detecting the mutations by Sanger sequencing were as follows: Fw, 5′-AGTACTTGTCTGGCAC-3′; Rv, 5′-GGAACACTACTGCCAGTT-3′.

Bioinformatics Analysis

Mutation Taster, PolyPhen-2, PROVEN, and SIFT were employed for the pathogenicity prediction of the variations in *EIF2AK3* gene. Evolutionary conservation of the altered amino acid residue was compared across different species.

RESULTS

Clinical Manifestations

The proband (II-3) was first diagnosed as diabetes when she was 1-year old. Later on, she developed emesis and hypersomnia at 4 years old; clinical examination showed that the blood C-peptide levels was 0.01 nmol/L, which was markedly lower than the normal range (0.40 ± 0.20 nmol/L). The patient's blood glucose level was 2.07 mmol/L (normal range, 3.9–6.1 mmol/L). The urinalysis showed that the patient had glucosuria, ketonuria, and proteinuria. After receiving insulin rejection to control the blood glucose and symptomatic treatment, the patient was discharge 1 day later. At the age of 8, she was admitted with liver dysfunction and pneumonia, the clinical examination revealed a blood glucose of 13.7 mmol/L, alanine aminotransferase (ALT) of 852 U/L (normal range, 7–40 U/L), aspartate aminotransferase (AST) of 750 U/L (normal range, 5–35 U/L), albumin of 31.0 g/L (normal range, 35–55 g/L), prothrombin time of 20 s (normal range, 9–13 s), lactic dehydrogenase (LDH) of 2,150 U/L (normal range, 120–250 U/L). The X-ray showed that the patient had

lobar pneumonia in the upper right lobe. After treatment with diammonium glycyrrhizinate, mezlocillin, and insulin, the patient was discharged 16 days later. The patient had no apparent skeletal deformities. The sister of the proband (II-2) was first diagnosed with diabetes and diabetic ketoacidosis at 4 years old and was then on insulin therapy. When the sister was 8 years old, she developed infection of the upper respiratory tract without apparent skeletal deformities. The brother (II-1) of the proband died of diabetes when he was 17 years old. The parents of the proband have no symptom of diabetes.

Identification of *EIF2AK3* Compound Heterozygous Variations

The pedigree of the family is shown in **Figure 1A**. By whole-exome sequencing, two potential mutations (c.2818C>T and c.2980G>C) were detected in *EIF2AK3* gene from the proband, which was reported to associate with WRS. Subsequently, these two compound heterozygous variations were verified by Sanger sequencing among her family.

The novel heterozygous single base substitution c.2818C>T in exon 13 of *EIF2AK3* gene was detected in the mother (I-1) of the proband but not in her father, which is predicted to cause an amino acid change at position 940 (**Figure 1B**). The other novel heterozygous single base substitution c.2980G>C in exon 13 was detected in the father (I-2) of the proband but not in her mother, which is predicted to cause an amino acid change at position 994 (**Figure 1B**). Both variations were predicted to be causative factors of the disease by Mutation Taster, PolyPhen-2, PROVEAN, and SIFT.

The results from whole-exome sequencing and Sanger sequencing of her sister (II-2) showed that these two novel mutations from the proband also existed in her sister (**Figure 1B**).

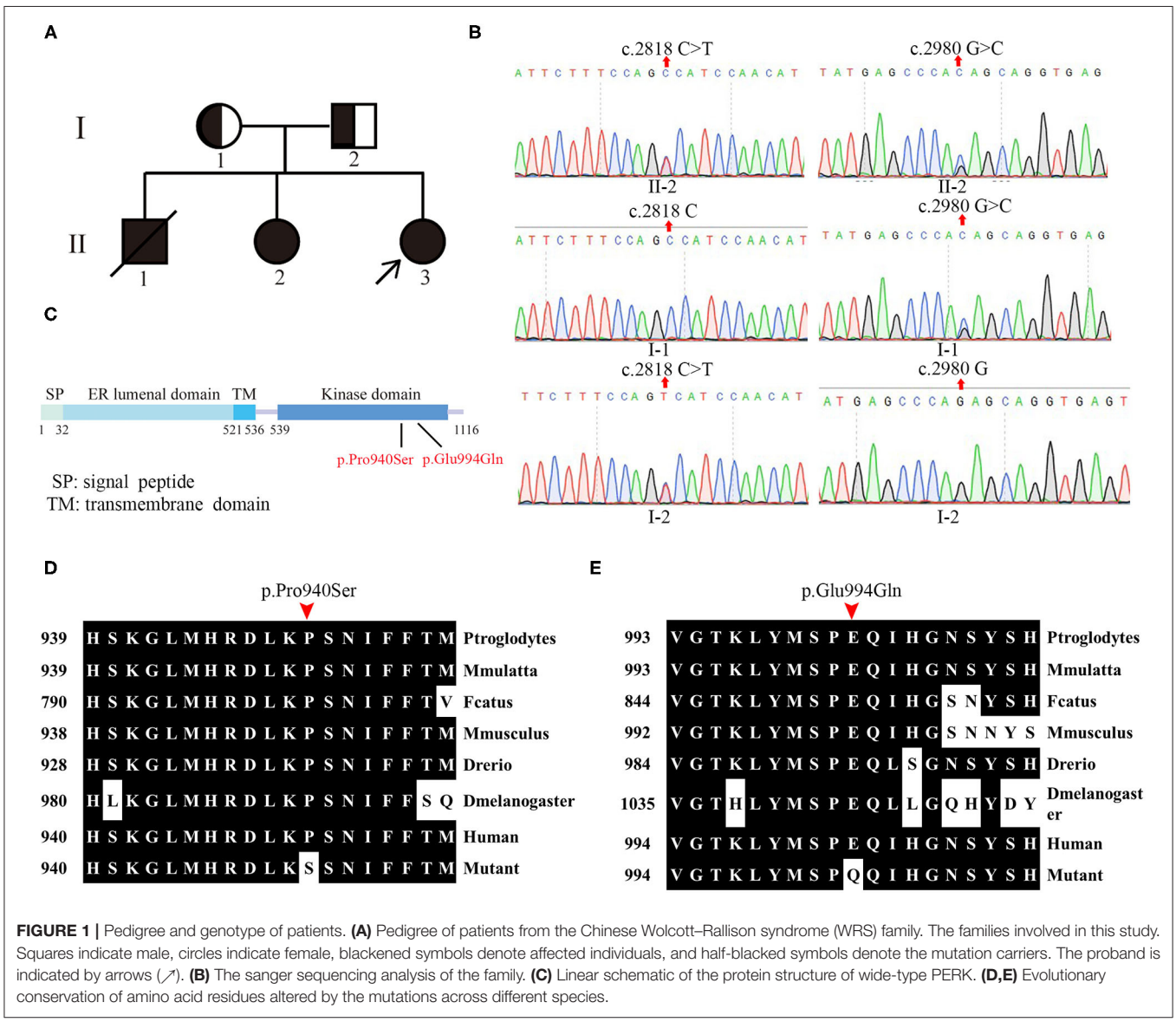

FIGURE 1 | Pedigree and genotype of patients. **(A)** Pedigree of patients from the Chinese Wolcott–Rallison syndrome (WRS) family. The families involved in this study. Squares indicate male, circles indicate female, blackened symbols denote affected individuals, and half-blacked symbols denote the mutation carriers. The proband is indicated by arrows (↗). **(B)** The sanger sequencing analysis of the family. **(C)** Linear schematic of the protein structure of wide-type PERK. **(D,E)** Evolutionary conservation of amino acid residues altered by the mutations across different species.

TABLE 1 | The variations and clinical information.

Mutation type	Skeletal dysplasia	Liver dysfunction	Kidney dysfunction	Liver and Kidney dysfunction	Non-liver or kidney dysfunction
Missense/Nonsense	27 (52)	23 (52)	8 (52)	7 (52)	28 (52)
Splicing	2 (9)	4 (9)	3 (9)	1 (9)	3 (9)
Small deletions	16 (24)	14 (24)	8 (24)	7 (24)	9 (24)
Small insertion	4 (8)	3 (8)	3 (9)	1 (8)	3 (8)
Small indel	1 (1)	0 (1)	1 (1)	0 (1)	0 (1)
Gross deletion	0 (2)	0 (2)	0 (2)	0 (2)	2 (2)
Total	50 (96)	44 (96)	23 (96)	16 (96)	45 (96)

**The numbers in brackets represent the total number of the variations.*

Molecular Analysis

These two novel mutations were both in the kinase domain of the PERK protein, which indicates that these mutations likely lead to WRS (**Figure 1C**). Evolution conservation analysis of amino acid showed that the impaired amino acid residues Pro940 and Glu994 in PERK protein were highly evolutionary conserved among different species (**Figures 1D**,**E**). Bioinformatic and biochemical assessment of the effect of the variations on the functions of PERK shows that both missense variations are located at the catalytic domain, a residue substation of Proline/P with Serine/S at the catalytic domain conserved region of PERK, which has the potential for phosphorylation, therefore affecting its functions. Glutamine replacing glutamic acid introduces charge change from negative and acidic residue to neutral residue, which may also bring about the functional change of PERK. In conclusion, the two novel compound heterozygous missense variations were identified by WES and verified further by Sanger sequencing in the *EIF2AK3* gene causing WRS in members of the family.

DISCUSSION

We identified two novel compound heterozygous variations in the *EIF2AK3* gene in a family, which included three patients with Wolcott–Rallison syndrome (WRS). So far, there are four families with WRS reported without homozygous variations in China (13). It seems that WRS has a low morbidity in China. Until April 2020, 98 variations were reported as the disease causative factors of WRS in HGMD, suggesting that the incidence of WRS is very low worldwide.

From previous reports, 96 variations in *EIF2AK3* involving 176 patients were identified for analysis in this study. Among the patients, there were 58 female patients, 68 male patients, and 50 patients without gender identification. Among the patients who indicated their gender, male patients accounted for 53.9%, and female patients accounted for 46.1%. It seems that there is no significant difference between genders on the morbidity of permanent neonatal diabetes mellitus (PNDM) caused by the variations in *EIF2AK3*. Functional analysis was performed in only nine variations. In addition to diabetes, the major symptoms of WRS are skeletal dysplasia and living and kidney dysfunction.

From **Table 1,** we find that among the clinical symptoms, skeletal dysfunction is the most common symptom in patients with WRS besides diabetes. The incidence of skeletal dysfunction is nearly 52.08% in this analysis. Base pair substitution mutation is the most common mutation type among all the variations. The percentage of small deletion that suffers both liver and kidney damage is the largest among all the mutation types. The patients with small deletion always suffer more symptoms. For example, the patients with variations c.1475-1476del (8), c.1567-1570del (14), c.1639-1642del (7), c.2791-2794del (4) suffered both living and kidney dysfunction.

However, different patients who had the same mutation may also show different symptoms; for example, the mutation c.560G>A (p.Trp163*) was found in a family with two patients; one patient had kidney dysfunction (acute), but the other had none (4). One of the three male patients with mutation c.1290G>A (p.Trp430*) from one family showed some epiphyseal dysplasia, but the other two had no obvious epiphyseal dysplasia (14). All these results indicate that there is no specific relationship between the mutation and clinical symptoms.

In this study, we recruited a family including three patients with WRS. WES and Sanger sequencing were performed to identify the mutations that may cause the disease in this family. From the liner structure (5), both of the affected amino acids are located in the kinase domain, indicating that the mutations caused defect on the kinase activity of the PERK. Evolutionary conservation analysis of amino acid residues showed that the amino acid residue Pro940 and Glu994 influenced by the novel mutations are most highly evolutionary conserved among PERK protein from different species, indicating that these mutations are likely pathological.

Because the variable of the clinical phenotype and the difference in gene penetrance except PNDM of the patients with WRS, the diagnosis of WRS was difficult. As the most common gene causing PNDM are *ABCC8* gene and *KCNJ11* gene (9), the analysis of *EIF2AK3* gene is always ignored in molecular diagnosis. It seems that WRS should be considered in the molecular diagnose of PNDM even when the incidence of WRS is very low.

These two mutations in this study were first reported, and it will be helpful for the diagnosis of WRS due to *EIF2AK3* mutations.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI [accession: PRJNA719691].

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committees of Shanxi University. Written informed consent to participate in this study was provided by the participants' legal guardian. Written informed consent was obtained from the minor(s)' legal guardian for the publication of any potentially identifiable images or data included in this article.

CONSENT FOR PUBLICATION

All patients participating in the study have given written informed consent.

AUTHOR CONTRIBUTIONS

YY performed the clinical investigations. NZ participated in the data analysis and drafted the manuscript. NZ carried out the molecular genetic studies. PL and QX helped with the study coordination and proofread the manuscript. CW and HX sponsored, conceived, and designed the study. All authors read and approved the final manuscript.

FUNDING

This study was supported by Shanxi 1331 Project Collaborative Innovation Centre, 1331 CIC (206541001).

ACKNOWLEDGMENTS

We thank the patients, their family, and control individuals for their support and participation.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 04 June 2021
doi: 10.3389/fped.2021.688287

Psychological Outcomes and Predictors of Weight Loss in Adolescents With Severe Obesity Following a Reversible Endoscopic Bariatric Procedure

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *30 March 2021*
Accepted: *10 May 2021*
Published: *04 June 2021*

Citation:
Klemenčič S, Bujišić A, Hribernik NŠ, Battelino T, Homan M, Orel R and Kotnik P (2021) Psychological Outcomes and Predictors of Weight Loss in Adolescents With Severe Obesity Following a Reversible Endoscopic Bariatric Procedure. Front. Pediatr. 9:688287. doi: 10.3389/fped.2021.688287

Introduction: Adolescent and children obesity is a growing concern worldwide. Bariatric surgery is used as an effective treatment for adolescents with obesity and provides physical and mental health benefits. Application of alternative, minimally invasive, safe, and reversible endoscopic procedures, such as the Duodenojejunal bypass liner (DJBL), has been recently suggested as an effective treatment for adolescents with obesity. We explored specific psychological outcomes of adolescents with obesity during a year of follow-up after undergoing a reversible endoscopic bariatric procedure, and a year after removal. We were also interested in identifying psychological factors that could predict successful weight loss after the procedure.

Methods: Nineteen adolescent with severe obesity undergoing DJBL procedure were psychologically assessed in an open-label, prospective clinical trial (NTC0218393), at the implantation of device and at the removal of device after 12 months. Control group of 26 adolescents with severe obesity were recruited from the same outpatient clinic undergoing only conservative treatment. In addition, adolescents from the intervention group were followed for 12 months after the removal of the device. The Youth Self Report (YSR) was used to assess adolescents' emotional and behavioural problems; The Multidimensional Body-Self Relations Questionnaire (MBSRQ) to assess body image and The Eating Disorder Examination Questionnaire (EDE-Q) to assess attitudes and behaviours related to eating disorder.

Results: Significant improvements in somatic complain ($F = 12.478$, $p = 0.001$), emotional and behavioural problems ($F = 7.169$, $p = 0.011$) and food restraining ($F = 9.605$, $p = 0.004$) were found in the intervention group at device removal compared to the control group. Moreover, at the time of device removal compared to baseline, improvements in several psychological outcomes were found ($F = 32.178$ $p = 0.000$ for emotional and behavioural problems). Adolescents also became more satisfied with their appearance ($F = 6.789$, $p = 0.019$). Majority of observed changes remained stable

at the next follow up a year after the device removal. Significant predictors of successful weight loss at device removal were fewer overeating episodes ($B = 0.147$, $p = 0.022$) and lower body satisfaction ($B = 0.932$, $p = 0.013$).

Discussion: Following a reversible bariatric procedure, improvements of psychological (emotional and behavioural) factors were found in adolescents with severe obesity. Psychological predictors of successful weight loss were identified, showing the greatest importance of eating behaviour and body satisfaction in successful weight loss.

Keywords: obesity, bariatric procedure, adolescents, psychological predictors, psychosocial functioning

INTRODUCTION

Despite the fact that obesity prevalence in Slovenian adolescents stabilised recently (1), obesity remains a major worldwide health problem (2). In adolescents and children obesity is related to numerous health complications such as diabetes type 2, dyslipidemia, non-alcoholic fatty liver disease, kidney diseases, hormonal changes, and increased risk of premature death (3–9). Youth obesity has also been linked to multiple psychological comorbidities, such as poorer quality of life, emotional and behaviour problems (ADHD, ADD, etc.), social stigma, and self-esteem issues from a very young age and later in adolescence (3, 10–12). More depressive and anxiety symptoms (12, 13) and somatoform disorders were observed in children that were overweight compared to their peers (10, 14). Adolescents with obesity often not only have negative body image and poor self-esteem, but also lower academic and social self-image compared to their peers, which is even more evident in girls (11, 15). Self-esteem issues were related to weight-based teasing by peers and family members, which is frequently reported by children that are overweight or obese (16, 17). Furthermore, adolescents with obesity and lower self-esteem are more likely to engage in high risk behaviour such us smoking and consuming alcohol (11).

There is increasing evidence for bariatric surgery as an effective treatment for severe obesity in adults and also for adolescents (18, 19). Adolescents undergone gastric bypass showed similar weight loss, mortality rate, remission of type 2 diabetes, and hypertension as adults after 5-year follow up (19). However, most of the surgical bariatric procedures are irreversible and are related to acute and chronic complication (20). Concerning, the rate of abdominal reoperations was even higher among adolescents than among adults (19). Therefore, recent trends suggest application of less invasive, reversible endoscopic procedures, such as Duodenojejunal bypass liner (DJBL). DJBL is an endoscopically placed intestinal liner that can be removed at any time leaving original gastrointestinal anatomy intact. It limits the absorption of nutrients and leads to weight reduction and metabolic improvements (21, 22). DJBL treatment was found to be safe and effective, and therefore more appropriate for adolescents with severe obesity (23).

Previous research has focused mainly on physical and mental health benefits of more invasive bariatric procedures (24). From mental health perspective bariatric surgeries were found to be associated with sustained improvement in quality of life for adolescents with severe obesity, specifically in weight related quality of life and physical health related quality of life (25–27). Some research studies have also found that after surgical procedure adolescents showed significantly fewer symptoms of anxiety and depression and significantly improved self-concept compared to baseline (27, 28). Similar results were found among adults, in addition to improvement in body image, social functioning, and also improved eating behaviour (29–31). However, some studies showed that more than half of adolescents with psychopathology prior to surgery reported sustained elevated symptomatology 2 years after (32–34), moreover treatment for mental and behaviour disorders did not differ after 5 years of surgery (35). There is still a lack of evidence regarding mental health benefits of minimally invasive and reversible procedures such as reversible endoscopic procedures.

There is also a growing interest in identifying possible predictors of weight loss in obesity treatment. The existing studies have identified a variety of pre- and post-surgical predictors of weight loss, which remain inconclusive (36). Eating behaviour, specifically emotional and external eating have been found as negative predictors for weight loss 6 months after bariatric surgery in adults (37). Binge eating status has also been reported to predict poorer weight loss in some studies in adults (38, 39), however, others have reported that binge eating, assessed before surgery does not predict weight loss outcome (35, 40–43). In a recent study, neither anxiety nor depression levels significantly predicted successful weight loss, although patients achieving successful weight loss were characterised by lower mean scores of anxiety and depressive symptoms (44). Conversely, in other study adults with psychiatric disorders, including depressive disorder, had poorer outcomes in terms of weight loss 30 months after the bariatric surgery (43). Advancing age, high BMI, and cognitive impairments before surgery were also found as significant negative predictors for total weight loss in adults (37, 45).

Existing data about psychological benefits of non-surgically placed reversible devices for weight loss in adolescents are limited. Therefore, we aimed to evaluate the specific psychological outcomes of adolescents with obesity during a year of follow-up after undergoing minimally invasive reversible endoscopic bariatric procedure, and a year after removal. Especially we examined emotional and behavioural problems in adolescents, self-image and self-esteem, disordered eating attitudes, and behaviours based on the results of previous studies

that adolescent with severe obesity have variety of psychological issues (10–14). We hypothesized that psychological burdens and negative eating patterns will decrease after bariatric procedure and remain relatively stable after bariatric procedure, as previously reported (28, 29, 37). The inclusion of control group of adolescents with obesity provided a critical comparison group.

Moreover, we aimed to identify the multiple psychological predictors of successful weight loss after the procedure. We hypothesised that adolescents with fewer emotional and behavioural problems, better self-image, and body satisfaction, would be more successful in weight loss due to more energy and coping strategies to follow instructions of bariatric team. Additionally, we predicted that adolescents fewer disturber eating attitudes and behaviours will achieve greater weight loss, according to results of other studies that examined effects of more invasive bariatric procedures (37–39, 46–48).

MATERIALS AND METHODS

Participants and Procedures

Nine-teen adolescents with severe obesity who were undergoing reversible endoscopic bariatric procedure were followed in the prospective, single-arm, open-label study at University Medical Centre Ljubljana. The procedure with Duodenojejunal Bypass Liner Placement (EndoBarrier®, GI Dynamics, Lexington, MA, USA) was previously reported (49). The procedure was offered to all eligible patients between July 2014 and April 2017 that visited outpatient clinic and met the inclusion criteria: BMI > 99 percentile for age and gender or > 35 kg/m^2, previously non-successful conservative treatment of obesity, age over 15 years, completed pubertal development, and at least one medical co-morbidity. Exclusion criteria were pregnancy (or pregnancy intention) and the following medical conditions: congenital gastrointestinal (GI) anomalies, previous GI surgery, inflammatory bowel disease, history of acute or chronic pancreatitis, coagulopathy, gallstones, severe GI reflux disease, active Helicobacter pylori infection, regular antithrombotic therapy, autoimmune connective tissue disorders. Prior to the procedure, all participants had complete psychological examination provided by the clinical psychologist to exclude patients with severe mental disorders (e.g., severe mood disorder, like major depression or psychotic disorders) or inability to follow instructions (e.g., intellectual disability).

Psychological evaluation was performed by a clinical psychologist for all participants with successful implantation at the time of implantation of the device T0 ($n = 19$) and at the removal of device 12 months after implantation (T1, $n = 19$).

Participants in the control group were recruited from the same outpatient clinic undergoing conservative treatment of obesity, after the intervention group was closed. Inclusion criteria were same as for the intervention group. Altogether 26 adolescents were followed up for up to 12 months. They received life style intervention by the same multidisciplinary team and were directed to the clinical psychologist to complete questionnaires (T0). Medical information for control group was obtained from medical records (T0). After 12 months (T1) participants from control group were asked to visit the clinic, where psychological examination and medical measures were taken again.

In addition, adolescents that underwent bariatric procedure were followed for 12 months after the removal of device—T2. Height, weight and general health status were also recorded for all participants at T0, T1, and T2 as previously described (49).

Ethical Approval

The study was registered at www.clinicaltrials.gov (NCT02183935) and approved by The Republic of Slovenia National Medical Ethics Committee (#39/03/14) and by the Competent Authority. All participants and their parents signed informed assents/consent.

Measures

Body Mass Index Standard Deviation Scores

Weight status was determined by Body Mass Index, calculated on a basis of measured height and weight data (kg/m^2). Height and weight measurements were taken by a trained nurse using validated stadiometers and electronic digital scales. Both were rounded to the 10th decimal place. Body Mass Index Standard Deviation Scores (BMI SDS) were calculated according to reference curves (50).

The Youth Self Report—YSR (51)

The Youth Self Report—YSR (51) is a widely used youth self-report measure for the assessment of emotional and behavioural problems among youth ages 11–18 in the past 6 months. It is divided in two parts (1) competencies and (2) problems. It contains 119 items: 14 socially desirable items and 105 problem items. All of the items are short sentences worded in first person, to be answered on a 3-point scale: 0 = not true, 1 = somewhat or sometimes true, 2 = very true or often true. The YSR consists of the following eight scales: Withdrawn/Depressed, Anxious/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Delinquent Behaviour, and Aggressive Behaviour. Withdrawn/Depressed, Somatic complaints and Anxious/Depressed together comprise a broad Internalising dimension, whereas Delinquent and Aggressive behaviours together constitute the Externalising dimension. Total Problems score can also be derived. Higher scores represent higher levels of problems. Good validity and test–retest reliability have been established (52).

The Multidimensional Body-Self Relations Questionnaire—MBSRQ-AS (53)

The Multidimensional Body-Self Relations Questionnaire—MBSRQ-AS (53) is a self-report inventory for evaluating attitudes related to body image. It is intended for use with adults and adolescents (15 years or older). It consists of 34 items that make up five subscales. Items are presented in a 5-point Likert format that range from 1 (very dissatisfied) to 5 (very satisfied). The subscales are Appearance Evaluation (7-item scale, high scorers indicating attractiveness/satisfaction with appearance, low scorers indicating unattractiveness/dissatisfaction with appearance), Appearance Orientation (12-item scale, higher

TABLE 1 | Sample characteristics for intervention and control group at baseline (T0), after 12 months (T1), and 12 months after the device removal (T2).

	EB group			Control group	
	T0	**T1**	**T2**	**T0**	**T1**
n	19	19	12	26	16
Age, ***M*** (*SD*)	**17.23** (1.24)			**16.09** (1.32)	
Gender (female)	12	12	6	13	11
BMI, ***M*** (*SD*)	**3.71** (0.31)	**3.21** (0.42)	**3.42** (0.37)	**3.35** (0.48)	**3.37** (0.64)

scorers indicating more investment in one's appearance), Overweight Preoccupation (4-item scale, higher scorers indicating more anxiety and vigilance related to weight), Self-classified Weight (2-items reflecting subject's perception of own weight from very underweight to very overweight), and the Body Satisfaction scale (9-item scale assesses satisfaction/dissatisfaction with specific areas of the body). All subscales possess acceptable internal consistency and stability, internal consistencies were based on normative samples and were in range from 0.73 to 0.89 for females and from 0.70 to 0.88 for males (53). Test–retest reliability coefficients were obtained from samples of college students ages 18 years or over ranging from 0.74 to 0.91 for females and from 0.79 to 0.89 for males (53). Body Satisfaction scale has been recognised as acceptable assessment of body image (54), moreover clean factor structure and adequate convergent validity of MBSRQ-AS was confirmed in several studies (55–57).

The Eating Disorder Examination Questionnaire—EDE-Q (58)

The Eating Disorder Examination Questionnaire—EDE-Q (58) is a 28-item self-report questionnaire. It is used to assess disordered eating attitudes and behaviours over previous 28 days. This measure provides a Global score and four subscale scores: Restraint, Eating Concern, Shape Concern, and Weight Concern. Responses are on a 7-point scale, with higher scores reflecting greater eating-related pathology. Frequencies of disordered eating behaviours including binge eating, overeating episodes, and various compensatory behaviours are also assessed. The instrument has generally received support of its reliability and validity. Subscales has acceptable internal consistency (alphas ranging from 0.70 to 0.93) (59, 60) and test–retest reliability (ranging from 0.66 to 0.94) (60). There is inconclusiveness about EDE-Q factor structure (59, 61–63).

Data Analysis

First, the analysis of covariance (ANCOVA) was used to determine the differences between two (treatment and control) groups (independent variable) for multiple independent variables, while still controlling for the starting value differences of the groups (64). The significance level was set for the two-tail hypothesis testing at $\alpha = 0.05$. The analysis of variance (ANOVA) was used to determine significance of the changes over time in intervention group. Mann–Whitney test was used for assessing the between-group differences when comparing responding vs. non-responding participants.

Next, the multivariate linear regression was used, where the dependent value was BMI Change from 12 months (T1) and starting point (T0) and the independent values were psychological variables. Before the analysis, all independent variables were centred. The creation of the linear regression model was done in accordance with the backward stepwise procedure, where we started with the model with all the main effect terms and all the combinations between pair variables as the interaction terms. Then the procedure of the removal of the terms was conducted, where the terms of a higher order with the highest statistical significance (p-values) were removed from the model and the model was re-evaluated with the calculation of Akaike Information Criterion (AIC). This procedure of backward stepwise removal of the terms continued if the AIC metric was decreasing, and the resulting model was one with the lowest AIC as the stepwise procedure finished. The logistic regression with two-tailed hypothesis testing and the significance level $\alpha = 0.05$ were used in the analysis.

RESULTS

Sample characteristics (age, gender and BMI SDS) of intervention and control group are presented in **Table 1** at baseline (T0), after 12 months (T1), and 12 months after the device removal (T2). Descriptive statistics of self-reported psychological outcomes are presented in **Table 2**. In the intervention group all participants participated at T1, while in control group 16 participants responded (drop-out rate: 38%). There were no significant differences in age, BMI, and all measured psychological characteristics between participants who responded and the one who did not in control group. Twelve participants from intervention group responded at T2 (dropout rate: 29%). Participant who did not responded did not differ from the ones who did according to age, BMI, and most of the measured psychological variables, except for the higher scores in Thought Problems at T0 ($MR = 14.50$ vs. 7.38, Mann–Whitney $U = 10.500$, and $p = 0.005$) and higher scores in Restraining at T1 ($MR = 13.36$ vs. 6.73, Mann-Whitney $U = 8.000$, and $p = 0.004$).

Improvements of Psychological Variables in Time

According to ANCOVA (**Table 2**), there were significant improvements in somatic complains (YSR), total emotional and behavioural problems (YSR), and food restraining (EDE-Q) in the intervention group at device removal compared to the control group (T0–T1). Those improvements were observed regardless

TABLE 2 | Measure outcomes at baseline (T0), after 12 months (T1), and 12 months after the device removal (T2); and between group and within group differences for psychological variables.

	EB group			Control group		ANCOVA (EB group vs. control group)				ANOVA (EB group)			
	T0 ($n = 19$)	T1 ($n = 19$)	T2 ($n = 12$)	T0 ($n = 26$)	T1 ($n = 16$)	Between group differences (T0–T1)*		Baseline measurements (T0)**		T0 vs. T1		T0 vs. T2	
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
YSR													
Anxious/depressed	**7.37** (6.16)	**5.26** (6.40)	**3.90** (4.25)	**5.25** (4.83)	**4.50** (3.20)	1.834	0.185	56.744	**0.000**	8.247	**0.010**	10.557	**0.010**
Withdrawn/depressed	**3.74** (2.85)	**2.79** (2.59)	**2.90** (2.56)	**5.17** (3.80)	**4.13** (2.28)	0.567	0.457	22.868	**0.000**	4.726	**0.043**	0.112	0.745
Somatic complaints	**3.89** (4.04)	**1.58** (1.68)	**1.80** (2.62)	**2.46** (2.40)	**3.69** (3.26)	12.478	**0.001**	10.923	**0.002**	7.703	**0.012**	2.609	0.141
Social problems	**4.63** (2.94)	**2.81** (2.95)	**2.00** (1.87)	**3.17** (2.60)	**3.50** (2.58)	2.472	0.127	7.470	**0.011**	9.121	**0.009**	14.253	**0.007**
Thought problems	**3.42** (3.73)	**1.95** (1.75)	**2.30** (2.91)	**2.04** (1.97)	**2.50** (2.03)	1.743	0.196	3.074	**0.089**	3.111	0.095	1.000	0.343
Attention problems	**6.37** (5.16)	**5.63** (4.72)	**4.60** (4.27)	**5.71** (3.06)	**5.13** (2.31)	0.004	0.950	65.060	**0.000**	2.025	0.172	5.000	**0.052**
Rule—breaking behaviour	**4.47** (4.16)	**3.79** (2.88)	**3.90** (2.85)	**4.25** (2.47)	**4.13** (2.92)	1.057	0.312	41.859	**0.000**	1.454	0.243	3.857	0.081
Aggressive behaviour	**7.84** (3.70)	**6.84** (3.61)	**5.70** (4.03)	**6.58** (3.88)	**5.50** (2.76)	0.015	0.903	34.373	**0.000**	2.631	0.122	5.597	**0.042**
Internalising problems	**15.00** (10.98)	**11.68** (12.23)	**8.60** (7.29)	**12.88** (9.94)	**14,.88** (12.95)	2.265	0.142	14.926	**0.001**	4.362	**0.051**	5.862	0.039
Externalising problems	**12.68** (7.17)	**12.89** (9.04)	**9.80** (6.37)	**10.83** (5.63)	**12.25** (11.75)	0.075	0.787	4.123	**0.051**	0.011	0.916	13.071	**0.006**
Total problems	**49.68** (25.35)	**37.37** (22.15)	**32.30** (17.99)	**40.33** (19.50)	**38.69** (16.45)	7.196	**0.011**	80.304	**0.000**	32.178	**0.000**	9.551	**0.013**
MBSRQ													
Appearance evaluation	**2.24** (0.82)	**2.76** (0.70)	**2.72** (0.67)	**2.73** (0.79)	**2.71** (0.91)	0.595	0.447	7.956	**0.009**	6.789	**0.019**	3.291	0.113
Appearance orientation	**3.24** (0.60)	**3.34** (0.52)	**3.16** (0.53)	**3.24** (0.70)	**3.18** (0.62)	0.077	0.783	14.164	**0.001**	0.231	0.637	1.482	0.263
Overweight preoccupation	**2.96** (0.69)	**3.13** (0.77)	**3.16** (0.81)	**2.78** (0.89)	**2.53** (0.46)	3.741	0.063	2.679	0.113	0.025	0.875	0.079	0.787
Self-classified weight	**4.74** (0.36)	**4.58** (0.61)	**4.55** (0.44)	**4.40** (0.57)	**4.50** (0.55)	0.204	0.655	9.899	**0.004**	1.000	0.332	2.032	0.197
Body satisfaction	**2.70** (0.50)	**2.87** (0.67)	**2.74** (0.77)	**3.04** (0.58)	**3.06** (0.72)	0.031	0.862	9.238	**0.005**	1.914	0.185	1.116	0.326
EDE-Q													
Restraint	**1.59** (0.94)	**2.16** (1.09)	**2.20** (1.57)	**1.75** (1.02)	**1.12** (0.87)	9.605	**0.004**	0.012	0.912	3.028	0.101	0.483	0.510
Eating concern	**1.18** (1.20)	**0.77** (0.73)	**1.16** (0.99)	**1.01** (0.96)	**0.83** (1.05)	0.081	0.778	2.424	0.130	1.705	0.210	1.371	0.280
Weight concern	**3.22** (1.22)	**2.98** (1.04)	**3.10** (1.36)	**2.70** (1.31)	**2.35** (1.23)	3.053	0.091	36.769	**0.000**	0.746	0.400	1.465	0.265
Shape concern	**3.25** (1.45)	**2.91** (1.36)	**2.97** (1.45)	**2.76** (1.48)	**2.33** (1.44)	1.051	0.314	32.173	**0.000**	2.928	0.106	3.124	0.120
Overeating episodes	**3.59** (6.92)	**1.11** (1.68)	**1.22** (1.20)	**2.83** (4.68)	**2.14** (2.04)	1.497	0.235	0.001	0.975	1.967	0.180	0.401	0.550

*EB Group, intervention group with device implantation; YSR, The Youth Self Report; MBSRQ, The Multidimensional Body-Self Relations Questionnaire; EDE-Q, The Eating Disorder Examination Questionnaire; Statically significant p-values appear in bold text. *Differences between experimental and control group after 12 months (T0-T1). **Differences between experimental and control at the baseline (T0).*

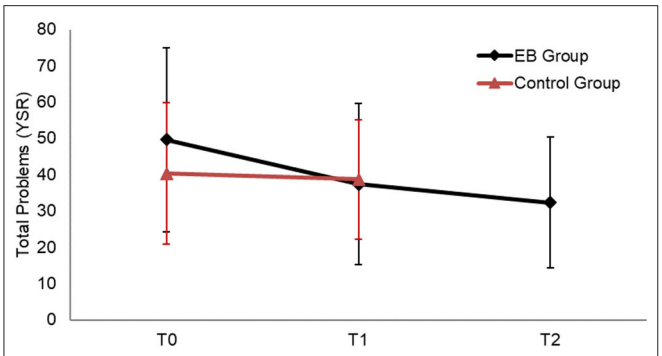

FIGURE 1 | Total emotional and behavioural problems at baseline (T0), after 12 months (T1), and after 24 months (T2) for experimental and control group.

of statistically significant differences between intervention and control group at baseline measurements.

In the intervention group, ANOVA showed statistically significant improvements in several psychological outcomes at the time of device removal compared to baseline (T0 vs. T1) namely Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social problems, Internalising Problems, Total emotional and behavioural problems (YSR). Moreover, adolescents became more satisfied with their appearance (MBSRQ). No improvements were found in variables referring to behaviours of eating disorders (EDE-Q). In addition, majority of observed changes remained stable at the next follow-up a year after the device removal (T0–T2).

The main statically significant improvement in Total emotional and behavioural problems (T0–T2) for experimental group (compared to the control group) is presented in **Figure 1**. **Figure 1** indicates that Total problems in the experimental group improved after the intervention (T1) compared to baseline (T0) of control group and remained stabled a year after removal.

Psychological Predictor of Weight Loss

Table 3 contains the final multivariate linear regression model predicting weight loss. Weight loss (BMI SDS Change) was calculated by subtracting BMI SDS_{T0} from BMI SDS_{T1} (lower BMI SDS Change indicating greater weight loss). The final model achieved adequate fit (global predictive capacity equal to $r^2 = 0.725$, adjusted $r^2 = 0.511$, $p = 0.046$). The model indicated that the significant predictors of successful weight loss at device removal were fewer overeating episodes and lower body satisfaction.

Three interactions between independent variables demonstrated statistical significance, namely interaction between depression-withdrawn and overeating episodes (1), interaction between body satisfaction and overeating episodes (2), and interaction between restraining and overeating episodes (3). Simple slope analyses were calculated for those three interactions and are presented in **Figure 2**.

Simple slope analysis presented in **Figure 2** shows that adolescents with the highest number of overeating episodes lost the least amount of weight, which was especially evident for those who reported more depressive symptoms (1), were more satisfied with their body (2) and reported higher levels of restraining with food (3). In other words, adolescents who reported more depressive symptoms (1), were more satisfied with their body (2) and reported higher levels of food-related restraining (3), lost more weight if they reported fewer overeating episodes.

DISCUSSION

In this preliminary and exploratory study, we aimed to investigated the specific psychological outcomes in adolescents with severe obesity undergoing reversible endoscopic bariatric procedure. We observed, according to our expectations, significant improvements in somatic complaints, total emotional and behavioural problems, and food restraint after 12 months of treatment compared to control group. Furthermore, within the experimental group additional improvements of depressive and anxiety symptoms and social problems were found. Adolescents were also more satisfied with their appearance. A year after device removal adolescents reported fewer externalising problems (especially aggressive behaviour) compared to baseline. Our results are comparable with studies investigating psychological outcomes of more invasive bariatric procedures that observed reduction in self-reported symptoms of anxiety, depression, anger and disruptive behaviour, and improved self-concept (27, 28). This can be explained with the growing feeling of competence after weight loss (65). Changes in psychological variables are considered favourable, are comparable with more invasive procedures, and are relatively stable a year after the procedure was finished, showing long term psychological effects of the treatment regardless of weight gain observed in some adolescents. Contrary to our hypothesis, we did not find changes in disordered eating attitudes and behaviours which could potentially contribute to weight gain. This is in contrast to findings of other studies, that found improved disordered eating after surgery (35, 66). Our findings suggest that bariatric surgery itself cannot be viewed as an intervention for disorder eating attitudes and behaviour, therefore psychological interventions designed to help adolescents in this matter could be useful. Some studies in adults found combination of lifestyle interventions and cognitive behaviour treatment as successful interventions for weight loss (67, 68).

We predicted that adolescents with fewer emotional and behavioural problems, better self-image and body satisfaction, and fewer disturber eating attitudes and behaviours will achieve greater weight loss. Our predictions were only partly confirmed by our results, that showed fewer overeating episodes and lower body satisfaction at baseline predicted successful weight loss 12 months after implantation of the device. Our findings are in accordance with previously published

TABLE 3 | Multivariate linear regression model indicating predictors of weight loss (BMI SDS Change).

Names	*B*	Exp *B*	*SE*	*t*	*p*
(Intercept)	−0.051		0.145	−0.354	0.732
Body Satisfaction (MBSRQ)	**0.932**	**1.458**	**0.301**	**3.097**	**0.013**
Withdrawn/Depressed (YSR)	0.052	0.440	0.033	1.562	0.153
Restraint (EDE-Q)	0.223	0.649	0.113	1.976	0.080
Overeating Episodes (EDE-Q)	**0.147**	**3.163**	**0.053**	**2.773**	**0.022**
Withdrawn/Depressed × Overeating Episodes	**0.044**	**0.458**	**0.014**	**3.131**	**0.012**
Body Satisfaction × Overeating Episodes	**0.292**	**0.374**	**0.117**	**2.497**	**0.034**
Restraint × Overeating Episodes	**0.086**	**0.252**	**0.036**	**2.397**	**0.040**

B, regression coefficient; Exp B, standardised regression coefficient; SE, standard error of coefficient; t, t-value; p, level of statistical significance. Values of variables considered as statically significant appear in bold text.

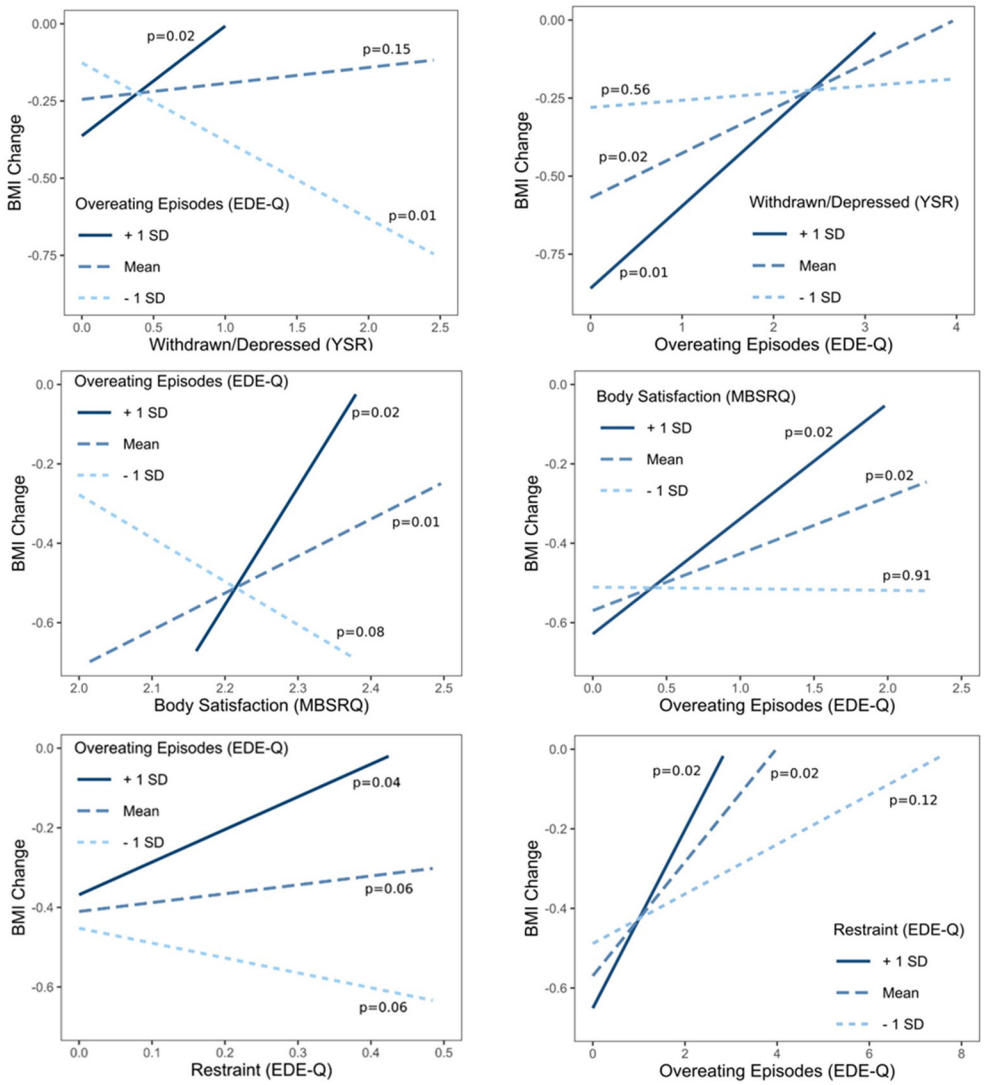

FIGURE 2 | Simple slope analysis for three statistically significant interactions in the multivariate regression model.

studies, that reported more frequent binge eating episodes as predictor of poorer weight loss after bariatric surgery (38, 46), but in contrast to others, where those association were not found (35, 40–43). We can assume that adolescents with fewer overeating episodes have more self-control than the ones with more overeating episodes, therefore they can control themselves better and are more adhere with dieting plans.

Unexpectedly, we observed that adolescents with higher body satisfaction lost less weight than others. Presented findings are in contrast with study where adolescents with better self-esteem have greater reduction of BMI 2 years post-surgery, while 5-years post-surgery relationship between self-esteem and weight loss was not significant any more (35). We suspect, that not overall self-esteem, but specifically but poorer satisfaction with body motivate adolescents to introduce some changes into their eating behaviours and other aspects of life, exercising, problem solving.

In addition, we observed that adolescents with more depressive symptoms, higher body satisfaction and more food restraining behaviour, showed lower probability of achieving weight loss if they had more overeating episodes. This finding suggests that some psychological variables, like depressive symptoms and food restraining, themselves does not have significantly strong effect on weight loss, but in combination overeating episodes does. Our results support the theory that mildly elevated symptoms of depression do not have effect on post-operative weight loss (44), while worse clinical manifestations (depression as psychiatric disorder) does (43). Similar, food restraining alone did not predict weight loss, but in combination with overeating become significant predictor. This raises the importance of evaluation of restraining feelings, especially as we observe higher levels of restraining at time of device removal in adolescent who did not respond for follow-up invitation year later.

Moreover, overeating episodes was one of the predictors with highest risk for unsuccessful weight loss and was moderating all significant interactions in simple slope analysis. Considering the fact, that overeating episodes did not improve after the procedure, which potentially shows how resistant these kind behaviours are, including evaluation and treatment of disorder eating in clinical setting is even of greater importance, specifically for adolescents with combined depressive symptomatology, higher food restraint, and higher body satisfaction.

Present findings should be considered within the context of several limitations. Firstly, groups were not randomised, which probably resulted in significant differences at baseline measures between control group and experimental group. Therefore, statistical analysis to correct baseline divergences was chosen. It is also not known whether the psychological characteristics of adolescents with severe obesity who decide for bariatric procedures are different in those seeking conservative treatment alternatives. Secondly, the small sample size might have prevented us from detecting important differences between the groups and reliable comparison on the basis of gender. Additionally, the attrition rate may have influenced our findings. Finally, psychological data were collected through self-report questionnaires, where there might be a risk of over- or under-reporting psychological distress.

We can conclude that specific psychological factors can predict how successfully adolescents will lose weight. Therefore, detailed and in-depth psychological evaluation, including estimation of disorder eating behaviours and attitudes, emotional and behavioural problems, and body satisfaction should be an essential part of pre-operative assessment, especially in adolescents. Presence of risk factors, like disturbed eating or depressive symptoms should not be necessary exclusion criteria for bariatric surgeries, rather the focus for additional psychological interventions (36). Some negative emotional states, like body dissatisfaction could be also a motivational factor, helping adolescents to healthier life. Integration of clinical psychologist or mental health professional, who recognised week points of individual adolescent, provide additional support, and promote post-operative adherence in the bariatric process could be crucial for success (24). In addition, psychological interventions specifically tailored to the adolescents, who are at risk for suboptimal weight loss in bariatric processes, should be included as part of the pre- and post-operative treatment plan to achieve successful weight loss.

Presented findings add to the knowledge in the research field of bariatric procedures in adolescents. Psychological outcomes in minimally invasive bariatric procedures are favourable and comparable to the outcomes of more invasive procedures in adolescents and adults. Results highlight the importance of psychological characteristic of the patients and multidisciplinary approach. Nonetheless, additional research with bigger sample size and longer follow-up is needed to evaluate the long-term effects of psychological aspect in minimally invasive bariatric procedures in adolescents.

In conclusion, following the reversible bariatric procedure, improvements of psychological (emotional and behavioural) factors were found in adolescents with severe obesity. Improvements were comparable to previously published results in non-reversible surgical bariatric procedures, adding a novel supplementary value to minimally invasive bariatric procedures. Reassuringly, most of the favourable psychological changes remained stable at follow-up, 12 months following device removal. Furthermore, psychological predictors of successful weight loss were identified, showing the greatest importance of eating behaviours and body satisfaction in successful weight loss. These findings highlight the importance of detailed psychological pre- and post-procedure assessment to identify potential difficulties, help achieve successful weight loss, and improve mental health status of adolescents with severe obesity.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Republic of Slovenia National Medical Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SK, PK, and TB designed the study. SK, AB, NH, TB, MH, RO, and PK were involved in the acquisition and interpretation of data. The first draught of the paper was written by SK, with the support of NH and PK. SK is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the conception of the work. All authors contributed to and approved the final version of the manuscript.

FUNDING

This study was supported by the Slovenian Research Agency grants P3-0343, J3-6798 and University Medical Centre Ljubljana grant No. 20100364.

ACKNOWLEDGMENTS

The authors express thanks to all the adolescents and their families, who participated in the study and all members of the endoscopic bariatric team, especially Miha Rutar, psychologist, and Tomaž Poredoš, dietitian, for their dedicated work. Authors also thank Eva Volk for the help in data acquisition and Sašo Karakatič for the statistical analysis, as well as the staff of the Department of Endocrinology, Diabetes, and Metabolism, University Children's Hospital, Ljubljana. Devices (DJDB) used were kindly provided by GI Dynamics Inc., Lexington, Mass, USA.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

MINI REVIEW
published: 09 June 2021
doi: 10.3389/fped.2021.696499

Preventing Cardiovascular Complications in Type 1 Diabetes: The Need for a Lifetime Approach

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Cardiovascular disease (CVD) remains the main cause of morbidity and mortality in individuals with type 1 diabetes (T1D). Adolescence appears to be a critical time for the development of early subclinical manifestations of CVD, with these changes likely driven by a deterioration in glycemic control during the progression through puberty, combined with the emergence of numerous other traditional cardiometabolic risk factors (e.g., hypertension, dyslipidemia, smoking, alcohol use, obesity, etc.) which emerge at this age. Although hemoglobin A1C has long been the primary focus of screening and treatment strategies, glycemic control remains poor in youth with T1D. Furthermore, screening for cardiovascular risk factors—which are often elevated in youth with T1D—is suboptimal, and use of pharmacological interventions for hypertension and dyslipidemia remains low. As such, there is a clear need not only for better screening strategies for CVD risk factors in youth, but also early interventions to reduce these, if future CVD events have to be prevented. Accumulating evidence has recently suggested that early increases in urinary albumin excretion, even within the normal range, may identify adolescents with T1D who are at an increased risk of complications, and results from pharmacological intervention with statins and ACE inhibitors in these individuals have been encouraging. These data join a growing evidence highlighting the need for a whole-life approach to prevention starting from childhood if efforts to improve CVD outcomes and related mortality in T1D are to be maintained.

Keywords: type 1 diabetes, cardiovascular, complications, adolescence, risk factors

OPEN ACCESS

Edited by:
Marco Marigliano, Integrated University Hospital Verona, Italy

Reviewed by:
Adriana Franzese, University of Naples Federico II, Italy
Elena Fornari, Integrated University Hospital Verona, Italy

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Pediatrics

Received: *16 April 2021*
Accepted: *17 May 2021*
Published: *09 June 2021*

Citation:
Chiesa ST and Marcovecchio ML (2021) Preventing Cardiovascular Complications in Type 1 Diabetes: The Need for a Lifetime Approach. Front. Pediatr. 9:696499. doi: 10.3389/fped.2021.696499

INTRODUCTION

Type 1 diabetes (T1D) is a key public health concern, because of the growing incidence and the increased morbidity and mortality associated with this chronic condition (1, 2). Recent estimates from the International Diabetes Federation indicate that worldwide there are over 1 million individuals younger than 19 years living with T1D, with around 100,000 new cases every year in this age group (3). Concern has been raised about the increasing incidence of T1D particularly in very young children (2), as this can lead to higher rates of long-term complications such as retinal and kidney disease, neuropathy, and cardiovascular disease (CVD)—all of which have a negative impact on the prognosis of young people (4).

Although over the last decades there have been key advances in diabetes management, T1D remains a major cause of morbidity, reduced quality of life and loss of productivity (5). In addition,

premature mortality in individuals with T1D still exceeds that of the background population by 2–4 fold (6–8). This burden is largely due to vascular complications, with CVD being the leading cause of mortality (9, 10). While significant improvements in the clinical management of T1D have made impressive inroads into these troubling figures in recent decades, ever-increasing population levels of obesity and its accompanying metabolic derangements mean that a failure to adequately address more "traditional" CVD risk factors may stall future progress (11).

The purpose of this review is to offer an update on cardiovascular risk in youth with T1D with a focus on (1) non-glycemic risk factors for CVD; (2) new markers/measures for the early detection of CVD, (3) new intervention strategies. Relevant research studies, primarily published in the last 10 years, and including pediatric populations, are reviewed.

THE BURDEN ASSOCIATED WITH CVD IN T1D

Premature atherosclerosis is the main cause of excess mortality in individuals with T1D, with a standardized mortality attributable to CVD of 5.7 for men and 11.3 for women (12). Individuals with T1D experience an earlier onset of cardiovascular events and a higher related mortality compared to their peers without diabetes, and women with T1D are at higher risk than men (13, 14).

The incidence of major coronary artery disease events in young adults (aged 28–38 years) with T1D is around 1% per year and increases over 3% per year after age 55 years (4). Evidence of premature atherosclerosis may be evident in as many as 50–70% individuals with T1D by the age of 45 years, and a significant proportion of young people will have clinical cardiovascular risk factors already detectable by the age of 12–19 years (15).

Great strides been made in recent decades to lower mortality in people with T1D (16), with most of this success attributed to improvements in glycemic control due to better insulin regimens along with the introduction of continuous subcutaneous insulin infusion and continuous glucose monitoring, and more recently hybrid closed loop systems (17). Nevertheless, individuals with T1D still experience significant excess morbidity and mortality, as evidenced by a recent Danish study assessing mortality in children and young adults (age 1–39 years) in which a 7-fold increase in all-cause mortality and 11-fold increase in cardiovascular mortality was observed compared to age-matched individuals without diabetes (18).

ADOLESCENCE IS A CRITICAL TIME FOR THE ONSET OF CARDIOVASCULAR DISEASE

Although the clinical manifestations of CVD are almost exclusively observed in later life, their incidence represents the end-result of decades-long subclinical disease process which is driven in large part by potentially modifiable lifestyle behaviors and risk factors (19) (**Figure 1**). In individuals with childhood-onset T1D, adolescence appears to be a particularly critical period for this process (20). This has been starkly illustrated by findings from the Swedish National Diabetes Register, in which T1D onset before the age of 10 years was associated with a loss of life expectancy of 17.7 years in women and 14.2 years in men, as compared with 10.1 and 9.4 years respectively in those with T1D onset at age 26–30 years (7). Furthermore, individuals with childhood-onset T1D were found to have a 30-times higher CVD risk than the general population (7). Further data from the US National Health Interview Survey support this, indicating that whereas deaths related to vascular complications declined over time in adults, they remained almost unchanged in young people with T1D aged 22–44 years (21). This higher risk of CVD in people with an early onset of T1D is likely related to a longer duration of risk factor exposure in individuals diagnosed in childhood, alongside a potentially more aggressive disease pathogenesis (22).

While suboptimal glycemic control likely plays a major role in this elevated risk, it is now well-established that early and sustained exposure to "traditional" CVD risk factors such as high BMI, blood pressure and cholesterol, physical inactivity, poor diet, and smoking (amongst others) likely accelerate the atherosclerotic disease process and further contribute to future CVD risk (19, 23) (**Figure 1**). These adverse risk factors frequently track across the lifespan, with individuals who are exposed to a high-risk factor burden already during adolescence more likely to carry this increased burden into later-life (24). This early and cumulative exposure to multiple risk factors throughout adolescence may therefore be particularly damaging in individuals with childhood-onset T1D, especially as it occurs alongside progressively worsening HbA1c levels (25). In support of this, multiple studies in individuals with T1D have demonstrated that it is during this period of life that the first signs of vascular damage often appear (26). These early vascular complications may be particularly aggressive in those with poor glycemic control (27), and have been shown to increase even further in those additionally exposed to other traditional risk factors (28).

The most common method for assessing these early subclinical manifestations are through the use of surrogate markers such as increased arterial wall thickness (carotid intima-media thickness, cIMT), accelerated arterial stiffening (pulse wave velocity, PWV), or endothelial dysfunction (flow mediated dilation, FMD) (15, 29). A recent systematic review and meta-analysis including 23 studies on cIMT and carotid-femoral PWV showed higher cIMT and PVW in youth with T1D than matched controls (30).

With recent evidence demonstrating a remarkable ability for even modest reductions in risk factors to substantially reduce cardiovascular events when sustained across the life-course (31), it is clear that efforts to mitigate risk factor exposure should start as early as possible and continue during the transition through adulthood, if maximal lifetime benefits are to be achieved.

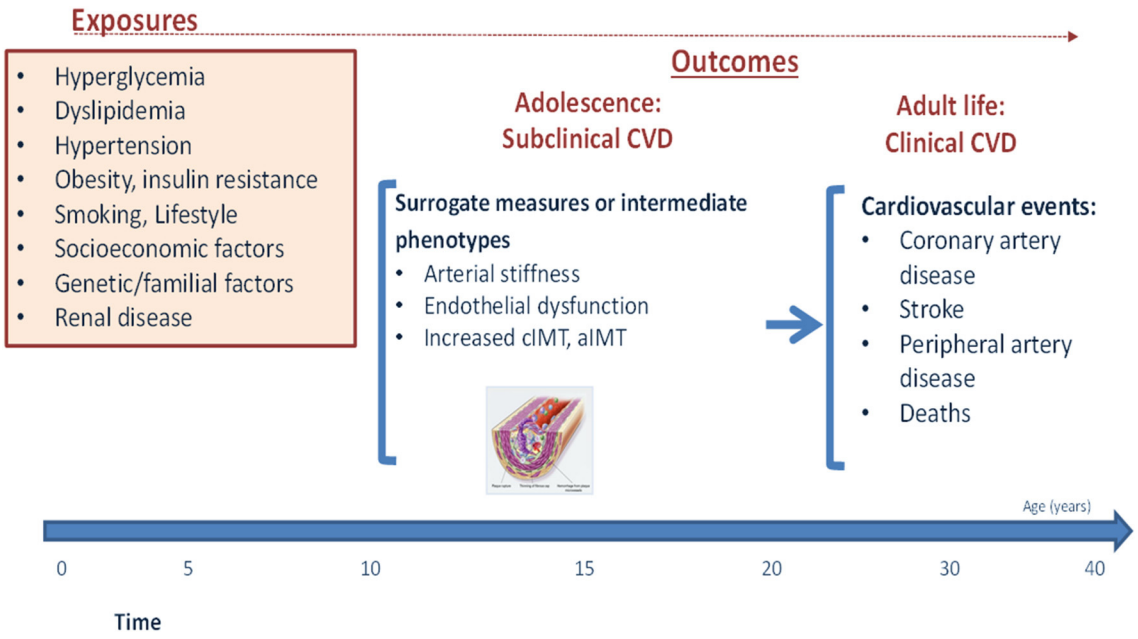

FIGURE 1 | Natural history of cardiovascular disease in youth with Type 1 diabetes.

NON-GLYCEMIC CVD RISK FACTORS IN YOUTH WITH T1D: A BURGEONING CONCERN

Poor glycemic control is the primary modifiable risk factor for CVD in T1D, as clearly highlighted by the results of the DCCT/EDIC studies and other more recent studies (32). However, other traditional cardiometabolic risk factors such as obesity, hypertension, and dyslipidemia; lifestyle factors such as smoking and physical inactivity, and comorbidities such as microalbuminuria, hyperfiltration/delcline in renal function can all independently and additively contribute to overall CVD risk (32).

Cardiometabolic Risk Factors

The increased prevalence of obesity in the past three decades has particularly alarmed the global health community, with up to 60% of the world's population now projected to become overweight or obese within 10 years if current trends continue (33). Worryingly, the most rapid increases in obesity during this time have been in the young—the vast majority of whom will go onto become obese adults (34). Although individuals with T1D were traditionally considered to be a relatively lean population, recent evidence has shown that obesity rates in this group are now similar or possibly even higher than in the general population, with an alarmingly high prevalence particularly in children and adolescents (35). Indeed, recent data based on large registries from the US, Australia and Europe have confirmed that overweight and obesity are common among youth with T1D at rates of 9–20% (36). Given the known causal relationship between obesity, its accompanying sequelae of cardiometabolic derangements, and CVD; these data suggest that recent improvements in CVD morbidity/mortality, which have been largely attributed to improved glycemic control, could now be at risk of stalling or even being reversed due to the emergence of these additional emerging risk factors (11).

Unfortunately, evidence suggests that youth with T1D are disproportionately affected by these risk factors when compared to their healthy peers, and the presence of many of these factors are therefore already common among youth with T1D. For example, recent studies have suggested that up to 60% of children may show evidence of cardiometabolic risk factors around the time of diagnosis (37, 38), and around 86% of individuals may have one and 14–45% more than two cardiovascular risk factors by the time they reach adolescence (15). Similar findings have been reported from the SEARCH study, in which 7% of T1D youth had two or more CVD risk factors and 1.7% had three or more. In these individuals, 26% participants were overweight, 14% obese, 13% hypertensive, and 29% dyslipidemic (32). In longitudinal follow-up, while the number of these risk factors did not significantly change over the 10-year study period [2002–2012], a strong relationship was found between BMI and CVD risk factors, and a clustering of CVD risk factors was associated with high rates of multiple vascular complications (39). Likewise, in a recent large Italian study, assessing 2021 young people with T1D aged 2–18 years, CVD risk factors were confirmed to be common, with 32.5% showing one CVD risk factor and 6.7% two risk factors (40).

Lifestyle Risk Factors

In addition to cardiometabolic derangements, lifestyle factors such as smoking, alcohol, sedentary behavior, and stress can

also contribute to CVD (15). Among teenagers with T1D, 10% reported alcohol consumption, 10% were smokers and 6% reported both alcohol and cigarette use (41). Both smoking and alcohol in youth are associated with suboptimal glycemic control, alongside a higher prevalence of other CVD risk factors (41).

Emerging Risk Factors

DKD is an independent risk factor for cardiovascular morbidity and mortality (42, 43). Albuminuria, the hallmark of DKD, is associated with two to four times greater risk for CVD and death (44), and there is strong evidence that urinary albumin excretion is a continuous CVD risk factor (45). Longitudinal studies of adolescents with T1D recruited to the Oxford Regional Prospective Study, Nephropathy Family Study and the Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT), have highlighted these observations (26). In these young cohorts, increases in the urinary ACR may occur as early as 1 year from T1D diagnosis in those who progress to microalbuminuria or macroalbuminuria (46). Furthermore, these modest increases in albumin excretion are not only linked to early risk for the development of microalbuminuria (46) but also to CVD risk factors such as carotid intima-media thickness, flow-mediated dilation, C-reactive protein, and over time changes in blood pressure (47, 48). Thus, urinary ACR is an important CVD risk marker in addition to traditional predictive risk factors in T1D. In addition, decline in glomerular filtration rates, which can occur already in youth with T1D, is an additional risk factor for CVD (49).

HOW BEST TO TACKLE CVD RISK FACTORS DURING ADOLESCENCE?

Collectively, the above findings have led to guidelines from organizations such as the International Society for Pediatric and Adolescent Diabetes and American Diabetes Association to recommend screening for CVD risk factors (primarily dyslipidemia, high blood pressure, obesity, and smoking) starting from the age of 10–11 years (50). Despite these recommendations, however, strategies on how to appropriately address these once identified remain suboptimal.

During adolescence, the primary focus of management of vascular complications risk is to improve glycemic control by intensifying insulin therapy (50). However, despite advances in insulin treatment, over 75% adolescents do not reach recommended targets for HbA1c (25).

In addition to issues with glycemic control, many adolescents also do not meet targets for blood pressure, cholesterol or BMI (15), potentially compounding the excess risk experienced in T1D that arises from high blood glucose levels. Guidelines recommend lifestyle interventions as first steps to control these risk factors before implementing drug interventions (50), despite a lack of strong evidence for their effectiveness. Weight management in youth with T1D presents its own challenges, with fear of hypoglycemia, difficult management of glycemic control during exercise and inadequate knowledge around exercise management representing potential barriers to increased levels of physical activity (51). Furthermore, while adherence to dietary guidelines is associated with improved glycemia, youth struggle to meet strict dietary recommendations—particularly during the critical transition through adolescence when newfound freedoms from parental oversight and other social pressures often have negative effects on diet or other health behaviors (52).

Despite these difficulties, however, robust evidence exists to suggest that the adoption of several relatively simple lifestyle changes in the early years of life are likely to significantly reduce risk of CVD in later years. In the general population, evidence from the CARDIA study has shown the remarkable effect that good health behaviors in adolescence/young adulthood have on cardiovascular risk in later life, with adolescents with 5 or more healthy lifestyle behaviors (as defined by the American Heart Association and comprising blood pressure, total cholesterol, glucose/HbA1c, BMI, physical activity, diet, smoking) over 20 times more likely to have a favorable cardiovascular health profile in 20 year-follow-up compared to those with 0 healthy behaviors (53). These findings have recently been replicated in individuals with T1D for the first time, suggesting that these metrics represent straightforward goals for health promotion that may reduce CVD risk in the T1D population (54). Together, these findings highlight the need for more focus on education and training on exercise and lifestyle change in diabetes to enable the implementation of early intervention programmes to tackle poor cardiovascular health from a young age.

DRUG INTERVENTIONS: WHERE ARE WE?

Similar to lifestyle changes, recent studies have highlighted a 'therapeutic inertia' related to treatment of CVD risk factors such as dyslipidemia and hypertension in youth with T1D, with low rates of use of antihypertensive or lipid-lowering medications even where there is an indication for their use (55, 56). Data from T1D registries from the United States and Germany/Austria have confirmed that most young patients are inadequately treated for hypertension and dyslipidaemia (57). Few young adults aged <26 years receive antihypertensive (3–5%) and lipid-lowering (1–3%) medications, highlighting the need for improved diabetes and cardiovascular risk management strategies in T1D.

Survey data suggest that clinicians endorse lifestyle recommendations for initial management of dyslipidemia and hypertension in 83–99% of cases, although only 6–17% of them believe that these are effective. In contrast, medications are rarely prescribed (58), partly due to limited data and guideline recommendations for the use of common drugs such as statins and ACE inhibitors use in adolescents with T1D.

Statins and ACE-Inhibitors in Adolescents With T1D

AdDIT was the first large randomized clinical trial evaluating the use of ACE inhibitors and statins during adolescence to protect against T1D vascular complications (59). The trial showed that statins can reduce exposure to high lipid levels and ACE-inhibitors can reduce new cases of microalbuminuria; changes that could potentially lead to protection against future

complications (59). In addition, a recent analysis of a subgroup of the trial population showed that ACE inhibitors improved endothelial function (assessed by flow mediated dilation) in high-risk adolescents transitioning through puberty, and may therefore offer long-term cardio-renal benefit during this potentially critical time period for the development of CVD (48).

The trial also provided reassuring data on the safety profile of both drugs in this age group (59). Overall adherence during the 2–4 year trial period was 75–80%, although it deteriorated over time, therefore highlighting the need of strategies to reinforce adherence to gain the maximum benefit from these interventions (60).

Participants from the AdDIT study are now entering their 2nd to 3rd decade from T1D diagnosis, a time when more pronounced arterial changes begin to emerge. Current follow-up of the study cohort up to 5 years from the end of the original trial, including detailed cardiovascular phenotyping, will provide invaluable information on the potential impact of cumulative exposure to acquired modifiable risk factors on CVD progression. The study will also determine whether a reduction in these risk factors during adolescence because of statin/ACE-inhibitors therapy results in long-term benefits for CVD health.

Metformin in Youth With T1D

Metformin is another potential treatment in youth with T1D. Although there is no strong evidence in terms of glycemic control following metformin treatment, its use has been shown to lead to small reductions in total daily insulin dose and BMI in youth with T1D (61). Furthermore, recent studies have shown a beneficial effect on insulin sensitivity and CVD risk profile. In one study, a 3-month intervention with metformin led to greater improvements in insulin sensitivity compared to placebo (62), while a second smaller study on 16 youth with T1D showed that 12-month treatment with metformin improved vascular smooth muscle function (63). In a more recent mechanistic study, 48 youth with T1D (aged 12–21 years) underwent a hyperinsulinemic euglycemic clamp and assessment of MRI-derived measures of aortic and carotid vascular health, before and after 3 months of metformin or placebo therapy. Treatment with metformin was associated with improved insulin sensitivity and aortic and carotid wall measures (64). Additional larger studies are required to confirm these findings and provide additional evidence before metformin could be recommended as an adjunct therapy in T1D.

Other Adjunct Medications in Youth With T1D

Non-insulin therapies, such as sodium-glucose co-transporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 receptor agonist and dipeptidyl peptidase-4 inhibitors, have been investigated to try to improve glycemic control, as well as for their potential effect in CVD risk factors (65).

A large body of evidence support a positive effect of SGLT2-inhibitors on cardiovascular and renal outcomes in adults with diabetes, although data for the pediatric population are lacking (65). While these medications appear promising, the risk for euglycemic diabetic ketoacidosis is still a potential limiting factor to consider before a wide implementation in the pediatric population (65).

MOTIVATIONAL AND PSYCHOLOGICAL INTERVENTIONS

Data based on semi-structured interviews have clearly shown poor awareness of complications among adolescents as well their reluctance to know about them (66). Even when patients are aware, however, a combination of insufficient support for how to implement lifestyle changes, lack of confidence to follow these changes, and lack of knowledge on other means for controlling blood pressure and lipid levels may all limit uptake of preventative measures (67). More effort is therefore required to not only raise awareness about complications in youth, but also to develop better ways to quantify and communicate this risk to patients and their families. To achieve this, potential barriers limiting adherence to weight management and lifestyle strategies should be identified and addressed (51), and reassurance about medical treatment of dyslipidemia and hypertension should be provided to both young patients and their parents.

Properly addressing physical activity, nutrition, pharmacotherapy, and psychosocial factors while emphasizing weight management may improve CVD risk factors and avoid their persistence during adulthood.

UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

Early Prediction

There is still a need for improved ways to identify people at risk at an early stage when pathology may be amenable to interventions.

Early screening for abnormal ACR may provide a valuable tool to identify adolescents at high risk for CVD in clinical practice. Risk stratification using urinary albumin excretion along with other traditional and new risk factors during early adolescence may be critical for the early identification of patients at risk and to guide the implementation of preventive and treatment strategies.

Early Prevention/Better Interventions

There is an urgent need to identify new targets for interventions to prevent CVD complications, and this can only be achieved through a better understanding of the mechanisms and key players implicated in the development and progression of vascular complications.

While the AdDIT trial has provided evidence that early interventions with ACE-Inhibitors and statins are both safe and effective in reducing exposure to CVD risk factors during adolescence, the impact of these early interventions on long-term cardiovascular outcomes needs to be further explored. For any intervention to provide maximum efficacy, strategies to promote adherence to both existing and new interventions will be required—especially given known issues surrounding adherence levels common during the transition from adolescence to adulthood. In addition, lifestyle interventions need to be more

widely promoted and made easy to be implemented for youth with T1D.

CONCLUSIONS

Despite significant improvements in the management of T1D during the past decades, vascular complications remain a major concern. Efforts to improve vascular outcomes and mortality in T1D should be a whole-life approach starting from childhood. Early investment in the understanding and care of youth with T1D from childhood will have substantial long-term benefits in terms of complications, quality of life, and life expectancy.

AUTHOR CONTRIBUTIONS

SC and MM reviewed literature and worked together on the manuscript draft and approved final version. Both authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 09 June 2021
doi: 10.3389/fendo.2021.689387

Two Cases With an Early Presented Proopiomelanocortin Deficiency—A Long-Term Follow-Up and Systematic Literature Review

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 31 March 2021
Accepted: 07 May 2021
Published: 09 June 2021

Citation:
Gregoric N, Groselj U, Bratina N, Debeljak M, Zerjav Tansek M, Suput Omladic J, Kovac J, Battelino T, Kotnik P and Avbelj Stefanija M (2021) Two Cases With an Early Presented Proopiomelanocortin Deficiency—A Long-Term Follow-Up and Systematic Literature Review. Front. Endocrinol. 12:689387. doi: 10.3389/fendo.2021.689387

Proopiomelanocortin (POMC) deficiency is an extremely rare inherited autosomal recessive disorder characterized by severe obesity, adrenal insufficiency, skin hypopigmentation, and red hair. It is caused by pathogenic variants in the *POMC* gene that codes the proopiomelanocortin polypeptide which is cleaved to several peptides; the most notable ones are adrenocorticotropic hormone (ACTH), alpha- and beta-melanocyte-stimulating hormones (α-MSH and β-MSH); the latter two are crucial in melanogenesis and the energy balance by regulating feeding behavior and energy homeostasis through melanocortin receptor 4 (MC4R). The lack of its regulation leads to polyphagia and early onset severe obesity. A novel MC4R agonist, setmelanotide, has shown promising results regarding weight loss in patients with POMC deficiency. A systematic review on previously published clinical and genetic characteristics of patients with POMC deficiency and additional data obtained from two unrelated patients in our care was performed. A 25-year-old male patient, partly previously reported, was remarkable for childhood developed type 1 diabetes (T1D), transient growth hormone deficiency, and delayed puberty. The second case is a girl with an unusual presentation with central hypothyroidism and normal pigmentation of skin and hair. Of all evaluated cases, only 50% of patients had characteristic red hair, fair skin, and eye phenotype. Central hypothyroidism was reported in 36% of patients; furthermore, scarce adolescent data indicate possible growth axis dysbalance and central hypogonadism. T1D was unexpectedly prevalent in POMC deficiency, reported in 14% of patients, which could be an underestimation. POMC deficiency reveals to be a syndrome with several endocrinological abnormalities, some of which may become apparent with time. Apart

from timely diagnosis, careful clinical follow-up of patients through childhood and adolescence for possible additional disease manifestations is warranted.

Keywords: proopiomelanocortin, POMC deficiency, obesity, adrenal insufficiency, type 1 diabetes, setmelanotide, systematic review

INTRODUCTION

Proopiomelanocortin (POMC) is a precursor polypeptide hormone secreted primarily in the hypothalamus. The post-translational process and cleavage give rise to several polypeptides, the melanocortins. Among these are adrenocorticotropic hormone (ACTH), alpha-, beta- and gamma-melanocyte-stimulating hormones (α-MSH, β-MSH, and γ-MSH), β-lipotrophin, and endorphins (1). These polypeptides bind to melanocortin receptors of different subtypes. The melanocortin 1 receptor (MC1R) regulates skin pigmentation; stimulation of MC2R induces adrenal steroidogenesis; MC3R and MC4R regulate energy balance through appetite regulation, whereas MC5R is expressed in sebaceous glands and is involved in sebogenesis (1). To add to the complexity, the POMC neurons in the central nervous system are highly heterogeneous in their regulation and action; furthermore, the POMC-derived peptides can have opposing effects on appetite regulation, α-MSH suppressing and β-endorphin, on the other hand, promoting appetite (2).

Major insights into POMC function in humans are derived by studying patients with POMC deficiency (OMIM#609734). Biallelic loss-of-function variants in the *POMC* gene give rise to a phenotype with a triad of clinical features: ACTH deficiency that is usually the first to be recognized, hypopigmented skin with red hair, and early onset obesity due to uncontrolled polyphagia. The cornerstone of treatment is glucocorticoid replacement and weight management, the latter being very challenging. Novel treatment, such as MC4R agonist, setmelanotide, has proven to be effective in reducing and maintaining body weight (3).

The condition is extremely rare; only a handful of cases have been reported since the first two cases have been described in 1998 (4). We gathered and analyzed the current experience on POMC deficiency by performing a systematic review of the literature. To increase the amount of information, we also included detailed clinical data on two unrelated patients with POMC deficiency from our center, a 25-year-long follow-up of a male patient with type 1 diabetes and a 4-year follow-up of a female patient with an unusual phenotype. It appears that POMC deficiency could also have important clinical consequences outside the classical triad, some of which could become apparent with time.

METHODS

The clinical information of the two patients who have been followed regularly at the University Medical Centre Ljubljana, Slovenia was gathered from the medical documentation. Written informed consent using local consent forms were obtained from patient 1 and parents of patient 2 for the publication of any potentially identifiable images or data included in this article. The pubertal stage was evaluated by a trained pediatric endocrinologist; testicular volume was estimated using a prader orchidometer. Anthropometric measurements were performed by trained nurses using professional certified digital devices. Gonadotropin-releasing hormone (GnRH) stimulation test was performed using gonadorelin (Relefact LH–RH, Sanofi-Aventis, Germany) 100 μg/m^2 body surface intravenously; blood samples were taken at 0, 20, 30, and 60 min, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immunoassay using Immulite 2000 (Siemens, Germany). Other dynamic tests, including arginine, levodopa (L-DOPA) growth hormone (GH) stimulation tests, oral glucose tolerance test, and standard and low-dose Synachten tests, were performed according to previously published test procedures (5). The Whole-Body Insulin Sensitivity Index (WBISI) was calculated as previously described (6). Genetic analysis of Case 1 was reported previously (7). In Case 2, genetic analysis was performed after obtaining informed consent approved by the Republic of Slovenia National Medical Ethics Committee (#132/03/15). Next-generation sequencing (NGS) was performed. The regions of interest were enriched using TruSight One library enrichment kit (Illumina, San Diego, CA, United States) according to the manufacturer's instructions and sequenced on the MiSeq desktop sequencer together with MiSeq Reagent kit v3 (Illumina, San Diego, CA, United States). A panel of 59 genes associated with obesity, including the *POMC* gene, was used for filtering of variants. The variant identified was subsequently confirmed by Sanger sequencing.

For the systematic review, we collected all the available scientific case report articles on POMC deficiency (**Figure 1**). The following search terms were used: "POMC" (AND) "deficiency". We found 287 research articles. By reading all the abstracts and titles, we excluded articles that did not meet the following conditions (i): articles in English published after 1998, when POMC deficiency was described for the first time; (ii) articles that were fully accessible; (iii) articles containing human data; (iv) original case report articles. Additional case reports were found through the articles' reference list. In the end, 16 articles and newly reported clinical data of our two patients were included.

RESULTS

Case Description Number 1

A male patient (#5 in **Table 1**), partially described in previous publications (3, 7), was diagnosed with POMC deficiency caused

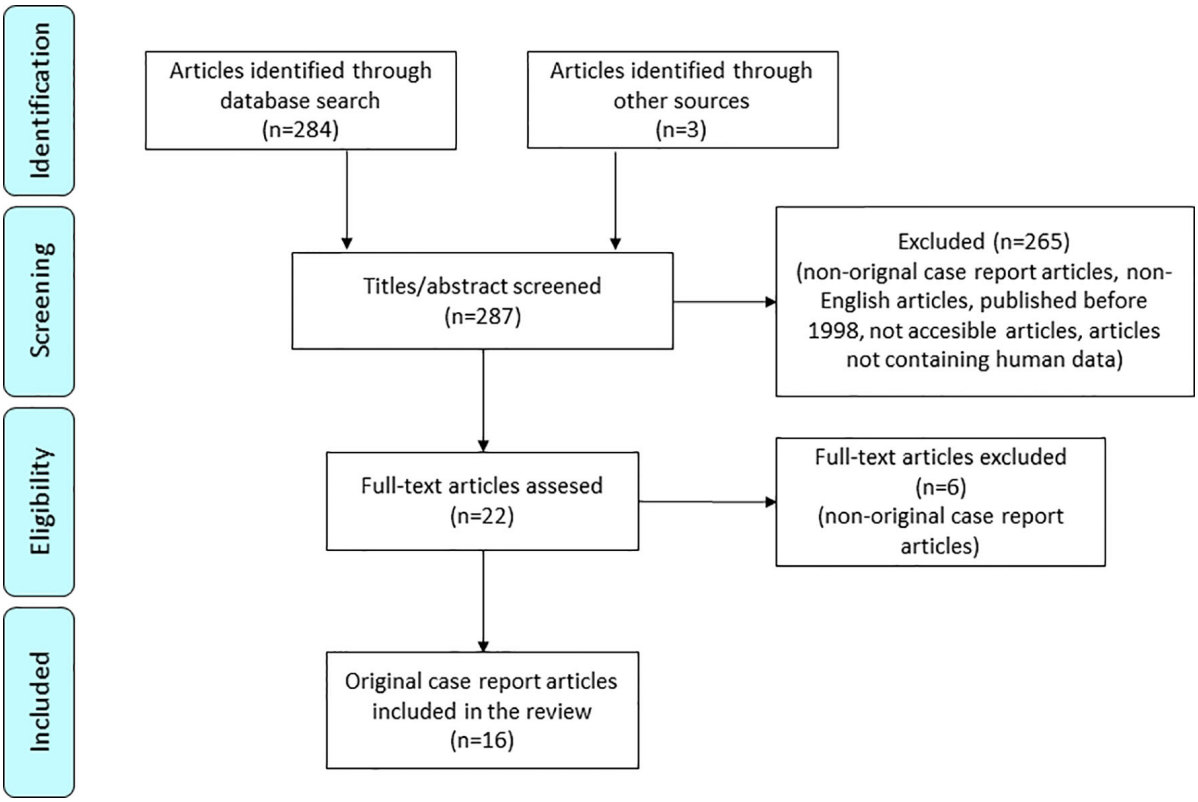

FIGURE 1 | PRISMA flow diagram for systematic case review of POMC deficiency (8).

by compound heterozygous variants c.151A>T and c.296del. The selected laboratory data is demonstrated in **Table 2**. He was born at term after an uneventful pregnancy to non-consanguineous parents, heterozygous carriers. His birth weight was 3,300 g. Neonatally, he developed hypoglycemia with convulsions and was treated in an intensive care unit where he required mechanical ventilation and parenteral feeding. Due to extremely low cortisol concentrations and ACTH levels, he was diagnosed with central adrenal insufficiency and put on substitutional treatment with hydrocortisone. No thyroid axis dysfunction was identified. The ultrasound and MRI later revealed atrophy of adrenal glands. Despite being treated with a low dose of hydrocortisone, his body weight rapidly increased due to uncontrollable polyphagia. At 24 months, he weighed 25 kg. Besides adrenal insufficiency and early onset obesity, another prominent physical feature was extremely pale skin and red hair that was in contrast to his immediate family members. His father was overweight; mother had normal weight. The patient followed a restriction diet program which success was only short-lived; the patient continued to gain weight (**Figure 2A**) until 7 years of age when he lost 4 kg due to symptomatic hyperglycemia and was hospitalized due to diabetic ketoacidosis. His C-peptide and insulin levels were low, and he was diagnosed with type 1 diabetes. The autoantibodies were not determined. In the following years, he was repeatedly hospitalized due to diabetes-related complications (severe hypoglycemia and diabetic ketoacidosis). Despite extensive efforts, the glycemia remained challenging due to poor diabetes management driven by behavioral and learning difficulties and uncontrolled hyperphagia. The patient often intentionally induced hypoglycemia in order to get an extra meal. Due to extreme obesity and high insulin requirement, indicating insulin resistance, he was treated with metformin 850 mg BID for a decade until 18 years of age, but the treatment failed to provide any significant effect. For a brief period, he was treated with appetite suppressant sibutramine. He lost 2 kg of body weight in 2 months but the success was short-lived as the drug was soon removed from the market. After the age of 20, he was receiving glucagon-like peptide-1 receptor agonist, liraglutide, titrated to 1.8 mg QD, again failing to produce any significant weight reduction or appetite suppression. At the age of 22 years, he was enrolled in the phase 3 trial with a novel MC4R agonist, setmelanotide (3). The trial lasted 12 months. During the trial, the polyphagia was well controlled, and the patient was able to lose approximately 27 kg of body weight from 113 kg (BMI 34 kg/m^2) to 86 kg (BMI 26 kg/m^2), demonstrated in **Figures 2A, B**. As the treatment continued, the patient was mostly able to maintain the body weight and curb the appetite. It is noteworthy that during

TABLE 1 | A list of reported cases of POMC deficiency so far.

Patient	Nucleotide change	AA change	Ancestry	Gender	Age of first symptoms	First presenting symptoms/ signs	Hair color	Other endocrine comorbidities	Reference
1	c.313G>T c.433delC	p.Glu105* p.Arg145fs	German	male	neonatal	hyperbilirubinemia	red	subclinical central hypothyroidism	(4)
2	c.-11C>A		German	female	12 months	hypoglycemia, hyponatremia	red	subclinical central hypothyroidism, GH deficiency, hypogonadism	(4, 7, 9)
3	c.-11C>A		Dutch	male	neonatal	hypoglycemia, convulsions, hyperbilirubinemia	red, but changed to brown at 2–3 years		(7)
4	c.-11C>A/ c.403_404dupGG	p.Glu134fs	Swiss	female	6 months	hypoglycemia, convulsions	red		(7)
5	c.151A>T c.296delG	p.Lys51* p.Gly99fs	Slovenian	male	neonatal	hypoglycemia, convulsions	red	type 1 diabetes, GH deficiency, hypogonadism	(3, 7), this report
6	c.206delC	p.Pro69fs	Turkish	male	not specified	not specified	brown, but dark red roots		(10)
7	c.223dupC	p.Arg75fs	North African	female	4 weeks	hypoglycemia	brown	GH deficiency, hypogonadism, central hypothyroidism	(9, 11)
8	c.296delG	p.Gly99fs	Turkish	male	neonatal	hypoglycemia, apnea attacks	red	mineralocorticoid deficiency	(12)
9	c.231C>A	p.Tyr77*	Hispanic	female	9 months	hypoglycemia, hyponatremia	dark brown to black		(13)
10	c.256C>T	p.Arg86*	Indian	male	neonatal	respiratory distress, convulsions, hypoglycemia, hyponatremia	skin and hair lighter than expected	central hypothyroidism	(14)
11	c.202C>T	p.Gln68*	Egyptian	male	neonatal	hypoglycemia	dark brown to black		(15)
12	c.206delC	p.Pro69fs	Turkish	male	neonatal	convulsions, apnea	red, but brown later	central hypothyroidism	(16)
13	c.-11C>A c.433C>T	p.Arg145Cys	French-Canadian	female	4.3 years	hypoglycemia, hyponatremia	red	elevated bioinactive ACTH	(17)
14	c.433C>T	p.Arg145Cys	French-Canadian	male	4 months	hypoglycemia, convulsions, hyponatremia	red	elevated bioinactive ACTH	(17)
15	c.-11C>A		Scottish/ German	male	neonatal	hypoglycemia, convulsions	red	type 1 diabetes	(18)
16	c.64delA	p.Met22fs	Turkish	female	neonatal	hyperbilirubinemia, hypoglycemia, convulsions	red		(19)
17	c.-11C>A c.251G>A	p.Trp84*	Russian	male	neonatal	hyperbilirubinemia, hypoglycemia	red	subclinical hypothyroidism	(20)
18	c.133-2A>C		Iraqi	female	neonatal	hyperbilirubinemia, frequent falls	light brown with a reddish hue	type 1 diabetes	(21)
19	c.133-2A>C		Iraqi	female	neonatal	hyperbilirubinemia	light brown with reddish hue		(21)
20	c.20_21ins25	p.Ser7fs	Hispanic	male	neonatal	hypoglycemia, hyperbilirubinemia, poor feeding	dark with a reddish tinge	central hypothyroidism	(22)
21	c.206delC	p.Pro69fs	Turkish	female	2.5 months	spasms, cyanosis, hypoglycemia, hyponatremia, elevated aspartate transaminase	red	no mini-puberty	(23)
22	c.296delG	p.Gly99fs	Slovenian	female	7 months	obesity, central hypothyroidism	brown	central hypothyroidism	this report

The reference numbers of POMC gene and POMC protein are NM_001035256.3 and NP_001030333.1, respectively.
AA, amino acid; GH, growth hormone; ACTH, corticotropin.

the setmelanotide treatment, his once pale skin and red hair gradually became hyperpigmented. At the time of writing this paper, he had dark brown hair and brown skin color as demonstrated in **Figure 3**. His blue eyes became brown.

During the first year of life, he suffered a couple of complex febrile convulsions. His psychomotor development was delayed; he began walking at 22 months, but gradually caught up. Later, as he acquired type 1 diabetes, he experienced very frequent

TABLE 2 | Laboratory values of case patient 1.

Biochemistry/Age	3 months	2.0 years	3.0 years	5.5 years	7 years	13.5 years	14.5 years	15 years	15.5 years	16.3 years	25 years
TSH [mU/L] (0.59–4.23)	3.83			NA	3.912	2.281	1.584			2.54	4.5
fT4 [pmol/L] (11.7–22.5)	14.9			16.5	13.2					13.57	16.0
fT3 [pmol/L] (3.79–6.05)	4.0			7	5.3					5.23	7.1
IGF-1 [μg/L]				312 (high)		141	103	89.4			128
IGFBP3 [mg/L]						4.6	5.02	5.89			2.67
GH (basal/peak) [μg/L]				2.9			0.199/2.76 (arginine)	0.515/1.76 (L-DOPA)			
LH [U/L]						<0.1	0.665	0.566		1.32	4.6
FSH [U/L]						0.919	2.2	2.27		1.64	2.2
Cortisol (basal/peak)* [nmol/L]	40.7/54	12/21	171†								
ACTH [pmol/L] (<10.20)			<2.2								
PRA [μg/L/h]				0.24 (low)	0.54						
Insulin (basal/2 h) [mU/L]		4.3		2.5/37.4							
Glucose (basal/2 h) [mmol/L]		3.7	3.4	3.1/7.0	22.7						
Androstendion [nmol/L] (0.7–10.8)											1.47
DHEA-S [μmol/L] (3.6–12.9)							0.0				1.6
SHBG [nmol/L] (18–114)											67.0
Testosterone, total [nmol/L]						0.0	0.0	0.05		2.5	33.7
Testosterone, free [pmol/L]											64.4
Total cholesterol [mmol/L] (<5.2)		4.1	4.5	4.2	3.2	5.6	4.1			6.1	4.5
LDL [mmol/L] (<3.4)		2.5	2.7	2.6	1.9	3.0	2.7			3.1	2.9
HDL [mmol/L] (>1.3)		0.5	0.9	1.0	0.7	0.7	0.7			0.8	1.0
Triglycerides [mmol/L] (<1.7)		2.1	2.0	1.2	1.4	4.0	2.8			10.7	1.4
Pubertal status				A1, P1		A1, P1	A1, P1	A1, P1	P2	A1, P2	
Testicular volume [mL]				1-2	2	2	3	1-2	8-10	15	

*Reference values are stated in brackets. *Peak values were obtained 60 minutes after stimulation with synthetic ACTH 125 μg and 250 μg intramuscularly at 3 months and 2 years, respectively, †value obtained while on substitution with hydrocortisone.*

hypoglycemic seizures, occasionally without noting symptoms of preceding hypoglycemia. The electroencephalography did not show any epileptiform activity.

In his teens, there was a significant growth delay, a drop from 95 percentile to 10th percentile in 5 years (**Figures 2A, B**). Further evaluation revealed GH deficiency, which was confirmed by arginine and L-DOPA GH stimulation tests and a decrease in insulin-like growth factor-1 (IGF-1) levels, which were, in childhood, above the normal range. The bone age was significantly delayed, 9 years, 4 months (−5.3 σ) at 14 years of age. Testosterone levels were low, and testicular volume started to develop spontaneously at 14–15 years up to 15 ml. He developed very poor body, pubic, and axillary hair, and adrenal androgens remained low into adulthood. A GH substitutional therapy was considered but never commenced as growth spurt occurred along with the slightly delayed puberty. His final height was 183 cm. It was only around 22 years of age, on setmelanotide for a year, that the patient developed notable body hair (pubic and chest hair) and began shaving his beard. Recently, at the age of 25, we evaluated his hypothalamic–pituitary–gonadal (HPG) axis and demonstrated normal function with high-normal testosterone levels. IGF-1 was within normal limits.

At the age of 15, he was evaluated for respiratory acidosis and narcolepsy and was diagnosed with obstructive sleep apnea.

In early childhood, he was also diagnosed with a small restrictive muscular ventricular septal defect that was persistent but did not require treatment.

Case Description Number 2

A 4-year-old girl (#22 in **Table 1**) who was born as a third child to distantly related parents, members of the Romani ethnic minority, was diagnosed with POMC deficiency caused by a homozygous frameshift variant in POMC (NM_000939.2: c.296delG). She was born at 35 weeks of gestation, with birth weight and length between the 10th and 50th percentiles. She had a complicated neonatal period. Paroxysmal tachycardia was noted already during pregnancy. After birth, paroxysmal intra-atrial tachycardia was defined, atrial undulation, and consequently decreased cardiac systolic function. She started therapy with amiodarone and metoprolol and needed cardioversion. Arrhythmia resolved completely in the first few months of life. Transiently, a small atrial septal defect type secundum and a small perimembranous ventricular septal defect were noted.

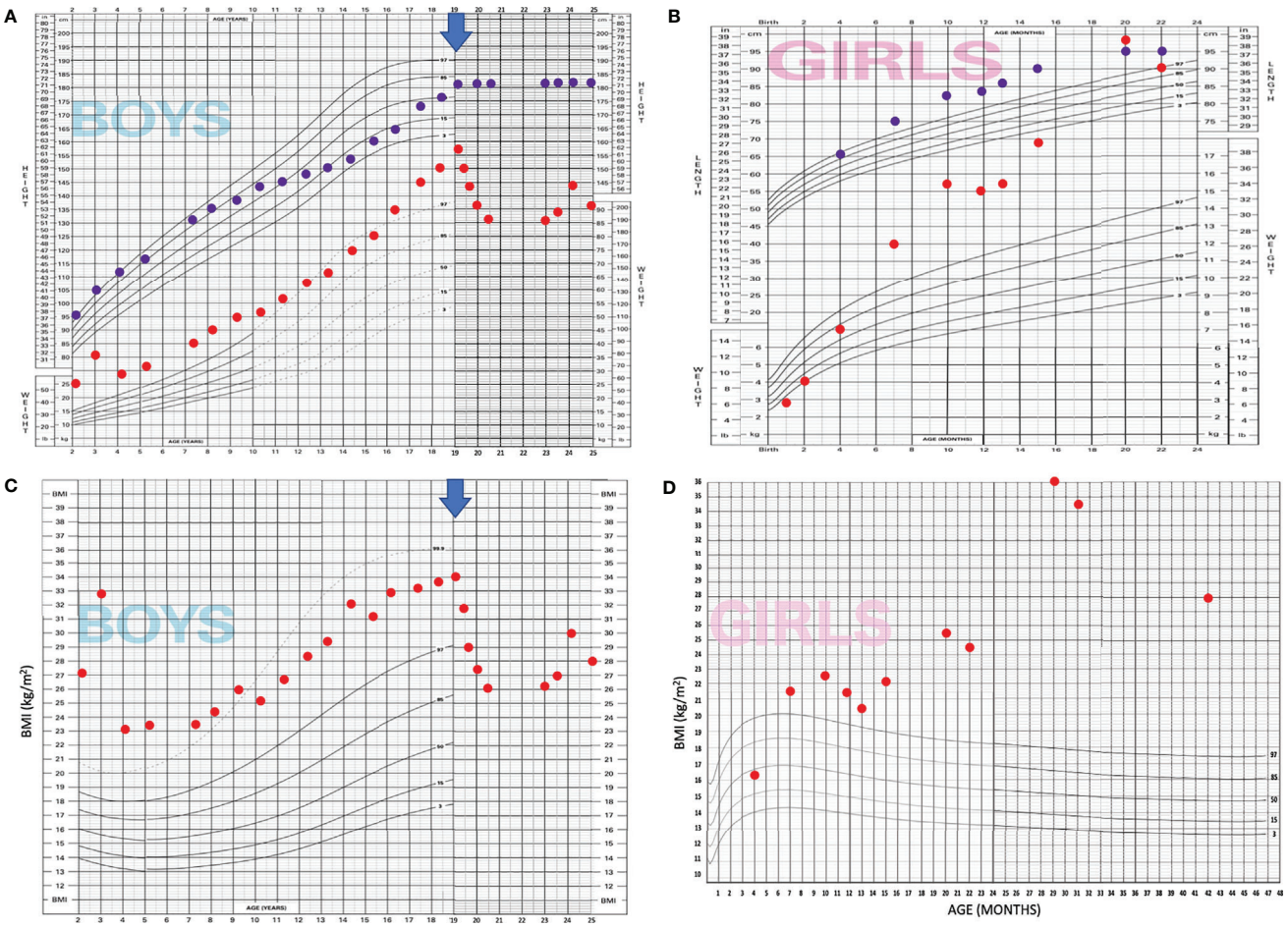

FIGURE 2 | Modified WHO growth charts* display both case patients. **(A)** Case patient 1—length for age and weight for age; **(B)**: Case patient 1—BMI for age, **(C)**: Case patient 2—length for age and weight for age, **(D)**: Case patient 2—BMI for age; BLUE ARROW marks the onset of setmelanotide treatment of case patient 1. *Original WHO growth charts were modified due to restrictions of scale and age.

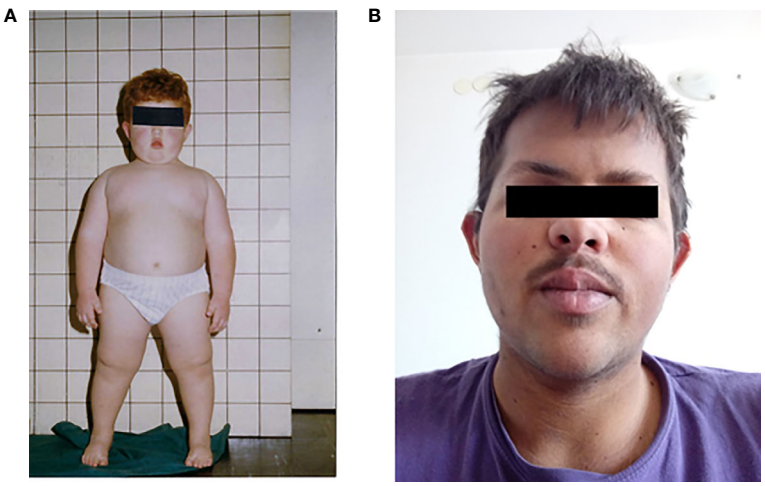

FIGURE 3 | Case patient 1 in childhood, before setmelanotide treatment **(A)** and after **(B)**.

She had signs of necrotizing enterocolitis, which was treated conservatively. During the first month, she had problems with obstipation; the feeding was difficult due to drowsiness. Hirschsprung disease was excluded by biopsy. The symptoms disappeared in the next months.

Neonatally, she had a prolonged requirement of phototherapy for indirect hyperbilirubinemia that started on the second day of life; slightly elevated was also conjugated bilirubin, which was attributed to ABO alloimmunization. No hypoglycemia was noted. Adrenal glands had a normal ultrasound appearance, and adrenal function at that time was not evaluated.

Since birth, she had suboptimal neurological development, delayed motor milestones, and muscular hypotonia. Brain imaging neonatally showed increased periventricular echogenicity, mild corpus callosum hypoplasia, mildly wider lateral ventricles, and signs of intracerebral micro bleedings, which were attributed to perinatal complications. By brain magnetic resonance imaging (age 1.5 years), slightly smaller pituitary and lower part of infundibulum were noted.

For the follow-up of thyroid function on amiodarone treatment, she came to the attention of an endocrinologist. The selected biochemical results are summarized in **Table 3**.

She had a normal thyroid function at age 9 days, mild latent primary hypothyroidism on amiodarone therapy at 3 weeks, while central hypothyroidism was identified at the age of 7 months after cessation of amiodarone and levothyroxine substitution. Subsequently, central hypocorticism was diagnosed in an asymptomatic state. She had elevated growth factors but peak stimulated GH at single testing was subnormal. She was, however, tall for her age. At 11 months, gonadotropins were appropriate for age.

She had normal weight at the age of 4 months (**Figures 2C, D**); at the age of 2.7 years she had an exponential weight gain up to 43 kg (height 111 cm, BMI 34.5 kg/m^2, +5.24 SDS) despite multiple dietary counseling. At 3.4 years, with improved parental control, she lost some weight (BMI 27.76 kg/m^2, +3.84 SDS). She had ultrasound signs of hepatic steatosis at the age of 2.7 years, elevated liver transaminases, and decreased HDL

TABLE 3 | Laboratory values of case patient 2.

Biochemistry/Age	9 days	3 weeks	7 months	11 months	20 months	2.5 years	3.5 years
TSH [mU/L] (0.59–4.23)	3.83	7.84	5.34	0.92*		3.24*	3.90*
fT4 [pmol/L] (11.7–22.5)	14.9	15.0	8.2	14.8*		11.3*	14.9*
fT3 [pmol/L] (3.79–6.05)	4.0	4.8	5.9	6.7*		5.74*	4.86*
IGF-1 [μg/L]				100 (20–159)	213 (19.5–132.3)	174 (22.2–145.4)	186 (25.9–164.2)
IGFBP3 [mg/L]				4.48 (1.37–4.30)	4.49 (1.22–3.72)	6.10 (1.39–4.15)	4.40 (1.55–4.56)
GH (basal/peak)† [μg/L]				0.73/3.49		0.34	
LH (basal/peak) [U/L]				0.2/8.5			
FSH (basal/peak) [U/L]				5.2/26.3			
Cortisol (basal/peak)‡ [nmol/L]				<27.5/<27.5			
ACTH [pmol/L] (<10.20)				<1.11			
PRA [μg/L/h]				8.72			
Insulin (basal/2 h)§ [mU/L]				9.4			3.5/238.0
Glucose (basal/2 h)§ [mmol/L]				4.2			4.0/6.7
AST [μkat/L] (<0.52)						1.25	0.55
ALT [μkat/L] (<0.57)						1.09	0.78
ϒGT [μkat/L] (<0.63)						1.40	0.21
Androstendion [nmol/L] (0.7–10.8)¶				<0.1		<0.1	
DHEA-S [μmol/L] (3.6–12.9)¶				0.09		<0.08	
SHBG [nmol/L] (18–114)¶						29	
17-OHP [nmol/L]						0.17	
Total cholesterol [mmol/L] (<5.2)							2.8
LDL [mmol/L] (<3.4)							1.7
HDL [mmol/L] (>1.3)							0.8
Triglycerides [mmol/L] (<1.7)							0.7

**thyroid function with L-thyroxin supplementation; † Peak value was obtained after stimulation with arginine; ‡ Peak value was obtained 30 min after intravenous stimulation with synthetic ACTH 1 μg; § values were obtained with oral glucose tolerance test; ¶ normal range in adults.*
The reference values are stated in brackets.

cholesterol. By the age of 3.5 years, she had normal glucose tolerance and HOMA index 0.6 but markedly elevated stimulated insulin, decreased WBISI 0.44, and acanthosis nigricans, indicative of insulin resistance. Simultaneously she had early signs of alveolar hypoventilation at nocturnal polysomnography. Interestingly, she had dark brown hair and eyes.

At the time of publication, she was receiving levothyroxine and hydrocortisone.

Review of Previously and Currently Reported Cases

All 22 reported cases, including our cases 1 and 2, are summarized in **Table 1**. All POMC deficient patients had early onset obesity and adrenal insufficiency, the latter being the first diagnosis to be established. The most common presenting sign, reported in 16 of 22 cases, was neonatal hypoglycemia with or without convulsions that usually prompted further workup that led to the diagnosis of adrenal insufficiency. It is worth noticing that in eight of 22 cases there was a preceding hyperbilirubinemia which may signify impending liver failure due to adrenal insufficiency. In five cases there were reports of siblings who had died from liver failure or sepsis within a few months of life (4, 10, 11, 21, 22). A *postmortem* analysis confirmed POMC deficiency in one of these cases (4), which suggests that some patients may succumb to complications of untreated adrenal insufficiency before the diagnosis of POMC deficiency is even made.

Due to lack of uniformity in case presentations, there is a considerable discrepancy in reported clinical features with a possibility of under-reporting, which is one of the limitations of this review article. The most notable discrepancy is the presenting age of signs or symptoms, ranging from the neonatal period to 4.3 years. The majority of the cases report only on childhood; three cases also include adolescence, and of these only one, our case, extends to adulthood.

Since present in all the cases early onset obesity and adrenal insufficiency were not included in the table. The summary of presenting symptoms and clinical characteristics is presented in **Figure 4**; however, the prevalence of characteristics that may appear with time could be underestimated due to the young age of patients having been reported.

All genetic variants associated with autosomal recessive POMC deficiency are listed in **Table 1** and marked in the *POMC* gene schematic in **Figure 5**.

DISCUSSION

POMC deficiency is an ultra-rare syndrome. At the time of writing this paper, there have been only 22 cases reported in the literature, including our two cases. Despite its extreme rarity, it should not be considered a mere exotic oddity. It is a treatable condition with three distinct features that enable early diagnosis. Adrenal insufficiency, early onset obesity, and red hair/fair skin are the most common and recognizable features, but as demonstrated in this review, there is a high variability of the phenotype that is often supplemented by additional endocrine disorders such as central hypothyroidism, hypogonadotropic hypogonadism, and type 1 diabetes that shed a new perspective on the pathophysiology of POMC deficiency. Furthermore, our long-term follow-up of a patient with POMC deficiency yielded some interesting insights into the natural course of the disease through the period of adolescence and early adulthood.

Present in all the cases, adrenal insufficiency is usually the first clinical feature and often occurs neonatally with hypoglycemia, hyperbilirubinemia, or signs of liver failure (**Table 1** and **Figure 4A**). The diagnosis of adrenal insufficiency, in most cases, is established within the first year, although in some cases, the clinical features are not evident, and the diagnosis can be delayed, even up to 9 years (21). The weight gain is independent of hydrocortisone replacement treatment. Newborns are usually of normal weight but gain weight at an accelerated pace and develop extreme obesity within a year. The infants are hyperphagic with uncontrollable hunger. At this point, POMC deficiency is very likely, and diagnosis should be considered.

The presence of red hair and fair skin makes the probability of diagnosis even more likely. The characteristic pigment changes in POMC deficiency are caused by the absence of MC1R stimulation by α-MSH (24, 25). When stimulated, MC1R shifts the expression of pheomelanin (red pigment) towards eumelanin (dark pigment). While α-MSH acts as the main MC1R agonist, Agouti-related protein (AgRP) acts as an MC1R antagonist (26). In reported POMC deficient patients (**Table 1** and **Figure 4B**), the whole spectrum of pigmentation phenotype can be observed. It ranges from characteristic red hair, fair skin, and eye phenotype described only in 11 out of 22 patients (50%), lighter than expected hair or reddish-brown hair (27%), up to dark brown hair and eyes described in five patients including our female patient (23%) (10, 11, 13, 15). Previously reported hair pigment analyses in the dark-haired patients demonstrated a markedly increased amount of pheomelanin and pheomelanin/eumelanin ratio (11, 15), signifying unopposed AgRP action on MC1R. Interestingly, eumelanin was either close to normal (15) or even elevated (11), likely a result of a constitutive, α-MSH independent MC1R signalization that was previously demonstrated in mice and humans (27, 28). While the complete deficiency of POMC results in the absence of all melanocortins and should provide a phenotype with red hair and fair skin, other gene variants that still permit the synthesis of γ-MSH might provide enough MC1R stimulation to allow some pigmentation. Despite this residual MC1R stimulation, these patients still have adrenal insufficiency since they lack ACTH which is the only ligand for MC2R that is present only in the adrenal cortex. In our dark-haired girl, the genetic variant was located downstream of γ-MSH (**Figure 5**). Yet, this mutation was reported in another patient, which presented with red hair (12). Actually, in most dark-haired patients, even γ-MSH synthesis is abolished (9, 10, 13, 15, 22); dark hair color phenotype in these patients, therefore, seems not to result from any residual POMC-derived melanocortin. Furthermore, there are few other reports of patients displaying different phenotypes with either red or

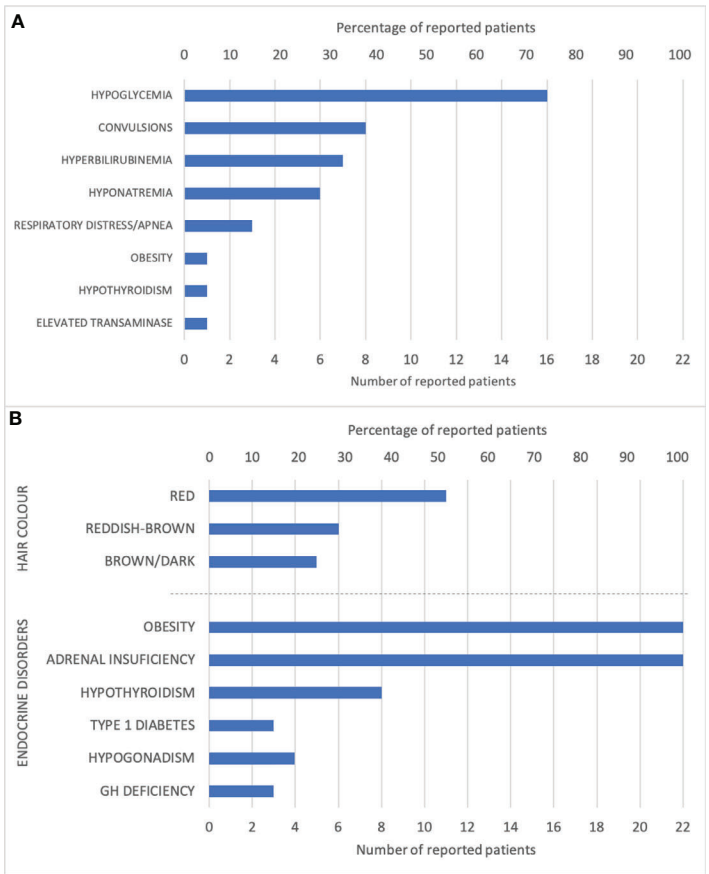

FIGURE 4 | Graphic representation of **(A)** the frequency of first presenting signs or symptoms and **(B)** the frequency of clinical features.

brown hair while having the same genotype (7, 10, 16, 23). The common denominator of patients with darker pigmentations seems to be a non-Caucasian ethnic origin, including our female patient that is of Romani descent. In clinical practice, therefore, one should not rely on pigmentation phenotype when confronted with a patient having other clinical signs of POMC deficiency. The skin phenotypes seem to depend on other inherited factors influencing skin pigmentation such as those characteristic of certain ethnic origins and probably associated with augmented constitutive MC1R signalization. Interestingly, setmelanotide, which is an MC4R agonist, increases pigmentation in all POMC deficient patients, including our male patient, but also in some leptin receptor (LEPR) and MC4R deficient patients and even in obese control subjects (4, 29). This observation likely at least partly results from a previously demonstrated ability of setmelanotide to also stimulate MC1R (29). However, human melanocytes express MC4R as well, whose stimulation increases melanogenesis (30).

Outside of the classical triad, other comorbidities are often found along with POMC deficiency. Most frequently occurring is subclinical central hypothyroidism, reported in eight out of 22 patients (36%) (7, 14, 16, 22). Whether there is a direct correlation between the absence of melanocortins and what predisposes a subset of patients to this phenotype remains to be determined. In our girl patient, hypothyroidism was not congenital, since she had confirmed adequate TSH secretion in the first months of life, while central hypothyroidism was evident by 7 months of age. A similar course of thyroid axis function was observed in the patient described by Hung et al. (14). The interrelationship between the hypothalamic–pituitary–thyroid (HPT) axis, nutrition, and energy balance has been well established. The thyrotropin-releasing hormone (TRH) secretion is normally reduced during starvation to reduce the basal metabolic rate to conserve fat and energy stores. The effect is then reversed by ingestion of food and surge of leptin (31), which stimulates the HPT axis and increases the energy consumption in an abundance of nutrients. It has been demonstrated that TRH secretion is stimulated by leptin directly and indirectly through melanocortin signaling pathways with binding the α-MSH to MC4R (32). Therefore, it would be expected to observe a well-stimulated HPT axis in obese hyperphagic individuals. Moderately elevated TSH values have indeed been consistently observed in obese populations where subclinical hypothyroidism has been excluded. It has been

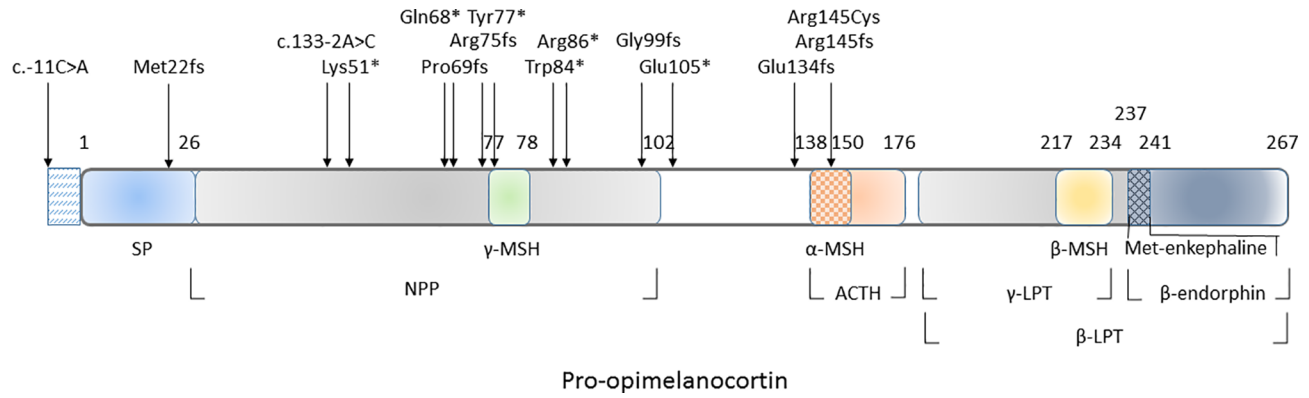

FIGURE 5 | Schematic representation of POMC-derived peptides and genetic variants associated with autosomal recessive POMC deficiency. ACTH, corticotropin; β-LPT, lipotropin β; γ-LPT, lipotropin γ; α-MSH, melanotropin α; β-MSH, melanotropin β; γ, MSH, melanotropin γ; NPP, N-terminal peptide of proopiomelanocortin; SP, signal peptide.

postulated that elevated thyroid hormone levels were an adaptive mechanism to increase the resting energy expenditure and reduce the conversion of energy into fat (33). Instead, in POMC deficiency, central hypothyroidism seems to be a common occurrence, and the absence of MC4R stimulation could very well be the culprit here. The diminished leptin effect on TRH stimulation is also evident in LEPR deficiency where central hypothyroidism is observed in 13% of cases (34). It would be interesting to observe whether the stimulation of MC4R with an agonist such as setmelanotide would improve central hypothyroidism to any extent, but unfortunately, there were no reports of such results in the literature to date.

The interrelation between melanotropins and somatotrophs is, to the best of our knowledge, unknown. Yet, in humans, MC4R deficiency is associated with increased height gain as compared to similarly obese control population, comparable and normal IGF-1 and IGF-2 values, and increased GH secretion (35). In MC4R knock-out mice, to the contrary, GH and IGF-1 suppression was observed recovered when hyperphagia was prevented and hyperinsulinemia reversed (36). Early growth acceleration was observed in both Slovenian patients (**Figure 2**) and is reported in all other case presentations. Nevertheless, GH deficiency (11) and decreased IGF-1 (9) were documented in three adolescents, including our male patient. While having increased IGF-1 in childhood, our patient had markedly decreased growth velocity at the time of anticipated puberty and confirmed GH deficiency. However, as the delayed puberty finally commenced, so did spontaneous growth acceleration. In childhood, the reported IGF-1 levels were within the normal range (13, 21, 22). As most patients were prepubertal when reported, the prevalence of growth hormone deficiency in adolescence in our review could be underestimated. Of note, setmelanotide therapy did not significantly affect IGF-1 levels in the two reported patients with GH deficiency, nor did a significant weight loss (9). On the contrary, after 3 years of setmelanotide treatment and significant weight loss, our male patient had a normal IGF-1.

Energy metabolism and reproduction are tightly linked, as the process of fertility requires proper energy reserves. Obesity, particularly when combined with insulin resistance and/or type 2 diabetes, is closely related to hypogonadotropic hypogonadism. There is an inverse correlation between free testosterone concentration and BMI as well as insulin resistance (37), and it is not restricted only to adult men. Even obese adolescent boys have 40% lower free testosterone concentrations compared to lean counterparts, and 40% of these obese individuals have subnormal testosterone concentrations (38). The circulating insulin and leptin levels, both deranged in POMC deficient patients (9), seemingly play a pivotal role in affecting the HPG axis (39). Currently, the most widely accepted mechanism of diabesity-related hypogonadism is that insulin and leptin resistance diminishes the stimulatory function of kisspeptin neurons on the secretion of GnRH. Furthermore, POMC neurons have direct synaptic connections with GnRH neurons (39), and α-MSH affects LH secretion depending on the ovulatory cycle in women (40). Kisspeptin, which is a regulator of GnRH neurons, has bidirectional communication with POMC neurons and is implicated in energy metabolism (41). This highly complex system involved in the regulation of GnRH secretion is required for evolutionary fitness. Some effects of POMC deficiency on the HPG axis should not be very surprising. The data on pubertal development in patients with POMC deficiency so far is scarce, particularly in males. Two girls lacked normal pubertal development with lower gonadotropins, which was not reversed by setmelanotide (9, 11). Another girl was reported to have spontaneous earlier normal puberty with late normal timing of menarche (17), indicating slower progressing puberty; however, one of the *POMC* gene variants in this girl targeted only ACTH and α-MSH and not the other POMC-derived peptides. Low gonadotropins during anticipated mini-puberty were observed in a 2.5-month-old girl, indicating congenital hypogonadotropic hypogonadism (23). The data suggest the follow-up for possible hypogonadism in girls with

POMC deficiency as reasonable. The only male-related data derives from our patient who underwent delayed, spontaneous puberty and achieved low normal testicular volume and scarce androgen-dependent hairiness in adolescence. The patient had noticed significant body hair growth and began shaving at age of 22, which was a year after setmelanotide treatment had commenced. At the age of 25, on therapy, he had fully developed body hair with normal levels of gonadotropins and testosterone. The positive effect of weight loss on HPG and testosterone concentration is well established. Whether, in our patient, it was a direct effect of setmelanotide treatment and/or indirect effect of weight loss remains to be determined.

Another curious phenomenon that came to be noticed in our review was a surprisingly high incidence of T1D. Of all 22 cases, there were three patients with T1D (18, 21), including our male patient, which indicates a possible pathogenetic association between the two diseases. The research on rodents has proven an association between melanocortin signaling pathways and insulin action where central stimulation of MC4R leads to inhibition of insulin secretion *via* the peripheral neuronal pathway (42). In contrast, in POMC deficiency, there is a complete absence of MC4R stimuli. In the presence of autoantibodies in two of the reported cases (18, 21), the autoimmune etiology is the more probable cause. The anti-inflammatory effects of melanocortins have been well established *in vitro* and *in vivo* (43). In this regard, it would be reasonable to expect more autoimmune occurrences; yet, apart from T1D there were no such reports in POMC deficient patients. However, given the young age of most reported patients, it is too early to draw any meaningful conclusions. Considering the severe obesity and overt clinical signs of insulin resistance in some reported cases, including our 4-year-old girl, it would be reasonable to anticipate the eventual occurrence of type 2 diabetes, that according to the published data at young ages, seems to be rare (3).

In the 22 POMC deficient patients reviewed here, 15 different pathogenic variants in the *POMC* gene were identified (**Table 1** and **Figure 5**). Except for a single missense variant that affects α-MSH and ACTH peptides (17), all other variants are null-variants abolishing at least α-MSH, ACTH, and all other C-terminal peptides. The most common variant identified in six unrelated patients of Caucasian ancestry (7, 17, 18, 20) is c.-11C>A, which introduces a premature translation signal and subsequent false translation (4). The variant c.133-2A>C affects a splice site of the third exon, which encodes POMC from the 45th amino acid onwards (21). The current genetic data suggest that α-MSH and ACTH deficiencies were sufficient for the main phenotypic features of the disease. The data on minor or less frequently reported phenotypes were too scarce to provide further estimations on the potential roles of other POMC-derived peptides.

The main limitation of our review is the small number of reported cases and particularly the young age of most reported patients, which prevented us from any firm conclusions on disease complications that could become evident in adolescence or adulthood.

CONCLUSIONS

As case reports continue to accumulate, the POMC deficiency reveals to be a more complex endocrine disorder than initially perceived and goes beyond its characteristic triad of adrenal insufficiency, early onset obesity, and red hair. The high hierarchal position of POMC and the multitude of melanocortin receptors and ligands explain its involvement in many neuroendocrinological functions, particularly in energy management, but also in skin and hair pigmentation, reproductive function, and growth. The absence of POMC may be associated with additional endocrine dysfunctions at least in a subset of patients. However, the exact role of POMC and the derived peptides in these endocrine functions remains to be determined. Ours and previously published experiences advocate for careful endocrine follow-up of POMC deficient patients through childhood and adolescence. Further collection of data for estimating the natural course of the disease and/or therapeutic effects of setmelanotide on hypothalamic-pituitary functions is needed.

ETHICS STATEMENT

Written informed consents using local consent forms were obtained from the patient 1 and parents of the patient 2 for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

UG and NG contributed to the study concept and design. MD and JK performed the molecular genetic analysis and data analysis with interpretation. NG, NB, MZ, JS, and MA collected the clinical data. NG, UG, and MA analyzed data obtained by the systematic review. NG and MA drafted the paper. PK and TB reviewed and edited the paper. MA is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

This work received the financial support from the Slovenian Research Agency (research core funding No. P3-0343).

ACKNOWLEDGMENTS

We are grateful to our adult patient and the parents of the girl for their kind participation in this work. We also gratefully thank Ms Tamara Grgic, Ms Jurka Ferran, and Ms Eva Đalić for their expert technical assistance.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 28 July 2021
doi: 10.3389/fendo.2021.727004

Profiling of Insulin-Like Growth Factor Binding Proteins (IGFBPs) in Obesity and Their Association With Ox-LDL and Hs-CRP in Adolescents

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Insulin-like growth factor binding proteins (IGFBPs) are critical modulators of metabolism. In adults, IGFBPs are associated with obesity and insulin resistance. However, the association of IGFBPs with metabolic homeostasis in children and adolescents is not yet fully characterized. In this study we investigated the association of plasma IGFBPs (IGFBP-1, 3 and 7) with weight, central adiposity and cardiovascular disease markers Hs-CRP and Ox-LDL. A total of 420 adolescents (age 11-14 years) were recruited from public middle schools in Kuwait. IGFBPs were measured using bead-based multiplexing while Hs-CRP and Ox-LDL were measured using ELISA. Results showed that levels of IGFBP-1 were significantly lower in obese and overweight children when compared to normal weight children. Correlation analysis showed negative association between the level of IGFBP-1 and waist circumference to height (WC/Ht) ratio. IGFBP-1 level was also negatively associated with Hs-CRP. It was also observed that the levels of IGFBP-3 and IGFBP-7 were negatively correlated with Ox-LDL. Our data demonstrate a strong negative association of IGFBP-1 with overweight/obesity, and the inflammatory marker Hs-CRP. This was not seen with the levels of IGFBP-3 and 7. The association of IGFBP-1 with central adiposity (WC/Ht ratio) was stronger than its association with BMI-for-age z-score. Therefore we suggest that IGFBP-1 could potentially be used as a sensitive biomarker for obesity and its subsequent effects in screening and monitoring of obesity-related metabolic complications in adolescents.

Keywords: adolescents, high sensitivity C-reactive protein, insulin-like growth factor binding proteins, obesity, oxidized low-density lipoprotein

OPEN ACCESS

Edited by:
Giulio Maltoni, Sant'Orsola-Malpighi Polyclinic, Italy

Reviewed by:
Jelena Kotur-Stevuljević, University of Belgrade, Serbia
Adriana Franzese, University of Naples Federico II, Italy

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: *17 June 2021*
Accepted: *15 July 2021*
Published: *28 July 2021*

Citation:
Rahman A, Hammad MM, Al Khairi I, Cherian P, Al-Sabah R, Al-Mulla F, Abu-Farha M and Abubaker J (2021) Profiling of Insulin-Like Growth Factor Binding Proteins (IGFBPs) in Obesity and Their Association With Ox-LDL and Hs-CRP in Adolescents. Front. Endocrinol. 12:727004. doi: 10.3389/fendo.2021.727004

INTRODUCTION

Childhood obesity is a public health concern. The Arabian Gulf region, including Kuwait, has one of the highest reported rates of childhood obesity (1). The prevalence of overweight and obesity among school children in Kuwait has been reported to be 45% (2, 3). Obesity has a complex etiology. The major contributing factors include both genetics and lifestyle behaviors such as poor dietary habits and lack of physical activity (4). Several reports show that childhood and adolescence obesity most likely persists into adulthood (5). Obesity is associated with many complications including dyslipidemia, hypertension, heart failure, and atherosclerotic cardiovascular diseases (6–8). Because of the increased health care cost to deal with these chronic diseases and the poor quality of life for patients, tackling obesity in early life should be a public health priority.

The need for a shift from reactive to predictive, preventive and personalized medicine (PPPM) is eminent (9, 10). While precision/personalized medicine has limited applications due to several challenges, tremendous effort is directed towards the advancement and use of this approach in the general clinical practice. One of the ways through which targeted therapy may be utilized is by screening for novel and easy-to-measure molecular biomarkers. The metabolic syndrome and its related pathologies, especially obesity, are in heightened need for such therapy options since they cause tremendous economic burdens on the healthcare systems (11). Identifying novel biomarkers, which could be used in detecting metabolic complications associated with obesity, is important for risk stratification and for monitoring and evaluating intervention programs. Personal variability related to genes, environment and lifestyle are taken into account when precision medicine approach is considered to be used (9).

Like insulin, insulin-like growth factors (IGFs) regulate diverse physiological functions related to growth, development and glucose homeostasis, which occur through common signaling pathways (12). When IGF-I and IGF-II were first described in the early fifties, they were presented as skeletal growth factors responsive to pituitary growth hormones (GH) and involved in the regulation of whole-body growth. Later investigations revealed that these molecules display homology to proinsulin and that they are regulated by multiple factors other than GH. The transport, metabolism and signaling by the IGFs are modulated by a family of binding proteins, which is comprised of six IGF binding proteins (IGFBP-1 – 6) as well as the IGFBP-related proteins (IGFBP-rP1, designated as IGFBP-7) (12–14). The different IGFBPs poses distinct features that allow them to specifically bind to certain receptors or translocate to various cellular compartments to mediate IGF-independent actions (14). Furthermore, different tissues produce different IGFBPs and their functions vary according to the metabolic conditions surrounding them (15). IGFBPs have been implicated in the development and pathogenesis of obesity and its related comorbidities like diabetes, metabolic syndrome and cardiovascular diseases (CVDs) through IGF-dependent as well as IGF-independent roles (12, 13).

Despite their significant sequence homology and their common ability to bind to IGFs with similar affinity, IGFBPs have unique structural features and distinct functions. This is mainly due to the different functional motifs of the family members. For examples, IGFBP-1 has an integrin-binding RGD (Arginine, Glycine, and Aspartate) motif and therefore can mediate cell migration. IGFBP-3 on the other hand does not have this RGD motif, but has many others including a nuclear localization sequence and a heparin binding domain (14).

The association of IGFBPs with obesity, metabolic syndrome and diabetes has been the subject of many investigations. Of these, IGFBP-1 has been consistently shown to be inversely associated with overweight and obesity (16–18), plasma insulin and glucose levels (19, 20), as well as fasting plasma leptin levels (21). Low serum IGFBP-1 levels have been reported to be predictive of the development of diabetes (22–24). The association of IGFBP-1 with metabolic homeostasis has been consistent across gender, different age groups and across various ethnicities (21, 25).

IGFBP-3 is the most abundant protein among all the IGFBPs and it transports more than 90% of IGF-I and IGF-II in circulation as a ternary complex with IGFs (12). However, its association with weight, metabolic syndrome and glucose homeostasis has not been consistent across studies. Some studies reported increased level of IGFBP-3 in overweight and obese subjects (16), while others reported no association with weight (26). In a prospective case-control study conducted on female nurses, a positive correlation of IGFBP-3 with the development of diabetes, BMI and waist circumference was reported (27).

IGFBP-7 is the most recent addition to the IGFBPs family. It has a similar amino acid sequence and structure to other human IGFBPs and can specifically bind to IGF-I and IGF-II. It is the least studied member of the IGFBP family in the context of its association with metabolic homeostasis. The available literature suggests that its serum levels are positively associated with BMI (28) and type 2 diabetes (29), fasting glucose levels (30), insulin resistance and metabolic syndrome (28, 31). A recent study reported higher levels of IGFBP-7 in coronary artery disease (CAD) patients compared to healthy subjects (32). In addition, IGFBP-3 was reported to be negatively associated with the levels of some inflammatory markers including C-reactive protein and interleukin-6 (33), whereas high levels of serum IGFBP-7 were associated with increased CRP levels (28).

The aim of this study was thus to investigate the association of IGFBP-1, 3 and 7 with weight status, waist circumference and well-established CVD risk factors, specifically Hs-CRP and Oxidized Low-Density Lipoprotein (Ox-LDL) in a group of healthy adolescents from Kuwait.

MATERIALS AND METHODS

Study Participants

This is a cross-sectional study that was conducted in selected public middle schools from the State of Kuwait as previously

described (34–36). Study participants were adolescents in the age range of 11–14 years. Data on socioeconomic status and other covariates were collected from the parents through a self-administered questionnaire, and from the adolescents using face-to-face interview.

Ethics Statement

The study was approved by The Ethics Committee at Ministry of Health, Kuwait (No: 2015/248), the Ethics Committee of the Health Sciences Centre, Kuwait University (No: DR/EC/2338) and the Ethical Review Committee at Dasman Diabetes Institute (RA2017-026). Written informed consent was obtained from the parents and verbal assent was obtained from all the study subjects. We certify that the work conducted in this research complies with the ethical standards recommended by the Helsinki Declaration.

Blood Collection and Biochemical Analyses

A sample of venous blood (5 mL) was collected from each child in EDTA-containing tubes. Plasma was separated and stored at −80 °C till analysis. IGFBPs levels were assessed using multiplexing immunobead array platform according to the manufacturer's instructions (R & D Systems). Median fluorescence intensities were collected on a Bioplex-200 system and data were processed using the Bio-Plex Manager Software version 6 (Bio-Rad), with five-parametric curve fitting. Hs-CRP concentrations were determined using ELISA (Hycult Biotech, Cat. # HK369) following manufacturer's instructions. Optimal dilution was found to be 1:1000. Ox-LDL concentrations were determined using ELISA (Immundiagnostik AG, Germany, Cat. # K 7810) following manufacturer's instructions. Optimal dilution was found to be 1:10.

Anthropometric Measurements and Other Covariates

Standing height and bodyweight of the study participants were measured in a standardized manner, using digital weight and height scale (Detecto, Webb City, MO, USA) with the participants standing erect without shoes and wearing light clothes. BMI-for-age z-scores were calculated using WHO growth charts. Obesity was defined as BMI-for-age ≥ +3 Standard Deviation (SD), while overweight was defined as BMI-for-age > +2 SD and < +3 SD. Waist circumference (WC) was measured in the horizontal plane at the superior border of the right iliac crest to the nearest 0.1 cm with a non-stretchable tape by a trained data collector. Measurements were taken at the end of normal expiration; three readings were taken, and the average of the three was recorded. Care was taken to ensure that the tape was horizontal to the floor and touched the skin without compressing it. The ratio of waist circumference (cm) to height in centimeters (WC/Ht ratio) was calculated and the obesogenic waist was defined as a WC/Ht ratio of > 0.5 (37).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows version 26 (IBM Corp., Armonk, N.Y., USA). Data were log-transformed and checked for normality using SPSS. Data for the IGFBP in different weight status groups and in male and female were presented as bar graphs showing mean with standard deviation (SD). Association between IGFBPs and weight status categories was assessed by both univariable and multivariable linear regression adjusting for age and sex. The association of IGFBPs with Hs-CRP and Ox-LDL (both log-transformed) was also assessed by linear regression analysis. Levels of IGFBPs were categorized into tertiles and the odds of overweight/obesity (combined) were determined in various tertiles of IGFBPs using binary logistic regression without and with adjusting for age and sex. Mean differences in the IGFBPs across weight status groups, age groups and gender were assessed by one-way ANOVA and t-test for independent samples. A p-value of < 0.05 was considered as statistically significant.

RESULTS

Study Population Characteristics

Table 1 summarizes the characteristics of the participants that were involved in the study. Data were analyzed for 420 participants of whom 192 (45.7%) were male. Mean (SD) age was 12.4 (1.5) years. Of the total samples, 47% adolescents were normal weight, 21% were overweight and 32% were obese. Median (IQR) for IGFBP-1 was 5.7 (3.5, 10.0) ng/mL, while median (IQR) for IGFBP-3 and IGFBP-7 were 835.0 (688.5, 1007.8) and 17.7 (15.2, 20.0) ng/mL, respectively. Female

TABLE 1 | Characteristics of the study population.

		N	Percentage
Gender	Male	192	45.7
	Female	228	54.3
Nationality	Kuwaiti	303	71.93
	Non-Kuwaiti	117	28.07
Age groups	10 - <12 years	179	42.5
	12 - <13 years	143	34.1
	13+ years	98	23.4
Weight Status	Normal weight	198	47.1
	Overweight	88	21.0
	Obese	134	31.9

adolescents had significantly lower level of IGFBP-1 compared to males (p<0.001) (**Figure 1A**), whereas the differences in plasma levels of IGFBP-3 and IGFBP-7 between male and female subjects were non-significant (data not shown). IGFBP-1 level was significantly higher in the age group 10-<12 year old when compared to 12-<13 years (p<0.01) and 13+ years group (p<0.001) (**Figure 1B**). On the other hand, no significant difference was observed among different age groups in IGFBP-3 and IGFBP-7 levels (data not shown).

Association of IGFBPs and Weight Status

IGFBP-1 level was significantly lower in participants classified in the obese and overweight groups compared to normal weight children (p<0.01) (**Figure 1C**). No significant difference was observed in the levels of IGFBP-3 and IGFBP-7 when comparing different BMI categories (data not shown). Parallel to these results, the plasma level of IGFBP-1 was negatively associated with weight status (BMI categories) in univariable regression analysis [β (95% CI) = -0.16 (-0.20, -0.13)]. This association remained significant in the multivariable regression when adjusted for age and sex [β (95% CI) = -0.17 (-0.20, -0.13)]. The association of weight status with either IGFBP-3 or IGFBP-7 plasma levels was not significant either in multivariable regression, or in the adjusted multivariable regression (**Table 2**). In binary logistic regression, the odds of being overweight/obese (combined category) were significantly higher in the lower tertile of IGFBP-1 level when compared to the upper tertile (reference) [(OR (95% CI) = 6.82 (2.29, 20.30)] in the unadjusted model and this association sustained in the model adjusted for age and sex [(OR (95% CI) = 7.22 (3.92, 13.32)] (**Table 2**). IGFBP-3 and IGFBP-7 tertiles did not show significant association with overweight/obesity in binary logistic regression models, either unadjusted or adjusted.

Association of IGFBPs With Waist Circumference

In the univariable regression analysis, the level of IGFBP-1 was negatively associated with WC/Ht ratio [β (95% CI) = -1.72 (-2.11, -1.33); p<0.001], and this association remained significant after adjusting for age and sex [β (95% CI) = -1.72 (-2.07, -1.37), p<0.001]. IGFBP-3 and IGFBP-7 levels did not show significant association with WC/Ht ratio either in the univariable or multivariable regression (**Table 3**). In the binary logistic regression, the odds of obesogenic waist, defined as a WC/Ht ratio of > 0.5, was significantly higher in the lower tertile of IGFBP-1 level when compared to the higher tertile [OR (95% CI) = 4.55 (2.74, 7.56)] in the unadjusted model. This remained significant in the model adjusted for age and sex [OR (95% CI) = 5.70 (3.26, 9.96)]. The tertiles of IGFBP-3 and IGFBP-7 levels did not show significant association with obesogenic waist in binary logistic regression models, whether they were adjusted or unadjusted (**Table 3**). When adjusted for age and sex, the negative association of IGFBP-1 level (log-transformed) with WC/Ht ratio [β (95% CI) = -1.73 (-2.08, -1.38)] was stronger than its association with the BMI-for-age z-scores [β (95% CI) = -0.11 (-0.13, -0.09)].

Association of IGFBPs With Markers of Oxidative Stress

In linear regression analysis, plasma level of Ox-LDL was not associated with IGFBP-1 level (β=0.06; p=0.40). However, the level of Ox-LDL was negatively associated with the levels of IGFBP-3 (β=-0.18; p=0.001) and IGFBP-7 (β=-0.02; p<0.001) (**Figures 2A–C**). On the other hand, plasma level of Hs-CRP was negatively associated with the level of IGFBP-1 (β=-0.30; p=0.001). No significant association was found between the level of Hs-CRP with either the level of IGFBP-3 or the level of IGFBP-7 in plasma (**Figures 2D–F**). We further analyzed mean differences of Ox-LDL and Hs-CRP levels across different tertiles of the levels of IGFBPs. The results are shown in **Figure 3**. The level of Ox-LDL was significantly higher in the lower tertiles of IGFBP-3 and IGFBP-7 levels compared to the middle and higher tertiles, whereas no differences were observed between Ox-LDL level in the middle and upper tertiles (**Figures 3B, C**). The level of Ox-LDL across the three tertiles of the level of IGFBP-1 was not significantly different (**Figure 3A**). On the other hand, the level of Hs-CRP across the three tertiles of IGFBP-3 and IGFBP-7 levels was not significantly different (**Figures 3E, F**). However,

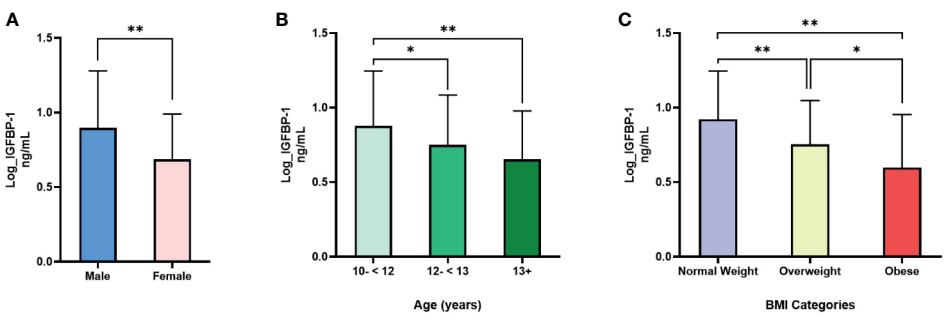

FIGURE 1 | Differences in IGFBP-1 levels based on **(A)** sex, **(B)** age and **(C)** weight status: **(A)** IGFBP-1 are significantly lower in females compared to males; N=191 males, 228 females; t=6.18, p<0.001; **(B)** IGFBP-1 levels are significantly reduced with age; N=178 (10-<12), 143 (12-<13) and 98 (13+); **(C)** IGFBP-1 is reduced in overweight and obesity compared to normal weight; N= 198 normal weight, 88 overweight, 135 obese. Data is presented as means ± SD. Males and females were compared by t-test for independent samples, whereas, age groups and weight status groups were compared with one-way ANOVA. *p < 0.01; **p < 0.001.

TABLE 2 | Association between IGFBPs and weight status in adolescents.

	Linear Regression					
	β[1]	95% CI	p	β[2]	95% CI	p
IGFBP-1 (log)	-0.16	-0.20, -0.13	<0.001	-0.17	-0.20, -0.13	<0.001
IGFBP-3	-0.18	-35.40, 36.04	0.99	-0.18	-35.53, 35,18	0.78
IGFPB-7	0.02	-0.47, 0.51	0.93	0.02	-0.47, 0.50	0.78
	Logistic Regression					
	OR[1]	**95% CI**	**p**	**OR[2]**	**95% CI**	**p**
IGFBP-1						
Lower Tertile	6.82	2.29, 20.30	<0.001	7.22	3.92, 13.32	<0.001
Middle Tertile	1.79	0.51, 6.26	0.25	1.53	0.83, 2.79	0.08
Upper Tertile	1.00	Ref		1.00	Ref	
IGFBP-3						
Lower Tertile	1.56	0.58, 4.18	0.38	1.57	0.58, 4.22	0.38
Middle Tertile	0.82	0.27, 2.51	0.50	0.83	0.27, 2.57	0.49
Upper Tertile	1.00	Ref		1.00	Ref	
IGFBP-7						
Lower Tertile	0.81	0.34, 1.94	0.37	0.80	0.33, 1.94	0.36
Middle Tertile	0.96	0.42, 2.22	0.67	0.98	0.42, 2.27	0.65
Upper Tertile	1.00	Ref		1.00	Ref	

[1]*Unadjusted.*

[2]*Adjusted for age categories and sex.*

In linear regression, IGF binding proteins (IGFBPs) were used as continuous variable (independent variable) and weight status (dependent variable) was categorized as normal weight, overweight or obese based on the WHO cutoffs of the BMI z-scores. N= 419 (IGFBP-1), 332 (IGFBP-3) and 428 (IGFBP-7). IGFBP-1 was log-transformed for normality.

Odds Ratios (OR) were calculated using binary logistic regression in which the response variable (weight status) was categorized into normal weight or overweight/obese, with the normal weight as the reference category.

the level of Hs-CRP was significantly higher in the lower tertile of IGFBP-1 level when compared to the middle and upper tertiles. Furthermore, the level of Hs-CRP was not significantly different between the middle and upper tertiles of IGFBP-1 level (**Figure 3D**).

DISCUSSION

In this study we investigated the association between obesity and the levels of various IGFBPs (particularly, IGFBP-1, 3 and 7) in a group of Kuwaiti adolescents. Our findings show that the plasma

TABLE 3 | Association of IGFBPs with waist-to-height ratio in adolescents.

	Linear Regression					
	β[1]	95% CI	p	β[2]	95% CI	p
IGFBP-1 (log)	-1.72	-2.11, -1.33	<0.001	-1.72	-2.07, -1.37	<0.001
IGFBP-3	86.13	-301.12, 473.37	0.66	89.21	-299.35, 477.78	0.65
IGFPB-7	1.24	-4.03, 6.50	0.65	1.19	-4.08, 6.47	0.66
	Logistic Regression					
	OR[1]	**95% CI**	**p**	**OR[2]**	**95% CI**	**p**
IGFBP-1						
Lower Tertile	4.55	2.74, 7.56	<0.001	5.70	3.26, 9.96	<0.001
Middle Tertile	1.26	0.78, 2.03	0.35	1.44	0.87, 2.39	0.16
Upper Tertile	1.00	Ref		1.00	Ref	
IGFBP-3						
Lower Tertile	1.05	0.62, 1.80	0.84	1.06	0.62, 1.80	0.84
Middle Tertile	1.06	0.62, 1.81	0.83	1.05	0.62, 1.80	0.85
Upper Tertile	1.00	Ref		1.00	Ref	
IGFBP-7						
Lower Tertile	0.77	0.48, 1.23	0.28	0.77	0.48, 1.24	0.28
Middle Tertile	1.10	0.69, 1.76	0.68	1.11	0.69, 1.77	0.68
Upper Tertile	1.00	Ref		1.00	Ref	

[1]*Unadjusted.*

[2]*Adjusted for age categories and sex.*

In linear regression, IGF binding protein (IGFBP) and WC/Ht ratio were used as continuous variables N= 419 (IGFBP-1), 332 (IGFBP-3) and 428 (IGFBP-7). IGFBP-1 was log-transformed for normality.

Odds Ratios (OR) were calculated using binary logistic regression in which the response variable (WC/Ht ratio) was categorized into non-obesogenic waist (WC/Ht ratio ≤ 0.5 and obesogenic waist (WC/Ht ratio > 0.5), with the normal non-obesogenic waist as the reference category.

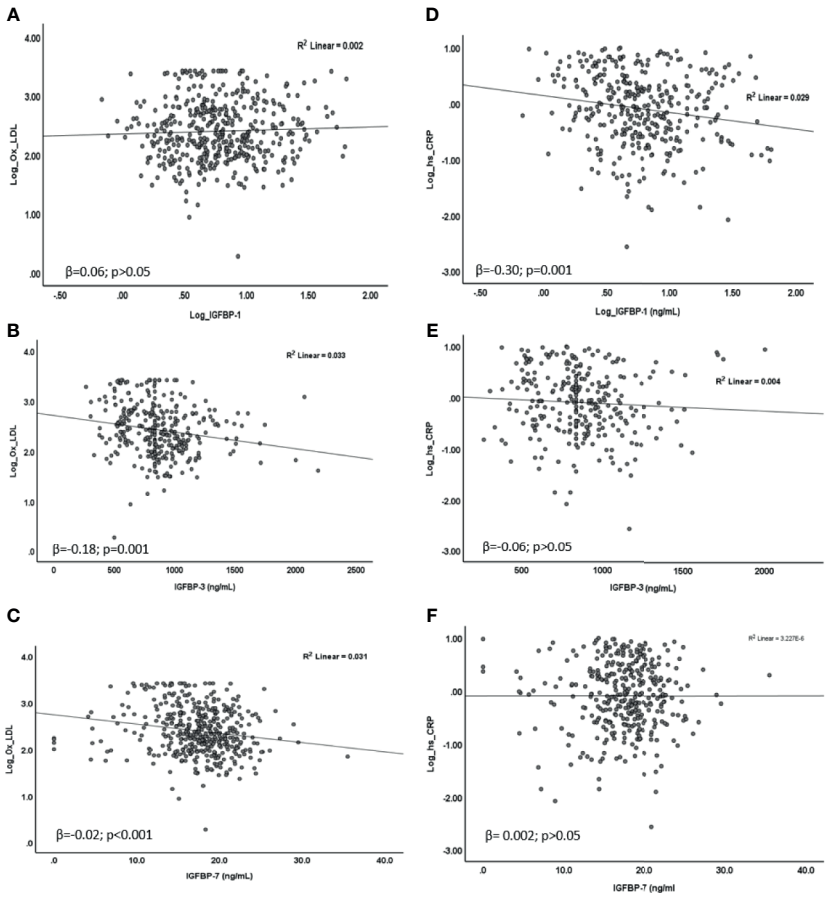

FIGURE 2 | Association of Ox-LDL **(A–C)** and Hs-CRP **(D–F)** with IGFBPs. **(A)** Ox-LDL was not associated with IGFBP-1 (β = 0.06; p = 0.40), **(B, C)** Ox-LDL was negatively associated with IGFBP-3 (β = -0.18; p = 0.001) and IGFBP-7 (β = -0.02; p < 0.001). **(D)** Hs-CRP was negatively associated with IGFBP-1 (β = -0.30; p = 0.001) **(E, F)** No significant association of Hs-CRP was found with either IGFBP-3 (β = -0.06; p > 0.05) or IGFBP-7 (β = 0.002; p > 0.05). Data were analyzed by linear regression.

level of IGFBP-1 was decreased in children who were classified in the overweight or obese group. On the other hand, plasma levels of IGFBP-3 and IGFBP-7 were not affected. Furthermore, the level of IGFBP-1 was negatively associated with WC/Ht ratio. This association was stronger than the association of IGFBP-1 level with BMI-for-age z-scores. Interestingly, a strong negative association between the level of IGFBP-1 and Hs-CRP was also observed.

Childhood obesity is on the rising trajectory, especially in the Gulf region. Early predictors of obesity complications can contribute to the reduction in obesity-related metabolic consequences. Upper body obesity (truncal obesity) has been consistently shown to be more strongly associated with obesity-related comorbidities like diabetes and CVD. Thus, we investigated the association of truncal obesity, measured by WC/Ht ratio, with IGFBPs. Only IGFBP-1 was lower in the children with obesogenic WC/Ht ratio and the other two IGFBPs were not different in the two categories of WC/Ht ratio. To our knowledge, this is the first report to specifically correlate central obesity with IGFBP-1. The overall pattern of association was similar whether we categorized the study group based on weight using BMI or based on WC/Ht ratio. However, this correlation is stronger with WC/Ht ratio when compared to the BMI-for-age z-scores, which suggests that central body adiposity has a stronger influence on the level of IGFBP-1 than the overall body adiposity. Several other studies has supported this notion that central adiposity is more strongly associated with the adverse health consequences of obesity (38–41) and that WC/Ht ratio is a better indicator of adiposity than BMI (42–44).

Hs-CRP and Ox-LDL are well established cardiometabolic risk factors. In this study we found a differential association pattern between the level of the various IGFBPs under study with markers of oxidative stress (Ox-LDL) and cardiovascular diseases (Hs-CRP). For instance, the level of IGFBP-1 showed a negative association with Hs-CRP but not Ox-LDL. On the other hand, the levels of both IGFBP-3 and 7 were significantly associated with Ox-LDL but not Hs-CRP. Obesity is a state of low-level chronic inflammation. Thus, high Hs-CRP level in individuals living with overweight or obesity is consistent with this notion. The functional as well as the causal relationship

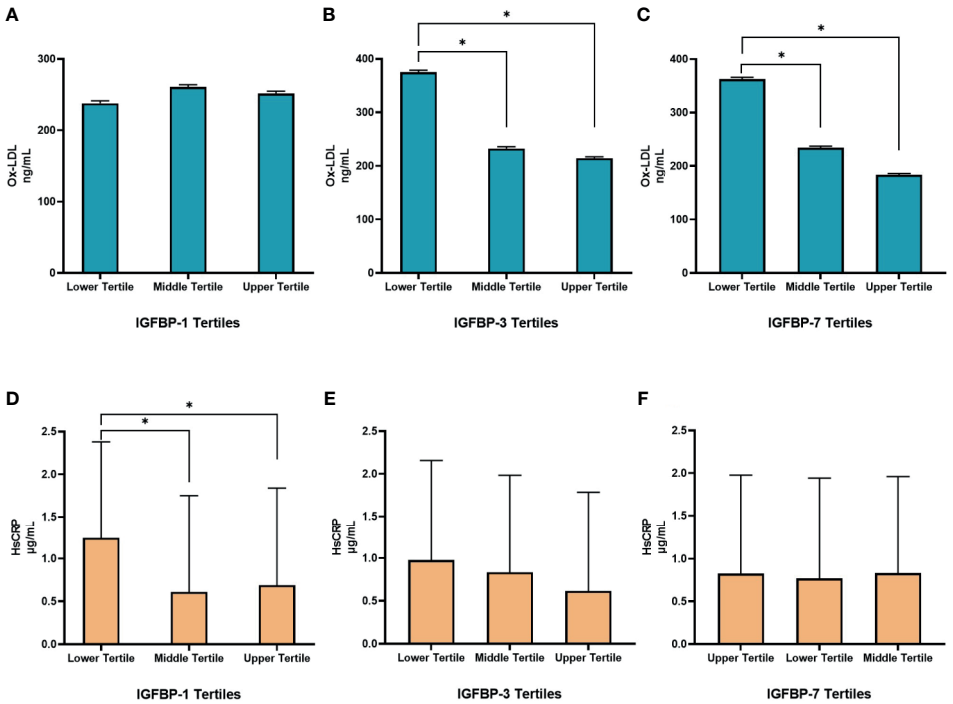

FIGURE 3 | Distribution of Ox-LDL **(A–C)** and Hs-CRP **(D–F)** in different tertiles of IGFBPs. **(A)** Levels of Ox-LDL across the three tertiles of IGFBP-1 were not significantly different. **(B, C)** The levels of Ox-LDL were significantly higher in the lower tertiles of IGFBP-3 and IGFBP-7 compared to the middle and higher tertiles, no differences were observed between Ox-LDL levels in the middle and upper tertiles. **(D)** Levels of Hs-CRP were significantly higher in the lower tertile of IGFBP-1 compared to the middle and upper tertiles but were not different between the middle and upper tertiles. **(E, F)** Levels of Hs-CRP across the three tertiles of IGFBP-3 and IGFBP-7 were not significantly different. N=144 in each group. Data is presented as geometric means ± SD. *p < 0.01 by one way ANOVA with Bonferroni post-hoc comparison.

between IGFBP-1 and Hs-CRP could not be deduced from this study. Therefore, a prospective study will possibly help delineate if the increased level of Hs-CRP are the cause or the consequence of lower level of IGFBP-1 in children with obesity.

A study conducted in Kuwait reported significantly lower levels of IGF-1 and IGFBP-3 in patients with coronary heart disease (CHD) and significant correlation between the level of IGFBP-3 and some metabolic markers including cholesterol, triglyceride (TG) and high-density lipoprotein (HDL) (45). Recently, a cross-sectional observational study conducted on 84 children under 10 years of age from two schools in Colombia demonstrated an inverse correlation of both IGFBP-1 and IGFBP-2 levels with the level of TG, as well as a direct correlation with HDL level (46). The study also reported lower level of IGFBP-1 with obesity. However, to the best of our knowledge, this is the first study to report an association of IGFBPs with Hs-CRP and Ox-LDL in adolescents. This is an important finding, since both markers are essential for detecting low inflammatory processes. It was interesting to observe that while the levels of both IGFBP-3 and -7 were not affected by obesity, they correlated negatively with Ox-LDL level. This suggests that the role of some IGFBPs in mediating inflammation and CVD can be independent of weight status and body fat composition. Further investigations are required to understand the role that both IGFBP-3 and -7 play in the development of CVDs. Our data further confirm that the different IGFBPs have distinct functional significance.

In this study the level of IGFBP-1 was negatively associated with age, which was not observed with the levels of both IGFBP-3 and 7. Although the age range of the study participants was very narrow (11-14 years), the difference in the level of IGFBP-1 was significant among various age groups. This suggests that IGFBP-1 is a sensitive biomarker when compared to the other IGFBPs. The age-dependent changes in IGFBP-1 level could be explained by changes in either body fat composition or in the level of sex hormones. The level of sex hormones is also affected by body fat composition. Therefore, body fat composition appears to be the major common determinant of IGFBP-1 level. The lower level of IGFBP-1 in girls compared to boys can also be explained by body fat composition, as girls generally have higher body fat percentage compared to boys at this age. However, the effect of changes in hormones should not be ruled out. Contrary to our results presented in this study, a Turkish group reported that serum level of IGFBP-3 are steadily increased in prepubertal children with age (47). However, due to lack of information regarding the pubertal stage of the subjects enrolled in our study, a direct comparison cannot be made. Nonetheless, such discrepancies emphasize the importance of personalized

medicine and group-specific interventions. Since therapies that could work with children from Turkish descendent might not necessarily work on children from Kuwaiti (Arab) descendent. Therefore, further studies that cover a larger population with a wider age range are necessary to determine the exact interplay between growth, the level of sex hormones, body fat composition and the levels of various IGFBPs.

To our knowledge, this is the first study that investigated the association of IGFBPs with markers of inflammation and oxidative stress (Hs-CRP and Ox-LDL). Also, for the first time, we investigated the association of IGFBPs with a measure of central body adiposity. Most studies in this field use BMI as a measure of adiposity. Although BMI is generally a good indicator of overall body fat composition, it does not discriminate between upper and lower body fat composition. It is well documented that upper body fat composition (central adiposity) is more closely associated with obesity-related comorbidities. Therefore, the fact that IGFBP-1 was more strongly associated with WC/Ht ratio than the BMI z-scores makes IGFBP-1 a sensitive biomarker for obesity-related metabolic abnormalities. Other strengths of this study include a large sample size, which was representative of the adolescent population in Kuwait, using several statistical approaches to minimize the bias in the association, and the narrow age range of the study subjects. The latter is particularly important, as it will minimize the age-dependent influence on the association between obesity and IGFBPs. Furthermore, this study is based on healthy subjects without any overt obesity-related complications. Thus it highlights the possible use of IGFBP-1 as a screening marker for metabolic disorders. However, the limitations of this study include, the cross-sectional design, which does not allow us to establish causality, and the narrow age range of the participants, which could be considered as both a strength and a limitation. The limitation could result from the inability to project a similar association between IGFBP-1 and obesity into other age groups. Finally, due to logistic restrictions, a fasting blood sample could not be obtained, and thus data on parameters of glucose homeostasis and lipid profile are lacking.

In conclusion, the study results presented demonstrate the importance of IGFBPs in childhood obesity and highlight the distinct functions of the different members of the IGFBPs family. Since, a strong negative association was detected between the level of IGFBP-1 with overweight and obesity in adolescents. This negative association was shown to be more pronounced with central adiposity when compared to overall increased body weight. Furthermore, IGFBP-1 was negatively associated with Hs-CRP, which is a marker of inflammation. On the other hand, the levels of both IGFBP-3 and 7 were not associated with body weight. Nevertheless, the levels of these proteins showed a significantly negative association with the level of Ox-LDL, which is a marker of oxidative stress. Together these data further illustrate the possibility of using IGFBP-1 as a sensitive marker for obesity-related inflammation and its related comorbidities. The identification of such markers, especially in younger subjects, is a step towards the advancement in the field of Predictive, Preventive and Personalized Medicine.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee at Ministry of Health, Kuwait (No: 2015/248), The Ethics Committee of the Health Sciences Centre, Kuwait University (No: DR/EC/2338), The Ethical Review Committee at Dasman Diabetes Institute (RA2017-026). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization, AR, MA-F, and JA. Methodology, MH, IA, and PC. Software, AR. Validation, MH, IA, and PC. Formal analysis, AR. Investigation, AR, MA-F, and JA. Resources, AR, MA-F, and JA. Data curation, AR, MH, IA, and PC. Writing—original draft preparation, MH and AR. Writing—review and editing, AR, MH, RA-S., FA-M, MA-F, and JA. Supervision, MA-F and JA. Project administration, AR, MA-F, and JA. Funding acquisition, AR, MA-F, and JA. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Dasman Diabetes Institute project No. RA 2017-026 and Kuwait University Project No. WF02/13.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 02 August 2021
doi: 10.3389/fendo.2021.684011

An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 22 March 2021
Accepted: 31 May 2021
Published: 02 August 2021

Citation:
Casertano A, Rossi A, Fecarotta S, Rosanio FM, Moracas C, Di Candia F, Parenti G, Franzese A and Mozzillo E (2021) An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. Front. Endocrinol. 12:684011. doi: 10.3389/fendo.2021.684011

Hypoglycemia is the result of defects/impairment in glucose homeostasis. The main etiological causes are metabolic and/or endocrine and/or other congenital disorders. Despite hypoglycemia is one of the most common emergencies in neonatal age and childhood, no consensus on the definition and diagnostic work-up exists yet. Aims of this review are to present the current age-related definitions of hypoglycemia in neonatal-pediatric age, to offer a concise and practical overview of its main causes and management and to discuss the current diagnostic-therapeutic approaches. Since a systematic and prompt approach to diagnosis and therapy is essential to prevent hypoglycemic brain injury and long-term neurological complications in children, a comprehensive diagnostic flowchart is also proposed.

Keywords: neonatal hypoglycemia, childhood hypoglycemia, inborn errors of metabolism, endocrine hypoglycemia, glucose homeostasis, congenital hyperinsulinism

Abbreviations: AAP, American Academy of Pediatrics; ACTH, Adreno–CorticoTropic Hormone; AI, Adrenal Insufficiency; BAPM, British Association for Perinatal Medicine; CAH, Congenital Adrenal Hyperplasia; CAI, Central Adrenal Insufficiency; CDG, Congenital Disorders of Glycosylation; CH, Congenital Hyperinsulinism; CoA, Coenzyme A; CPT, Carnitine Palmitoyl–Transferase; CRH, Corticotropin–Releasing Hormone; DZX, Diazoxide; FAOD, Fatty Acid Oxidation Disorders; FBPase, Fructose 1,6 bisphosphatase; FFA, Free Fatty Acids; G6Pase, Glucose–6–Phosphatase; GALT, Galactose–1–phosphate uridyltransferase; GDH, Glutamate Dehydrogenase Enzyme; GH, Growth Hormone; GHD, Growth Hormone Deficiency; GHRH, Growth Hormone–Releasing Hormone; GKD, Glycerol Kinase Deficiency; GLP–1, Glucagon–Like Peptide–1; GSD, Glycogen Storage Disease; HFI, Hereditary Fructose Intolerance; HY, Hypoglycemia; IGF1, Insulin Growth Factor1; IMD, Inborn Metabolic Disorder; IVA, Isovaleric Acidemia; KB, Ketone Bodies; LGA, Large for Gestational Age; MMA, Methylmalonic Acidemia; MRI, Magnetic Resonance Imaging; NBS, Newborn Screening; NGS, Next Generation Sequencing; OA, Organic Acidemias; OXPHOS, Oxidative Phosphorylation; PA, Propionic Acidemia; PAI, Primary Adrenal Insufficiency; PC, Pyruvate Carboxylase; PEPCK, Phosphoenolpyruvate Carboxykinase; PES, Pediatric Endocrine Society; PET, Positron Emission Tomography; PG, Plasma Glucose; SGA, Small for Gestational Age; UCCS, Uncooked cornstarch; UDP, Uridine Di–Phosphate; UOA, Urine Organic Acids.

INTRODUCTION

Hypoglycemia (HY) in pediatric age shows some peculiarities regarding its diagnosis and management, mostly linked to age dependent features in glucose homeostasis and to the broad spectrum of causes. Such causes can initially present with the same unspecific picture, but they require different treatment (1). While being frequent but hard to detect in neonatal age, it is less common in infants and toddlers, even rarer in older children (1–3). In childhood, HY is a common metabolic-endocrine emergency possibly causing permanent neurological consequences. It is therefore essential to promptly detect and treat children with HY as well as those at risk. It is crucial to appropriately investigate its specific etiology for providing adequate and specific therapy (3, 4). In this review, we present current knowledge on management of HY in neonates and children including difficulties in establishing thresholds for both definition and therapeutic intervention and providing a comprehensive overall diagnostic approach through the use of a simple practical flowchart (5–8).

CONTROVERSIES ABOUT CLINICAL AND BIOCHEMICAL DEFINITION OF HYPOGLYCEMIA

Glucose is the primary energy source for central nervous system metabolism, independently from the feeding state (1). Several metabolic pathways cooperate to ensure normal blood glucose concentrations in the fasted state (**Figure 1**). Such pathways are tightly regulated by the hormonal (insulin, glucagon, cortisol, and growth hormone) and autonomic (catecholamines) response. In case of impaired metabolic pathways and/or altered hormonal regulation, glucose could become too low to satisfy neuronal demand, causing classical symptoms of HY. In pediatric age, both glucose homeostasis and clinical presentation of HY show peculiarities compared to adults. In newborns, the adaptation to extrauterine life, characterized by immature hormonal and enzymatic pathways, and the higher glucose requirement of the brain, lead to a higher HY risk compared to older children and adults (9). Infants and children, have less glycogen storage and a higher substrates demand.

HY definition remains controversial in neonates and children. Some approaches define HY on the basis of symptoms, others on the plasma glucose value. In adolescents and adults, HY definition is based on the so-called "Whipple triad" [(I) symptoms of HY, (II) blood glucose level below 60 mg/dl, (III) resolutions of symptoms after glucose intake]. This definition appears inadequate for neonates and children in which symptoms are often subtle, and with the child being unable to communicate them (10). In addition, it is difficult to identify a single plasma glucose (PG) value below which symptoms of HY appear: in fact, symptoms appearance depends on additional factors, including the availability of alternative energy substrates (e.g. ketone bodies) and the severity, duration, and recurrence of low PG (7). Neurogenic symptoms are secondary to the neuroendocrine response, while neuroglycopenic symptoms are due to the low glucose availability to the brain. In neonates and infants, neurogenic and neuroglycopenic symptoms are not specific for HY. Therefore HY can be defined as the individualized condition in which PG concentration is low enough to cause symptoms and/or signs of impaired brain function (11).

Based on the above-mentioned considerations, three age-based different clinical scenarios exist:

i. *Neonates <48 h of life*: signs or symptoms of neonatal HY may vary from severe (e.g. lethargy, tachypnea, hemodynamic instability, apnea, seizures, or even cardiac arrest) to milder (e.g. abnormal cry, decreased feeding, jitteriness, irritability, pallor, cyanosis, hypothermia, or diaphoresis) (12). In neonates showing specific symptoms, HY is diagnosed when PG is lower than a specific threshold: 47 mg/dl according to American Academy of Pediatrics (AAP) (13, 14), and 50 mg/dl according to Pediatric

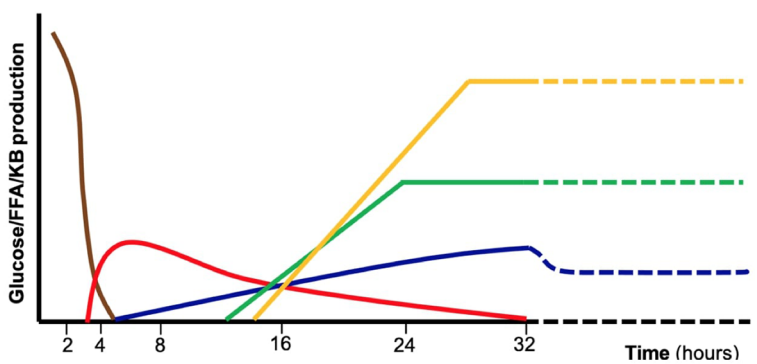

FIGURE 1 | Schematic representation of the major metabolic pathways involved in glucose homeostasis during absorptive phase and fasting including exogenous carbohydrates (brown), glycogenolysis (red), gluconeogenesis (blue), fatty acid oxidation (green), ketogenesis and ketolysis (yellow). These mechanisms are tightly controlled by hormonal regulation. Defects in specific enzymes or transporters involved in those pathways as well as endocrine disorders may result in fasting intolerance and hypoglycemia. FFA, free fatty acids, KB, ketone bodies.

Endocrine Society (PES) (7). A different glucose threshold has been proposed for pre-term newborns (15).

ii. *Neonates >48 h of life, infants, and younger children unable to communicate*: HY is defined as PG <50–70 mg/dl (i.e. the normal threshold for neurogenic responses). Notably, recurrent PG levels in this range may cause the HY-associated autonomic failure, that in turn can attenuate HY autonomic symptoms (HY unawareness). Conflicting results on the definition of a safety glucose target level have emerged (16, 17). Currently, an acceptable threshold for this group is still considered 60 mg/dl (7).

iii. *Older children able to communicate their symptoms*: for children who are able to communicate their symptoms, Whipple's triad could be adopted. In this age the clinical presentation is characterized by more specific symptoms as compared to neonates. In particular, the neuroglycopenic symptoms, due to the scarce availability of glucose for the central nervous system, and so indicative for lower PG level, are more clearly recognizable.

Signs and symptoms of HY are summarized and distinguished in neonatal and infant setting (**Table 1**). Neonatal HY cut off needing for intervention according with AAP, PES, BAPM are summarized in **Table 2**.

MANAGEMENT

During the first 48 h of life many healthy neonates could experience low PG, as a consequence of the physiologic adaptation to the extrauterine life. Sometimes it could last up to weeks being clinically relevant. So, it is crucial to identify as soon as possible neonates at risk of developing pathological low PG, and possibly distinguish between persistent and transient forms. Even though there is no consensus recommendation, HY persisting beyond the first 48 h, suggests a high risk of permanent pathology. Some authors consider the persistence over 7 days of HY and/or the need for more than 10 mg/kg/minute of intravenous glucose infusion as indicative for persistent HY and anamnestic and clinical features could help to identify neonates at risk of persistent HY (18). Various glucose monitoring schemes have been proposed (**Table 3**) (18). A more practical and concise indication is that infants of diabetic mothers and LGA should be screened for 12 h after birth while SGA and preterm neonates should be screened for the first 24 h (14). Besides receiving a close glucose monitoring, at-risk neonates should be fed as soon as possible preferably with breast milk because it promotes ketogenesis, an alternate source of energy for the brain (19). Despite several interventional studies support the efficacy of the preventive use of dextrose oral gel, prior to the first hour (20–23), there is conflicting evidence regarding its ability to prevent the need for intravenous glucose infusion (24–27). Indeed, prompt treatment of HY is needed to quickly restore brain demand. Based on the severity of clinical manifestations, glucose should be administered intravenously or orally. Similarly to the diagnosis, several interventional thresholds for HY have been proposed (18, 28).

Two major age-based groups can be distinguished:

i. *Neonates*: asymptomatic neonates who cannot maintain PG above 50 mg/dl (threshold for neuroglycopenic symptoms) after the first 48 h could be at risk of a persistent HY disorder. A structured intervention can only be indicated if the patient is symptomatic or has PG <60 mg/dl (threshold for neurogenic symptoms).

ii. *Infants and children*: in case of mild to moderate HY, in infants/children able to take simple sugars orally, oral glucose has the quickest response (even in case of unknown etiology). Compared to sucrose, glucose leads to higher and earlier glycemic peak (29). Notably, in some metabolic conditions [e.g. hereditary fructose intolerance or defects in neoglucogenesis as Fructose 1,6 bisphosphatase (FBPase) deficiency] the administration of sugary drinks containing sucrose could considerably worsen the metabolic decompensation. In such conditions or in case of unknown etiology 10–20 grams of oral glucose are

TABLE 1 | Symptoms and signs of HY.

Age	Neuroglycopenic (PG < 30 mg/dl)	Neurogenic (PG < 55–65 mg/dl)
Newborn	Poor suck or poor feeding, weak or high–pitched cry, change in level of consciousness (lethargy, coma), seizures, hypotonia.	Jitteriness/tremors, pallor, sweating, irritability, tachypnea.
Infant–Child	Warmth, weakness, difficulty thinking, confusion, tiredness, drowsiness, coma, death.	*Cholinergic system*: sweating, hunger, tingling. *Adrenergic system*: shakiness, tremulousness, heart pounding, nervousness, anxiety.

TABLE 2 | Neonatal HY Cut Off needing for intervention according with AAP, PES, BAPM.

Guidelines	Asymptomatic	Symptomatic
American Academy of Pediatrics 2011	0–4 h: <25 mg/dl on two consecutive occasion 4–24 h: <35 mg/dl on two consecutive occasion	40 mg/dl
Pediatric Endocrine Society 2015	0–48 h: <50 mg/dl >48 h: >60 mg/dl	No clear cut off
British Association of Perinatal Medicine	Single value <18 mg/dl or two consecutive values <36 mg/dl	45 mg/dl

Modified from Kallem et al. (18).

TABLE 3 | Glucose monitoring for neonates at risk for HY.

Type of HY	Onset	Duration	HY degree	Response to glucose	Duration of monitoring	Examples
Early transitional adaptive	<6–12 h	12–24h	Mild	Good	24–48 h	Preterm infants, infant of diabetic mother, intrapartum glucose infusion, hypothermia
Secondary	12–24 h	24–48 h	Mild	Good	24–48 h	Asphyxia, sepsis, intraventricular bleeding
Classic transient neonatal	24–48 h	48–72 h or more	Moderate to severe. About 80% are symptomatic	Requires often higher glucose infusion rate	48–72 h	Small for gestation
Severe recurrent	Variable	>7 d	Severe	Requires higher glucose infusion rates >10–12	May be days to weeks	Congenital hyperinsulinism, metabolic and endocrine forms

Modified from Kallem et al. (18).

recommended, followed by a snack of starchy carbohydrates or a milk feed in infants (1, 30). In severe cases (when the patient is unconscious/unable to take anything orally) in an out of hospital setting and/or in the case of unavailable venous access, unless a diagnosis of a specific Inborn Metabolic Disorder (IMD) [e.g. Glycogen storage disease (GSD) type I] has been performed, glucagon (1 mg for children aged more than 12 years and/or weighing at least 25 kg, while 0.5 mg for younger/leaner) (1) should be used, due to its fast counter-insular action. Glucagon should be administered carefully because repeated/excessive doses may induce vomiting and so aggravate HY. Furthermore, it could be ineffective in case of long lasting HY or fasting, when liver glycogen stores may have already been depleted. In this case glucose must be infused starting with a bolus of 200–500 mg/kg (2–5 ml/kg of 10% glucose solution) followed by an infusion with 10% glucose adjusted to maintain euglycemia based on the age requirements (1).

ETIOLOGICAL DIAGNOSIS

Endocrine Causes

Congenital Hyperinsulinism

Congenital Hyperinsulinism (CH) represents the most common cause of persistent HY in infants and children, with an estimated incidence of 1:40.000–50.000 in general population. It is a heterogeneous and complex biochemical disorder characterized by the dysregulated insulin secretion from pancreatic β-cell causing random HY associated with low/normal ketones and absence of metabolic acidosis. Besides the classical neonatal onset, there are also late-onset forms that can appear in adolescence/adulthood (0.5–5.0% of cases) and could exhibit glycemic fluctuations from HY to hyperglycemia (31). In CH one or more steps of insulin secretion are disrupted due to a genetic defect, resulting into an insulin release that is independent from PG levels; sometimes, it is triggered by peculiar events, such as meal and exercise (31). CH genetic diagnosis could be achieved in about half of the patients. Besides syndromic conditions, currently about 14 genes are known to cause monogenic forms. Considering the known pathogenic mechanisms, currently CH could be grouped into four categories (32):

i. *Channel Defects (ChD; genes ABCC8, KCNJ11, KCNQ1, CACNA1D)*: among these mutations, ABCC8 and KCNJ11 (KATP channel subunits Kir6.2 and SUR1, respectively) causes the most common and severe forms of CH especially in case of biallelic mutation, although there have been reported patients carrying *ABCC8* biallelic mutations with optimal response to Diazoxide (DZX) (33, 34) even showing progressive resolution of hypoglycemia (35).
ii. *Metabolic Defects (MeD; genes GLUD1, GCK, HADH, UCP2, HK1, PMM2, PGM1)*: this class includes enzyme defects causing abnormal intracellular levels of specific metabolites regulating insulin release. Among these mutations, GLUD1 activating result into increased glutamate dehydrogenase enzyme (GDH) activity and cause the Hyperinsulinism/Hyperammonemia syndrome (the second most common cause of CH). Since GDH is allosterically enhanced by Leucine, protein load can induce HY. HY due to dominantly inherited by GCK activating mutations is clinically heterogeneous with respect to severity and age of onset.
iii. *Transcription factors Defects (TfD; genes HNF1 α, HNF4 α, FOXA2)*: this class includes molecular defects in the transcriptional factors that regulate the glucose-induced secretion of insulin. Patients affected by these mutations are subjected to transient hyperinsulinemic HY followed by the development of Maturity Onset Diabetes of the Young during adolescence (36, 37).
iv. *Syndromic conditions*: CH could be a manifestation of several syndromic conditions (**Table 4**).

From the histopathological point of view, CH is classified into three variants (31, 38). In diffuse forms, all β-cells share the same molecular defect and show the same morphology. In focal forms, a β-cell cluster develops as a nodular adenomatous hyperplasia because of a confined molecular defect in the 11p15.1-11p15.5 imprinted region, that involve the ABCC8/KCNJ11 genes. These forms usually develop sporadically in a patient carrying a recessive ABCC8/KCNJ11 paternally inherited mutation, when a somatic loss of the maternal allele occurs ("double hit"). ABCC8 mutations can cause phenotypes who switch from HY in infancy to hyperglycemia in adolescence and even adulthood (39–41). Late onset forms are mostly linked to dominant mutations of ABCC8/KCNJ11 genes or to activating mutation of GCK gene (40). CH diagnosis may be suspected at any insulin

TABLE 4 | Syndromic causes of CH.

Overgrowth syndromes	*Gene*	*Chromosome*	*Inheritance*
Beckwith–Wiedemann syndrome	IGF2/H19	11p15.5–15.4	Autosomal dominant
	CDKN1C		Sporadic
	KCNQ1OT1		Paternal uniparental disomy
Sotos syndrome	NSD1	5q35.2–35.3	Autosomal dominant
	NFIX	19p13.3	Sporadic
Simpson–Golabi–Behmel syndrome	GPC3	Xq26	X–linked
Perlman syndrome	DIS3L2	2q37	Autosomal recessive
Chromosomal abnormality syndromes			
Turner syndrome	KDM6A	Xp11.2	Sporadic
Trisomy 13	CDX2, IPF	Trisomy 13	Sporadic
Postnatal growth failure syndromes			
Kabuki syndrome	KMT2D	12q13.12	Autosomal recessive
	KDM6A	Xp11.3	Sporadic
Costello syndrome	HRAS	11p15.5	Autosomal dominant Sporadic
Contiguous gene deletion affecting the ABCC8 gene			
Usher syndrome	USH1C	11p15.1	Autosomal recessive
Timothy syndrome	CACNA1C	3p21.1	Autosomal dominant Sporadic
Insulin receptor mutation			
Insulin resistance syndrome (Donohue syndrome)	INSR	19p13.2	Autosomal recessive
Congenital Disorders of Glycosylation (CDG)			
CDG Type Ia	PMM2	16p13.2–13.3	Autosomal recessive
CDG Type Ib	PMI	15q22–24	Autosomal recessive
CDG Type Ic	hALG3	3q27	Autosomal recessive
Other causes			
Poland syndrome	UCMA	10p13–14	Sporadic
CHARGE syndrome	CHD7	8q12	Autosomal dominant

Modified from Galcheva S, et al. (32).

concentration detectable in a hypoglycemic plasma sample thus as a marker of inappropriate insulin secretion (1, 42). Indirect signs of this phenomenon are the absence of ketonemia (excepted for HADH deficit) and fatty acidemia. Adjunctive diagnostic criteria for CH could be a positive response to glucagon or octreotide injection (glucose levels >1.5 mmol/L) and the need for more than 8 mg/kg/min of glucose infusion to maintain euglycemia (1, 32, 38, 40, 41). Some authors (42) have proposed a classification of CH diagnostic criteria as reported in the **Table 5**. Once the diagnosis of CH has been established, genetic test should be performed; as a general rule, ABCC8/KCNJ11 mutations must be investigated first. Concurrently to genetic tests, a prompt treatment should be started with diazoxide (DZX) and then, in case of unresponsiveness, with octreotide (38, 41, 42, 44). In fact, many ABCC8/KCNJ11 mutations cause refractoriness to DZX. These cases require an 18-Fluoro-DOPA-Positron Emission Tomography (PET) to search for any focal forms, and need to be treated with octreotide. Dosage adjustment could be required due to possible tachyphylaxis. Side effects include abdominal discomfort, diarrhea (rarely necrotizing enterocolitis), and in long term, bile sludge/gallstones and suppression of pituitary hormones. Long-acting release (LAR) octreotide analogues, administered monthly, have also been successfully tried in children (45, 46) and even preferable for better compliance and safety (47), however they take a long time to achieve the steady state (lanreotide needs 23–30 days and sandostatin 3 months to gain therapeutic blood concentration). They should initially be administered together with octreotide: the starting

TABLE 5 | Diagnostic criteria of CH.

Cardinal diagnostic criteria	Low plasma glucose (<3 mmol/L) with Detectable serum insulin Detectable C–peptide
Biochemical criteria	Suppressed/low beta–hydroxybutyrate and acetoacetate Suppressed/low serum free fatty acid
Clinical criteria	Increased requirement of glucose infusion rate (>8 mg/kg/min) Positive response to i.m./i.v. glucagon (glycemic response >1.5 mmol/L)
Supportive criteria	Positive response to s.c./i.v. octreotide Low serum levels of IGFBP–1 (suppressed by insulin) Suppressed branch chain amino acids Normal lactic acid Normal plasma hydroxybutyrylcarnitine Normal ammonia Appropriate counterregulatory hormone response (cortisol >20 mcg/dl, GH > 7 ng/ml) Provocation test (leucine loading or exercise testing) may be needed in some patients

Modified from Vora et al. (43).

dose is 30–60 mg for lanreotide (subcutaneously) while for sandostatin-LAR (intramuscularly) the dose is equivalent to the cumulative 31-day subcutaneous octreotide dose, calculated by multiplying the daily dose of octreotide (5–25 μg/kg) for 31. Other drugs proposed for the treatment of DZX un-responsive cases include Nifedipine, Sirolimus, and Glucagon-like peptide-1 (GLP-1) receptor antagonist "Exendin" (1, 42). In particular, Sirolimus, is an antiproliferative drug that reduces insulin secretion probably by lowering the mammalian target of rapamycin (mTOR), a serine/threonine kinase that is overexpressed in the diffuse variant of CHI and which enhances insulin secretion; moreover, Sirolimus causes depletion of intracellular Calcium decreasing insulin release (48). This drug has shown variable efficacy and safety being even able to avoid surgery up to 18 months, but sometimes uneffective both for duration and power to reduce hypoglycemic events (49). Although there haven't been reported major side effects, it should be used with caution for its immunosuppressive action and long-term follow-up studies are needed for chronic toxicity (42). Therapy effectiveness in CH patients is based on blood glucose monitoring. The tools used for this monitoring are the same used for the glycemic monitoring of type 1 diabetes patients (50) such as blood glucose sampling and continuous subcutaneous glucose monitoring (51). According with the most recent evidences, 18-Fluoro-DOPA PET should be offered not only in case of refractoriness to DZX, but also when no mutation is identified or in case of a single recessive paternal inherited mutation in ABCC8/KCNJ11, that could reveal focal forms, even if responsive to DZX. While surgery could be definitively curative for focal forms, near-total pancreatectomy, reserved for diffuse forms unresponsive to available drugs, could cause iatrogenic diabetes and allocate patients to life-long pancreatic enzyme replacement and insulin therapy. Moreover, the residual pancreatic tissue left near the common bile duct and along the duodenum could be responsible for persistent HY. Data from long-term follow-up show efficacy in prevention of severe hypoglycemic episodes but with only few cases of remission (52).

Adrenal Insufficiency

Adrenal insufficiency (AI) is a life-threatening condition in which the adrenal cortex is unable to adequately produce steroid hormones. AI can be distinguished in Primary (PAI) or Central (CAI), depending on the impairment of adrenal cortex or hypothalamus/pituitary gland respectively. The most common etiology of PAI in children is the Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency. In PAI both glucocorticoids and mineralocorticoids synthesis are affected, while in CAI only glucocorticoid synthesis is compromised (53, 54).

Glucocorticoids, mostly cortisol, play an essential role in glucose metabolism (55): in the liver they promote glucose output by activating gluconeogenesis and triglycerides accumulation (56–59); in the muscle they suppress glucose uptake and glycogen synthesis, accelerate protein breakdown, and inhibit protein synthesis; in adipose tissue they promote lipolysis and so increase serum FFA and glycerol (60) thus, together with amino-acids coming from protein catabolism, they provide substrates for gluconeogenesis. Cortisol deficiency results in incapacity to raise up glucose levels in stressful conditions, causing HY associated with low/normal ketones and absence of metabolic acidosis.

Clinical presentation of PAI may be non-specific with anorexia, weight loss, fatigue, abdominal pains, headache, nausea, arthralgia, myalgia, joint pain in chronic forms (61) or with an "adrenal crisis," characterized by a cardiovascular decompensation due to massive impaired electrolyte and fluid balance, in case of acute onset (61). The critical sample collected during an adrenal crisis will show hyponatremia, metabolic acidosis (normal anion gap, increased serum chloride), HY, hyperkalemia, and low cortisol level.

Congenital Hypopituitarism and Growth Hormone Deficiency

Congenital Hypopituitarism is a pathologic condition characterized by a partial or a total deficiency in one or more pituitary hormones (62). Among those conditions, ACTH and GH deficiency (GHD) may present with HY associated with low/normal ketones and absence of metabolic acidosis. ACTH deficiency has been already described above.

Although the cause of HY in hypopituitarism is still debated, it is known that GH and cortisol, in physiologic doses, act synergistically to elevate the blood glucose, and that the replacement of both hormones is necessary to normalize insulin secretion and maintain normal glucose homeostasis in children with hypopituitarism; probably GHD is responsible of HY because of loss of amino acid mobilization to support gluconeogenesis (63, 64).

Congenital Hypopituitarism should be suspected in neonates carrying dysmorphic features with midline defects, ocular and craniofacial anomalies, and in males, micropenis often with undescended testes. Detection of persistent HY and jaundice reinforces the suspect (65–68). GHD could also appear in evolutive age mostly with its auxological consequences: short stature, delayed bone age, decrease in the growth rate (69).

Metabolic Disorders

Glycogen Storage Diseases (GSD)

All Metabolic disorders are resumed in **Table 6** and **Supplementary Table 1**.

GSD are secondary to defects of the enzymes and transporters involved in glycogen breakdown and synthesis. Their overall incidence is 1:25,000 births. More than 12 GSD types are recognized. Based on clinical presentation, they are classified as hepatic GSD (e.g. GSDI) and muscle GSD (e.g. GSDII, GSDV). HY and hepatomegaly are the primary manifestation of the hepatic GSD (GSD0a, GSDI, GSDIII, GSDVI, GSDIX, GSDXI). GSDIII is the only GSD presenting with concomitant liver and muscle involvement. Based on the ketone levels hepatic GSD are traditionally defined as ketotic (GSD0a, GSDIII, GSDVI, GSDIX, GSDXI) or non-ketotic (GSD I). Genetic studies are the preferred method for diagnosing hepatic GSD (enzyme tests are performed in selected cases). Dietary plan with frequent feedings and uncooked cornstarch (UCCS) and/or tube feeding are the cornerstone of the treatment for hepatic GSD; carbohydrates are given to maintain euglycemia, but excessive carbohydrate

TABLE 6 | Main clinical and biochemical features of major metabolic causes of childhood HY.

Disorder	Timing	Lactate	Ketones	Additional biochemical abnormalities	Clinical features
Ketotic hypoglycemia	Fasting >6 h	+/– M.A.	+	Low alanine	Fever Vomiting Diarrhea
Glycogen storage disease type I	Fasting (2–4 h)	+ M.A.	(–)	Elevated lipids Elevated uric acid Elevated transaminases Neutropenia (GSDIb)	Hepatomegaly Doll–like face
Glycogen storage disease type III/VI/IX	Fasting (2–6 h)	–	+	Elevated lipids Elevated transaminases Elevated CK (GSDIIIa)	Hepatomegaly Cardiomyopathy (GSDIIIa)
Hereditary Fructose Intolerance	1–2 h after the ingestion of fructose, sucrose, sorbitol	+ M.A.	+/–	Elevated transaminases	Vomiting Diarrhea Hepatomegaly Liver failure Fatty liver
Galactosemia	1–2 h after the ingestion of galactose, lactose	– M.A.	+/–	Elevated bilirubin Elevated transaminases Abnormal clotting tests	Vomiting Hepatomegaly Liver failure Cataract Sepsis
Fructose 1,6 bisphosphatase deficiency	Fasting >6 h	+ M.A.	+ 	Elevated alanine Elevated pyruvate and glycerol 3–phosphate	Intercurrent disease Hepatomegaly
Pyruvate carboxylase deficiency	Variable	+ M.A.	+ 	Hyperammonemia Elevated citrulline	Severe encephalopathy Seizures Movement disorders
Organic acidemias	Prolonged fasting or after an initial symptom–free period (neonatal)	+ M.A.	+ 	Hyperammonemia Elevated branched chain amino acids and glycine Elevated acylcarnitines UOA abnormalities	Encephalopathy Movement disorders Renal disease Cardiomyopathy
Fatty acid oxidation disorders	Fasting >8 h	+ M.A.	(–) FFA/KB > 2.5	Elevated acylcarnitines Dicarboxylic aciduria Hyperammonemia	Exercise intolerance Cardiomyopathy Arrhythmias
Ketogenesis defects	Prolonged fasting or after an initial symptom–free period (neonatal)	+ M.A.	– FFA/KB > 2.5	Hyperammonemia Dicarboxylic aciduria UOA abnormalities	Hepatomegaly Seizures Cardiomyopathy
Ketolysis defects	Prolonged fasting	–	+ FFA/KB < 0.3	UOA abnormalities	Intercurrent disease Hepatomegaly
Disorders of Oxidative Phosphorylation	Variable	+ M.A.	+/–	UOA abnormalities	Multisystem involvement
Congenital Disorders of Glycosylation	Variable	+/–	+/–	High insulin (mostly)	Psychomotor retardation Dysmorphic features Multisystem involvement

M.A., metabolic acidosis.

intake may result in hyperinsulinemia with consequent complications (70). Carbohydrates restriction with protein supplementation or ketogenic diets are recommended to avoid glycogen storage and to minimize insulin secretion in some forms (71). Restricted fructose and galactose intake aims at avoiding acidosis in GSDI. Major hepatic GSD are discussed.

GSD I is the most common and severe GSD (both glycogenolysis and gluconeogenesis are impaired). It is due to a defect of either the catalytic (GSDIa, 80% of cases) or the microsomal glucose 6-phosphate transporter (GSDIb, 20% of cases) of the G6Pase system. GSDI patients usually present at 3–6 months of age with fasting HY, lactic acidosis and hypoketosis

(usually 2–4 h after meal), hepatomegaly, doll-like face, failure to thrive, hyperlipidemia, and hyperuricemia. Additionally, GSDIb patients show neutropenia and recurrent infections (72). Long-term complications include liver neoplasms, renal disease, and increased risk of inflammatory bowel disease (73) and autoimmune (74, 75) or endocrine disorders (76–78).

GSDIII is due to glycogen debrancher enzyme deficiency. Two main subtypes are recognized: GSDIIIa (85% of the cases, mixed liver and muscle involvement) and GSDIIIb (15% of the cases, isolated liver involvement). As gluconeogenesis is intact, HY is usually less severe than GSDI showing prominent fasting ketosis without lactic acidosis. Transaminases concentrations are usually higher (may exceed 1,000 U/L) with less severe hyperlipidemia compared to GSDI. Bone disease (78) and benefit of a high-fat diet on muscle symptoms have been reported (79).

GSDVI and GSDIX are secondary to liver glycogen phosphorylase and glycogen phosphorylase kinase defect, respectively. They are generally mild disorders improving with age. However, they can also present with symptomatic fasting ketotic HY, hyperlipidemia, increased transaminases, hepatomegaly, growth retardation, and hypotonia (80).

GSD0 is caused by a deficiency of hepatic glycogen synthase resulting in inadequate production of hepatic glycogen. The clinical manifestations include fasting ketotic hypoglycemia accompanied by low levels of alanine and lactate and postprandial hyperglycemia and hyperlactatemia. Unlike other GSDs, patients with GSD0 usually do not develop hepatomegaly (81, 82).

GSDXI (Fanconi-Bickel syndrome) is caused by deficiency in a solute carrier family 2 protein (GLUT-2) that is expressed in hepatocytes and proximal renal tubule. Patients typically present at 3–10 months of age with hepatomegaly, Fanconi syndrome (e.g. severe glycosuria, polyuria, hyperaminoaciduria, hypophosphatemic rickets, acidosis, hypokalemia, hypochloremia), failure to thrive, fasting HY, and postprandial hyperglycemia. Only symptomatic treatment is available (frequent feeds with complex carbohydrates, electrolytes replacement, vitamin D) (5).

Hereditary Fructose Intolerance

HFI is caused by deficiency of Aldolase B, resulting into inhibition of gluconeogenesis (inhibition of Aldolase A) and glycogenolysis (inhibition of glycogen phosphorylase A) secondary to fructose 1-phosphate accumulation. Symptoms usually present at weaning, after the ingestion of food containing fructose, sucrose, or sorbitol (e.g. fruit, vegetables) and include post-prandial HY, with ketosis and lactic metabolic acidosis, hepatomegaly, vomiting, pallor, sweating, lethargy, failure to thrive, convulsions, and eventually coma. Acute liver failure and renal dysfunction (proteinuria, glycosuria, hyperaminoaciduria) are also observed. Most patients develop a natural aversion to fruit/sweets. Therapy includes avoidance of dietary fructose, sucrose, and sorbitol (5), although mild signs of liver injury, without progression on a long-term follow-up could be detected in patients on a FSS-free diet, particularly with specific genotypes (83).

Galactosemia

Classical galactosemia is caused by deficiency of galactose-1-phosphate uridyltransferase (GALT) (the enzyme converting lactose into glucose and galactose) resulting into accumulation of galactose 1-phosphate, galactitol, and galactonate in blood and tissues. Symptoms usually appear a few days after the ingestion of breast or formula milk and include vomiting, diarrhea, poor feeding, nuclear cataract, jaundice, hepatomegaly, and high transaminases evolving to liver failure (HY, bleeding tendency) and renal failure; *Escherichia coli* sepsis is common.

Inherited Disorders of Gluconeogenesis

The conversion of pyruvate into glucose is the central pathway of gluconeogenesis. Overall, disorders of gluconeogenesis present with recurrent HY and lactic acidosis with or without ketosis. Major inherited disorders of gluconeogenesis are described.

FBPase deficiency is a disorder of gluconeogenesis characterized by episodic acute crisis of HY, lactic acidosis (lactate may rise up to 25 mmol/L), and (usually) ketosis manifesting with hyperventilation, apneic spells, hepatomegaly (with normal transaminases), seizures, coma, and brain damage. The crises are likely to occur when glycogen reserves are limited (as in newborns or after ingestion of large amount of fructose) or exhausted (e.g. fasting, intercurrent illness) and are reversed by high glucose infusion rates (about 1.5 times maintenance). The frequency of the attacks decreases with age and patients are usually well between attacks. Treatment includes frequent feedings and avoidance of prolonged fasting (84).

Pyruvate carboxylase (PC) deficiency is a defect of both gluconeogenesis and Krebs cycle. Although fasting HY can occur, this disorder usually presents with severe encephalopathy, developmental delay, seizures, movement disorders, failure to thrive, and metabolic acidosis. A high lactate to pyruvate ratio with a low hydroxybutyrate to acetoacetate ratio is suggestive of the diagnosis. Treatments include intravenous glucose infusion, bicarbonate, dietary management, and supplementation with citrate, aspartate, dichloroacetate, biotin, and thiamine (85).

Phosphoenolpyruvate carboxykinase (PEPCK) deficiency affects gluconeogenesis and can cause HY, failure to thrive, lactic acidosis, and lipid accumulation in the kidney and liver. Only six patients have been reported in the literature and its clinical relevance is currently disputed (86).

Glycerol kinase deficiency (GKD) can present either isolated or together with congenital adrenal hypoplasia or Duchenne muscular dystrophy (partial deletion of Xp21). Patients with isolated GKD can develop episodic vomiting with HY, hyperketonemia, metabolic acidosis, and coma. Typically, high glycerol excretion in the urine is found by gas chromatography-mass spectrometry. Metabolic crises should be avoided by providing an adequate supply of fluid, calories, and glucose during intercurrent illness (87).

Congenital Disorders of Glycosylation

CDG constitute a group of conditions due to defects in the glycoprotein synthesis. Around 90 CDG types are currently recognized. Phosphomannomutase 2 Deficiency and Glucosyltransferase 1 Deficiency are the most common CDG.

A broad spectrum of symptoms including psychomotor retardation, failure to thrive, hypotonia, deafness, bleeding tendency, cerebral hemorrhage, cardiomyopathy, hypogonadism, and HY (hyper- or normoinsulinemic) is known (88).

Fatty Acids Oxidation Disorders

FAODs constitute a group of conditions characterized by hypoketotic HY and presenting with great variability. Three typical presentations are known for FAOD:

i. Acute hypoketotic HY with lactic acidosis and encephalopathy with hepatomegaly and liver dysfunction (including hyperammonemia); symptoms usually present under catabolic circumstances (e.g. newborn, prolonged fasting, intercurrent illness)
ii. (Hypertrophic) cardiomyopathy and arrhythmias
iii. Myopathy presenting with weakness and/or acute rhabdomyolysis with symptoms precipitated by exercise or intercurrent illness (89).

Diagnosis can be suggested by acylcarnitine profile and confirmed by enzyme testing or gene sampling (90). Treatment includes a high carbohydrate diet to maintain euglycemia and to avoid prolonged fasting or stress induced states. Carnitine supplementation can be used for specific FAOD (91).

Disorders of Ketone Body Metabolism

Disorders of KB metabolism can present either in the first days of life or later in childhood. Similarly to FAOD, prolonged fasting and intercurrent illness are triggers to metabolic decompensation. Ketogenesis defects are characterized by hypoketotic HY with or without hyperammonemia, metabolic acidosis, and liver disease. Decompensations lead to encephalopathy, vomiting, and coma. Conversely, ketolysis defects present with episodes of hyperketotic HY and severe ketoacidosis in childhood; patients are healthy between episodes (92).

Disorders of Oxidative Phosphorylation

Disorders of Oxidative Phosphorylation (OXPHOS) are clinically, biochemically, and genetically heterogeneous. They are due to mutations in nuclear genes coding for respiratory complexes subunits and can present at any age with a wide range of possible symptoms, including fasting HY with lactic acidosis and variable ketone bodies levels. Children often suffer from encephalomyopathic disease (93).

Organic Acidemias

OA are disorders of intermediary metabolism due to defect of enzymes involved in branched-chain amino acid catabolism. They are characterized by the mitochondrial accumulation of CoA metabolites causing metabolic acidosis, elevated lactate, ketotic HY, and hyperammonemia. The most common OA are Methylmalonic acidemia (MMA), Propionic acidemia (PA), and Isovaleric acidemia (IVA).

Three clinical presentations are recognized:

i. Neonatal (intoxication type): lethargy, poor feeding, encephalopathy, myoclonic jerks, multiorgan failure.
ii. Chronic intermittent: episodes of ketoacidosis, lethargy, cerebral involvement.
iii. Chronic progressive: vomiting, failure to thrive, psychomotor retardation, hypotonia, renal disease.

OA are diagnosed by their specific urinary organic acid profiles or abnormal plasma acylcarnitines.

The diagnosis is confirmed with enzymatic studies and/or molecular DNA testing (94). OA are included in NBS programs in several countries with an increasing number of patients diagnosed pre-symptomatically (95). Treatment of the acute phase is aimed at correcting hyperammonemia (by temporary stopping protein intake, promoting anabolism, and administering ammonia scavengers), metabolic acidosis, and HY. The cornerstone of chronic treatment are protein-restricted diet, long-term ammonia scavengers, vitamin cofactors, and carnitine supplementation (carnitine transforms toxic CoA esters into less toxic carnitine esters).

Idiopathic Ketotic HY

Ketotic HY is the most common cause of childhood HY. It usually presents between 18 months and 5 years and resolves spontaneously by the age of 9 years. Typically, the child presents with symptomatic HY in the morning after long fast often precipitated by an intercurrent illness. Glucose <55 mg/dl and massive ketosis are observed. Metabolic acidosis can also develop. The child improves dramatically on dextrose infusion (conversely glucagon injection elicits little or no increase in glucose concentrations) and is usually restored to normal health within hours. Despite being the most common cause of HY in childhood, there are no specific diagnostic tests for ketotic HY. Therefore, all possible causes of HY must be ruled out (diagnosis of exclusion). Treatment measures include avoidance of prolonged fasting, UCCS, and close monitoring of oral intake when in stressed states (such as illness) to avoid HY (2).

DIAGNOSTIC PATHWAY: PRACTICAL APPROACH TO HY IN CHILDHOOD

The diagnostic path of HY results from the combination of medical history and clinical, dietary, and biochemical data. A systematic approach is necessary to collect relevant information.

Personal History

The first step consists in collecting information on the timing of the hypoglycemic event, including:

Age of onset (neonatal, infant, child)

- Fasting tolerance (e.g. feeding frequency, night snack, morning ketosis)
- Temporal relation with meals (fasting, post-prandial, random)
- Relation to/Avoidance of food [e.g. protein, fruit, fruit (juice), (ga)lactose]

- Associated conditions/triggers
- Recurrence (e.g. intercurrent disease, fatigue)

The following information should also be carefully detailed:

- Perinatal history: birth weight, gestational age, gestational diabetes, and any other form of perinatal distress and perinatal glucose requirements (e.g. >10 mg/kg/min glucose intravenously).
- Growth and developmental milestones (e.g. intellectual disability, movement disorders, epilepsy)
- Family history: relatives with HY/hyperglycemia or IMD, previous miscarriages or deaths, consanguinity, medications, and social history.

Physical Examination

Physical examination can reveal signs pointing to:

- Endocrine dysfunction, such as micropenis, short stature, midline anomalies (hypopituitarism), skin hyperpigmentation, abdominal pain, muscle pain, weight loss, signs of hyperandrogenism [Adrenal Insufficiency (AI)]
- Inherited metabolic disease, such as hepato(spleno)megaly (since the liver and spleen size become larger with age, patients' age and height should be considered for adequate assessment), jaundice, spider angiomas (e.g. GSD), cataract (e.g. galactosemia), absence of dental caries [e.g. Hereditary Fructose Intolerance (HFI)], arrhythmias and/or heart murmur [e.g. Fatty Acid Oxidation Disorders (FAOD)], movement disorders [e.g. Organic Acidemias (OA)], hypotonia and inverted nipples and/or bleeding tendency [e.g. Congenital Disorders of Glycosylation (CDG)], multisystem involvement (e.g. mitochondrial disorders)
- Dysmorphic features, macrosomia, hemihypertrophy (e.g. CH, genetic syndromes of overgrowth)

Laboratory Investigations

Biochemical tests can provide crucial information to reach a final diagnosis. Indeed, specific biochemical patterns can point to specific defects. In particular, low ketones at the time of hypoglycemia may immediately suggest a diagnosis of hyperinsulinism or FAOD. Contextual levels of Free Fatty Acids (FFA) could help to distinguish between endocrine or metabolic etiology, as low ketones together with low FFA suggest hyperinsulinism, while low ketones together with increased FFA suggest FAOD or defects of ketolysis (when FFA/KB ratio results below 0.3) (**Table 6**).

A "critical sample" (i.e. a sample obtained during HY) must be collected. Laboratory investigations should include: blood glucose, lactate, ketones (mainly beta-hydroxybutyrate), blood gases, FFA, insulin, C-peptide, cortisol, GH, Insulin, Growth Factor1 (IGF1), acylcarnitines, amino acids, as well as urinary organic acids (UOA). Some investigations may not be performed in all hospitals. Therefore, one or two spare tubes should also be collected for any additional tests to be performed afterwards. However, appropriate blood samples might be missed when an immediate treatment is required (e.g. severe HY). Still, collecting (and store frozen) the first urine sample after HY might provide helpful information in such cases (e.g. increased/undetectable ketones, lactate, tricarboxylic acids).

Additional tests can be considered: ammonia, toxicology tests, urine reducing substances test (to assess fructosuria, galactosuria). Transferrin electrophoresis/isoelectric focusing should be required if a CDG is suspected. Over the past years, a number of minimally invasive continuous glucose monitoring systems have also become available, possibly providing additional information on the extent, timing, and duration of PG fluctuations.

Fasting test. Evaluation of metabolic changes after fasting may be helpful to reach the diagnosis and to assess patients' fasting tolerance to tailor the treatment. Since fasting can lead to the accumulation of toxic metabolites and sometimes fatal complications in some defects, a fasting challenge should only be performed in specialized metabolic units and only after less risky investigations have been performed without reaching a clear diagnosis (FAOD must be ruled out before a fasting test). The maximal duration of the fasting is based on the clinical suspicion and on the children's age (usually 12–16 h at 6–12 months, 18 h at 1–2 years, 20 h at 2–7 years, 24 h in children >7 years). The fasting should be stopped at any time if glucose concentration is below 2.6 mmol/L (47 mg/dl). Since newer diagnostic strategies (biochemistry and molecular biology) are rapidly becoming available, fasting test is not performed routinely; however, it can be helpful in selected cases (96).

Glucagon test explores the response of glucagon injection during HY to assess the availability of glycogen for compensation of low blood glucose. Typically, HY due to GSDI does not benefit from glucagon injection (with worsening hyperlactatemia) while an exaggerated glucose response to glucagon could be observed in case of CH. Due to its possible risks (prolonged HY) it has been largely superseded by enzyme or mutation analysis.

Alternative causes of abnormality of the test results should always be ruled out (e.g. lactate elevation secondary to laborious sampling or increased pCO2 secondary to apnea during blood collection).

Additional Investigations

Imaging tests can provide additional information. Abdominal ultrasound and Magnetic Resonance Imaging (MRI)/Computed tomography/Scintigraphy scan can define liver, spleen, pancreas, and kidneys morphology and structure (e.g. liver steatosis, liver adenomas, focal hyperplasia). Left hand and wrist X-ray can be helpful in patients with growth retardation. Specific additional investigations may be performed based on the accompanying clinical features (e.g. cardiac ultrasound, brain MRI).

HY COMPREHENSIVE FLOWCHART

The combination of the aforementioned information enables reaching a working diagnosis in most of the children presenting with HY. To date no consensus exists on a standardized diagnostic flowchart. Several algorithms with various starting points and workflow have been proposed (5, 7, 8). Although such algorithms can provide metabolic or endocrine specialists with

specific pathophysiological insight, the two main groups of causes (namely endocrine and metabolic) may appear not clearly suited to the understanding and use of generalist pediatricians, which, on the other hand, are often the first level of observation of HY phenomena. Our center has a long-standing experience of cooperation between metabolic and endocrine experts in the management of childhood HY. In this respect, a comprehensive diagnostic flowchart is proposed (**Figure 2**). The major advantage of such flowchart is the ability to orient the diagnose, distinguishing both (main) metabolic and endocrine causes of childhood HY by using simple, routinely available tests such ketone bodies, emogas analysis, and lactate. As a matter of fact, data included in this flowchart can be easily implemented by physicians in a hospital setting, to obtain biochemical findings at the time of hypoglycemia, that should not be missed and that could be very useful to hypothesize the diagnosis. In order to provide adequate information, physicians should be aware that the flowchart applies to results collected on a "critical sample." Possible limitations include the lack of rarer disorders (e.g. PEPCK deficiency, GKD, CDG) and the inability to diagnose uncommon presentations of common disorders.

Only the main diagnostic features that guide bedside diagnosis about the most common causes of pediatric HY are shown in the flowchart (e.g., hyperlactatemia is also found in OXPHOS defects and GSD I; hepatomegaly is also found in FBPase deficiency and GSD I). Firstly: the timing of HY is the starting point; the patients fasting tolerance can provide an essential clue to the diagnosis in children with fasting HY (e.g. HY after a short fast suggests hepatic GSD, HY after moderate to long fast suggests gluconeogenesis defects or FAOD/KB defects). Secondly: laboratory investigations play a pivotal role to reach a working diagnosis. In this respect, assessing the presence of (un) detectable ketones (as well as metabolic acidosis, hyperlactatemia and, if possible, FFA) on a "critical sample" is of paramount importance. Thirdly: the presence of hepatomegaly can help differentiating disorders causing fasting ketotic HY.

So far, the resultant flowchart seems to facilitate the logical process leading to the diagnostic suspicion and help to address the biochemical and clinical elements that need to be sought. The subsequent diagnostic process is up to the specialists of the two endocrine and metabolic sectors.

In case of HY in otherwise healthy children and/or with no recognizable pattern, intoxications/factitious causes should always be ruled out by toxicological tests on blood and urine (most common drugs include insulin, sulfonylurea, beta-blockers, salicylates).

Diagnosis can be confirmed through enzymatic and/or molecular testing for IMD and CH and challenge tests for endocrine disorders. Enzyme diagnostics is generally performed on blood cells or skin fibroblast (e.g. debranching enzyme or very-long chain acetyl-CoA dehydrogenase activity). However, some enzymes (e.g. G6Pase) are not expressed in these mediums and require a liver biopsy. Since liver biopsy is invasive, it has been largely superseded by DNA analysis.

THE RECENT ROLE OF NGS

DNA analysis has become increasingly sophisticated and rapid in recent years. Various techniques are used to search for mutations in IMD/CH genes; single gene analysis (Sanger sequencing) has been traditionally used to confirm a specific diagnostic suspicion, after a traditional work-up. When a group of disease is considered, the traditional diagnostic approach would involve a long process with subsequent gene-by-gene molecular analyses.

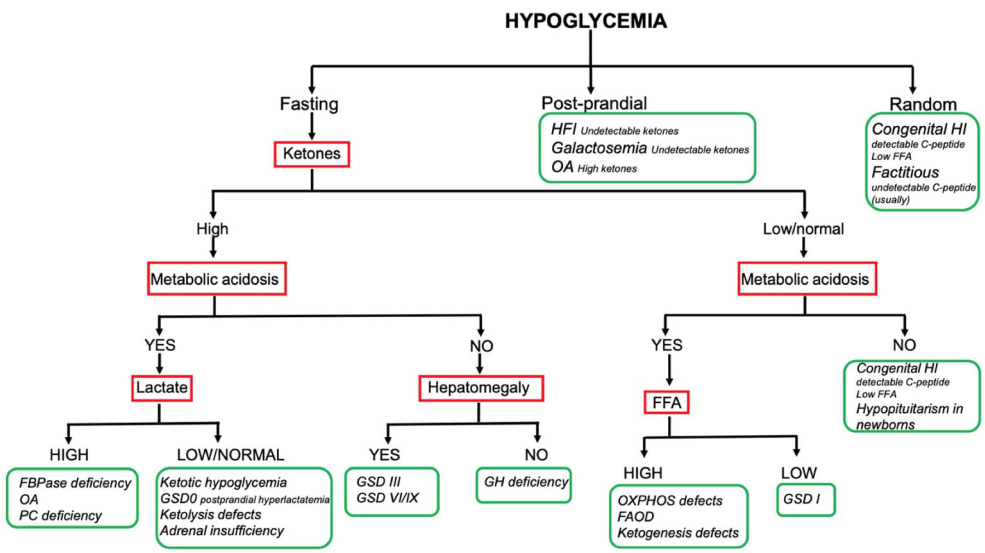

FIGURE 2 | Hypoglycemia diagnostic flowchart.

The gene-by-gene technique has now been superseded and replaced by the analysis of panels with NGS techniques. The introduction of NGS represents a major advancement in the diagnostic approach, allowing in parallel sequencing of millions of small fragments of DNA. Given the difficulties in the diagnostic workup in HY and due to the overlapping of clinical manifestations in several disorders of glucose metabolism, patients showing recurrent undiagnosed HY could be further investigated with an NGS-based approach. This modern technique has the potential to identify underrecognized rare disorders in the wide group of children with ketotic hypoglycemia, clinically diagnosed in the past as affected by benign hydiopatic hypoglycemia. In addition to a targeted approach with gene panels, the NGS technology can be used through untargeted strategies based on whole-exome sequencing, having this approach also the potential to identify new genes involved in disorders of glucose metabolism (97).

DISCUSSION AND CONCLUSIVE REMARKS

Despite being a common emergency in pediatrics (3, 4), there are still controversies on the definition and management of HY in neonates and children. Neither the standard diagnostic PG threshold nor the operative threshold are defined. Also, no consensus on the definition of at–risk neonates exists. Such uncertainties together with the broad spectrum of causes, make the approach to HY in childhood complex and time consuming. Irrespective of its cause, prompt recognition and treatment of acute HY are critical to prevent its complications (namely brain damage). Bolus administration of dextrose (either intravenously or orally) is the cornerstone of the treatment. Glucose requirements may vary depending on the patient's age (e.g. higher in neonates) and disease (e.g. up to 10–15 mg/kg/min of glucose or more can be required in children with CH). Since additional treatments can be necessary for specific disorders (e.g. specific dietetic approach, drugs and cofactors in OA and FAOD or DZX in CH) a timely etiological diagnosis is crucial. Once acute HY has been managed, pending the results of confirmatory tests (e.g. enzyme/DNA diagnostics) specific actions should be taken in order to prevent HY relapse. As a general recommendation, fasting must be avoided and adequate carbohydrate intake must be maintained during any metabolic stress. Tailored dietary treatment plan with frequent feedings and UCCS and/or tube feeding are the most common interventions, such a plan aims at ensuring glucose concentrations as stable as possible and is generally sufficient in patients with ketotic HY, hepatic GSD, and disorders of KB metabolism. Additional dietary interventions may be required for specific IMD (e.g. life–long fructose–, sucrose– sorbitol–restricted diet regimen in HFI, or low–protein diet in OA). Irrespective of their final diagnosis, in acute situations (e.g. intercurrent illness, prolonged fasting) patients can become catabolic, due to (the combination of) high fever, a reduced intake, and/or increased losses. Therefore, it is important to know what to do in emergency situations. An emergency protocol is designed at this purpose (98). Patients (and caregivers) should be encouraged to always carry an emergency protocol with them and follow its instructions. As HY can be secondary to a variety of different disorders, a systematic multidisciplinary approach is ideal in caring for neonates and children with HY. Interestingly, there is no consensus on standardized diagnostic algorithms for childhood HY. Therefore, a comprehensive practical diagnostic flowchart (including the main endocrine and metabolic causes) is proposed to guide the diagnostic suspicion, highlighting a minimal set of clue clinical and biochemical findings at the time of HY, that can be easily investigated in any hospital, at any time of the day. In fact, it is of paramount importance that samples are collected during HY (i.e. "critical sample"), otherwise the diagnosis can be missed (biochemical investigations might result normal when euglycemia has been reached). As shown in the proposed diagnostic flowchart, the minimal set of biochemical findings in children presenting with of HY includes ketones, lactate (both in blood and urine), and blood gases (i.e. metabolic acidosis). Such findings can help reaching a provisional diagnosis, which can be confirmed with additional (biochemical and/or genetic) tests. In this respect, collecting (and store adequately) additional samples at the time of HY is crucial. Laboratory data must also be appropriately integrated with anamnestic, dietary, clinical, and imaging information. The proposed flowchart aims at guiding the diagnostic management of a such common manifestation in pediatric age that can be due to a wide spectrum of causes. A double level usefulness is expected for the proposed flowchart: the first one is addressed to general pediatrician by providing the clinical–anamnestic and laboratory findings to be sought in order to refer the patient to the most appropriate Tertiary Center (Endocrinological/Metabolic/Genetic disease), the second level is for specialists in pediatric endocrine–metabolic diseases in order to remind them the wider etiological spectrum of pediatric HY by giving the essential elements of the differential diagnosis involving different areas (genetic, endocrine, and metabolic). In other word our flowchart aims to be a quick scheme to help pediatricians of every setting in managing HY, attempting to be comprehensive of the main disorders and differential diagnosis. Of course, mostly compared to the current available flowcharts focused on peculiar fields (metabolic or endocrinological), some rare conditions or rare presentations cannot be included. Sometimes, clinicians are not able to reach a final diagnosis, despite multiple efforts, due to the lack of specific biochemical pattern or atypical presentation of some disorders. In such cases, innovative diagnostic techniques can be considered. Even if much progress has been made over past years, many things remain to be discovered and clarified for diseases causing HY in childhood. Advances in diagnostic techniques (e.g. NGS) will identify specific defects or even new entities in a subgroup of patients who have been diagnosed with ketotic HY, likely resulting in a change in the disease epidemiology or in the discovery of new conditions (97).

In conclusion, future studies are also needed to optimally define normal glucose thresholds in neonates and children (10). In addition, irrespective of the specific diagnosis prompt recognition and treatment of acute HY are critical to prevent its complications,

the diagnostic work–up should start at the emergency hospital, collecting critical sample at the time of HY and providing specialists the clued results from simple tests that are available at any hospital at any time and that are very useful to address the clinical suspicion.

Based on the recognized risks of some tests the traditional diagnostic process, including fasting or dynamic tests, is presently controversial and probably superseded by modern molecular diagnostic techniques. NGS approach has also the potential to diagnose disorders with mild biochemical abnormalities or atypical presentations or even to identify new diseases, changing the epidemiology of many disorders. In this respect, the development of extended collaboration networks for rare diseases is worthy (43).

AUTHOR CONTRIBUTIONS

AC, AR and EM wrote the manuscript. EM, AF, SF and GP reviewed the manuscript. FMR, CM and FDC edited the manuscript and collected data. EM and SF are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors were responsible for drafting the article and revising it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors are grateful to the European Reference Network dedicated to Rare Endocrine Conditions (Endo–ERN) for useful discussions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.684011/full#supplementary-material

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frontiers in Endocrinology

ORIGINAL RESEARCH
published: 09 September 2021
doi: 10.3389/fendo.2021.727083

Molecular Genetics, Clinical Characteristics, and Treatment Outcomes of K_{ATP}-Channel Neonatal Diabetes Mellitus in Vietnam National Children's Hospital

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Specialty section:
This article was submitted to Pediatric Endocrinology, a section of the journal Frontiers in Endocrinology

Received: 18 June 2021
Accepted: 16 August 2021
Published: 09 September 2021

Citation:
Ngoc CTB, Dien TM, De Franco E, Ellard S, Houghton JAL, Lan NN, Thao BP, Khanh NN, Flanagan SE, Craig ME and Dung VC (2021) Molecular Genetics, Clinical Characteristics, and Treatment Outcomes of K_{ATP}-Channel Neonatal Diabetes Mellitus in Vietnam National Children's Hospital. Front. Endocrinol. 12:727083. doi: 10.3389/fendo.2021.727083

Background: Neonatal diabetes mellitus (NDM) is defined as insulin-requiring persistent hyperglycemia occurring within the first 6 months of life, which can result from mutations in at least 25 different genes. Activating heterozygous mutations in genes encoding either of the subunits of the ATP-sensitive K^+ channel (K_{ATP} channel; *KCNJ11* or *ABCC8*) of the pancreatic beta cell are the most common cause of permanent NDM and the second most common cause of transient NDM. Patients with NDM caused by K_{ATP} channel mutations are sensitive to sulfonylurea (SU) treatment; therefore, their clinical management can be improved by replacing insulin with oral agents.

Patients and Methods: Seventy patients were diagnosed with NDM between May 2008 and May 2021 at Vietnam National Children's Hospital, and molecular genetic testing for all genes known to cause NDM was performed at the Exeter Genomic Laboratory, UK. Patients with *ABCC8* or *KCNJ11* mutations were transferred from insulin to oral SU. Clinical characteristics, molecular genetics, and annual data relating to glycemic control, SU dose, severe hypoglycemia, and side effects were collected. The main outcomes of interest were SU dose, SU failure (defined as permanent reintroduction of daily insulin), and glycemic control (HbA1c).

Results: Fifty-four of 70 patients (77%) with NDM harbored a genetic mutation and of these; 27 (50%) had activating heterozygous mutations in *ABCC8* or *KCNJ11*. A total of 21 pathogenic mutations were identified in the 27 patients, including 13 mutations in *ABCC8* and 8 mutations in *KCNJ11*. Overall, 51% had low birth weight (below 3rd percentile), 23 (85%) were diagnosed before 3 months of age, and 23 (85%) presented with diabetic ketoacidosis. At diagnosis, clinical and biochemical findings (mean ± SD) were pH

7.16 ± 0.16; HCO_3^-, 7.9 ± 7.4 mmol/L; BE, –17.9 ± 9.1 mmol/L; HbA1C, 7.98% ± 2.93%; blood glucose, 36.2 ± 12.3 mmol/L; and C-peptide median, 0.09 (range, 0–1.61 nmol/l). Twenty-six patients were successfully transferred from insulin to SU therapy. In the remaining case, remission of diabetes occurred prior to transfer. Glycemic control on SU treatment was better than on insulin treatment: HbA1c and blood glucose level decreased from 7.58% ± 4.63% and 19.04 ± 14.09 mmol/L when treated with insulin to 5.8 ± 0.94% and 6.87 ± 3.46 mmol/L when treated with SU, respectively.

Conclusions: This is the first case series of NDM patients with *ABCC8/KCNJ11* mutations reported in Vietnam. SU is safe in the short term for these patients and more effective than insulin therapy, consistent with all studies to date. This is relevant for populations where access to and cost of insulin are problematic, reinforcing the importance of genetic testing for NDM.

Keywords: neonatal diabetes mellitus, *ABCC8* mutations, *KCNJ11* mutations, sulfonylureas treatment in neonatal diabetes mellitus, diabetes mellitus in infants

INTRODUCTION

Neonatal diabetes mellitus (NDM) is defined as uncontrolled hyperglycemia with onset in the first 6 months of life. It is estimated to affect one in 90,000 newborns (1). NDM can be divided into three forms based on phenotypic characteristics; transient NDM (TNDM), permanent NDM (PNDM), and syndromic NDM (2). TNDM and PNDM account for 90% of NDM cases. NDM can result from mutations in at least 25 different genes (2). Most cases of TNDM are caused by imprinting defects on chromosome 6q24, with presentation in infancy, remission, and subsequent relapse in later life. Activating heterozygous mutations in the genes encoding either of the subunits of the ATP-sensitive K^+ channel (K_{ATP} channel; *KCNJ11* or *ABCC8*) of the pancreatic beta cell are the second most common cause of TNDM (26% of TNDM cases) and the most common causes of PNDM (44% of PNDM cases) (2). The *KCNJ11* (MIM # 600937) and *ABCC8* (MIM # 60059) genes are located on the short arm of chromosome 11 (11p15.1) and encode the Kir6.2 subunit and the SU receptor 1 (SUR1) regulatory subunit of the K_{ATP} channel, respectively. In the normal pancreatic beta-cell, increased glucose enters the cell *via* a glucose transporter and is metabolized by the enzyme glucokinase, resulting in increased production of ATP. This causes closure of the K_{ATP} channel, which, in turn, depolarizes the cell membrane, activating the influx of calcium through voltage-gated calcium channels that subsequently allows exocytosis of insulin granules. Activating *KCNJ11* and *ABCC8* mutations cause the K_{ATP} channels to remain inappropriately open even in the presence of hyperglycemia. Without channel closure, the cell membrane is not able to depolarize effectively; thus, insulin cannot be released from the beta-cell (3).

In the clinical setting, insulin is the immediate choice for establishing glycemic control in NDM patients because it will be effective in all cases where an insulin deficit is involved. If a diagnosis of diabetes is made before 6 months of age and genetic screening is undertaken, the identification of mutations in *KCNJ11* or *ABCC8* provides an alternative therapeutic strategy. This is because if NDM results from overactive K_{ATP} channels, closing these channels is a key step to suppress insulin release, and sulfonylureas (SU) are well-studied K_{ATP} channel inhibitors (4). Moreover, SU have proven to be an effective treatment for individuals with NDM resulting from *KCNJ11* or *ABCC8* mutations (5, 6).

In 2020, De Franco et al. (7) collected and summarized a total of 748 *ABCC8* and 205 *KCNJ11* pathogenic and likely pathogenic mutations associated with congenital hyperinsulinism and NDM from various countries. In the present study, we report *KCNJ11/ABCC8* pathogenic mutations in Vietnamese patients with NDM diagnosed at Vietnam National Children's Hospital between May 2008 and May 2021, and the outcomes of SU therapy transfer.

RESEARCH DESIGN AND METHODS

Study Design and Individuals

The patients were diagnosed with NDM and were admitted to Vietnam National Children's Hospital from May 2008 to May 2021. Inclusion criteria were hyperglycemia onset before 6 months of age and fasting blood glucose ≥126 mg/dl (7.0 mmol/L). Fasting was defined as no caloric intake for at least 4 h in children aged 0–1 years or random plasma glucose concentration ≥11.1 mmol/L (200 mg/dl). Hyperglycemia lasting at least 2 weeks required insulin for treatment. All patients with *KCNJ11* or *ABCC8* mutations and their parents agreed to participate in the study. Exclusion criteria were hyperglycemia due to glucose infusion, infection, stress, drugs, and other factors.

Data Collection and Biochemical Analyses

Clinical phenotype and biochemical tests were performed at Vietnam National Children's Hospital. Data included pedigree, sex, date of birth, gestational age, birth weight, date of diabetes

diagnosis, natural history, and examination at diagnosis such as weight, height, and symptoms of diabetic ketoacidosis (DKA) including tachypnea, dehydration, lethargy, coma, and other symptoms such as convulsion. Blood glucose and HbA1C were measured by the automated Beckman Coulter AU2700/AU680 system. The specimen was collected in the early morning at schedule visits. Hexokinase technology with ORS 6221 reagent of OLYMPUS and ORS6192 reagent were used for blood glucose and HbA1c testing, respectively. Insulin and C-peptide were measured using immunoassay chemiluminiscent technology by automated biochemistry Hitachi 704. Arterial blood gas was measured by spectrometry, using GEM primer 300. Capillary glucose levels were measured at home by One Touch Ultrain in all patients five times/day (before breakfast, lunch, dinner, 22 h and 2 h) or whenever there was an abnormality. Continuous glucose monitoring (CGM) over 7 days was monitored by using the Medtronic Ipro^{TM}2 Professional. HbA1C was checked at 3-month intervals. Blood glucose level and HbA1C targets were determined according to the International Society of Pediatrics and Adolescent Diabetes (ISPAD) 2018 guideline (8).

Mutation Analysis

Mutation analysis was performed at the Exeter genomic laboratory, UK. Blood samples were taken with informed consent obtained from the patients and their parents. Genomic DNA was extracted from peripheral blood using phenol/chloroform methods at Vietnam National Children's Hospital.

The single exon of the *KCNJ11* gene was amplified in three overlapping fragments, as previously described (9). The *ABCC8* gene was analyzed at the same time as *KCNJ11*. The 39 exons of *ABCC8* were amplified in 38 fragments using previously described primers (10). PCR products were sequenced on an ABI 3100 or ABI 3730 (Applied Biosystems, Warrington, UK). Sequences were compared with the reference sequences (*KCNJ11*, NM_000525.3; *ABCC8*, NM_001287174.1) using Staden or Mutation Survey or software version 2.61.

The identified mutations were checked in common databases such as dbSNP154 database, ClinVar database, Leiden open variation database (LOVD), human gene mutation database (HGMD), and the genome aggregation database (gnomAD). *In silico* analysis was performed using Alamut Visual. Pathogenic variants identified after 2018 were classified using the ACMG best practice guidelines (11). Protein visualization was generated using the Protter website (http://wlab.ethz.ch/protter/start/)

Transfer to Sulfonylureas From Insulin

For this study, clinicians were provided with two recommended protocols for the transfer to the SU glyburide (also known as glibenclamide) for Kir6.2 and SUR1 patients [see www.diabetesgenes.org and (12)]. One was for a rapid inpatient transfer, where the glyburide dose was increased by 0.2 mg/kg/day every day and the other for a slower outpatient transfer, where the glyburide dose was increased by 0.2 mg/kg/day every week. Both involved the gradual withdrawal of insulin, as the SU was introduced depending on blood glucose levels. These protocols were modified by the treating clinicians (5).

Statistical Analysis

Data analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL). Data are expressed as frequency (%), mean ± SD, or median and range. Continuous variables were analyzed using ANOVA or Kruskal–Wallis tests. Proportions were compared using Fisher's exact tests. Statistical significance was defined as $p < 0.05$ or the corresponding Bonferroni-adjusted p value for multiple comparisons.

RESULTS

A total of 70 patients were diagnosed with NDM before 6 months of age. Pathogenic variants were identified in 54 (77%). The most common genetic causes were mutations in *ABCC8* (26%) and *KCNJ11* (24%). The current study focused on the analysis of the 27 patients with *ABCC8/KCNJ11* mutations.

Clinical Features of NDM Patients With *KCNJ11/ABCC8* Mutations

Of the 27 patients with a K_{ATP} channel mutation, 14 had a mutation in *ABCC8*, and 13 had a *KCNJ11* mutations. Clinical characteristics of these patients are provided in **Table 1**. Five of the 27 patients had TNDM, which was diagnosed before 3 months of age and onset with severe/moderate DKA. The average birth weight was 2758.3 ± 419.0 g with gestational age of 39.2 ± 1.28 weeks. Fifty-two percent (14/27) of the subjects were small for gestational age with a birth weight below the third centile. The average onset age in 27 cases was 60 ± 36.4 days, of which 85% were diagnosed before 3 months of age. At diagnosis, biochemical studies showed blood glucose, 36.2 ± 12.3 mmol/L; HbA1C, 7.16 ± 0.16% (normal range, 4–6.5%); HCO_3^-, 7.9 ± 7.4 mmol/L; BE, −17.9 ± 9.1 mmol/L; and C-peptide, median of 0.09 (range, 0–1.61 nmol/L; normal range, 0.26–0.62). Twenty-three patients (85.2%) presented with DKA. Nine patients (33.3%) had a convulsion at diagnosis.

Molecular Genetic Analysis

A total of 21 pathogenic variants were identified, including 13 *ABCC8* and 8 *KCNJ11* mutations (**Table 2** and **Figure 1**). These mutations were predicted to be disease causing by the Mutation Taster tool and evaluated as pathogenic variants in the ClinVar database. Four mutations, namely, *ABCC8* (p.E747X), *ABCC8* (p.R1183W), *KCNJ11* (p.R201C), and *KCNJ11* (p.R201H), were found in multiple patients. Except for the *ABCC8* (p.E747X) mutation, which was homozygous in patient 4 (13), the remaining mutations were heterozygous.

Twelve patients inherited mutations from their parents, while in 14 patients, the mutation had arisen *de novo*. Patient 33 inherited the *ABCC8* (p.R598Q) mutation from his mother and also had a *de novo* mutation *ABCC8* (p.R826W). Three patients 5, 14, and 33 had compound heterozygous mutations in *ABCC8*. Among 21 pathogenic mutations, four were unpublished, including *KCNJ11* (p.S331P), *ABCC8* (p.E1141G), and *ABCC8* (p.A1153G) (**Table 2**).

TABLE 1 | Clinical features of 27 NDM Vietnamese patients with *KCNJ11/ABCC8* mutations.

Pt	Age (days)	BW (percentile)	DKA	Glucose (mmol/L)	pH	HCO_3^- (mmol/L)	BE (mmol/L)	HbA1C (%)	C-peptide (nmol/L)	Neurological symptoms	Type	Mutation
1	44	<3	Severe	49.5	7.12	–	−22.7	9.7	0.009	Convulsion	PNDM	*KCNJ11*: p.R201H
2	37	<3	Severe	31.2	6.9	1	Very low	8.4	0.05	DEND	PNDM	*KCNJ11*: p.R201C
3	45	<3	Mild	28.2	7.34	14.5	−9.5	5.8	0.3	No	PNDM	*ABCC8*: p.R1183W
4	36	<3	No	30.9	7.36	4	−18	8.0	0.2	DEND	PNDM	*ABCC8*: p.E747X
5	44	50	Severe	26.2	7.03	3.7	−25.1	10.3	0.03	No	PNDM	*ABCC8*: p.E128K/p.E747X
10	160	70	Severe	37.2	6.9	1.9	−28.2	13.7	–	No	TNDM	*KCNJ11*: p.R50Q
12	7	3	Moderate	20.6	7.2	20.6	−5.7	5.4	0.003	No	PNDM	*KCNJ11*: p.R201C
13	15	<3	Mild	22.4	7.3	12.8	−15.4	3.5	0.52	Mild mental development delay	PNDM	*ABCC8*: p.A1153G
14	96	<3	Severe	47.7	6.99	4.3	−26	6.7	0.04	No	PNDM	*ABCC8*: c.3403-1G>A/p.E1507Q
15	45	10	No	39.3	7.35	28.9	3.2	6.0	0.09	Convulsion	PNDM	*KCNJ11*: p.E292G
16	52	3	Severe	43.1	6,9	4	−28.7	5.1	0.0001	No	TNDM	*KCNJ11*: p.E229K
23	71	>10	Severe	25.6	7.1	6.3	−22.2	7.2	0.19	No	PNDM	*ABCC8*: p.C435R
24	36	>10	Severe	31.7	7,08	3.3	−26	7.6	0.17	No	TNDM	*ABCC8*: p.R1183W
25	48	3	No	13.08	7.44	23	−0.8	8.2	0.03	No	PNDM	*ABCC8*: p.P1199L
26	62	10	Severe	41.6	7.06	3.7	−26.6	10.2	0.1	No	PNDM	*KCNJ11*: p.R201H
27	82	10	Severe	30.0	6.89	5,1	Very low	11.5	0.01	No	TNDM	*ABCC8*: p.R1183W
28	72	3	Severe	27.7	6.86	3.6	-28.5	9.31	0.08	Convulsion	PNDM	*KCNJ11*: p.K185Q
30	100	<3	Moderate	56	7.2	5.4	−19.9	11.0	0.27	No	PNDM	*KCNJ11*: p.G53S
32	72	>10	severe	53.11	6.9	3	Very low	8.17	0.07	No	PNDM	*ABCC8*: p.R1380H
33	33	10	Mild	50.1	7.29	17.8	−8.8	4	0.09	No	PNDM	*ABCC8*: p.R598Q/p.R826W
35	62	50	No	27.8	7.38	10.1	−15	4.5	1.61	Convulsion	PNDM	*KCNJ11*: p.S331P
36	50	<3	Moderate	Very high	7.19	8.0	−19	7.58	0.41	Convulsion	TNDM	*ABCC8*: p.E1141G
37	81	>10	Severe	26.87	7.15	3.5	−25.8	12	0.36	Convulsion	PNDM	*KCNJ11*: p.R201H
40	30	<3	Mild	53.3	7.24	3.9	−21	5.1	0.27	Convulsion	PNDM	*ABCC8*: p.G833S
48	23	<3	Severe	59.56	7.05	2.6	−25.5	4.05	0.04	No	PNDM	*KCNJ11*: p.R201C
49	90	>10	Severe	27.8	7.04	3.0	NA	8.3	0.27	No	PNDM	*KCNJ11*: p.R201C
52	152	>10	Mild	40.2	7.31	NA	NA	14.3	0.33	No	PNDM	*ABCC8*: p.R1183W

(-), Not detected; NA, Not analysis.

Transfer to Sulfonylureas

Of the 27 patients with mutations in *KCNJ11*or *ABCC8*, 26 were successfully transferred from insulin to SU treatment. Patient 24 remitted after 5 months of insulin treatment and before transfer to SU. Glycemic fluctuations reduced when the patients were on SU treatment as compared with insulin treatment (**Figure 2A**, p = 0.000001). The mean HbA1C level dropped from 7.75% ± 3.60% on insulin treatment (13.3 ± 25 months) to 5.69% ± 1.02% on SU treatment (86.0 ± 56.9 months, p = 0.0000038) (**Figure 2B**). While on insulin treatment, there was one case of DKA and one case with convulsion due to severe hypoglycemia. After transfer to SU (86.0 ± 56.9 months), there were no patients with DKA or hypoglycemia.

Two of the 27 patients (patients 2 and 4) were diagnosed with developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome (**Table 1**). Both individuals successfully transferred to SU, which resulted in improved mental and motor development. Patient 2, who is heterozygous for the *KCNJ11* p.R201C mutation, is currently 14 years of age and can understand simple sentences and has good eye contact. Patient 2 showed a decrease in HbA1c from 8.25% ± 0.20% (insulin treatment) to 6.65% ± 0.07% (SU treatment). During insulin treatment, patient 2 was admitted to the hospital twice (due to DKA and convulsion due to severe hypoglycemia). After transfer to SU, there were no further episodes of severe hypoglycemia or DKA. Patient 4 with a homozygous nonsense mutation in *ABCC8* p.(E747X) is currently 16 years of age and can walk, speak in short sentences, and understand and answer some simple questions. When patient 4 was treated with insulin, HbA1C was 8.5% ± 0.4%, and blood glucose ranged from 4 to 20 mmol/L; however, HbA1c decreased to 5.3% ± 0.2%, and blood glucose fluctuation ranged from 5 to 10 mmol/L when he was treated with SU.

Two TNDM patients with *KCNJ11* mutations (p.(R50Q) and p.(E229K)) and three TNDM patients with *ABCC8* mutations [two patients with p.(R1183W) and one patient with p.(E1141G)] remitted at 6, 50, 6, 14, and 24 months of ages, respectively (**Table 2**).

Treatment requirements (insulin or SU dose) did not show significant differences between the patients with *KCNJ11* mutations and those with *ABCC8* mutations (data not shown).

Side Effects

None of the patients reported side effects during SU treatment such as diarrhea, nausea, or vomiting. Renal and liver function tests of all patients were checked every 6–12 months; all were within the normal range. No severe hypoglycemic episodes were reported while on SU treatment, and no other side effects were noted.

TABLE 2 | Molecular analyses of 27 Vietnamese patients with NDM.

Gene	cDNA change	Protein change	Zygosity	Inheritance	dbSNP	ClinVar	Reference	Type (Patient No.)	Treatment
ABCC8	c.382G>A	p.E128K	Het	Maternal	rs781617345	RCV001058712 Pathogenic	(13)	PNDM (5)	Ins→SU
ABCC8	c.1303T>C	p.C435R	Het	Paternal	–	Pathogenic	(14)	PNDM (23)	Ins→SU
ABCC8	c.1793G>A	p.R598Q	Het	Maternal	rs1344172059	VCV000523361.1 Likely pathogenic	unpublished	PNDM (33)	Ins→SU
ABCC8	c.2239G>T	p.E747X	Hom (4) Het (5)	Paternal and maternal (4) Paternal (5)	–	RCV001051901.2 Pathogenic	(13)	PNDM (4) PNDM (5)	Ins→SU
ABCC8	c.2476C>T	p.R826W	Het	*De novo*	–	Pathogenic	(15)	PNDM (33)	Ins→SU
ABCC8	c.2497G>A	p.G833S	Het	*De novo*	–	Pathogenic	(16)	PNDM (40)	Ins→SU
ABCC8	c.3403-1G>A	Splicing	Het	Maternal	rs576684889	VCV000370935.5 Likely Pathogenic	(17)	PNDM (14)	Ins→SU
ABCC8	c.3422A>G	p.E1141G	Het	Paternal	–	Pathogenic	unpublished	TNDM (36)	Ins→SU Remission 24 months
ABCC8	c.3458C>G	p.A1153G	Het	Maternal	–	Pathogenic	unpublished	PNDM (13)	Ins→SU
ABCC8	c.3547C>T	p.R1183W	Het	Paternal (3, 24) *De novo* (27, 52)	rs797045209	VCV000210076.2 Pathogenic	(18)	TNDM (24, 27) PNDM (3, 52)	Ins→SU Remission 6 months (24) Remission 14 months (27)
ABCC8	c.3596C>T	p.P1199L	Het	*De novo*	rs1554909277	VCV000434047.1 Pathogenic	(19)	PNDM (25)	Ins→SU
ABCC8	c.4139G>A	p.R1380H	Het	Maternal	rs193922401	VCV000585346 Likely Pathogenic	(15)	PNDM (32)	Ins→SU
ABCC8	c.4519G>C	p.E1507Q	Het	Paternal	–	Pathogenic	(20)	PNDM (14)	Ins→SU
KCNJ11	c.149G>A	p.R50Q	Het	*De novo*	rs80356611	VCV000036431.1 Pathogenic	(21)	TNDM (10)	Ins→SU Remission 6 months
KCNJ11	c.157G>A	p.G53S	Het	*De novo*	rs80356613	VCV000008681.1 Pathogenic	(22)	PNDM (30)	Ins→SU
KCNJ11	c.553A>C	p.K185Q	Het	*De novo*	–	Pathogenic	(23)	PNDM (28)	Ins→SU
KCNJ11	c.601C>T	p.R201C	Het	Maternal (2) De novo (12, 48, 49)	rs80356625	VCV000008668.3 Pathogenic	(24)	PNDM (2) PNDM (12, 48, 49)	Ins→SU
KCNJ11	c.602G>A	p.R201H	Het	*De novo*	rs80356624	VCV000008666.4 Pathogenic	(25)	PNDM (1, 26, 37)	Ins→SU
KCNJ11	c.685G>A	p.E229K	Het	*De novo*	rs587783673	RCV000146117 Pathogenic	–	TNDM (16)	Ins→SU Remission 50 months
KCNJ11	c.875A>G	p.E292G	Het	Maternal	–	Pathogenic	(26)	PNDM (15)	Ins→SU
KCNJ11	c.991T>C	p.S331P	Het	Paternal	–	Pathogenic Variant #0000497821 LOVD database	Unpublished	PNDM (35)	Ins→SU

Het, heterozygous; Hom, homozygous; PNDM, permanent neonatal diabetes mellitus; TNDM, transient neonatal diabetes mellitus; Ins, insulin; SU, sulfonylureas.

DISCUSSION

In our comprehensive mutation analysis of a large cohort of 70 patients with NDM enrolled at Vietnam National Children's Hospital, we identified gene mutations in 54 cases (77%). In these 54 patients with a confirmed genetic diagnosis, mutations in the K_{ATP} channel genes was the most common cause of NDM with a rate of 50%. Overall, 51% had low birth weight (below third percentile), 23 (85%) were diagnosed before 3 months of age, and 23 (85%) presented with DKA. Twenty-six patients were successfully transferred from insulin to SU therapy, and glycemic control subsequently improved.

The mutation rate of 77% in our study was lower than that in Ukrainian (12) and Chinese studies but similar to the University of Chicago Monogenic Diabetes Registry (n = 88) (27). In contrast, Russo et al. (28) found the most common genes causing NDM diagnosed during the first 6 months of life were *KCNJ11* and *ABCC8* (70%), but mutations in *KCNJ11* were more common than *ABCC8*. These differences may be due to ethnicity, race, or size of the study cohort. In our study, we only investigated patients with NDM onset before 6 months of age who have mutations in the genes encoding the K_{ATP} channel.

The rate of patients with low birth weight (under third percentile) was 52%, which is similar to the results reported by Besser et al. (29). In the study reported by Russo et al. (28), the patients diagnosed with PNDM before 6 months of age but without mutations in *KCNJ11*, *ABCC8*, or *INS* had higher birth weight than those with mutations in these genes.

The majority of our cohort (85%) presented in DKA. Similarly, Letourneau et al. (27) reported that 66% of patients with neonatal diabetes (and 79% of patients with *KCNJ11*/*ABCC8* mutations) presented with DKA. While this is slightly

less than observed in our cohort, there may have been a delay in diagnosis in some of our cases, which is reflected in the later age of diagnosis. This delay may be related to the challenge of diagnosing diabetes in infants who cannot communicate symptoms and in whom polydipsia and polyuria may not be readily apparent—indeed, this could even be reassuring to clinicians.

The numbers of Vietnamese patients with *ABCC8* and *KCNJ11* mutations were similar (14 versus 13), which is consistent with the findings in the Indian population (30). In contrast, Hashimoto et al. (31) reported more patients with *KCNJ11* than *ABCC8* mutations (16 versus 8). Recurrent mutations *KCNJ11* p.(R201C), *KCNJ11* p.(R201H), and *ABCC8* p.(R1183W) in Vietnamese patients have been reported in NDM patients in Jordan (32), India (30), Ukraine (12), The United States (33), Japan (34), and China (35). Therefore, these mutations may be considered as common mutations in different ethnic groups. The high rate of *de novo* mutations, which can arise either during gametogenesis or embryogenesis in NDM patients (15/27), identified in the current study, is consistent with the findings of Edghill et al. (36).

None of the 12 mutations identified in *ABCC8* are located in the glibenclamide binding pocket of SUR1. However, four mutations [*ABCC8* p.(C435R) and p.(R598Q), p.(E1141G), and p.(A1153G)] are located in the transmembrane domains TMD1 and TMD2, respectively (**Figure 1A**). Interestingly, p.(C435R) was previously reported in two TNDM patients (15, 37), while patient 23 in our study had PNDM after treatment on SU 72 months (**Table 1**). Three *ABCC8* mutations p.(R598Q), p.(E1141G), and p.(A1153G) are unpublished (**Table 2**). Patient 33 had a compound heterozygous *ABCC8* mutation, p.(R598Q) and p.(R826W) (**Tables 1**, **2**).

Eight *KCNJ11* mutations identified in this study are located in the N- and C-terminal regions (**Figure 1B**), which form the ATP binding pocket of Kir6.2 (38). The p.(R50Q), p.(K185Q), p.(R201C), and p.(R201H) mutations may reduce the response of the channel to ATP, as they lie at the main binding pocket (23, 24, 39). The *KCNJ11* p.R50Q mutation has been reported to

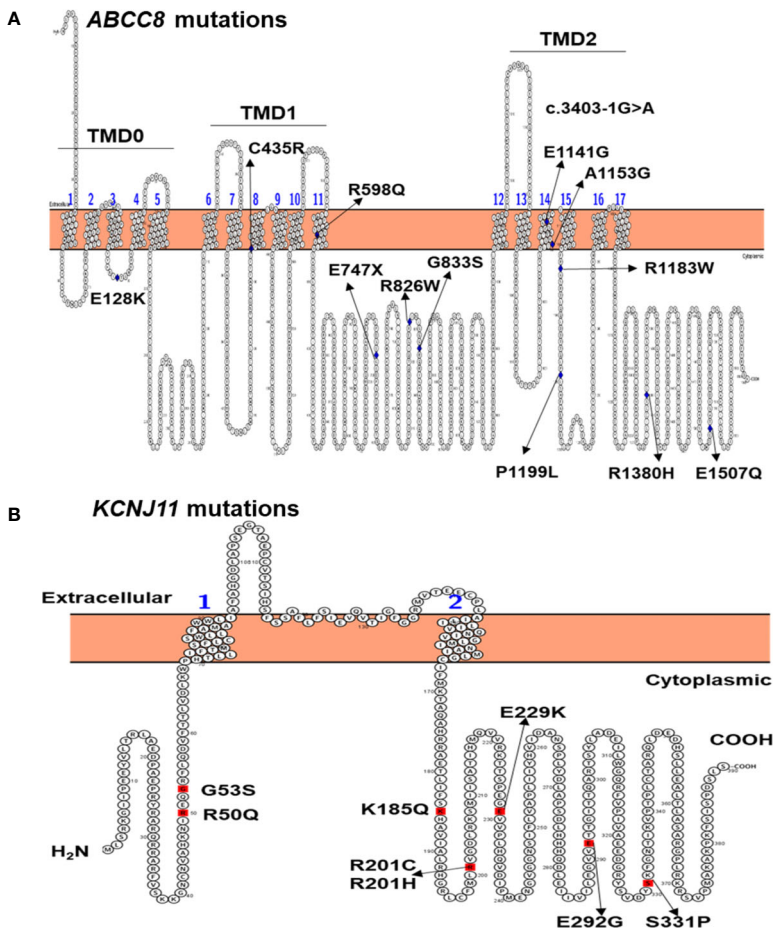

FIGURE 1 | Location of *ABCC8* mutations in the SUR1 protein **(A)** and *KCNJ11* mutations in the Kir6.2 protein **(B)** identified in 27 Vietnamese patients with NDM. TMD, transmembrane domain.

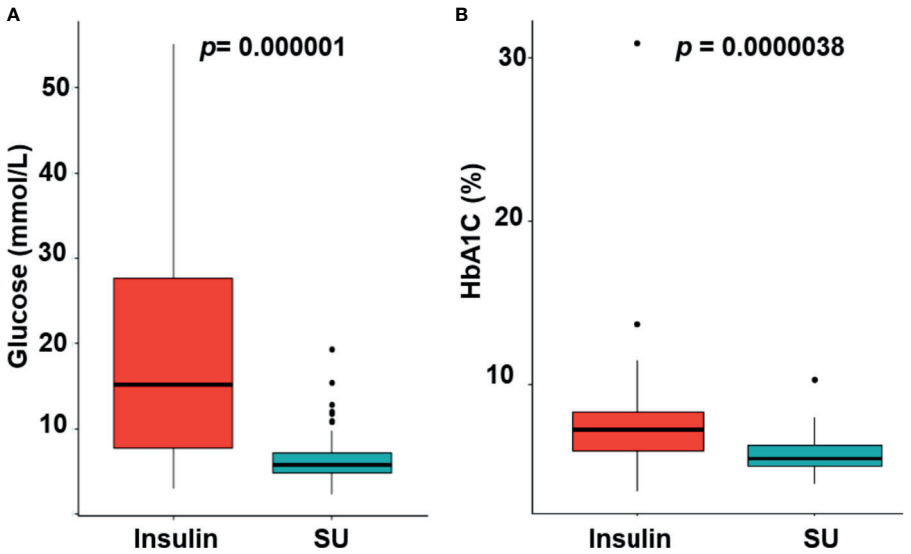

FIGURE 2 | The glycemic control **(A)** and reduction of HbA1C levels **(B)** with switching from insulin to sulfonylurea therapy. SU, sulfonylurea.

cause both TNDM (34) and PNDM (9); therefore, patients with this mutation can present with different phenotypes as suggested by Suzuki et al. (34). The *KCNJ11* p.(K185Q) mutation was identified in PNDM patient 28 (**Table 1**). This mutation have been reported previously in a 3-year-old girl with PNDM, and after treatment with insulin, her HbA1C was between 6.8% and 7.8% (23). In our study, patient 28 was also treated with insulin; however, she was transferred to SU, resulting in an HbA1C of 7.6% and blood glucose level of 7.9 mmol/L. Functional studies indicated that this mutation reduced ATP binding to Kir6.2, resulting in a reduction in ATP sensitivity of the K_{ATP} channel, leading to PNDM in the patient (23).

SU therapy is effective in the treatment of hyperglycemia in patients with NDM who have a mutation in *KCNJ11* or *ABCC8*. Up to 90–95% of patients with NDM caused by mutations in these genes can cease insulin therapy after initiation of SU therapy (3, 5). In our study, the rate was higher, at 96.3%. The one remaining case (3.7%) had a remission of diabetes at 5 months of age before transfer to SU could be initiated. SU acts on the K_{ATP} channel to promote closure, allowing insulin to be released from the beta cells. Since SU therapy increases insulin release, there is a risk for hypoglycemia to occur. However, there was no severe hypoglycemia reported in our study. Excellent glycemic control was maintained after commencing SU therapy (**Figure 2A**), which is similar to other reports (6, 40). In the study of Bowman et al. (40), there were no reports of severe hypoglycemia in 809 patient-year follow up for the whole *KCNJ11* cohort, and 93% of the participants remained on SU therapy for the 10-year duration; however, a high rate (14%) of patients presented with mild and transient side effects of SU such as diarrhea, nausea, weight loss due to reduced appetite, and abdominal pain (40). Whereas, in our study, there was no side effects of SU, continued follow-up will be required to determine the long-term outcome of SU therapy in this group of patients. Interestingly, two patients (patients 2 and 4) with DEND syndrome were successfully transferred to SU treatment, which is consistent with previous studies (41, 42).

In conclusion, we found that all patients in our cohort with *ABCC8* and *KCNJ11* mutations could be successfully treated with oral SU treatment even if they had previously been treated with insulin. It is essential to perform rapid genetic testing for *ABCC8/KCNJ11* in any patient diagnosed with diabetes before 6 months of age, particularly given issues regarding access to and cost of insulin in some populations. On SU treatment, we observed that this therapy is safe in the short term for patients with K_{ATP} channel NDM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Vietnam National Children's Hospital IRB#1, 18/879 Lathanh, Dongda, Hanoi, Vietnam. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CN, TD, SE, and VD conceptualized, designed the study, and wrote and reviewed the manuscript. VD, CN, BT, and NNK

provided patients' clinical information, and MC reviewed/edited the manuscript. EF, SF, JH, and NNL analyzed data and wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This project was supported by grants from the Wellcome Trust and the Royal Society (Grant Number: 105636/Z/14/Z). EDF is a Diabetes UK RD Lawrence fellow. SF has a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (105636/Z/14/Z). MC supported by a NHMEC practitioner fellowship (#1136735).

ACKNOWLEDGMENTS

The authors thank the patients and their family members for their time and support.

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