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Novel Insights into Causal Effects of Lipid and Lipid-Lowering Targets with Autoimmune Thyroid Disease: A Mendelian Randomization Study

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Background: Dyslipidemia has been implicated in the pathogenesis of several diseases, including thyroid dysfunction and immune disorders. However, whether circulating lipids and long-term use of lipid-lowering drugs influence the development of autoimmune thyroid disease (AITD) remains unclear. This study aims to evaluate the effects of lipid-lowering drugs on AITD and explore their potential mechanisms.

Methods: Two-sample and two-step Mendelian randomization (MR) studies were performed to assess the causal relationships between circulating lipids (LDL-C, TC, TG, and ApoB) and seven lipid-lowering drug targets (*ApoB, CETP, HMGCR, LDLR, NPC1L1, PCSK9*, and *PPARα*) with AITD. Mediation analyses were conducted to explore potential mediating factors.

Results: There was no clear causality between circulating lipids (ApoB, LDL-C, TC, and TG) and AITD ($p > 0.05$). *ApoB* inhibition is related to a reduced risk of autoimmune thyroiditis (AT) (OR = 0.462, p= 0.046), while *PCSK9* inhibition is related to reduced Graves' disease (GD) risk (OR = 0. 551, $p = 0.033$). Moreover, *PCSK9* inhibition (OR = 0.735, $p = 0.003$), *LDLR* inhibition (OR = 0.779, $p = 0.027$), and *NPC1L1* inhibition (OR = 0.599, $p = 0.016$) reduced the risk of autoimmune hypothyroidism (AIH). Mediation analysis showed that *NPC1L1* inhibition and *PCSK9* inhibition exerted effects on AIH through IL-4 and FGF-19 levels. And the effect of *PCSK9* inhibition on GD through TNF-β levels.

Conclusion: There was no clear causality between circulating lipids (ApoB, LDL-C, TC, and TG) and AITD. Lipid-lowering drug target gene inhibitors reduced the AITD risk by modulating inflammatory factors.

Keywords: Mendelian randomization, drug targeting, thyroid autoimmune disease, lipid trait, inflammatory factors

Introduction

Autoimmune thyroid disease (AITD), including Graves' disease (GD) and autoimmune thyroiditis(AT), is one of the most common autoimmune diseases.[1] Approximate 3–5% of worldwide individuals have been affected by AITD and this incidence continues to increase.[2] AITD has been listed as a major cause of abnormal thyroid function, and the latter further leads to lipid metabolic disorder.[3,4] Hyperlipidemia have been considered an independent risk factor for thyroid diseases.[5,6] The prevalence of thyroid diseases is significantly increased in hyperlipidemia patients.[7,8] Interestingly, several recent studies indicated that lipotoxicity correlated with an increased risk of hypothyroidism.[5,9] Moreover, Graves' ophthalmopathy (GO), one of the most serious complications of GD, has been proven to be related with dyslipidemia.[10] Lipid-lowering agents are the mainstay of treatment for dyslipidemia, and many studies have proven their anti-inflammatory and antioxidant properties, besides their lipid-lowering effects.[11,12] Based on the clinical correlation of the interplay between dyslipidemia and AITD, the association between lipid and lipid-lowering drugs with AITD deserves further exploration.

Mendelian randomization (MR) stands as an analytical approach that utilizes genetic variations in humans to study the causal impacts of modifiable disease exposures. Due to the random segregation of alleles of a single nucleotide polymorphism (SNP) following Mendelian laws, MR presents an advantage in mitigating confounding factors compared

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to other research methods.[13] Drug target MR analysis has emerged as a potent technique for assessing the influence of drugs, antagonists, agonists or inhibitors targeting protein-coding genes on disease risk, which can be an important aid in addressing the potential for drug therapy.[14] Therefore, this study aims to comprehensively investigate the causal relationships between circulating lipids (low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG) and apolipoprotein B (ApoB)) and seven lipid-lowering drug targets (Apolipoprotein B (*ApoB*), Cholesteryl Ester Transfer Protein (*CETP*), 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGCR*), Low-Density Lipoprotein Receptor (*LDLR*), Niemann-Pick C1-Like1 (*NPC1L1*), Proprotein Convertase Subtilisin/Kexin Type 9 (*PCSK9*) and Peroxisome Proliferator Activated Receptor-alpha (*PPARα*)) with AITD using MR analysis. This study will provide novel insights into the risk of AITD associated with lipid traits and lipid-lowering drugs.

Materials and methods

Study Design

Figure 1 shows the flowchart of this study. Firstly, we performed two-sample univariable MR (UVMR) analyses to investigate the causal effects of circulating lipid traits on AITD using genetically predicted LDL-C, TC, TG, and ApoB levels as exposures and AITD including GD, GO, AT, and autoimmune hypothyroidism (AIH) as outcomes. Secondly, multiple drug target MR analysis was conducted to investigate the association between lipid-lowering drug targets and AITD. Seven drug target genes were included in the analysis: *ApoB, HMGCR, NPC1L1, PCSK9, CETP, LDLR*, and *PPARα*. The effectiveness of lipid-lowering drug targets was verified by their impact on coronary heart disease (CHD). Thirdly, mediation MR analysis was used to explore the potential mediation effect of inflammatory factors on the association between lipid-lowering drug targets

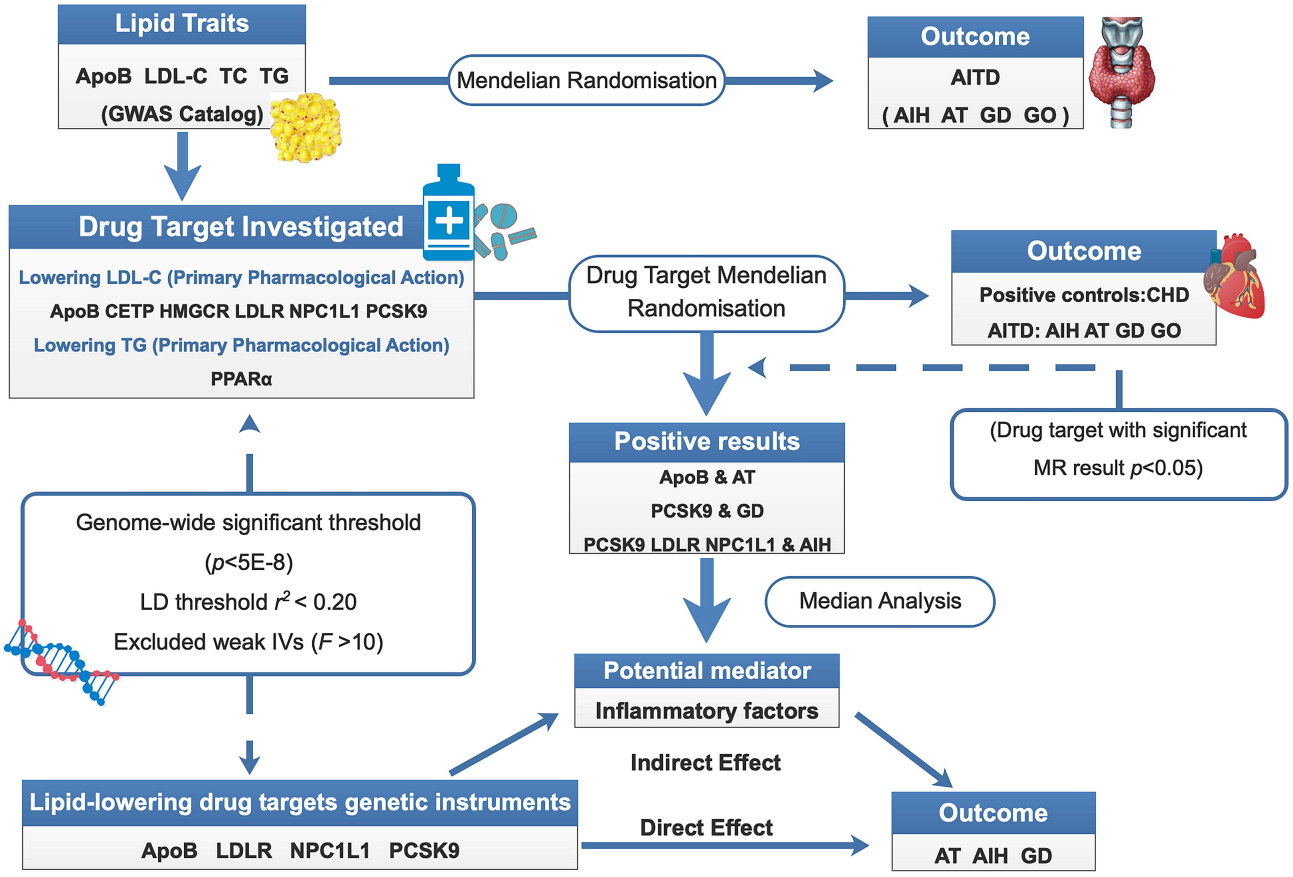

Figure 1 Overview of study design and analysis strategy. ApoB, Apolipoprotein B; LDL-C, Low-Density Lipoprotein Cholesterol; TC, Total cholesterol; TG, Total triglyceride; GD, Graves' disease; GO, Graves' ophthalmopathy; AT, Autoimmune thyroiditis; AIH, Autoimmune hypothyroidism; CHD, Coronary heart disease; *CETP*, Cholesteryl Ester Transfer Protein; *HMGCR*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *LDLR*, Low-Density Lipoprotein Receptor; *NPC1L1*, Niemann-Pick C1-like 1; *PCKS9*, Proprotein Convertase Subtilisin/Kexin Type 9; *PPARα*, Peroxisome ProliferatorActivated Receptor-alpha.

with AITD. The reporting of this study adhered to the guidelines outlined in Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR).[15] This MR study employed publicly accessible summary statistics for its analysis, and the ethical approval on it can be traced back to the original article.

Selection of genetic Instruments

To construct instrumental variables (IVs) representing lipid traits, we included GWAS data of 4 lipoproteins, including ApoB, LDL-C, TC, and TG, from a large-scale study that included up to 249 metabolic biomarkers in 88,329 European individuals.[16] We extracted full-gene significant variations of 4 lipid traits using $p < 5\times10^{-8}$ and linkage disequilibrium (LD) $r^2 \leq 0.001$ and actual distance ≥ 10 Mb as extraction criteria. The characteristics of each GWAS dataset are detailed in Supplementary Table 1.

Based on the dyslipidemia management guidelines, we identified commonly prescribed lipid-lowering drugs,[17] and queried their respective target genes through Drugbank (https://go.drugbank.com/). We then identified SNPs located within the target genes that were significantly associated with LDL-C and TG, respectively (Table 1). These genetic instruments were derived from the Global Lipids Genetics Consortium (GLGC) GWAS data on LDL-C, TG, which includes 1,320,658 European individuals.[18] We selected SNPs within each target gene that exhibited genome-wide significant associations with LDL-C, TG ($p < 5\times10^{-8}$) and the LD parameter was set at $r2<0.2$ within a range of 100 kb. We removed SNPs with palindromic structures to ensure the reliability of the results. The IVs we obtained were significantly associated with exposure ($p < 5\times10^{-8}$) in *cis*-expression Quantitative Trait Loci (*cis*-eQTL).

We calculated the *F*-statistic for selected IVs and excluded SNPs with an *F*-statistic <10 that represents minimal weak instrument bias. If the SNP was not present in the resultant GWAS, it was replaced with a surrogate SNP in the high LD ($r^2 > 0.80$) using SNiPA (https://snipa.helmholtz-muenchen.de/snipa3/index.php). If no suitable surrogate SNP was available, it was discarded.

Genetic Instruments for Inflammatory Factors

We have chosen 91 inflammatory factors from an analysis of 11 cohorts, encompassing 14,824 individuals of European descent, the original publications detailed the entire procedure for measuring inflammatory factors.[19] Complete per-protein GWAS summary statistics can be downloaded at https://www.phpc.cam.ac.uk/ceu/proteins and the EBI GWAS Catalog (accession numbers GCST90274758 to GCST90274848).

Table 1 Summary Information of Lipid-Lowering Drug Classes, Targets, and Encoding Genes

Drug class	Drug target (Drug Bank)	Encoding genes	Gene region (in GRCh37 from Ensembl)	Drug substance
ASO targeting ApoB mRNA	mRNA of ApoB-100	*ApoB*	chr 2:21,225,354–21266932	**Mipomersen**
ASO targeting CETP mRNA	Cholesteryl ester transfer protein	*CETP*	chr 16:56,996,104–57017662	**Torcetrapib**
HMGCR inhibitors	HMG-CoA reductase	*HMGCR*	chr 5:74,632,193–74657918	**Atorvastatin Rosuvastatin etc.**
Key Modulator	LDL Receptor	*LDLR*	chr 19:11,200,139–11,244,496	–
TC absorption inhibitors	Niemann-Pick C1-Like 1 (NPC1L1) protein	*NPC1L1*	chr 7:44,553,349–44580706	**Ezetimibe**
PCSK9 inhibitors	Proprotein convertase subtilisin/kexin type 9	*PCSK9*	chr 1:55,505,371–55530503	**Evolocumab Alirocumab**
Fibrates	Peroxisome Proliferator-Activated Receptor-alpha	*PPARα*	chr 22:46,546,429–46639653	**Fenofibrate Gemfibrozil**

Abbreviations: *ApoB*, Apoprotein B; *CETP*, Cholesteryl Ester Transfer Protein; *HMGCR*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; *LDLR*, Low-Density Lipoprotein Receptor; *NPC1L1*, Niemann-Pick C1-like 1; *PCKS9*, Proprotein Convertase Subtilisin/Kexin Type 9; *PPARα*, Peroxisome ProliferatorActivated Receptor-alpha.

Outcome Data

Taking into account the well-established benefits of lipid-lowering drugs on coronary heart disease, we performed a positive control analysis using coronary heart disease as the outcome data. The GWAS data for CHD were sourced from the IEU GWAS database (60,801 cases and 123,504 controls). Then, we selected GD, GO, AT, and AIH as the primary outcomes of our study. In our research, GWAS data for GD (3176 cases and 409,005 controls), GO (598 cases and 411,583 controls), AT (539 cases and 349,717 controls) and AIH (45,321 cases and 298,847 controls) were obtained from the FinnGen database (Release 10), as described in Supplementary Table 1.

Statistical Analysis

MR Analysis to Estimate the Effects of Lipid Traits Targets on AITD

We applied UVMR to assess the effects of lipid traits on AITD. The main analysis method is inverse variance weighting (IVW).[20] Heterogeneity testing was used to determine whether to choose a random effects model or a fixed effects model for IVW. Specifically, when heterogeneity was observed (Q_*pval* < 0.05 and I^2 > 50%), the random effects model was selected as it provides more precise estimates and confidence interval (CI) than the fixed effects IVW method, and was tested using Cochran's Q, Otherwise, a fixed effects model is used. In addition to the IVW method, we also used four MR methods as supplementary analysis, namely MR-Egger, weighted median, weighted mode, and simple mode. Furthermore, to assess relative pleiotropy, the MR-Egger intercept test and MR pleiotropy residuals and outliers (MR-PRESSO) were used. Outlier SNPs were detected using the MR-PRESSO outlier test using a level *p* index of 0.05. The MR results were evaluated using a leave-one-out approach to check their robustness.

MR Analysis to Estimate the Effects of Lipid-Lowering Drug Targets on AITD

First, we used CHD as a control outcome to evaluate the reliability of the extracted SNPs as alternatives to lipid-lowering drugs. IVW was also used for estimating the impact of genetic tools and lipid-lowering drugs on CHD. Subsequently, we continued to use IVW as the primary method, with the above 4 methods as complementary methods to determine the association between validated IVs and the risk of AITD. MR-Egger intercept test MR pleiotropy residuals and MR-PRESSO assessed pleiotropy. In addition to this, MR.RAPS provides our results with robust estimates corrected for systematic and idiosyncratic pleiotropies.[21]

Mediation MR Analysis Linking Lipid-Lowering Drug Targets with AITD via Inflammatory Factors

To evaluate the mediating role of 91 inflammatory factors on the relationship between lipid-lowering drug targets and AITD, we performed two-step MR (Figure 1). First, we used UVMR to estimate the impacts of lipid-lowering drug targets on 91 inflammatory factors ($\beta 1$). We selected *cis*-eQTL genetic variation as IV, gene expression as exposure, and 91 inflammatory factors as outcomes for MR analysis. Then, we selected inflammatory factors significantly correlated with gene expression as exposures to conduct MR analysis ($\beta 2$) on AT, GD, and AIH respectively. Since the number of SNPs in some inflammatory factors is small, we selected SNPs that were significantly associated with inflammatory factors at the genome-wide level ($p < 1\times10^{-5}$) as the corresponding IVs. The LD parameter was set to $r^2 < 0.001$ within 100 kb. To note, the IVs variables of the two-step MR analysis cannot be repeated, so the IVs used in the second step need to exclude those used in the first step. Finally, the mediating proportion of each inflammatory factor in the association between the lipid-lowering drug target and AITD was calculated as the product of $\beta 1$ and $\beta 2$ divided by the total effect of the lipid-lowering drug targets on AITD. The 95% CI for the mediation proportion was calculated using the delta method.[22]

Sensitivity Analysis

We used the intercept term of the MR-Egger regression to represent the mean pleiotropy of IVs, and the likelihood of horizontal pleiotropy was estimated using MR-Egger regression. In addition, we used MR-PRESSO as a supplement to assess horizontal pleiotropy.[23] The purpose of detecting horizontal multivariate validity, correcting horizontal multivariate validity by removing outliers, and determining whether the causal effects have substantially changed before and after removing outliers in MR analysis can all be achieved through MR-PRESSO. To improve the accuracy and robustness of the genetic instrument, we quantified heterogeneity using Cochran's Q statistic, where $p > 0.05$ indicates no effect heterogeneity.

Results

Selection and Validation of Genetic Instruments

By applying the thresholds we set in the method analysis, among 88,329 European individuals, 44 SNPs represented ApoB, 46 SNPs represented LDL-C, 49 SNPs represented TC, and 55 SNPs represented TG. In addition, we selected 12 SNPs proxied *ApoB*, 6 SNPs proxied *CETP*, 6 SNPs proxied *HMGCR*, 13 SNPs proxied *LDLR*, 5 SNPs proxied *NPC1L1*, 11 SNPs proxied *PCSK9* and 3 SNPs proxied *PPARα* in 1,320,658 European individuals. Among the IVs studied, *F*-statistics ranged from 29.9 to 9740.0, suggesting that weak instrumental bias has little impact on our analysis. We then performed UVMR analyses of lipid-lowering drug targets and CHD using gene proxies with CHD as positive controls. All lipid-lowering drug target genes involved in this study showed significant associations with CHD risk. No significant heterogeneity or multiple effects were observed in the results, suggesting that these genetic tools are effective. Details of all included SNPs can be found in Supplementary Tables 2 and 3.

Association of Lipid Traits with Genetic Proxies for AITD

We conducted a two-sample MR analysis on the association between lipid traits (including ApoB, LDL-C, TG, and TC) and AITD. Although no evidence of pleiotropy was detected in our results, the presence of heterogeneity was observed. Therefore, the IVW model was conducted using random effects. We found that there was no clear causality between ApoB, LDL-C, TC, TG, and AITD ($p > 0.05$) (Supplementary Table 4).

Association of Lipid-Lowering Drugs Targets with Genetic Proxies for AITD

In our preliminary analyses using the IVW approach, we observed strong evidence that LDL-C-derived *ApoB* inhibition (OR = 0.462, 95% CI = 0.216,0.986; $p = 0.046$) reduced the risk of AT and that *PCSK9* inhibition (OR = 0.551. 95% CI = 0.319,0.953; $p = 0.033$) reduced the risk of GD, while *PCSK9* inhibition (OR = 0.735, 95% CI = 0.598,0.903; $p = 0.003$) were also found to reduce the risk of AIH. In addition to this, we found that both *LDLR* inhibition (OR = 0.779, 95% CI = 0.624,0.972; $p= 0.027$) and *NPC1L1* inhibition (OR = 0.599, 95% CI = 0.412,0.872; $p = 0.016$) similarly reduced the risk of AIH (Table 2, Figure 2). No pleiotropy or heterogeneity was found for any of the above gene inhibitors ($p>0.05$) (Supplementary Table 5).

Mediation MR of Lipid-Lowering Drug Targets, Inflammatory Factors and AITD

We estimated the impacts of lipid-lowering drug targets on 91 inflammatory factors and observed that a total of 30 inflammatory factors were significantly associated with *ApoB* inhibition, *NPC1L1* inhibition, and *PCSK9* inhibition,

Table 2 MR Analyses of Lipid-Lowering Drugs on AITD by Different Methods

Exposure	Outcome	IVW		MR.RAPS	
		p	OR (95% CI)	*p*	OR (95% CI)
ApoB	AT	0.046	0.462 (0.216,0.986)	0.046	0.461 (0.216,0.986)
PCSK9	GD	0.033	0.551 (0.319,0.953)	0.034	0.551 (0.318,0.955)
PCSK9	AIH	0.003	0.735 (0.598,0.903)	<0.001	0.734 (0.624,0.863)
LDLR	AIH	0.027	0.779 (0.624,0.972)	0.009	0.778 (0.644,0.939)
NPC1L1	AIH	0.007	0.599 (0.412,0.872)	0.008	0.597 (0.409,0.874)

Abbreviations:MR, Mendelian randomization; OR, odds ratio; CI, confidence interval; MR.RAPS, Mendelian randomization robust adjusted profile score. OR, 95% CI, and *p*-values were calculated for the respective method of MR analysis.

Exposure	Outcome	Method	nSNP	P	OR_CI
ApoB	GD	IVW	12	0.154	0.794(0.578-1.09)
	GO	IVW	12	0.797	0.904(0.419-1.951)
	AT	IVW	12	0.046	0.462(0.216-0.986)
	AIH	IVW	12	0.093	0.924(0.842-1.013)
	CHD	IVW	11	<0.01	0.69(0.566-0.841)
CETP	GD	IVW	6	0.36	0.586(0.186-1.842)
	GO	IVW	6	0.562	0.466(0.035-6.154)
	AT	IVW	6	0.085	0.091(0.006-1.396)
	AIH	IVW	6	0.058	0.675(0.45-1.013)
	CHD	IVW	6	<0.01	0.429(0.281-0.654)
HMGCR	GD	IVW	6	0.179	0.493(0.176-1.382)
	GO	IVW	6	0.146	0.174(0.016-1.84)
	AT	IVW	6	0.471	0.479(0.039-5.811)
	AIH	IVW	6	0.993	0.998(0.677-1.473)
	CHD	IVW	6	0.019	0.608(0.401-0.922)
LDLR	GD	IVW	13	0.811	0.923(0.476-1.787)
	GO	IVW	13	0.302	0.475(0.116-1.952)
	AT	IVW	13	0.571	0.64(0.136-3.000)
	AIH	IVW	12	0.027	0.779(0.624-0.972)
	CHD	IVW	11	<0.001	0.454(0.353-0.583)
NPC1L1	GD	IVW	5	0.961	0.969(0.272-3.448)
	GO	IVW	5	0.697	0.562(0.031-10.28)
	AT	IVW	5	0.941	0.89(0.041-19.107)
	AIH	IVW	5	0.007	0.599(0.412-0.872)
	CHD	IVW	5	0.006	0.459(0.263-0.800)
PCSK9	GD	IVW	11	0.033	0.551(0.319-0.953)
	GO	IVW	11	0.688	0.774(0.222-2.697)
	AT	IVW	11	0.927	0.922(0.163-5.210)
	AIH	IVW	11	0.003	0.735(0.598-0.903)
	CHD	IVW	9	<0.001	0.519(0.417-0.646)
PPARα	GD	IVW	3	0.2	(0.005,3.035)
	GO	IVW	3	0.435	(0,83.440)
	AT	IVW	3	0.98	(0,1956.070)
	AIH	IVW	3	0.756	(0.335,2.214)
	CHD	IVW	3	0.001	(0.036,0.446)

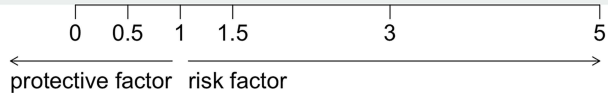

Figure 2 Forest plot of the effects of lipid-lowering drugs on autoimmune thyroid disease. OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted; $p < 0.05$ was considered significant. OR>1 is a risk factor, and OR<1 is a protective factor.

respectively (Supplementary Table 6). We did not observe a significant correlation of inflammatory factors on *LDLR* inhibition.

We further estimated the effects of 30 inflammatory factors significantly associated with lipid-lowering drug targets on AITD and found that 3 inflammatory factors were significantly associated with AIH and one inflammatory factor was significantly associated with GD (Supplementary Table 7). We observed a significant correlation between Interleukin-4 (IL-4) levels (OR=1.119, 95% CI=1.020,1.228; $p = 0.018$), Osteoprotegerin levels (OR = 0.924, 95% CI = 0.862,0.992; $p = 0.029$), Fibroblast growth factor-19 (FGF-19) levels (OR = 0.934, 95% CI = 0.890,0.980; $p = 0.006$) and AIH; Tumor

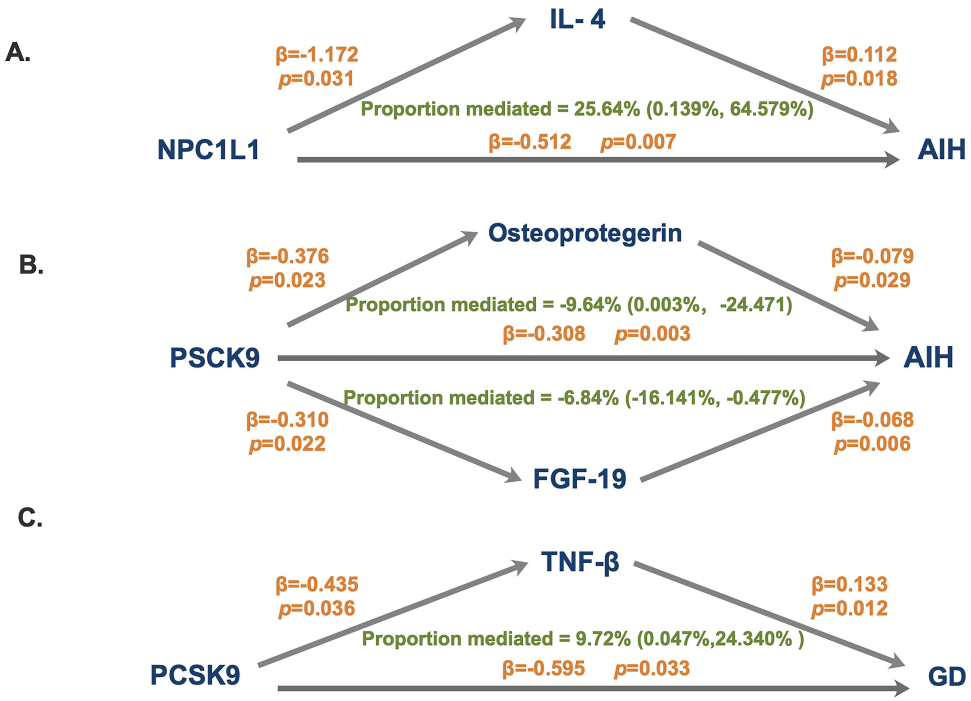

Figure 3 The potential causal evidence summarized from the two- step MR analysis IL-4, F GF-19, and TNF-β. IL-4, Interleukin-4; FGF-19, Fibroblast growth factor-19; TNF-β, Tumor necrosis factor-beta. The 95% CI for the mediation proportion was calculated using the delta method. (**A**) Mediation analysis of the effects of *NPC1L1* inhibition on AIH via potential mediators. (**B**) Mediation analysis of the effects of *PCSK9* inhibition on AIH via potential mediators. (**C**) Mediation analysis of the effects of *PCSK9* inhibition on GD via potential mediators.

necrosis factor-beta (TNF-β) levels (OR =1.142, 95% CI = 1.029,1.268; p = 0.012) was significantly associated with GD. There was no evidence of horizontal pleiotropy, and although some of the results were heterogeneous, we used a random effects IVW approach for analysis.[24] The IVs for the 30 inflammatory factors were all strong (F-statistics >38.55) (Supplementary Table 8).

We found that *NPC1L1* inhibition through IL-4 levels had an indirect effect on AIH, with a mediated proportion of the total effect of 25.64% (95% CI =0.139%,64.579%, p=0.008); *PCSK9* inhibition through FGF- 19 levels have an indirect effect on AIH, and the mediated proportion of the total effect is −6.84% (95% CI =−16.141%,-0.477%, p=0.036); *PCSK9* inhibition has an indirect effect on GD through TNF-β levels, and the mediated proportion of the total effect of 9.72% (95% CI = 0.047%,24.340%, p=0.045). However, we observed that although Osteoprotegerin levels were significantly related to AIH, the 95% CI crossed the invalid line (95% CI = 0.003%, −24.471%, p=0.625), indicating that the mediating effect of this result was not established (Figure 3).

Discussion

In this study, we systematically evaluated the causal relationship between 4 blood lipid traits,7 lipid-lowering gene inhibitors, 91 inflammatory factors, and the risk of AITD through drug-targeted MR analysis and mediation MR analysis. There was no clear causality between circulating lipids and AITD. *ApoB* inhibition is related to a reduced risk of AT, while *PCSK9* inhibition is related to reduced GD risk. Moreover, *PCSK9* inhibition, *LDLR* inhibition, and *NPC1L1* inhibition reduced the risk of AIH. Mediation analysis indicated that the effect of *NPC1L1* inhibition and *PCSK9* inhibition on AIH through IL-4 and FGF-19 levels. And the effect of *PCSK9* inhibition on GD through TNF-β levels.

Thyroid hormone plays a crucial role in the modulation of energy metabolism.[25] The causal relationship that thyroid dysfunction caused dyslipidemia is a well-accepted clinical finding.[26] Interestingly, some recent studies have demonstrated that lipotoxicity resulted in the pathogenesis of multiple diseases, including thyroid dysfunction and immune disorders.[9] A prospective cohort study showed that the subclinical hypothyroid patients with hypercholesterolemia were more vulnerable to developing overt hypothyroidism during a 3-year follow-up.[27] Statins, the most commonly used of the

lipid-lowering drugs, have been observed to be correlate with reduced GO risk in patients with Graves' hyperthyroidism.[28] Based on the clinical correlation of the interplay between dyslipidemia and AITD, the association between lipid and lipid-lowering drugs with AITD deserves further exploration. The present study showed that there was no clear causality between circulating lipids and AITD, however, lipid-lowering targets reduced the AITD risk. Therefore, the underlying mechanisms may extend beyond the lipid-lowering effect.

Further mediation MR analysis found the effect of *NPC1L1* inhibition and *PCSK9* inhibition on AIH through IL-4 and FGF-19 levels. And the effect of *PCSK9* inhibition on GD through TNF-β levels. Fortunately, the anti-inflammatory effects of lipid-lowering drug target gene inhibitors have long been demonstrated in other studies. Combining ezetimibe with a statin is a more effective way to lower CRP levels.[29,30] *PCSK9* inhibition also exerts anti-inflammatory effects and is positively correlated with levels of inflammatory biomarkers such as leukocytes, hsCRP, and fibrinogen.[31] The occurrence and development of AITD are also closely related to inflammatory factors. The key to the occurrence of AITD is the activation of T cells,[32] and the generation of IgG1 isotype response is stimulated by Th1 cytokines,[33] which is the main pathogenic TSH receptor autoantibody observed in AITD.[34,35] IL-4 can stimulate the expression of HLA class II antigens and oppose Th1 cell inflammatory responses through signal transducer and activator of transcription 6 (*STAT6*).[36] A cohort study demonstrated that patients with AITD had lower overall IL-4 activity, which may contribute to the propensity to produce IgG1 autoantibodies.[37] However, in another study it was demonstrated that ectopic expression of IL-4 in thyroid tissue increases the incidence of spontaneous AT, the eventual evolution of which can lead to hypothyroidism.[38] Furthermore, FGF-19, a promising lipid modulator, exhibited a notable decrease in the serum of individuals suffering from hypothyroidism and subclinical hypothyroidism.[39,40] This may be related to the fact that TSH triggers hepatic sterol regulatory element-binding protein (SREBP) through its receptor to negatively regulate the transcription of FGF19 in human intestinal cells.[41,42] However, the impact of FGF-19 on AITD has not been completely confirmed. TNF-β exerts a crucial influence on regulating inflammatory responses, apoptosis, and immune cell activity. TNF-β alleles may mark a specific immune response state with altered immune responses to mitogens and suppressor T cells and may contribute to the development of GD in a predisposing manner.[43,44] Interestingly, a positive association between high levels of TNF-β and GD risk was also observed in a recent MR analysis.[45]

Although there are some similar cross-sectional studies, the methods of drug-target MR and mediation analysis we used are more rigorous.[46] MR analysis is a natural randomized controlled trial that studies large sample sizes, minimizes confounding and reverse causation, and provides accuracy and convenience. Our study evaluated the relationship between circulating lipids and AITD and focused on the risk of AITD caused by long-term use of lipid-lowering drugs. Moreover, we pointed out the role of inflammatory factors more accurately by analyzing them as mediators rather than as exposures.[45,47] This result illustrates the innovative use of lipid-lowering drugs and expands their therapeutic use in clinical practice. However, our study has some limitations. First, drug-targeted MR analysis cannot capture the short-term effects of lipid-lowering drugs. Second, the databases we used were not stratified by sex, age, or disease severity. Finally, this study utilized European population data, raising uncertainty regarding generalizability to other ethnic groups. Further investigation through laboratory studies and clinical trials is necessary to confirm and elucidate these findings.

Conclusions

The incidence of AITD is reduced after taking lipid-lowering drugs in hyperlipidemia patients. Lipid-lowering drug target gene inhibitors reduced the AITD risk by modulating inflammatory factors. This study will help to expand the use of this class of drug in clinical practice.

Abbreviations

AIH, Autoimmune hypothyroidism; AITD, Autoimmune thyroid disease; ApoB/*ApoB*, Apolipoprotein B; AT, Autoimmune thyroiditis; *CETP*, Cholesteryl Ester Transfer Protein; CHD, Coronary heart disease; CI, Confidence interval; *cis*-eQTL, *cis*-expression Quantitative Trait Loci; FGF-19, Fibroblast growth factor-19; GD, Graves' disease; GLGC, Global Lipids Genetics Consortium; GO, Graves' ophthalmopathy; *HMGCR*, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IL-4, Interleukin-4; IVs, Instrumental variables; IVW, Inverse variance weighting; LD, Linkage disequilibrium; LDL-C, Low-density lipoprotein cholesterol; LDLR, Low-Density Lipoprotein Receptor; MR, Mendelian

randomization; MR-PRESSO, Mendelian randomization pleiotropy residuals and outliers; *NPC1L1*, Niemann-Pick C1-Like1; *PCSK9*, Proprotein Convertase Subtilisin/Kexin Type 9; *PPARα*, Peroxisome Proliferator Activated Receptor-alpha; SNP, Single nucleotide polymorphism; SREBP, Sterol Regulatory Element-binding Protein; *STAT6*, Signal transducer and activator of transcription 6; STROBE-MR, Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization; TC, Total cholesterol; TG, Triglyceride; TNF-β, Tumor necrosis factor-beta; UVMR, Univariable Mendelian Randomization.

Data Sharing Statement

Original data generated and analyzed during this study are included in this published article or in the data repositories listed in References. The FinnGen database is an open access resource and researchers need to obtain approval from the FinnGen database (https://www.finngen.fi/fi). Lipid-related data come from the IEU database (https://gwas.mrcieu.ac.uk/), GWAS Catalog database (https://www.ebi.ac.uk/gwas/) and GLGC database (www.lipidgenetics.org/#data-downloads-title), the above databases are all open access resources.

Ethics Approval and Consent to Participate

The data in this study were obtained from published studies, of which all data had been approved by the institutional review committee. The ethical application for this study was approved by the Ethics Committee of Beijing Chaoyang Hospital Affiliated to Capital Medical University.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflict of interest to disclose. This paper has been uploaded to ResearchSquare as a preprint:https://doi.org/10.21203/rs.3.rs-4428352/v1.

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