frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 30 August 2024
DOI 10.3389/fendo.2024.1419964

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OPEN ACCESS

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RECEIVED 19 April 2024
ACCEPTED 05 August 2024
PUBLISHED 30 August 2024

CITATION
Wang Q, Wang L, Hao R, Zhang L, Wang W and Xia L (2024) Health condition and socioeconomic status mediate the causal effect of reproductive traits on nonalcoholic fatty liver disease: evidence from Mendelian randomization study.
Front. Endocrinol. 15:1419964.
doi: 10.3389/fendo.2024.1419964

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Health condition and socioeconomic status mediate the causal effect of reproductive traits on nonalcoholic fatty liver disease: evidence from Mendelian randomization study

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Background: Observational data posits a correlation between reproductive traits and nonalcoholic fatty liver disease (NAFLD), but their causal inference is still unclear. This investigation seeks to elucidate the causal influence of reproductive traits on NAFLD and determine the intervening role of health condition and socioeconomic status in these connections.

Methods: Utilizing a Mendelian Randomization (MR) approach, this research leveraged a comprehensive dataset from the Genome-wide Association Study (GWAS) database. The study incorporated body mass index, major depression, educational level, household income and Townsend deprivation index as intermediary variables. Initially, a bidirectional two-sample MR study was conducted to explore the genetic associations between reproductive traits and NAFLD. Then, two-step MR analyses were implemented to quantify the extent of mediation by these indicators. The weighted inverse variance method was the primary analytical approach, complemented by several sensitivity analyses to affirm the robustness of the MR assumptions. Finally, these findings were validated in the FinnGen research.

Results: The bidirectional MR analysis indicated that earlier reproductive traits (age at menarche, age at first sexual intercourse, and age at first birth) were associated with an elevated risk of NAFLD, absent any evidence of the reverse relationship. Body mass index accounted for 35.64% of the association between premature menarche and NAFLD. Additionally, body mass index, major depression, educational level and household income mediated 41.65%, 14.35%, 37.88%, and 18.59% of the connection between early sexual intercourse and NAFLD, respectively. Similarly, these same variables elucidated 36.36%, 15.58%,

41.56%, and 22.73% of the correlation between younger age at first birth and NAFLD.

Conclusion: Our study elucidated the causal relationships between reproductive traits and NAFLD. Potential underlying mechanisms may involve factors such as body mass index, major depression, educational attainment and household income.

KEYWORDS

reproductive traits, nonalcoholic fatty liver disease, body mass index, major depression, socioeconomic status, mendelian randomization, mediation

1 Introduction

The escalating prevalence of nonalcoholic fatty liver disease (NAFLD), affecting an estimated 30% of the global population (1, 2), cannot be disregarded. Metabolic syndrome, closely associated with obesity, insulin resistance, and hyperlipidemia (3), is recognized as the predominant cause of NAFLD. These conditions contribute to chronic inflammation, poor lipid, and hepatocellular carcinoma (4, 5). Regrettably, there is no approved treatment for NAFLD (6). Hence, it becomes imperative to promptly detect and address the risk elements linked to NAFLD.

Reproductive health and evolutionary adaptability are significantly influenced by female reproductive behaviors. This behavioral aspect encompasses various key factors, such as age at menarche (AAM), age at first sexual intercourse (AFS), age at first birth (AFB), age at last birth (ALB), and age at menopause (AMP). Numerous studies have proposed a correlation between AAM and NAFLD (7–10). Similarly, a meta-analysis evidence indicates that menopause is associated with approximately 2.4 times higher odds of NAFLD (11). Another study has highlighted the impact of menopause on the severity of fibrosis among individuals with non-alcoholic steatohepatitis (12). Nonetheless, the genetic links between AMP and NAFLD remain to be fully understood.

Recent evidence from the NHANES study verified the interaction between AFB and NAFLD (13). Meanwhile, Zuo et al. observed a connection between AFB/ALB and the risk of metabolic syndrome in women (14). However, the causal relationships between these reproductive factors remain uncertain due to constraints in traditional observational research. Moreover, research investigating the correlation between AFS and NAFLD is scarce. Thus, the current understanding of the genetic association between reproductive factors and NAFLD is limited.

Mendelian randomization (MR) analysis has become increasingly popular in genetic research for assessing causal relationships between exposure and outcome variables (15, 16). This method effectively mitigates confounding factors and infers causal relationships by randomly assigning alleles exposed to genetic variation (17, 18). These advantages also extend to intermediary analysis (19, 20). The potential mediating factors can be accurately estimated using the two-step MR approach.

Premature menarche is associated with the occurrence of NAFLD, although there are divergent viewpoints regarding the role of body mass index (BMI) in this association (7–10). Emerging research suggests that major depression (MD) correlates with NAFLD and reproductive behaviors (21–23). In addition, some cross-sectional studies have shown that lower household income and educational levels are associated with an increase the risk of suffering hepatic steatosis in U.S. adolescents (24, 25). However, there is currently no research that fully reveals the relationship between health conditions, socioeconomic status (SES), and reproductive factors. Utilizing a two-step, two-sample Mendelian randomization study, we aimed to investigate the causal effect between reproductive traits and NAFLD and explore the potential roles of health condition and SES.

2 Materials and methods

2.1 Study design

This research aggregated GWAS data and adhered to the MR analysis guidelines as outlined in previous studies (15), while working based on three fundamental assumptions: first, the instrumental variables (IVs) in this study should demonstrate a robust correlation with the exposures. Second, the IVs should be uncontaminated by confounding factors. And finally, the IVs should influence the outcome exclusively through their impact on

Abbreviations: AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth; ALB, age at last birth; AMP, age at menopause; BMI, body mass index; CI, confidence interval; EA, educational attainment; GWAS, genome-wide association studies; IV, instrumental variable; IVW, inverse variance weighted; LD, linkage disequilibrium; MR, Mendelian randomization; MD, major depression; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PPD, postpartum depression; SNP, single nucleotide polymorphism; SES, socioeconomic status; TDI, Townsend deprivation index.

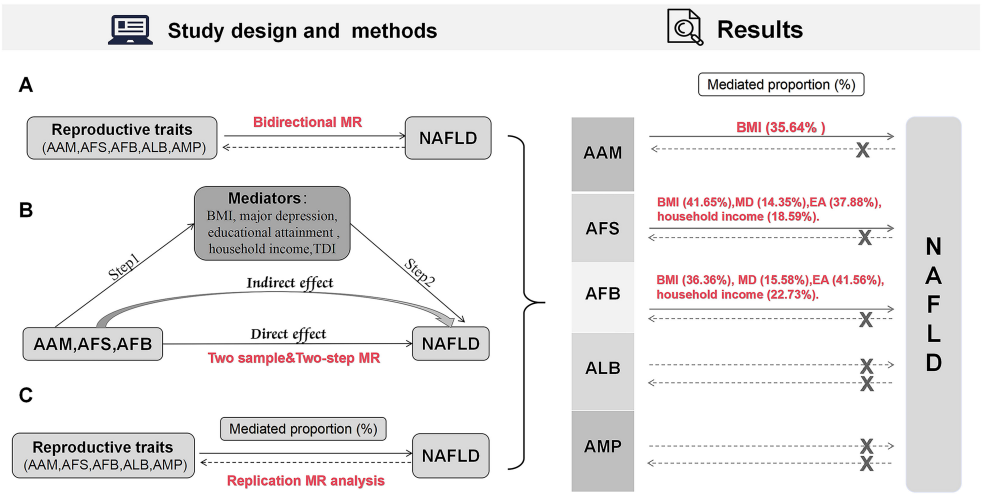

FIGURE 1
Design and results of the MR analysis. **(A)** Bidirectional MR analysis; **(B)** Mediation MR analysis; **(C)** Replication MR analysis. MR, Mendelian randomization; AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth; ALB, age at last birth; AMP, age at menopause; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; MD, major depression; EA, educational attainment; TDI, Townsend deprivation index.

the exposure. The research design and results are depicted in Figure 1.

2.2 Data sources and ethics

2.2.1 Reproductive traits

The AAM-related IVs were acquired from a survey encompassing 182,416 female subjects (26). Summary statistics for AFS and AFB were obtained from research conducted by Mills MC (27). This study identified a whopping number of 16,292,532 single nucleotide polymorphisms (SNPs) for AFS and 10,766,720 SNPs for AFB. SNPs for ALB and AMP were downloaded from the IEU Open GWAS database (https://gwas.mrcieu.ac.uk/), with 170,248 and 143,819 individuals, respectively.

2.2.2 NAFLD

Summary-level data for NAFLD were gathered from the largest GWAS meta-analysis, which included 8,434 NAFLD cases and 770,180 controls (discovery stage) (28). The validation dataset consists of 894 cases and 217,898 controls, sourced from the FinnGen Research Project (https://www.finngen.fi/en).

2.2.3 Mediators

The summary data of BMI were obtained from the IEU OpenGWAS database, encompassing 461,460 Europeans. MD related genetic variations were extracted from the Psychiatric Genomes Consortium (PGC), including 500,199 individuals (29).

Three factors of SES were considered at three levels: individual-level SES was assessed by educational attainment (EA), specifically, in years of schooling; household-level SES by average pre-tax household income; and community-level SES by the Townsend deprivation index (TDI) (30). IVs for EA, household income, and TDI were derived from the IEU OpenGWAS project with sample sizes of 99,970, 397,751, and 461,242, respectively.

2.2.4 Ethics

All sample populations are Europeans, which excluded racial variability. Details for datasets are listed in Table 1. All data were collected from publicly available databases and published studies, which hence obviated the requirement for any additional ethical clearance.

2.3 IVs selection

SNPs with $p < 5\times10^{-8}$ were prioritized as the potential IVs. For greater statistical robustness, SNPs ($p < 5\times10^{-7}$) were chosen to include more IVs for ALB. And SNPs ($p < 5\times10^{-6}$) were used for the reverse analysis (Supplementary Tables S1–S3). Parameters were set to exclude SNPs affected by linkage disequilibrium (LD), with $r^2 = 0.001$ and a distance of kb=10,000 (31). Subsequently, we excluded weak instruments with F-statistics below 10. The computational formula for F-statistic used here is $Beta^2/SE^2$. Harmonization processes were applied to align datasets on exposure and outcome, minimizing the inclusion of palindromic and ambiguous SNPs with non-concordant alleles (Supplementary Tables S1–S3).

2.4 Mendelian randomization

The standard inverse variance weighted (IVW) method was primarily employed for this estimation. Supplementary analyses included MR-Egger, weighted median, simple mode, and weight mode methods. The IVW is validated a reliable method under the

TABLE 1 Data sources used in the MR analyses for the current study.

Traits	Consortium/ Author	Sample size	Population	Year	Pubmed ID
Exposures					
Age at menarche	ReproGen	182,416	European	2014	25231870
Age at first sexual intercourse	Mills MC	182,791	European	2021	34211149
Age at first birth	Mills MC	418,758	European	2021	34211149
Age at last birth	MRC-IEU	170,248	European	2018	/
Age at menopause	MRC-IEU	143,819	European	2018	/
Outcome					
Nonalcoholic fatty liver disease	Ghodsian N	778,614	European	2018	34841290
Nonalcoholic fatty liver disease	FinnGen	218,792	European	2021	/
Mediators					
Body mass index	MRC-IEU	461,460	European	2018	/
Major depression	PGC	500,199	European	2019	30718901
Years of schooling	Within family GWAS consortium	99,970	European	2022	/
Average total household income before tax	MRC-IEU	397,751	European	2018	/
Townsend deprivation index at recruitment	MRC-IEU	462,464	European	2018	/

/ represented PubMed id not retrieved in IEU database.

condition that the SNP being used is valid and does not exhibit significant pleiotropy (32). In addition, MR-Egger provides an assessment of potential horizontal pleiotropic effects (33). If no less than 50% of the data from valid instruments is accessible, the weighted median method provides precise and resilient effect estimates (34). The reliability and consistency of the results are verified using both weighted mode and simple mode analyses.

2.5 Mediation MR analysis

In a two-step MR process, three β values were obtained: β0, β1, and β2. β0 represents the initial MR of exposures on outcome, β1 represents the impact MR of exposures on mediators, and β2 represents the effect of mediators on outcomes. The calculation of the indirect effect determines the mediation proportion of each mediator, which can be achieved through the formula: (β1×β2)/β0. Delta methods were employed to compute standard errors and confidence intervals (CIs) (35).

2.6 Sensitivity analysis

Heterogeneity computation was evaluated by Cochran's Q statistic (36). The MR results were analyzed using a random-effects model of IVW if the p-value is less than 0.05. If not, a fixed-effects model was used (37). The heterogeneity of IVs was evaluated by examining the p-value of MR-Egger's intercept. To further validate the potentially abnormal SNPs and address the issue of horizontal pleiotropy, the MR-PRESSO approach was integrated (38).

2.7 Replication MR analysis

In order to improve the statistical ability and accuracy of our causal estimation, replication bidirectional MR analysis was performed between reproductive characteristics and NAFLD using the FinnGen Research Project. SNPs with a significance threshold of $p < 5\times10^{-8}$ were prioritized as potential IVs. Given the relatively small sample size of NAFLD, the SNP threshold for AAM and MD was adjusted to $p < 5\times10^{-6}$. In the reverse analysis, the SNP threshold for NAFLD and AAM analysis was set at $p < 5\times10^{-5}$, while the thresholds for other analyses were maintained at $p < 5\times10^{-6}$. Finally, the mediation effect was estimated using the replication analysis datasets.

2.8 Statistical analysis

All data analyses were performed using the Two Sample MR packages (39) in R version 4.2.1. Results were presented as an odds

ratio (OR) with 95%CI per standard deviation. P < 0.05 was deemed to indicate statistical significance.

3 Results

3.1 Causal effect of reproductive traits on NFLD

3.1.1 Causal effect of AAM and AMP on NAFLD

The initial analysis explored the impact of AAM on NAFLD. The results, as depicted in Figure 2, the IVW analysis indicated a negative causal relationship between AAM and NAFLD with an odds ratio (OR=0.828, 95%CI: 0.749 - 0.916, $p=2.557\times10^{-4}$). The Cochran's Q test revealed the absence of significant heterogeneity (Q=54.110, p =0.656). Moreover, no evidence of pleiotropy was observed (MR-Egger intercept = -5.821×10^{-3}, p =0.528). The robustness of these findings was further validated through the Leave-one-out sensitivity analysis (Figure 3A).

Next, we assessed the causal effect of AMP on NAFLD (Figure 2). The IVW method indicated no significant effect of AMP on NAFLD (OR=1.028, 95%CI: 0.940 - 1.124, p=0.545). The presence of heterogeneity was not detected by Cochran's Q test (Q=97.774, p=0.653). And no pleiotropy was observed in the study (MR-Egger intercept=0.008, p=0.051). The Leave-one-out test was shown in Figure 3E.

3.1.2 Causal effect of AFS and AFB on NAFLD

Additionally, the IVW analysis revealed a negative causal relationship between AFS and NAFLD (OR=0.654, 95% CI:0.518 - 0.825; $p=3.484\times10^{-4}$) (Figure 2). Cochran's Q test did not indicate any heterogeneity (Q=43.018; p=0.677). Additionally, there was no evidence of pleiotropy based on the MR-Egger intercept (MR-Egger intercept = −0.003; p=0.828). A "leave-one-out" test was conducted to confirm the reliability and consistency of the results, which can be seen in Figure 3B.

Similarly, the causal link between AFB and NAFLD was assessed using IVW (Figure 2), which indicated a negative causal effect (OR=0.857, 95%CI:0.801 - 0.918; $p=8.741\times10^{-6}$). Heterogeneity was not observed through Cochran's Q test (Q =53.078; p=0.471), and no pleiotropy was detected (MR-Egger intercept= 0.004; p=0.712). The results remained stable based on the Leave-one-out test (Figure 3C).

3.1.3 Causal effect of ALB on NAFLD

Finally, we investigated the potential association between ALB and NAFLD. As shown in Figure 2, IVW results revealing no significant causal connection between ALB and NAFLD (OR=0.698, 95% CI:0.426 - 1.144; p=0.154). Then, neither heterogeneity nor pleiotropy were detected. Leave-one-out analysis for ALB on NAFLD is depicted in Figure 3D, with detailed results of sensitivity analyses were depicted in Supplementary Table S4.

3.2 Causal effects of NAFLD on reproductive traits

The analysis on MR in reverse revealed that the presence of genetic predisposition towards NAFLD did not influence any of the reproductive characteristics. And no heterogeneity or pleiotropy were detected. The results maintained robust based on the Leave-one-out and MR-PRESSO test (Figure 4 and Supplementary Table S5).

Exposures	Outcome	Method	SNPs	Forest Plot	p-Value	OR	95%LCI	95%UCI	*P*-het	*P*-intercept
AAM	NAFLD	MR Egger	60		0.740	0.936	0.633	1.383	0.636	0.528
		WM	60		0.015	0.827	0.710	0.963		
		IVW	60		2.557E-04	0.828	0.749	0.916	0.656	
		Simple mode	60		0.531	0.904	0.660	1.238		
		Weighted mode	60		0.387	0.893	0.692	1.152		
AFS	NAFLD	MR Egger	49		0.587	0.736	0.246	2.202	0.640	0.828
		WM	49		0.005	0.634	0.461	0.870		
		IVW	49		3.484E-04	0.654	0.518	0.825	0.677	
		Simple mode	49		0.172	0.547	0.233	1.284		
		Weighted mode	49		0.148	0.565	0.264	1.209		
AFB	NAFLD	MR Egger	54		0.194	0.809	0.589	1.110	0.438	0.712
		WM	54		1.845E-04	0.829	0.751	0.915		
		IVW	54		8.741E-06	0.857	0.801	0.918	0.471	
		Simple mode	54		0.052	0.799	0.640	0.997		
		Weighted mode	54		0.038	0.812	0.671	0.983		
ALB	NAFLD	MR Egger	16		0.466	0.336	0.019	5.810	0.133	0.618
		WM	16		0.087	0.578	0.308	1.083		
		IVW	16		0.154	0.698	0.426	1.144	0.162	
		Simple mode	16		0.142	0.434	0.151	1.248		
		Weighted mode	16		0.132	0.475	0.190	1.188		
AMP	NAFLD	MR Egger	105		0.157	0.875	0.729	1.051	0.729	0.051
		WM	105		0.768	0.979	0.851	1.127		
		IVW	105		0.545	1.028	0.940	1.124	0.653	
		Simple mode	105		0.608	0.915	0.653	1.283		
		Weighted mode	105		0.117	0.865	0.722	1.035		

FIGURE 2
The causal effects of reproductive traits on NAFLD. NAFLD, nonalcoholic fatty liver disease; AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth; ALB, age at last birth; AMP, age at menopause; WM, weighted median; IVW, inverse-variance weighted; OR, odds ratio; LCI, lower confidence interval; UCI, upper confidence interval; P-het, P value for heterogeneity using Cochran Q test; P-intercept, P value for MR-Egger intercept.

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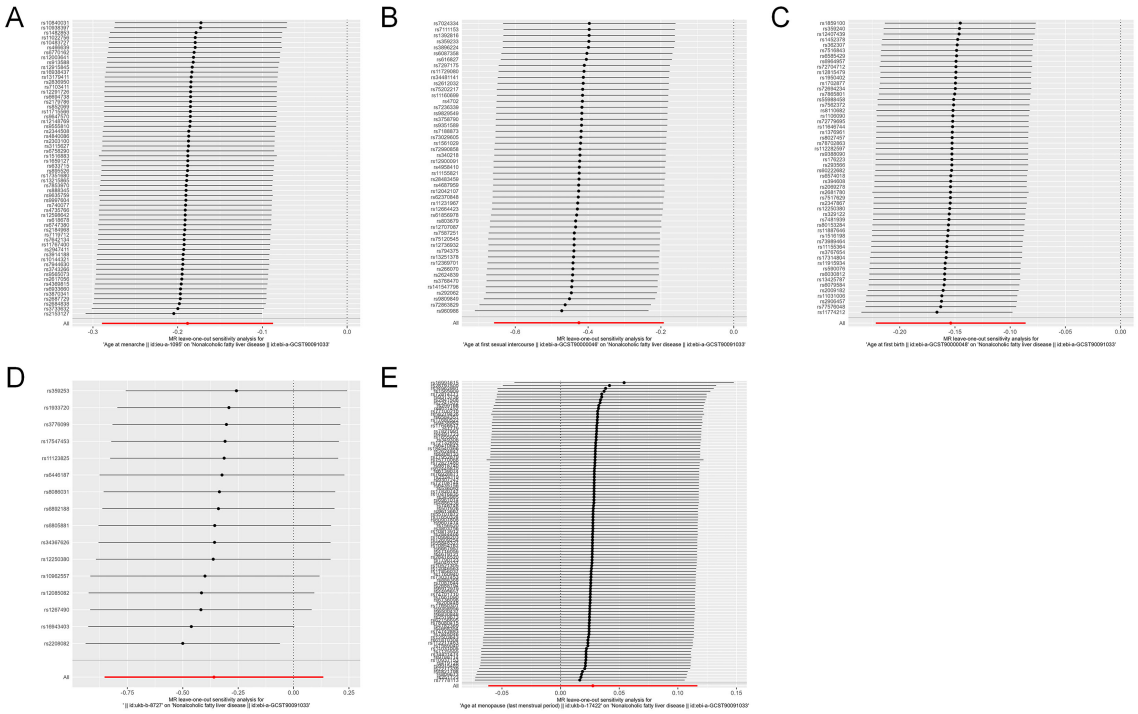

FIGURE 3
Leave-one-out analysis for causal effects of reproductive traits on NAFLD. **(A)** Leave-one-out analysis plots for age at menarche on NAFLD. **(B)** Leave-one-out analysis plots for age at first sexual intercourse on NAFLD. **(C)** Leave-one-out analysis plots for age at first birth on NAFLD. **(D)** Leave-one-out analysis plots for age at last birth on NAFLD. **(E)** Leave-one-out analysis plots for age at menopause on NAFLD. NAFLD, nonalcoholic fatty liver disease.

Exposure	Outcomes	Method	SNPs	Forest Plot	p-Value	OR	95%LCI	95%UCI	*P*-het	*P*-intercept	Outliers
NAFLD	AAM	MR Egger	6		0.950	0.995	0.867	1.142	0.193	0.937	/
		WM	6		0.965	0.999	0.939	1.062			
		IVW	6		0.985	1.001	0.946	1.058	0.297		
		Simple mode	6		0.854	1.010	0.912	1.119			
		Weighted mode	6		0.921	0.996	0.924	1.073			
NAFLD	AFS	MR Egger	9		0.613	1.011	0.970	1.054	0.297	0.746	/
		WM	9		0.420	1.009	0.987	1.033			
		IVW	9		0.612	1.005	0.986	1.025	0.381		
		Simple mode	9		0.695	0.991	0.951	1.034			
		Weighted mode	9		0.555	1.008	0.983	1.033			
NAFLD	AFB	MR Egger	9		0.622	1.044	0.887	1.229	0.102	0.956	/
		WM	9		0.269	1.046	0.966	1.132			
		IVW	9		0.313	1.040	0.964	1.121	0.153		
		Simple mode	9		0.156	1.146	0.966	1.360			
		Weighted mode	9		0.274	1.052	0.967	1.144			
NAFLD	ALB	MR Egger	12		0.958	0.999	0.950	1.049	0.046	0.906	/
		WM	12		0.783	0.996	0.972	1.022			
		IVW	12		0.727	0.996	0.974	1.019	0.069		
		Simple mode	12		0.507	0.983	0.937	1.032			
		Weighted mode	12		0.950	1.001	0.974	1.028			
NAFLD	AMP	MR Egger	4		0.587	1.037	0.927	1.161	0.070	0.822	/
		WM	4		0.062	1.026	0.999	1.053			
		IVW	4		0.157	1.023	0.991	1.056	0.139		
		Simple mode	4		0.265	1.031	0.987	1.077			
		Weighted mode	4		0.179	1.028	0.997	1.061			

0.5 OR(95%CI) 1.5

FIGURE 4
The causal effects of NAFLD on reproductive traits. NAFLD, nonalcoholic fatty liver disease; AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth; ALB, age at last birth; AMP, age at menopause; WM, weighted median; IVW, inverse-variance weighted; OR, odds ratio; LCI, lower confidence interval; UCI, upper confidence interval; P-het, P value for heterogeneity using Cochran Q test; P-intercept, P value for MR-Egger intercept.

3.3 Two-step MR analyses

3.3.1 Causal effects of reproductive traits on mediators

Our investigation explored the causal relationships between AAM, AFS, and AFB, as ALB and AMP were found to have no causal effect on NAFLD. Additionally, due to the lack of a significant correlation between TDI and NAFLD (OR =1.641, 95% CI =0.871 - 3.089; p=0.125), it was not considered as a mediator in the relationship. Figure 5 presents the results of the IVW analysis.

Through a two-step MR analysis, we discovered that AAM was causally related to BMI (OR=0.878, 95% CI =0.827 - 0.932; $p=1.881\times10^{-5}$). Additionally, AFS exhibited a causal relationship with BMI (OR =0.709, 95% CI =0.651-0.771; $p=1.341\times10^{-15}$), MD (OR=0.738, 95% CI=0.659 - 0.826; $p=1.272\times10^{-7}$), EA (OR =1.468, 95% CI =1.341 - 1.608; $p=9.959\times10^{-17}$), and household income (OR =1.282, 95% CI =1.206 - 1.363; $p=1.890\times10^{-15}$). Finally, the causal effects of AFB on mediators is as follows: BMI (OR =0.896, 95% CI: 0.874 - 0.919; $p=6.782\times10^{-18}$), MD (OR =0.886, 95% CI =0.858 - 0.915; $p=1.546\times10^{-13}$), EA (OR =1.166, 95% CI =1.138 - 1.194; $p=3.000\times10^{-35}$), and household income (OR =1.115, 95% CI =1.096 -1.135; $p=3.728\times10^{-33}$).

3.3.2 Causal effects of mediators on NAFLD

Figure 5 also presents the assessment of the potential mediators' impact on NAFLD. Our findings indicated a positive association between these mediators and NAFLD, encompassing BMI (OR =1.673, 95% CI: 1.496 - 1.870; $p=1.376\times10^{-19}$), MD (OR =1.221, 95% CI =1.026 - 1.453; p =0.025), EA (OR =0.658, 95% CI =0.545 - 0.968; p =0.020), and household income (OR =0.726, 95% CI =1.142 - 1.706; p =0.029). Despite the existence of heterogeneity was observed, the random-effects IVW method we selected remains reliable (37). Moreover, the consistency of these associations was confirmed through MR-PRESSO analysis after removing outlier data. the detailed results and sensitivity analyses are listed in Supplementary Tables S6–S9.

3.3.3 Mediation proportion

As shown in Table 2, BMI (35.64%, 95%CI : 18.62%, 54.79%) is considered mediator of the impact of AAM on NAFLD. Four mediating factors revealed the correlation between AFS and NAFLD as follows: BMI (41.65%, 95%CI : 28.94%, 56.24%), MD (14.35%, 95%CI : 1.65%, 29.18%), EA (37.88%, 95%CI : 5.88%, 72.71%), and household income (18.59%, 95%CI : 1.88%, 37.18%). Regarding the link between AFB and NAFLD, the mediators identified include BMI (36.36%, 95%CI : 25.97%, 48.70%), MD (15.58%, 95%CI : 1.95%, 31.17%), EA (41.56%, 95%CI : 6.49%, 77.92%), and household income (22.73%, 95%CI : 2.60%, 44.16%).

3.4 Replication MR analysis

A replication analysis conducted using the FinnGen database yielded consistent results. The findings revealed that earlier AAM, AFS, and AFB were associated with an increased risk of NAFLD, with no evidence supporting the reverse relationship. Figure 6 presents the results of the IVW analysis, and no indications of pleiotropy were detected. Specified results and sensitivity analyses are provided in Supplementary Tables S10–S12.

Subsequently, a two-step mediation analysis was undertaken. Unfortunately, efforts to replicate the link between household income and NAFLD was unsuccessful, attributed to the limited sample size of the Finnish database. Nevertheless, the influence of education level on personal income remains significant, and a clear correlation exists between financial income and NAFLD (24, 25, 40, 41), thereby reinforcing the validity of our findings. As illustrated in Figure 6, BMI played a significant role in mediating 37.90% of the relationship between AAM and NAFLD. Moreover, BMI, MD, and EA acted as mediators for 35.52%, 15.98%, and 51.93% of the

Exposures	Outcomes	SNPs	p-Value	OR	95%LCI	95%UCI	*P*-intercept
AAM	BMI	61	1.881E-05	0.878	0.827	0.932	0.625
	MD	28	0.717	0.986	0.916	1.062	0.625
	EA	62	0.392	1.018	0.977	1.062	0.418
	Household income	41	0.223	1.024	0.986	1.065	0.510
AFS	BMI	50	1.341E-15	0.709	0.651	0.771	0.559
	MD	52	1.272E-07	0.738	0.659	0.826	0.128
	EA	50	9.959E-17	1.468	1.341	1.608	0.972
	Household income	50	1.890E-15	1.282	1.206	1.363	0.180
AFB	BMI	55	6.782E-18	0.896	0.874	0.919	0.721
	MD	53	1.546E-13	0.886	0.858	0.915	0.575
	EA	54	3.000E-35	1.166	1.138	1.194	0.480
	Household income	55	3.728E-33	1.115	1.096	1.135	0.602
BMI	NAFLD	423	1.376E-19	1.673	1.496	1.870	0.920
MD		48	0.025	1.221	1.026	1.453	0.252
EA		18	0.020	0.658	0.463	0.935	0.676
Household income		43	0.029	0.726	0.545	0.968	0.780
TDI		18	0.125	1.641	0.871	3.089	0.604

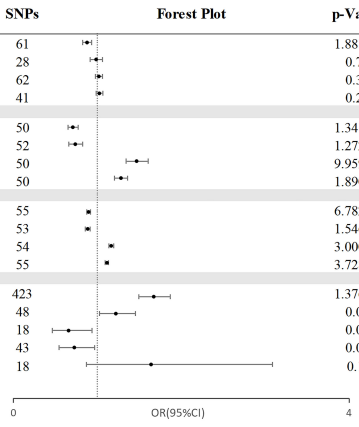

FIGURE 5
The causal effects of mediating factors in the relationship between AAM, AFS, and AFB and NAFLD. NAFLD, nonalcoholic fatty liver disease; AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth; BMI, body mass index; MD, major depression; EA, educational attainment; TDI, Townsend deprivation index; OR, odds ratio; LCI, lower confidence interval; UCI, upper confidence interval; P-intercept, P value for MR-Egger intercept.

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TABLE 2 The detailed mediation proportions of mediators.

Exposures	Mediators	Outcome	β0(95% CI)	β1(95% CI)	β2(95%CI)	Mediation effect(95%CI)	Mediated Proportion (%) (95%CI)
AAM	Body mass index	NAFLD	-0.188 (-0.289,-0.087)	-0.130(-0.190,-0.070)	0.514(0.403,0.626)	-0.067(-0.103,-0.035)	35.64(18.62,54.79)
AFS	Body mass index	NAFLD	-0.425 (-0.658,-0.192)	-0.345(-0.429,-0.260)	0.514(0.403,0.626)	-0.177(-0.239,-0.123)	41.65(28.94,56.24)
	Major depression	NAFLD	-0.425 (-0.658,-0.192)	-0.304(-0.417,-0.191)	0.200(0.025,0.374)	-0.061(-0.124,-0.007)	14.35(1.65,29.18)
	Educational attainment	NAFLD	-0.425 (-0.658,-0.192)	0.384(0.294,0.475)	-0.419(-0.770,-0.067)	-0.161(-0.309,-0.025)	37.88(5.88,72.71)
	Household income	NAFLD	-0.425 (-0.658,-0.192)	0.248(0.187,0.310)	-0.320(-0.607,-0.033)	-0.079(-0.158,-0.008)	18.59(1.88,37.18)
AFB	Body mass index	NAFLD	-0.154 (-0.222,-0.086)	-0.109(-0.135,-0.085)	0.514(0.403,0.626)	-0.056(-0.075,-0.040)	36.36(25.97,48.70)
	Major depression	NAFLD	-0.154 (-0.222,-0.086)	-0.121(-0.153,-0.089)	0.200(0.025,0.374)	-0.024(-0.048,-0.003)	15.58(1.95,31.17)
	Educational attainment	NAFLD	-0.154 (-0.222,-0.086)	0.153(0.129,0.178)	-0.419(-0.770,-0.067)	-0.064(-0.120,-0.010)	41.56(6.49,77.92)
	Household income	NAFLD	-0.154 (-0.222,-0.086)	0.109(0.091,0.127)	-0.320(-0.607,-0.033)	-0.035(-0.068,-0.004)	22.73(2.60,44.16)

β0, the initial MR of exposures on outcome; β1, the step one MR of exposures on mediators; β2, the step two MR of mediators on outcome; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth. Although the β1 value of AFB and BMI was -0.1096, we marked it as -0.109 here to ensure the accuracy and consistency of the mediation analysis results.

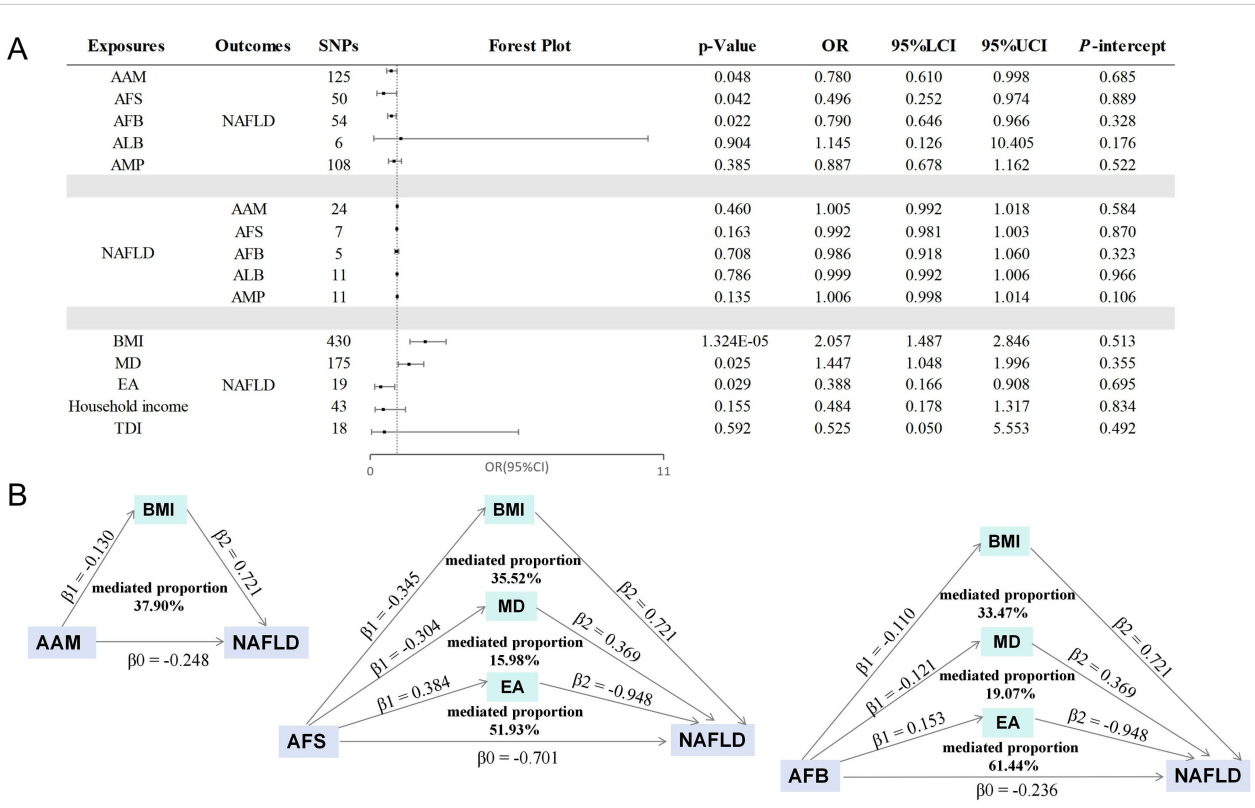

FIGURE 6
The results of replication MR analysis. **(A)** The causal effects of stepwise MR mediation analysis; **(B)** The mediation proportions of mediators. AAM, age at menarche; AFS, age at first sexual intercourse; AFB, age at first birth; ALB, age at last birth; AMP, age at menopause; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; MD, major depression; EA, educational attainment; TDI, Townsend deprivation index; LCI, lower confidence interval; UCI, upper confidence interval; P-intercept, P value for MR-Egger intercept.

correlation between AFS and NAFLD. Similarly, the mediators for the association between AFB and NAFLD included BMI (33.47%), MD (19.07%), and EA (61.44%). The results of the stepwise MR analysis can be found in Supplementary Table S13.

4 Discussion

Our investigation represents the initial endeavor to analyze the causal relationships between reproductive factors (AAM, AFS, AFB, ALB, AMP) and NAFLD using a bidirectional two-sample, two-step MR analysis. Initially, we discovered that individuals experiencing early AAM, AFS, and AFB face an elevated risk of NAFLD development. And there is no evidence establishing a causal connection between ALB, AMP and NAFLD. Furthermore, two-step mediation analyses elucidated that BMI acts as a mediator in the association between AAM and NAFLD. The relationships between AAM, AFS, AFB, and NAFLD are partially mediated by four factors: BMI, MD, EA and household income.

Consistent with prior studies, we found a positive correlation between early menarche age and NAFLD (7–9, 42). Research has demonstrated that early menarche tends to be more prevalent among girls living in urban areas, associated with higher BMI, and intake of high-energy nutrients (43, 44). Moreover, obesity in childhood accounts for much of the cross-national variation in age at menarche (45). Young women who experience early menarche may exhibit pre-existing metabolic abnormalities, such as insulin resistance, poor lipids and blood pressure change (46–49). Therefore, obesity or subsequent weight gain may mediate the linkage between AAM and NAFLD. Our research further indicated that BMI mediates the connection between menarche and NAFLD, which emphasized that we should pay attention to the BMI of adolescents in early menarche age and be alert to the risks of adult obesity and NAFLD.

Then, our research demonstrated an association between AFS and an increased risk of NAFLD. A finding applicable to AFB, as girls who experience AFS at a young age may predispose to earlier AFB. However, the literature currently lacks studies examining the connection between AFS and NAFLD. Previous findings suggest a causal protective effect of education on NAFLD, with individuals of lower socioeconomic status (SES) exhibiting higher incidence rates of NAFLD, particularly in regions such as the United States and Europe (50, 51). It is worth noting that the role of educational attainment and household income on the AFS/AFB-NAFLD relationship also can be interpreted through risky behaviors. Individuals with early reproductive behavior frequently exhibit externalizing behaviors like smoking and alcohol misuse (52–55), which are also recognized predisposing factors for NAFLD (56). In addition, lower educational and income levels among young women who become mothers for the first time often contribute to mental disorders, as well as smoking and alcohol abuse during pregnancy (13, 57). These behaviors further increase the vulnerability to developing NAFLD. Additionally, emerging evidence indicated that early sexual activity is a risk factor for MD, with our MR analysis corroborating this association (58). MD is believed to facilitate NAFLD development through various mechanisms, including inflammation, chronic stress, and gut microbiota (59, 60). Significantly, adolescents experiencing NAFLD exhibited a significant prevalence of clinically diagnosed anxiety and depression (61). Therefore, MD could be a critical metabolic companion in the development of NAFLD caused by AFS. Nevertheless, the exact mechanisms through which AFS impacts BMI remain uncertain, potentially including factors such as AFB.

Previous research has provided evidence linking AFB with an increased risk of NAFLD in women (13). A plausible explanation may involve the influence of weight gain and retention during pregnancy. Even for women who gain weight within expected limits during pregnancy, the accumulated weight during this period and the sustained postpartum weight can contribute to future obesity (62, 63). In obese individuals, abdominal fat accumulation in obese individuals disrupts lipid and glucose metabolism, fostering insulin resistance, thought to be involved in the advancement of NAFLD (64). In terms of the relationship between AFB and MD, a negative correlation has been observed (65). There is a heightened possibility of postpartum depression (PPD) among younger mothers due to a notable decline observed in all opregnanolone levels (66, 67). Our MR analysis further supports the causal influence of early AFB on increasing PPD risk, thereby enhancing the likelihood of NAFLD. Therefore, it is crucial to focus on women's reproductive health to reduce the incidence of NAFLD.

Numerous factors, including physiological, genetic, environmental, and social influences, contribute to the development of NAFLD (68). Research indicates that individuals from lower socioeconomic backgrounds are disproportionately affected by liver disease, underscoring it as a significant issue of health inequality (69). A cohort study conducted in Iran has demonstrated that a more vulnerable SES is associated with an increased risk of NAFLD (70). Furthermore, a protective effect of higher educational attainment on the risk of NAFLD has been reported (71). Our findings also suggest that social status plays a critical role in the relationship between AFS/AFB and NAFLD. These insights are crucial and imply that guidelines for the prevention and management of NAFLD should be formulated with consideration for the disparities among different socioeconomic status groups.

Our study is characterized by several strengths. Firstly, we introduced a total of five distinct reproductive traits for the first time. The examination of these traits and their correlation with NAFLD was rigorously analyzed using extensive data from many GWAS. By integrating genetic instruments and deploying diverse MR approaches, our investigation enables a comprehensive investigation into the causal inference between reproductive characteristics and the susceptibility to NAFLD. Furthermore, our application of a two-step MR analysis, thereby enhancing our understanding of the underlying mechanisms and provided solid evidence to substantiate prevention strategies. This pioneering effort holds substantial significance in understanding the potential impact of female reproductive characteristics on the risk of NAFLD.

This research acknowledges specific restrictions that must be considered. Firstly, our study was confined to participants of European descent. Further studies should include a broader ethnic range to validate the universality of our findings. Secondly,

the reliance on recall data for reproductive age introduces the possibility of bias. This dependence on memory has the potential to introduce recollection partiality, which must be considered while interpreting the outcomes (72, 73). Thirdly, the utilization of GWAS data restricted our ability to investigate potential non-linear connections or variations in stratification effects relating to age or gender. Meanwhile, the sample size from the Finnish database constrained the outcomes of our replication analysis, highlighting the need for larger datasets in future studies. Although we observed pleiotropy in the causal effect of AAM on NAFLD risk in our repeated MR analysis, the magnitude and direction of this causal effect remained consistent with the findings from the primary analysis. In conjunction with previous literature reports, we believe that these results are still significant (74). Finally, given that NAFLD encompasses multiple subtypes, and whether the subtype analysis results are consistent with our research requires more study to understand how reproductive characteristics modulate the risk of NAFLD and its subtypes.

In conclusion, this groundbreaking research indicates early AAM, AFS, and AFB as risk factors for NAFLD. Factors such as BMI, MD, educational level, and house income may mediate these causal relationships, offering valuable insights for targeting interventions at obesity, mental health, and educational disparities to mitigate NAFLD's burden.

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/s.

Author contributions

QW: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. LW: Data curation, Investigation, Software, Writing – original draft. RH: Data curation, Formal analysis, Software, Validation, Writing – original draft. LZ: Funding acquisition, Resources, Supervision, Writing – review & editing. WW: Project administration, Resources, Supervision, Writing – review & editing. LX: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

Summary-level data were extracted from the IEU Open GWAS database (https://gwas.mrcieu.ac.uk/) and the FinnGen Research Project (https://www.finngen.fi/en). We would like to thank all the researchers and study participants for their contributions.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1419964/full#supplementary-material

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