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Shared Genetic Basis and Causal Relationship Between Television Watching, Breakfast Skipping and Type 2 Diabetes: Evidence From a Comprehensive Genetic Analysis

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Background: Epidemiological investigations have established unhealthy lifestyles, such as excessive leisurely sedentary behavior (especially TV/television watching) and breakfast skipping, increase the risk of type 2 diabetes (T2D), but the causal relationship is unclear. We aimed to understand how single nucleotide variants contribute to the co-occurrence of unhealthy lifestyles and T2D, thereby providing meaningful insights into disease mechanisms.

Methods: Combining summary statistics from genome-wide association studies (GWAS) on TV watching (N = 422218), breakfast skipping (N = 193860) and T2D (N = 159208) in European pedigrees, we conducted comprehensive pairwise genetic analysis, including high-definition likelihood (HDL-method), cross-phenotype association studies (CPASSOC), GWAS-eQTL colocalization analysis and transcriptome-wide association studies (TWAS), to understand the genetic overlap between them. We also performed bidirectional two-sample Mendelian randomization (MR) analysis for causal inference using genetic instrumental variables, and two-step MR mediation analysis was used to assess any effects explained by body mass index, lipid traits and glycemic traits.

Results: HDL-method showed that T2D shared a strong genetic correlation with TV watching (r_g = 0.26; P = 1.63×10^{-29}) and skipping breakfast (r_g = 0.15; P =2.02×10^{-6}). CPASSOC identifies eight independent SNPs shared between T2D and TV watching, including one novel shared locus. TWAS and CPASSOC showed that shared genes were enriched in lung, esophageal, adipose, and thyroid tissues and highlighted potential shared regulatory pathways for lipoprotein metabolism, pancreatic β-cell function, cellular senescence and multi-mediator factors. MR showed TV watching had a causal effect on T2D (β_{IVW} = 0.629, P_{IVW} = 1.80×10^{-10}), but no significant results were observed between breakfast skipping and T2D. Mediation analysis provided evidence that body mass index,

fasting glucose, hemoglobin A1c and high-density lipoprotein are potential factors that mediate the causal relationship between TV and T2D.

Conclusions: Our findings provide strong evidence of shared genetics and causation between TV watching and T2D and facilitate our identification of common genetic architectures shared between them.

Keywords: TV watching, breakfast skipping, type 2 diabetes, Mendelian randomization, genome genetic correlation

HIGHLIGHTS

- The strongest positive genetic correlation was observed between TV watching and type 2 diabetes.
- Cross-trait meta-analysis identifies eight independent genomic loci shared between type 2 diabetes and television watching, one of which is novel.
- Implicated genes suggest potential treatment targets and signaling pathways for type 2 diabetes and television watching.
- Transcriptome-wide association studies and cross-trait meta-analysis support the role of lipoprotein metabolism, cellular senescence and multi-mediator factors may account for the shared metabolic pathway and causes between TV watching and T2D.
- Mendelian randomization study showed TV watching had strong causal effect on T2D (β_{IVW} = 0.629, P_{IVW} = 1.80×10^{-10}).

INTRODUCTION

Type 2 diabetes (T2D) is a global epidemic that affects more than 463 million people and is a leading cause of morbidity and mortality worldwide. Family-based studies have shown that T2D is highly heritable, with an estimated heritability range of 20%-80% (1, 2). Currently, worldwide prevalent unhealthy lifestyles (especially TV watching and breakfast skipping) are also considered to be the key contributors to T2D. However, whether such an unhealthy lifestyle is causally associated or shares a genetic basis with T2D remains largely unknown.

A growing body of evidence from observational studies suggests that the risk of T2D is positively associated with prolonged TV watching (3–6) and breakfast skipping (7–9). A prospective study showed that TV watching is always related to higher energy intake than expenditure and leads to higher BMI (10), which affects metabolism by releasing non-esterified fatty acids (NEFAs) (11). Increasing plasma NEFA levels then leads to inadequate insulin secretion and insulin resistance (low insulin sensitivity), together contributing to the development of T2D (11). The association between breakfast skipping and T2D is also reported to be partially mediated by body mass index (BMI) (9). Furthermore, breakfast skippers are more likely to have lower serum HDL cholesterol levels (12), which is widely confirmed to be associated with an increased risk of T2D in Mendelian randomization studies (13). Therefore, we hypothesized that a common genetic etiology and the mediating role of BMI or HDL may at least partially explain the association between T2D and TV watching and breakfast skipping.

Evidence from observational studies is limited for making causal inferences, as such associations may be due to (residual) confounding and/or reverse causality (14). Considering that genetics is unlikely to be influenced by these factors, it is informative to use genetic variants as instrumental variables to investigate the causal relationships behind these associations. To date, genome-wide association studies (GWAS) have been able to detect 145, 128 and 6 genome-wide significant independent SNP signals for T2D, TV watching and breakfast skipping, respectively. Many of the significant loci for TV watching are also susceptibility loci for T2D, suggesting a possible common genetic etiology between them (15–17). Meanwhile, a growing number of Mendelian randomization studies based on strong instrumental variables (IVs) have shown a causal relationship between TV watching and numerous adverse outcomes, such as cerebrovascular diseases (18), coronary artery disease (17), chronic kidney disease (19) and lung cancer (20). However, Mendelian randomization cannot deal with pleiotropy, where genetic variation is associated with multiple traits, since it will break the single pathway hypothesis of MR (21). Research suggests that cross-phenotypic (CP) associations can recognize genetic pleiotropy in human diseases and highlight shared biological pathways compared to single-trait analysis (22). However, little research has been done on CP association analysis between T2D with TV watching and breakfast skipping.

Therefore, to increase our understanding of potential causality and shared genetic architecture between TV watching, breakfast skipping and T2D, we conducted a comprehensive genetic analysis. We performed a bidirectional MR and mediation analysis using summary statistics from public external URL (https://data.mendeley.com/datasets/mxjj6czsrd/1), the Common Metabolic Diseases Knowledge Portal (CMDKP) website (for exposures) and the Diabetes Genetics Replication And Meta-analysis (DIAGRAMv3) Consortium (for type 2 diabetes). To further identify genomic loci shared between T2D and exposures,

Abbreviations: T2D, Type 2 diabetes; BMI, body mass index; SNV, Single nucleotide variant; SNP, Single nucleotide polymorphism; GWAS, genome-wide association study; HDL, High density lipoprotein; HDL-method, High-definition likelihood; LDSC, Linkage disequilibrium score regression; CPASSOC, Cross phenotype association study; eQTL, expression quantitative trait loci; MR, Mendelian randomization; TWAS, Transcriptome wide association study; GTEx, Genotype-tissue expression portal.

we used cross-phenotype association (CPASSOC) analysis and transcriptome-wide association (TWAS) studies to explore shared genetic components among these complex phenotypes.

MATERIALS AND METHODS

Data Source and Study Population

The study was conducted using publicly available GWAS summary data. Details on the study characteristics, participants, and ethics declarations for each dataset can be found in the original publications (16, 17, 23). The hitherto largest GWAS of self-reported TV watching was conducted based on the United Kingdom Biobank (UKB) population cohort (N = 422218) (17). A total of 45.7% of participants were male, with a mean age of 57.4 [standard deviation (SD) 8.0] years at the first assessment of the cohort, and the mean daily reported leisure TV watching was 2.8 h (SD 1.5). The most recent summary results for breakfast skipping were based on a proxy-phenotype (breakfast cereal skipping) GWAS obtained from the Common Metabolic Diseases Knowledge Portal website (16), which included 193860 participants with 24-hour retrospective dietary data from the UKB. We used the T2D GWAS summary statistics from the 2017 report of the DIAGRAMv3 Consortium, consisting of 26676 T2D cases and 132532 control individuals (23). All participants were of European ancestry and had no overlap between exposure (TV watching, breakfast skipping) and outcome (T2D) samples. The location of SNPs is based on the Genome Reference Consortium Human Build 37 (GRCh37).

Genetic Correlation Analysis

The more recent high-definition likelihood (HDL-method) (24) method and conventional cross-trait linkage disequilibrium score (LDSC) regression (25) were conducted to evaluate the genetic correlation ($\boldsymbol{r}_g$) between T2D and TV watching and breakfast skipping. HDL-method extends the LDSC method by modeling the relation between covariances among Z statistics for pairs of traits across multiple SNPs and a full matrix of cross-SNP LD scores. As the HDL-method yields more precise estimates of genetic correlations than LDSC, we chose the HDL-method as the primary result. The HDL-method uses the LD reference computed from 335265 genomic British individuals in the UKB.

Cross Trait Meta-Analysis

Genetic correlation depicts the genome-wide average sharing of genetic effects between traits. To identify genetic variants shared between traits, we applied cross-trait GWAS meta-analysis using the cross-phenotype association (CPASSOC) (26) method to combine the association evidence for TV watching and breakfast skipping with T2D based on the criteria of both $\boldsymbol{r}_g > 10\%$ and $P_{bonferroni} < 0.05$ from HDL-method. CPASSOC combines effect estimates and standard error of GWAS summary statistics to test the hypothesis of association between a SNP and two traits and assumes that effects may exist only within a subset of traits (27). We used the heterogonous version of cross-phenotype association (SHet), which is based on a sample size-weighted, fixed-effect model and is more powerful when there is a heterogonous effect present between studies (26).

We applied PLINK1.9 clumping function (parameters: –clump-p1 2.5e-8 –clump-p2 1e-5 –clump-r2 0.4 –clump-kb 500) to determine index loci that are independent of each other, i.e., variants with P value less than 1×10^{-5} have an r^2 greater than 0.4 and less than 500 kb away from the peak will be assigned to that peak's clump. We identified all genes falling within each clump region. A P value of 2.5×10^{-8} ($5\times10^{-8}/2$) was used as genome-wide significance level for cross-trait meta-analysis to account for 2 meta-analyses. SNPs with a meta-analysis P value less than 2.5×10^{-8} and trait-specific P value less than 1×10^{-5} were selected for downstream analysis.

GWAS-eQTL Coloclization Analysis

To investigate whether the shared index SNPs from CPASSOC and their expression quantitative trait loci (eQTLs) co-localized with candidate causal variants, we performed colocalization analysis, COLOC, which uses Bayesian posterior probability to assess colocalization (28). We extracted cis-eQTL data from the Genotype-Tissue Expression (GTEx) Portal v7 for 48 single tissues (29). The SNP-associated locus was defined as within a 1-Mb window for each of the shared SNPs. The posterior probability H4 hypothesis was calculated to determine whether shared SNPs are associated with two traits. In our study, loci with posterior probability H4 > 0.9 were considered to be co-localized.

Transcriptome-Wide Association Studies

For TV watching, skipping breakfast and T2D, we used transcriptome-wide association studies (TWAS) to identify genes whose cis-regulated gene expression was associated with the corresponding traits. Then, we further evaluated shared tissue-gene pairs between different traits. We performed TWAS analysis using FUSION software and its precomputed transcript expression reference weights, as well as eQTL data from GTEx v.7 (30). Bonferroni correction was applied to determine significant association results after multiple comparisons for all tissue-gene pairs tested for each trait ($\boldsymbol{P}_{Bonferroni} < 0.05$). To increase the significance of the TWAS results, we used the most recent and authoritative summary data for T2D obtained from DIAGRAM. This study was performed in 2018 by Mahajan et al., who mined additional novel T2D susceptibility SNP loci by combining data from 898130 (including UKB sample) individuals of European descent (31).

Mendelian Randomization Analysis

Finally, we implemented a bidirectional MR using TwoSample MR package to test the causal relationship between T2D and unhealthy lifestyles, where the associations for IV-exposure and IV-outcome came from two nonoverlapping groups of participants. Since different MR methods have different degrees of explanation and contexts of application and differ in statistical efficiency, we adopt many MR methods to estimate causal effects. The causal effect estimates from the multiplicative random effects inverse variance weighted (IVW) model were used as the primary result. We conducted a range of sensitivity analyses

using multiplicative random effects inverse variance weighted heterogeneity test, weighted median, MR–Egger regression, MR-Steiger, MR-Robust Adjusted Profile Scores (MR-RAPS), MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) analysis and leave-one-out cross-validation analysis. The weighted median approach provides consistent and robust estimates even if more than 50% of the IVs are invalid (32). The intercept of MR–Egger regression can be used to evaluate the directional pleiotropy of IVs (33). We applied MR-Steiger to assure that the causal direction between the hypothesized exposure and outcome was correctly assigned (34). Considering the measurement error in SNP exposure effects, MR-RAPS is unbiased when there are many weak instruments and is robust to systematic and idiosyncratic pleiotropy (35). MR-PRESSO and leave-one-out cross-validation analysis are mainly used to detect anomalous IVs (36, 37).

Furthermore, the effect allele frequency reported in the corresponding GWAS was used to detect and exclude all palindromic SNPs to determine the corresponding strand between two GWAS in harmonization section. For trait pairs with significant causal relationships, we searched the GWAS catalog (https://www.ebi.ac.uk/gwas/) to exclude IVs with genome-wide significance for potential confounding traits (e.g., educational attainment, cognitive performance, smoking behavior, alcohol consumption, hypertension, BMI, waist-to-hip ratio, body fat percentage, cardiovascular disease, etc.) and reran the MR to obtain more robust MR estimates. For TV watching, breakfast skipping and T2D, independent genetic instruments were selected at GWAS p value < 5×10^{-8} and LD r^2 < 0.001 based on the 1000 Genomes European phase 3 reference panel. Given the multiple comparisons, in this study, we considered a P threshold < 0.05 as suggestive significance, while Bonferroni-corrected P threshold was used as statistically significant (P<0.05/6 = 0.008).

To further assess the direct effects of TV watching on T2D, we performed two-step MR mediation analysis. We selected body mass index (BMI), 4 lipid traits [including high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride (TG), total cholesterol (TC)], and 6 glycemic traits [including fasting glucose (FG), fasting insulin (FI), 2-h postprandial glucose (2hGlu), hemoglobin A1c (HbA1c), homeostatic model assessment of beta cell function (HOMA-β), homeostatic model assessment of insulin resistance (HOMA-IR)] as potential mediators of liability to TV watching in T2D. Two-step MR is based on the coefficient product method to calculate indirect (or mediator) effects (**Figure 1**). This process involves calculating two MR estimates, one for the causal effect of exposure on the mediator and the other for the causal effect of the mediator on the outcome. These two estimates are then multiplied together to estimate the indirect effect (38). GWAS summary statistics for BMI, lipid traits, and glycemic traits were obtained from the Genetics of ANthropometric Traits (GIANT) Consortium, the Meta-Analysis of Glucose and Insulin-related traits Consortium (MAGIC), and the Global Lipid Genetics Consortium (GLGC), respectively. The source literature corresponding to the three mediated traits can be found here (39–42). There was no sample size overlap between exposures and mediators and little overlap between mediators and outcomes in the selected GWAS data. Bonferroni-corrected P threshold (P<0.05/11) was used as statistical significance accounting for the 11 mediation analyses.

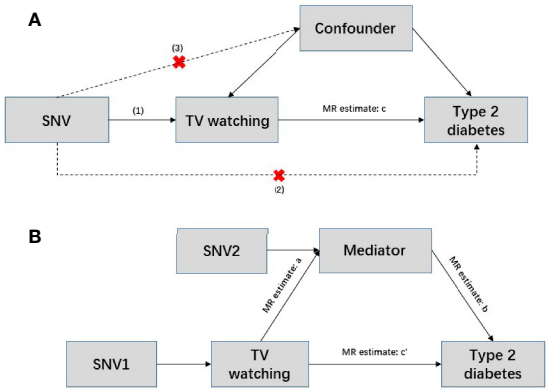

FIGURE 1 | Conceptual diagram of Mendelian randomization and mediation analysis. **(A)** Mendelian randomization is based on the following three assumptions. (1) Genetic variants are strongly associated with exposure ($p<5\times10^{-8}$); (2) instrumental variables can only act on the outcome through exposure, and there is no direct association with the outcome; and (3) instrumental variables are independent of any confounding factors. In this situation, c represents the total effect, SNV: single nucleotide variant. **(B)** Two-step Mendelian randomization, where a represents the effect of the exposure on the mediator; b represents the effect of mediator on the outcome; c' represents the direct effect; and a and b are estimated separately using separate genetic instrumental variables for both the exposure and mediator. These estimates are then multiplied together to estimate the indirect effect of the mediator (a * b), and the direct effect c' = c – a*b.

RESULTS

Genetic Correlations

T2D showed a strong positive genetic association with TV watching (r_g = 0.26; P = 1.63×10^{-29}) and skipping breakfast (r_g = 0.15; P =2.02×10^{-6}). The results suggested a potential common genetic basis and thus warranted further investigation of the underlying mechanisms using cross trait meta-analysis and instrumental variable analysis (**Table 1**).

Cross Trait Meta-Analysis

We identified eight index loci shared between T2D and TV watching (P_{meta} < 2.5×10^{-8} and single-trait P < 1×10^{-5}). However, we did not find any shared loci between T2D and breakfast skipping. GWAS-eQTL colocalization analysis had no significant results, but it identified a specific region at 12q14.3 that might be an expression quantitative trait locus between T2D and TV watching (tissue: lung, mapped gene: *HMGA2*, $P_{nominal}$ = 1.79×10^{-4}, H4 = 1.29×10^{-3}). Two of our CPASSOC index SNPs are located at the 12q14.3 region mapping to *HMGA2* gene. *HMGA2* encodes a protein belonging to the non-histone

TABLE 1 | Genetic correlation of type 2 diabetes with TV watching and breakfast skipping, estimated by high-definition likelihood method (HDL-method) and linkage disequilibrium score regression (LDSC).

Method	Trait	r_g	SE	r_g, 95%CI	pvalue	h^2(SE)
HDL-method	TV watching	0.26	0.023	0.21 to 0.31	1.63E-29	0.13(0.004)
	breakfast skipping	0.15	0.032	0.09 to 0.21	2.02E-6	0.05(0.002)
LDSC	TV watching	0.28	0.030	0.22 to 0.34	1.28E-21	0.13(0.004)
	breakfast skipping	0.14	0.043	0.06 to 0.22	1.30E-3	0.05(0.003)

Summary statistics for each trait were merged with Hapmap3 SNPs excluding the HLA region to estimate r_g; p value < 0.05/2; h^2 indicates the heritability of the corresponding phenotype.

chromosomal high-mobility group (HMG) protein family, and the protein contains structural DNA-binding domains and may act as a transcriptional regulating factor. Significantly higher expression of *HMGA2* mRNA in white adipose tissue has been reported in patients with T2D (43).

More importantly, we identified one novel locus shared between T2D and TV watching (11q13.1, index SNP: rs78028320, mapped gene: *CFL1*, $P_{meta} = 2.68\times10^{-9}$). *CFL1* is a typical protein-coding gene that encodes cofilin-1, an intracellular actin regulatory protein that plays an important role in regulating the organization of the actin cytoskeleton. Phosphorylated (inactive) cofilin-1 is upregulated in diabetic glomeruli, suggesting alterations in actin dynamics (44). In addition, podocytes in glomeruli are the key structure for maintaining the selective filtration barrier of the kidney. Its loss and structural abnormalities contribute to the progression of diabetic nephropathy (45). It has also been reported that mice deleted of *CFL1* in podocytes developed increased albuminuria and developed renal dysfunction, as indicated by a rise in creatinine (46).

The most significant locus overall was index SNP rs4420638 (mapped gene: *APOC1*, $P_{meta} = 2.42\times10^{-14}$). The mapped gene *APOC1* (apolipoprotein C1) is a protein-coding gene engaged in the inhibition of cholesteryl ester transfer protein (CETP). A study showed that *APOC1* was highly expressed in clear cell renal cell carcinoma (47), and a variant of *APOC1* called T45S led to elevated rates of T2D (48). The second strongest SNP was rs4565329 (mapped gene: *CENPW*, $P_{meta} = 7.64\times10^{-14}$). *CENPW* encodes a centromere protein that plays a central role in the assembly of kinetochore proteins, mitotic progression and chromosome segregation. The association between *CENPW* and T2D has been reported in previous genome-wide meta-analysis (49). SNP rs74333814 was also shared between TV watching and T2D (mapped gene: *ARAP1*, $P_{meta} = 3.84\times10^{-13}$). *ARAP1* encodes protein that is thought to regulate the cell-specific trafficking of a receptor protein involved in apoptosis. Findings suggest that *ARAP1* engages in islet insulin content and secretion and is thus likely to mediate the effects on diabetes susceptibility (50). Significantly, previous studies also showed that *APOC1* (51) and *ARAP1* (52) had a significant effect on BMI.

Transcriptome-Wide Association Studies

We next delved into the genetic level and examined shared TWAS genes between TV watching, breakfast skipping and T2D. After Bonferroni correction, a total of 10127 gene-tissue pairs were found to be significantly associated with T2D in 48 GTEx tissues, in addition to 7540 and 143 gene-tissue pairs associated with TV watching and breakfast skipping, respectively. We found 365 TWAS-significant genes shared between T2D and TV watching, with significant system-wide overlap, especially in the endocrine system, cardiovascular system, digestive system and nervous system (**Figure 2**). Intriguingly, 6 of the 365 shared TWAS-significant genes were also identified in CPASSOC, including *CENPW*, *ARAP1*, *CFL1*, *HMGA2*, *ABO* and *ATG16L2*. The functions of the first four genes have been described in detail in the CPASSOC section, and here, we focus on the two genes *ABO* and *ATG16L2*. The *ABO* (9q34.2) gene encodes the blood group ABO systemic transferase and is ubiquitously expressed in many tissues and cell types (53). Genetic variation at the ABO locus and ABO blood group have been found to be associated with the risk of venous thromboembolism (54) and type 2 diabetes (55). *ATG16L2* (11q13.4) is a protein-coding gene whose function is not fully understood, and it has been shown to play a unique function in autophagy. Analysis of transcriptomic data shows that autophagy plays a major role in the molecular pathology of T2D and AD (56).

However, for T2D and breakfast skipping, we observed only 12 shared TWAS-significant genes, mainly enriched in the endocrine system (**Figure 2**). Notably, we found that *EIF2S2P3* was the most enriched and significant among the 12 shared genes. *EIF2S2P3* is located at 10p23.33 and is a pseudogene. It has been reported to be associated with T2D (56), but its function remains unclear.

Mendelian Randomization Analysis

In our MR study, for T2D, TV watching, and breakfast skipping, we selected 35, 127 and 5 SNPs as IVs, respectively. The detailed characteristics of the IVs are shown in **Tables S1**-**S4**, and the screening flow of IVs is shown in **Figure 3**. F statistics provide an indication of the strength of the instrument and can be calculated using formula $F = \frac{n-k-1}{k} \cdot \frac{r^2}{1-r^2}$ (n is sample size, k is the number of IVs, and r^2 refers to how much variation in the trait can be explained by the set of genetic instruments used) (57). Given that r^2 is not generally provided in GWAS summary data, we used the formula $r^2 = \sum \left[\frac{\beta^2 \cdot 2 \cdot f \cdot (1-f)}{\beta^2 \cdot 2 \cdot f \cdot (1-f) + se^2 \cdot 2 \cdot n \cdot f \cdot (1-f)}\right]$ (f is effect allele frequency, n is sample size, β is effect estimate for each SNP and *se* is standard error for each SNP) (58) to obtain r^2 estimates. The F statistics for T2D, TV watching and breakfast skipping IVs are 69.86, 142.42 and 49.69, respectively (F >10 demonstrates that the analysis is unlikely to be affected by weak instrumental bias) (59).

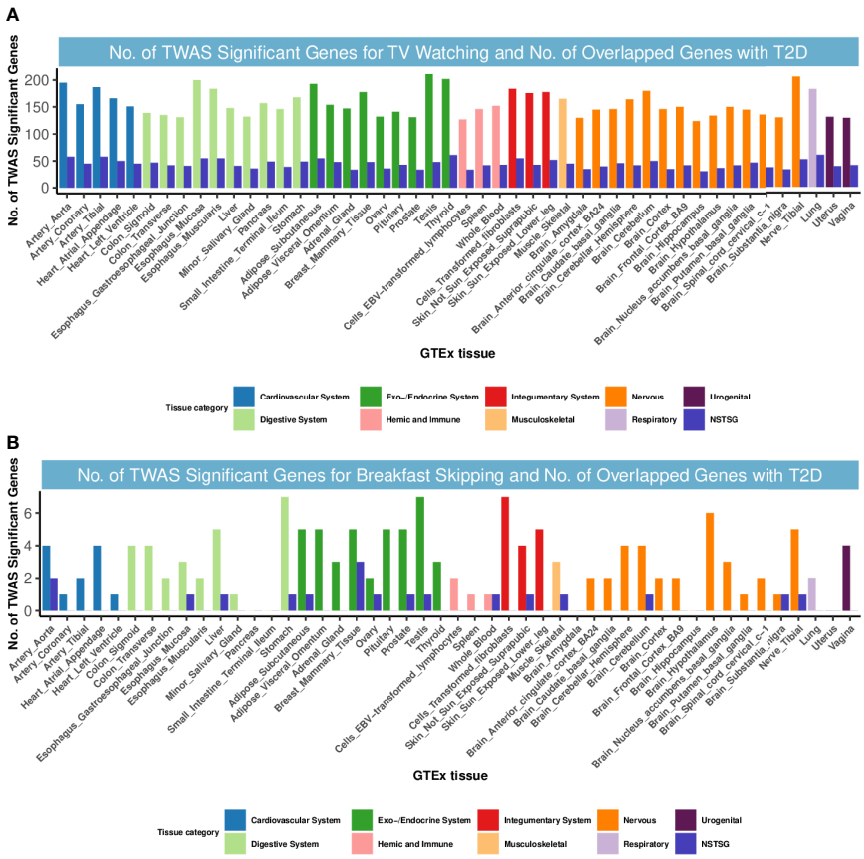

FIGURE 2 | Numbers of significant genes related to TV watching and breakfast skipping and the number of shared genes with T2D. Significant genes were identified by $P_{Bonferroni} < 0.05$. GTEx, genotype-tissue expression project; GWAS, genome-wide association studies; TWAS, transcriptome-wide association study; NSTSG, Number of shared TWAS significant genes between traits; T2D: type 2 diabetes. **(A)** No. of TWAS Significant Genes for TV watching and No. of Overlapped Genes with T2D. **(B)** No. of TWAS Significant Genes for breakfast skipping and No. of Overlapped Genes with T2D.

As shown in **Table 2**, TV watching was positively associated with the risk of type 2 diabetes [OR (95% CI)$_{IVW}$ = 1.86 (1.54, 2.26), $P = 1.80\times10^{-10}$; OR_{WM} = 1.82 (1.43, 2.32), $P = 1.12\times10^{-6}$; $OR_{MR\text{-}RAPS}$ = 1.78 (1.50, 2.11), $P = 3.13\times10^{-11}$; $OR_{MR\text{-}PRESSO\ Outlier\text{-}corrected}$ = 1.84 (1.56, 2.16), $P = 1.22\times10^{-11}$], with all P values reaching the Bonferroni-corrected threshold and without any evidence of pleiotropy ($P_{MR\text{-}Egger\text{-}intercept}$ = 0.41). This causal effect became more significant in the sensitivity analysis

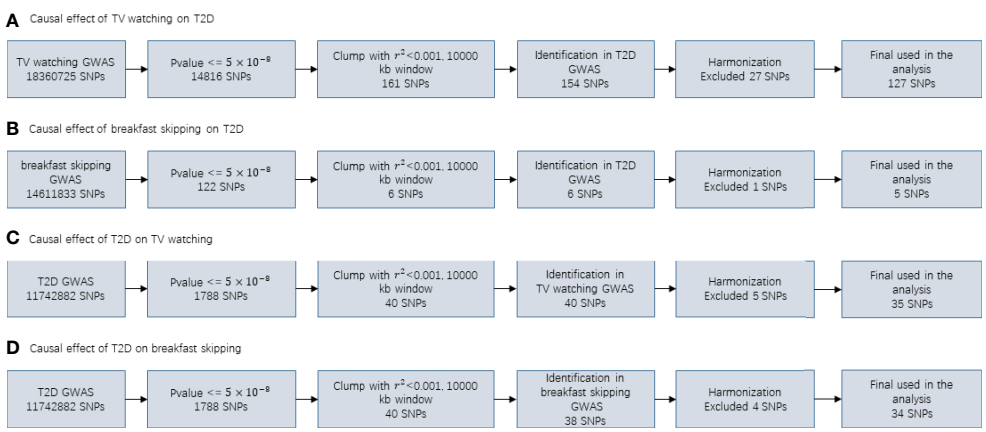

FIGURE 3 | Flowcharts visualizing the process for instrument definition, extraction and harmonization for the two-sample MR analyses conducted in the present study.

TABLE 2 | Causal relationships between TV watching, skipping breakfast and T2D (findings adjusted for multiple comparisons).

Exposure	Outcome	N_snp	Method	beta	OR	95%CI[#]	SE	p_value	Heterogeneity_P_value	Intercept_P_value	Steiger_P_value
TV watching	T2D	127	IVW	0.629	1.86	(1.54,2.26)	0.098	**1.80E-10**	1.66E-05	NA	1.12E-168
			WM	0.599	1.82	(1.44,2.3)	0.12	**6.36E-07**	NA	NA	
			MR–Egger	0.253	1.29	(0.52,3.17)	0.46	5.83E-01	1.60E-05	0.41	
			MR-RAPS	0.577	1.78	(1.5,2.11)	0.087	**3.13E-11**	NA	NA	
			MR-PRESSO: raw	0.569	1.77	(1.49,2.09)	0.086	**6.07E-10**	NA	NA	
			MR-PRESSO : Outlier-corrected	0.609	1.84	(1.56,2.16)	0.083	**1.22E-11**	NA	NA	
skipping breakfast	T2D	5	IVW	0.232	1.26	(0.51,3.14)	0.465	6.18E-01	0.11	NA	3.67E-16
			WM	0.752	2.12	(0.89,5.07)	0.444	8.99E-02	NA	NA	
			MR–Egger	2.111	8.25	(0.31,219.57)	1.674	2.97E-01	0.16	0.33	
			MR-RAPS	0.255	1.29	(0.63,2.67)	0.37	4.90E-01	NA	NA	
			MR-PRESSO: raw	0.239	1.27	(0.6,2.69)	0.383	5.61E-01	NA	NA	
			MR-PRESSO: Outlier-corrected	NA	NA	NA	NA	NA	NA	NA	
T2D	TV watching	35	IVW	-0.003	NA	(-0.017 0.011)	0.007	6.16E-01	3.04E-09	NA	2.87E-290
			WM	0.001	NA	(-0.011,0.013)	0.006	8.79E-01	NA	NA	
			MR–Egger	0.012	NA	(-0.021,0.045)	0.017	4.82E-01	4.78E-09	0.34	
			MR-RAPS	-0.002	NA	(-0.016,0.012)	0.007	7.55E-01	NA	NA	
			MR-PRESSO: raw	-0.002	NA	(-0.014, 0.010)	0.006	8.07E-01	NA	NA	
			MR-PRESSO: Outlier-corrected	-0.001	NA	(-0.011, 0.009)	0.005	8.85E-01	NA	NA	
T2D	skipping breakfast	34	IVW	-0.002	NA	(-0.016, 0.012)	0.007	7.72E-01	1.29E-03	NA	1.28E-203
			WM	-0.001	NA	(-0.017, 0.015)	0.008	9.25E-01	NA	NA	
			MR–Egger	0.009	NA	(-0.024, 0.042)	0.017	5.99E-01	1.15E-03	0.49	
			MR-RAPS	0.004	NA	(-0.010, 0.018)	0.007	5.24E-01	NA	NA	
			MR-PRESSO: raw	0.002	NA	(-0.010,0.014)	0.006	7.58E-01	NA	NA	
			MR-PRESSO : Outlier-corrected	-0.001	NA	(-0.013,0.011)	0.006	9.08E-01	NA	NA	

T2D, type 2 diabetes; CI, confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; NA, not applicable; N_snp: number of instrumental variables; OR, odds ratio; SE, standard error; SNP, single nucleotide polymorphism; WM, weighted median. When T2D is used as the outcome, there is an OR value.

[#]: 95% CIs of ORs are presented for the analysis of T2D as outcome, while 95% CIs of β values are presented for the analysis of the other outcomes.

p_value in bold refers to achieving statistical significance (p_value < 0.05/6).

excluding 16 SNPs associated with potential confounders (**Table 3**) [OR (95% CI)$_{IVW}$ = 1.94 (1.60, 2.36), $P = 3.74\times10^{-11}$; OR_{WM} = 1.82 (1.41, 2.35), $P = 3.27\times10^{-6}$; $OR_{MR\text{-}RAPS}$ = 1.78 (1.50, 2.11), $P = 3.13\times10^{-11}$; OR $_{MR\text{-}PRESSO\ :\ Outlier\text{-}corrected}$ = 1.84 (1.56, 2.16), $P = 1.22\times10^{-11}$]. The confounding traits associated with the 16 SNPs can be found in **Table S5**. However, there was no significant causal effect estimate from breakfast skipping to T2D. Due to shared biological pathways, T2D may further influence unhealthy lifestyles. To explore whether there is reverse causality, we performed an inverse MR analysis. We did not observe any significant association between genetic predisposition to T2D with TV watching and breakfast

TABLE 3 | The association between TV watching and risk of type 2 diabetes after remove 16 SNPs associated with confounding traits.

Exposure	Outcome	N_snp	Method	beta	OR	CI	SE	p_value	Heterogeneity_P_value	Intercept_P_value	Steiger_P_value
TV watching	**T2D**	111	IVW	0.66	1.94	(1.6,2.36)	0.1	3.74E-11	1.66E-05	NA	1.1E-168
		111	WM	0.59	1.82	(1.41,2.35)	0.129	3.27E-06	NA	NA	1.1E-168
		111	MR Egger	0.60	1.83	(0.71,4.69)	0.481	0.21	1.60E-05	0.41	1.1E-168
		111	MR-RAPS	0.58	1.78	(1.5,2.11)	0.087	3.13E-11	NA	NA	1.1E-168
		111	MR-PRESSO:raw	0.57	1.77	(1.49,2.09)	0.086	6.07E-10	NA	NA	1.1E-168
		111	MR-PRESSO: Outlier-corrected	0.61	1.84	(1.56,2.16)	0.083	1.22E-11	NA	NA	1.1E-168

T2D, type 2 diabetes; CI, confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; NA, not applicable; N_snp, number of instrumental variables; OR, odds ratio; SE, standard error; SNP, single nucleotide polymorphism; WM, weighted median. When T2D is used as the outcome, there is an OR value.

skipping (**Table 2** all $P > 0.05$). The leave-one-out cross-validation analysis showed that the overall estimates were not overdriven by any particular SNP (**Figures S1**-**S4**). The MR Steiger results showed that all causal estimates were in the intended direction (all $\boldsymbol{P}_{\text{MR Steiger}} \ll 0.05$, **Table 2**). The nearly symmetric funnel plots indicate no evidence of pleiotropy in the analysis (**Figures S5**-**S8**). In summary, instrumental variable analysis suggests a potential causal effect of increased TV watching time on an increased risk of T2D.

Epidemiological studies have shown that prolonged TV watching leads to increased BMI (60), lower HDL cholesterol (61), and higher fasting glucose concentrations (62) and that BMI and blood glycolipid traits are known risk factors for T2D (63), suggesting a potential mediating role for these traits in the association between TV watching and T2D. We performed a two-step MR mediation analysis to explain the mediation proportion for BMI, 4 lipid traits, and 6 glycemic traits. As shown in **Table 4**, the results revealed that four potential mediators produced a significant mediating effect. After adjusting for HbA1c, FG, and HDL, the estimates of causal effects produced moderate attenuation (OR: 1.78 adjusted for HbA1c, 1.71 adjusted for FG and 1.75 adjusted for HDL). In contrast, the association between TV watching and the risk of T2D was much more attenuated after adjusting for BMI (OR: 1.55 adjusted for BMI). Mediation analysis showed that the causal association between TV watching and T2D risk was partially mediated by BMI (mediation percentage = 29.10%), FG (mediation percentage = 13.51%), HDL (mediation percentage = 9.86%) or HbA1c (mediation percentage = 7.31%). Adjusting for these four factors simultaneously and adjusting for each factor separately produced results that were in the same direction as the results without adjustment, although the effect size was attenuated. In addition, we did not observe significant mediating effects for the other 7 glycemic-lipid traits.

Finally, we calculated the statistical power of this study using the mRnd website (64) (https://shiny.cnsgenomics.com/mRnd/). With the current sample size of T2D and the phenotypic variance of TV watching explained by IVs (4.1%, **Table S3**), at an alpha level of 0.05, we had 99% power to determine that each standard deviation increase in TV watching time increased the overall risk of T2D by 86% (i.e., an OR_{IVW} of 1.86, **Table 2**).

DISCUSSION

In the present study, we conducted a comprehensive genetic analysis to explore causal relationships and genetic overlap between T2D and TV watching and breakfast skipping by using summary statistics from GWAS. In the first instance, we showed that there was a strong positive genetic correlation between T2D and both exposures. Second, shared genetic structure at the locus level was identified between T2D and TV watching in cross-trait association analysis. Third, in the TWAS study between T2D and TV watching, we identified TWAS-significant genes, especially in tissues from the endocrine system, cardiovascular system, digestive system and nervous system. Finally, and most importantly, bidirectional MR showed that TV watching was positively associated with the risk of T2D. Mediation analysis identified four different traits as potential mediating factors between TV watching and T2D. Our results in the present study highlighted that TV watching plays an important role in the risk of T2D. The genetic overlaps elucidate potential shared biological pathways, thus providing new ideas and opportunities for T2D treatment and drug design.

The results of genetic correlation analysis are highly consistent with observational studies showing that breakfast skipping (8) and TV watching are significantly associated with an increased risk of T2D (4). These findings do not necessarily imply that TV watching per se causes T2D; rather, we believe that prolonged TV watching and breakfast skipping significantly affect the risk of developing diabetes in the future. There are two possible explanations for the observed positive association between TV watching and the risk of T2D. First, prolonged TV watching may result in lower energy expenditure and higher caloric intake, which are directly associated with obesity and weight gain (65, 66). Second, individuals who spend more time watching TV tend to eat more processed meats, snacks, and sweets and fewer vegetables and fruits, and such a diet may inversely affect diabetes risk (67). The average time spent watching TV is significantly associated with elevated levels of leptin and LDL cholesterol and lower levels of HDL cholesterol and apolipoprotein, which are important plasma biomarkers of T2D (68). Similarly, skipping breakfast may also trigger hyperglycemia and high glycated hemoglobin after lunch and dinner, further leading to impaired insulin response and thus increasing the risk of T2D (69). For these possible mechanistic pathways, we made

TABLE 4 | Two-step Mendelian randomization mediation analysis of the association between TV watching (exposure) and type 2 diabetes (outcome).

Mediator	Exposure → Mediator			Mediator → Outcome			Indirect causal effect by coefficient product	Direct causal effect	Adjust OR	Proportion of mediation
	IVW causal effect	IVW p value	MR Egger Intercept p value	IVW causal effect	IVW p value	MR Egger Intercept p value				
Adjust for BMI	0.315	**2.76E-06**	0.195	0.581	**5.14E-04**	0.563	0.183	0.439	1.55	29.10%
Adjust for TC	0.112	1.08E-01	0.119	-0.1	4.21E-02	0.307	NA	NA	NA	NA
Adjust for TG	0.24	**3.18E-06**	0.207	0.106	1.61E-01	0.028	NA	NA	NA	NA
Adjust for HDL	-0.289	**1.22E-05**	0.002	-0.213	**7.15E-04**	0.008	0.062	0.561	1.75	9.86%
Adjust for LDL	0.171	6.58E-03	0.197	-0.033	4.96E-01	0.344	NA	NA	NA	NA
Adjust for FG	0.053	**9.45E-04**	0.589	1.602	**4.03E-08**	0.015	0.085	0.537	1.71	13.51%
Adjust for FI	0.088	**1.09E-06**	0.898	1.318	6.19E-02	0.253	NA	NA	NA	NA
Adjust for HOMA-β	0.074	**4.01E-03**	0.862	-2.595	1.76E-01	0.221	NA	NA	NA	NA
Adjust for HOMA-IR	0.176	**2.13E-08**	0.596	0.346	2.03E-01	0.405	NA	NA	NA	NA
Adjust for 2hGlu	0.063	3.16E-01	0.506	0.921	1.78E-02	0.823	NA	NA	NA	NA
Adjust for HbA1c	0.038	**5.92E-04**	0.944	1.223	**3.08E-03**	0.183	0.046	0.576	1.78	7.31%
Adjust for ALL	NA	NA	NA	NA	NA	NA	0.376	0.253	1.29	59.78%

*BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; FG, fasting glucose; FI, fasting insulin; HOMA-β, homeostatic model assessment of beta cell function; HOMA-IR, homeostatic model assessment of insulin resistance; 2hGlu, 2-h postprandial glucose; HbA1c, hemoglobin; NA, not applicable; The IVW causal effect size was the beta coefficient estimated by IVW models for corresponding outcome; Direct causal effect: this value is obtained by subtracting the indirect effect from 0.629 as show in **Table 5**; IVW p values < 0.05/11 indicate statistical significance and are marked in bold font, and mediation analysis is significant only if both MR steps reach statistical significance; Proportion of mediation = Indirect causal effect by coefficient product/0.629.*

TABLE 5 | Cross-trait meta-analysis results between type 2 diabetes and television watching ($P_{meta} < 2.5\times10^{-8}$ and single-trait $P < 1\times10^{-5}$).

Index.SNP	CHR	Genome position	EA	NEA	EAF	T2D		TV watching		P_{meta}	Genes	variant annotation
						BETA	P	BETA	P			
rs4420638	19	19q13.32	A	G	0.84	0.110	1.50E-09	0.014	3.60E-07	2.42E-14	[APOC1,APOE,PVRL2,TOMM40]	downstream
rs4565329	6	6q22.32	T	C	0.48	0.073	4.40E-09	0.010	1.50E-06	7.64E-14	[CENPW]	intron
rs74333814	11	11q13.4	T	C	0.86	-0.095	5.80E-09	-0.014	3.50E-06	3.84E-13	[ARAP1,ATG16L2,FCHSD2,MIR4692, STARD10]	intron
rs243024	2	2p16.1	A	G	0.55	0.066	3.90E-08	0.011	1.00E-06	4.39E-12	[AC007381.3]*	upstream
rs2258238	12	12q14.3	A	T	0.88	-0.110	1.60E-07	-0.016	7.40E-06	1.23E-11	[HMGA2,RPSAP52]	intron
rs10400419	12	12q14.3	T	C	0.57	-0.067	1.70E-07	-0.010	9.60E-06	1.34E-10	[HMGA2]*	intergenic
rs550057	9	9q34.2	T	C	0.76	0.065	3.40E-06	0.012	3.00E-06	1.81E-09	[ABO]	intron
rs78028320	11	11q13.1	A	G	0.82	0.069	5.80E-06	0.013	3.90E-06	2.68E-09	[CFL1]*	intergenic

*EA, effect allele; NEA, noneffect allele; P_{meta} is the cross-trait meta-analysis P value. CHR, chromosome; T2D, type 2 diabetes; genes in * are the nearest genes to this locus.*

presumptions and validated them in the subsequent shared genetic structure analysis and MR-mediated analysis.

CPASSOC and TWAS showed that the shared genes between TV watching and T2D were mostly enriched in the endocrine system and cardiovascular system, suggesting an underlying correlation between the biological pathway and these tissues. Study shows that the *CFL1* gene, which controls cell proliferation and cell death, is overexpressed in the subcutaneous adipose

tissue of subjects who have gained weight, suggesting that the *CFL1* gene affects the risk of T2D through a mediating pathway of BMI (70). Reports have demonstrated that elevated *APOC1* gene expression is significantly associated with the risk of T2D and TG levels; also, apoC1 glycosylation has been observed in patients with T2D, which impairs the ability of *APOC1* to inhibit plasma cholesteryl ester transporter protein activity, suggesting that elevated apoC1 expression may increase the risk of T2D through lipoprotein metabolic pathways (71, 72). *APOC1* has also been reported to activate lecithin-cholesterol acyltransferase (LCAT), which in turn promotes HDL cholesterol esterification and increases HDL levels (73). Furthermore, increased *HMGA2* expression can be expected to lead to increased expression of $p14^{Arf}$, an inducer of cellular senescence, and the accumulation of senescent cells triggers inflammation associated with insulin resistance, driving the development of T2D, predicting that TV watching induces a signaling pathway linked to cellular senescence to increase the risk of T2D (43). Of additional interest to us is the fact that individuals who watch television for long periods of time consume more food and energy, increasing the burden on the digestive system (74). Additionally, patients with T2D often experience gastrointestinal disturbances, suggesting that gastrointestinal disturbances play a collider role in the association between TV watching and T2D (75). The exact mechanism of the digestive system in this association needs to be further elaborated. Moreover, previous research shows that *APAR1* affects the function of pancreatic β-cells and that the proinsulin-raising allele of *ARAP1* is related to a decreasing risk of T2D (76). The opposite conclusion was also reported: T2D pathogenic activity is mediated by *STARD10* expression instead of *ARAP1* (77), but both genes are located in a specific region, 11q13.4, which was identified in our cross-trait analysis, implying that pancreatic β-cell and proinsulin processing may be located in the biological pathway between TV watching and T2D. Our study suggests that multisystem, multitissue, polygenic effects may have a synergistic effect on the risk of T2D, but this needs more experimental evidence for further clarification.

Overall, using the MR study design, we found strong causal relationship between TV watching time and an increased risk of T2D. The observed causal effect was greatly attenuated when the mediating role of BMI, glycemia, and lipids was taken into account, suggesting that BMI, glycemia, and lipids play a key role in the association. Our finding is consistent with most previous observational studies and meta-analyses showing that prolonged TV watching is associated with an increased risk of T2D. A recent systematic review and dose–response meta-analysis based on 11 prospective studies published from 2001-2016 showed a linear association between TV watching and T2D (78), which was again validated in a recent meta-analysis (79). Our results are also supported by previous epidemiological studies that used Cox proportional hazards regression, controlling for multiple time-independent (i.e., constant across all cycles) and time-related (i.e., varying from cycle to cycle) covariates, to clarify that watching more than 4 hours of television and video per day at age 16 increases the risk of developing T2D (80). Moreover, this association was also verified in a multivariate logistic regression study based on an East Asian population that took into account gender differences (6). In addition, cross-sectional and longitudinal studies assessing the association between TV watching time and cardiometabolic biomarkers among multiple ethnic groups corroborated the plausibility of our choice of mediating variables and provided some potential mechanistic pathways that act through these mediators (62, 68, 81). However, a recent MR analysis of sedentary behavior with T2D and glycemic traits contradicts our results, finding no causal relationship between sedentary behavior and T2D. Two reasons may explain this discrepancy, one of which is that sedentary behavior is assessed by accelerometers, which is not conducive to measuring posture and sedentariness and estimating energy expenditure (82). In addition, the presence of the Hawthorne effect makes it possible for subjects to change their habituation (83). Second, although they also used data from UKB, the sample size was so small ($N = 91084$) that they could not select enough IVs to improve the statistical power (number of IVs = 6 in their study) (84). We also acknowledge the discrepancy between the results of breakfast skipping and T2D, and the findings of traditional epidemiological investigations may be partly due to fewer IVs for breakfast skipping.

In contrast to traditional observational studies and randomized controlled trials, the highlight of this study is the MR approach, which allows estimation of the causal effect of unhealthy lifestyles on T2D with a large sample size and high precision, controlling for potential reverse causality and confounders to the maximum extent possible. In addition, this study used various methods for sensitivity analysis, especially excluding SNPs related to potential confounders, to enhance the strength of instrumental variables and improve the robustness of estimation. Two-step MR mediation analysis was used in our study. When the results are binary variables (e.g., T2D), the estimation accuracy obtained by this method is higher than that obtained by multivariate Mendelian randomization (MVMR) (85). However, several potential shortcomings need to be acknowledged. First, in TWAS and GWAS-eQTL analysis, small eQTL samples are not sufficient to detect relatively weak signals, reducing the efficacy of the method. Second, our study is limited to individuals of European ancestry and cannot be generalized to other ethnicities. Third, no sex-specific MR analysis was conducted for the association between TV watching and T2D in our study. In addition, the analysis of breakfast skipping was limited to a few IVs and could not produce results with high power and reliability. Finally, further exploration of unhealthy lifestyle and T2D association mechanisms in the future, such as larger replication studies, sex-specific studies based on individual data, and more studies of mediating factors (hypertension, physical activity, education attainment, diet, leptin level, etc.), would greatly benefit our findings.

Our comprehensive genetic analysis identified shared genetic similarities between TV watching and T2D, suggesting a strong intrinsic genetic link between this trait pair. We further used MR to find convincing evidence supporting a putative causal role between TV watching and T2D, but mediation analyses suggest that this effect is largely mediated by BMI, HbA1c, FG, and HDL. As obesity, hyperglycemia, and hyperlipidemia are recognized as established risk factors for T2D, our findings underscore the importance of actionable prevention strategies for T2D. However, to date, the complex interactions between TV watching and T2D do not appear to be fully understood, and further studies are needed to deepen our

understanding of the biological pathways by which TV watching influences T2D.

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. Custom ***R*** scripts used to generate results in this study can be made available upon request.

ETHICS STATEMENT

The study was conducted using publicly available summary-level genetic data, and no ethical approval was requested.

AUTHOR CONTRIBUTIONS

JJ and TH conceived and designed the study. DC and HW performed the data preparation and statistical analysis. DC and HW wrote the manuscript. DC and HW contributed equally to this article. All authors helped interpret the data, reviewed and edited the final paper and approved the submission.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.836023/full#supplementary-material

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