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Association of thyroid disease and risk of fatty liver disease: an exposure-wide Mendelian randomization study

H.-F. ZHANG, Q.-X. ZHANG, Y.-H. FENG, S.-J. LI, B.-Y. XIE

Department of Medicine, Internal Medicine, The First People's Hospital of Fuyang, Hangzhou, Zhejiang, China

H.-F. Zhang, Q.-X. Zhang, and Y.-H. Feng contributed equally to this work

Abstract. – OBJECTIVE: Previous studies have often observed a possible association between thyroid and fatty liver diseases. The pathogenesis of both diseases is complex, with many confounding factors and controversies. We used a two-sample Mendelian randomization (MR) analysis to test the causality between thyroid disease and the risk of developing fatty liver disease.

MATERIALS AND METHODS: All data were obtained from the genome-wide association studies (GWAS) Catalog database. Thyroid disorders include hypothyroidism, hyperthyroidism, autoimmune thyroiditis, and Hashimoto's thyroiditis. Fatty liver diseases include alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD). The inverse variance weighting (IVW) method was used for MR analysis, and sensitivity analysis was further performed to test its robustness.

RESULTS: We discovered no causal relationship between thyroid disease and alcoholic fatty liver disease after excluding weak instrumental variables (IVs). Hyperthyroidism and hypothyroidism had a significant causal relationship with NAFLD. Hypothyroidism increased the risk of NAFLD using the IVW method (OR=7.62, 95% CI: 2.61-22.25, $p<0.001$). MR-Egger regression did not suggest potential evidence of directional pleiotropy (intercept, $p=0.698$). Hyperthyroidism also significantly increased the risk of NAFLD (OR=11.83, 95% CI: 2.9-22.54, $p=0.026$). MR-Egger regression did not suggest any potential directional pleiotropy (intercept, $p=0.295$).

CONCLUSIONS: Hypothyroidism can significantly increase NAFLD incidence, and hyperthyroidism may be a risk factor for NAFLD.

Key Words:

Thyroid disease, Fatty liver disease, Mendelian randomization.

Introduction

Fatty liver diseases, including alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), are histologically characterized by abnormal fat accumulation in the liver[1]. NAFLD is a chronic liver disease associated with metabolic syndrome with a global prevalence of 25%[2]. The mortality rate associated with death from chronic liver disease increased by 46% globally between 1980 and 2010, which is closely related to the increasing NAFLD prevalence[3]. ALD is also an important cause of chronic liver disease worldwide and a common factor in the progression of other liver disease[4]. Health institutions have paid more attention to fatty liver disease because of its increasing incidence and huge economic burden.

Thyroid hormones, such as glucose and lipid, regulate energy metabolism[5]. Thyroid dysfunction is associated with liver problems due to low or high thyroid hormone levels in the blood[6]. In an observational study[7] of 20,289 participants, NAFLD patients had thyroid hormone resistant-like features, with high levels of thyroid hormone-free triiodothyronine (FT3) and thyroid stimulating hormone (TSH). However, some studies[8] have demonstrated that hypothyroidism may cause metabolic disorders of blood glucose and lipids, which play an important role in the occurrence and development of NAFLD. Thyroid hormone supplementation reduces free fatty acid and triglyceride levels and has been used to reduce hepatic steatosis and improve the NAFLD patient prognosis[9,10]. Overlapping histological signs with an autoimmune liver can be observed in 48% of patients with non-alcoholic steatohepatitis[11]. Therefore, abnormal immune

Corresponding Author: Biyun Xie, MD; e-mail: angisxie@163.com

function in autoimmune thyroiditis patients may be involved in developing NAFLD inflammation[12]. Consequently, it is necessary to explore the effects of different thyroid diseases on the occurrence of fatty liver disease and its possible mechanisms.

Mendelian randomization (MR) is an approach used to investigate the causality between exposures and outcomes of interest[13]. This method uses single nucleotide polymorphisms (SNPs) as unconfounded proxies for exposures, thereby avoiding the residual confounding and reverse causality commonly present in conventional observational studies[14]. MR design is an important strategy for causal inference without randomized clinical trials (RCTs) because genetic variants are randomly sorted during meiosis, miming an RCT[15]. Additional studies[7,8] have reported an association between thyroid disease and fatty liver disease, and numerous clinical confounding factors exist. Therefore, an MR study must be conducted to investigate this association.

Materials and Methods

In this study, all data were derived from the Genetic Alliance's publicly available compilation of statistical data from genome-wide association studies (GWAS). All original studies received a specific ethical review and informed consent.

Study Design

Summary statistics were collected for thyroid and fatty liver diseases from published GWAS. We aimed to explore the causal effect of thyroid disease on the risk of fatty liver using two-sample MR.

MR approach was based on three main assumptions: (1) genetic variants as instrumental variables (IVs) should be robustly associated with the risk factor of interest; (2) the genetic variants used should not be associated with potential confounding factors; and (3) selected genetic variants affect the risk of outcome only *via* risk factors and not *via* other pathways (Figure 1).

Outcome and Exposure Data Source

Summary statistics were collected for thyroid and fatty liver diseases from published GWAS. The GWAS Catalog database is publicly available for download at https://www.ebi.ac.uk/gwas/. Among them, the exposure group had hypothyroidism[16], hyperthyroidism[17], autoimmune thyroid disease[18], Hashimoto's thyroiditis[19], and the outcome group had alcoholic fatty liver disease[20] and non-alcoholic fatty liver disease[21]. All the participants were of European ancestry. The details of GWAS outcomes are presented in Table I.

Genetic Variants Selection Criteria

Genetic instruments for each exposure trait or disease were selected at the genome-wide significance threshold ($p<5\times10^{8}$) from corresponding GWASs. Independent single nucleotide polymorphisms (SNPs) were defined by $R^2<0.001$ and clump window >10 kb, and correlated SNPs (linkage disequilibrium, LD) with the lowest *p*-value were retained. Linkage disequilibrium

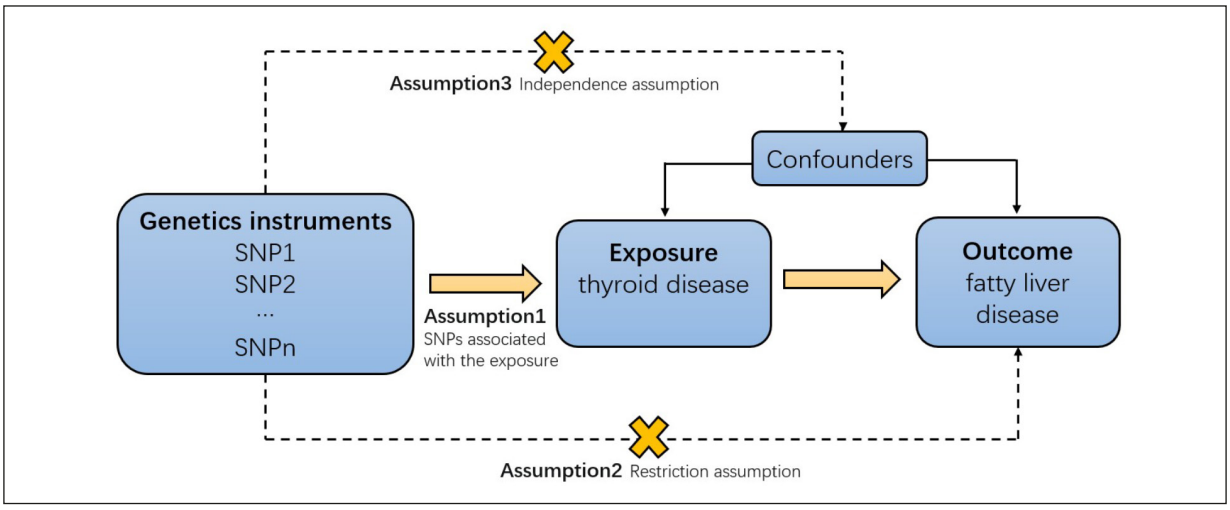

Figure 1. The overall design of Mendelian randomization analyses in the present study. SNP: single nucleotide polymorphism.

Table I. Detailed information of the genome-wide association studies in our analysis.

Exposure/Outcome	Year	PMID	First author	Population	Sample size	
					Cases	Controls
Alcoholic liver disease	2021	34737426	Jiang et al[20]	European	451	455,897
Non-alcoholic fatty liver disease	2021	34841290	Ghodsian et al[21]	European	8,434	770,180
Hypothyroidism	2018	29892013	Loh et al[16]	European	473,703	
Hyperthyroidism	2021	33959723	Dönertas et al[17]	European	3,731	480,867
Autoimmune thyroid disease	2021	34278373	Glanville et al[18]	European	607	324,074
Hashimoto's thyroiditis	2021	34594039	Sakaue et al[19]	European	15,654	379,986

among SNPs for each risk factor was calculated based on 1,000 genomes LD reference panel (European population)[22] using the PLINK clumping approach[23].

Statistical Analysis

The variance in thyroid disease explained by the IVs was calculated, and weak IV bias was tested using F-statistics.

R^2 was calculated[24] based on the effect estimates (β) and allele frequencies (EAF) of each SNP using $2\times EAF(1\text{-}EAF)\times\beta^2$. The F-value was calculated as $R^2*(N\text{-}2)/(1\text{-}R^2)$[25]. An F-value >10 was considered a strong genetic IV; otherwise, the SNP was discarded.

Cochran's Q statistics were performed to assess heterogeneity across individual SNPs. The random-effect inverse-variance-weighted (IVW) model was used as the primary analytical method to examine causal association, *p*-values <0.05 were deemed statistically significant[26]. The MR-Egger method was used to determine whether the instrumental SNPs were multi-effect, and *p*-value <0.05 indicated possible pleiotropy[27]. Sensitivity analyses were performed using several approaches to investigate the potential pleiotropic bias: IVW-RE MR, MR-Egger regression, weighted median MR, funnel plots, and leave-one-variant-out analysis for IVW-RE, where one variant at a time was left out. All analyses were implemented in R software (version 4.2.0).

Results

Selection of Instrumental Variables

We calculated the F-value for each SNP individually and kept the SNPs greater than 10, suggesting that there could be no bias due to weak IVs (Table II). Finally, 43 SNPs were excluded because F<10.

Mendelian Estimations

Cochran's Q test revealed no significant heterogeneity among SNPs. ALD outcome measures included hypothyroidism (p=0.057), hyperthyroidism (p=0.797), autoimmune thyroid disease (p=0.666), and Hashimoto's thyroiditis (p=0.507). NALFD outcome measures included hypothyroidism, p=0.364; hyperthyroidism, p=0.712; and Hashimoto's thyroiditis, p=0.380 (Table III). Finally, we chose the random-effects IVW model for MR Analysis.

Table II. Details of variance explained by the selected instruments and F-statistics for the MR analysis based on the sample size of autoimmune diseases.

Exposure	Outcome	Case	Control	R^2 of instrument	F-statistic
Hypothyroidism	Alcoholic liver disease	451	455,897	0.0027	1,224.83
Hypothyroidism	Non-alcoholic fatty liver disease	8,434	770,180	0.0002	160.86
Hyperthyroidism	Alcoholic liver disease	451	455,897	0.0013	591.99
Hyperthyroidism	Non-alcoholic fatty liver disease	8,434	770,180	0.0002	121.11
Autoimmune thyroid disease	Alcoholic liver disease	451	455,897	0.0008	363.00
Autoimmune thyroid disease	Non-alcoholic fatty liver disease	8,434	770,180	0.0009	343.69
Hashimoto's thyroiditis	Alcoholic liver disease	451	455,897	0.0157	653.62
Hashimoto's thyroiditis	Non-alcoholic fatty liver disease	8,434	770,180	0.0002	120.98

Table III. Mendelian randomization analysis of thyroid disease with fatty liver disease risk.

Risk factors	Number of SNPs	OR	95% CI	*p* for association	*p* for MR-Egger intercept	*p* for heterogeneity
Autoimmune thyroid disease and ALD						
MR Egger	3	13.05	0.01-31.40	0.501	0.535	
Weighted median	3	5.40	0.01-13.92	0.582		
Inverse variance weighted	3	4.87	0.01-9.69	0.580		0.666
Simple mode	3	0.01	-9.84-8.26	0.393		
Weighted mode	3	12.01	1.78-28.90	0.546		
Autoimmune thyroid disease and NALFD						
MR Egger					/	
Weighted median						
Inverse variance weighted	2	0.14	0.01-1.61	0.540		/
Simple mode						
Weighted mode						
Hashimoto's thyroiditis and ALD						
MR Egger	8	0.78	0.35-1.71	0.553	0.398	
Weighted median	8	1.12	0.78-1.60	0.534		
Inverse variance weighted	8	1.09	0.83-1.45	0.527		0.507
Simple mode	8	1.06	0.65-1.72	0.823		
Weighted mode	8	1.07	0.71-1.60	0.766		
Hashimoto's thyroiditis and NALFD						
MR Egger	6	1.23	0.98-1.55	0.150	0.279	
Weighted median	6	1.08	0.97-1.21	0.157		
Inverse variance weighted	6	1.08	0.98-1.18	0.121		0.380
Simple mode	6	1.08	0.92-1.26	0.393		
Weighted mode	6	1.10	0.97-1.24	0.204		
Hyperthyroidism and ALD						
MR Egger	10	15.43	-20.11-21.04	0.562	0.404	
Weighted median	10	1.37	-4.5-23.68	0.469		
Inverse variance weighted	10	-1.22	-12.07-23.68	0.540		0.797
Simple mode	10	16.86	-26.81-32.50	0.708		
Weighted mode	10	18.14	-1.33-37.64	0.587		
Hyperthyroidism and NALFD						
MR Egger	7	15.69	0.32-24.08	0.121	0.295	
Weighted median	7	16.75	0.30-30.02	0.086		
Inverse variance weighted	7	11.83	2.90-22.54	0.026		0.712
Simple mode	7	8.40	0.06-18.20	0.165		
Weighted mode	7	17.12	0.23-25.26	0.133		
Hypothyroidism and ALD						
MR Egger	115	0.48	0.01-539.14	0.838	0.916	
Weighted median	115	0.31	0.01-45.08	0.642		
Inverse variance weighted	115	0.67	0.02-19.01	0.815		0.057
Simple mode	115	0.01	-4.28-16.04	0.169		
Weighted mode	115	0.49	0.01-82.63	0.784		
Hypothyroidism and NALFD						
MR Egger	86	11.50	1.11-118.98	0.044	0.698	
Weighted median	86	6.88	1.31-36.26	0.023		
Inverse variance weighted	86	7.62	2.61-22.25	< 0.001		0.364
Simple mode	86	83.94	3.30-2,135.06	0.009		
Weighted mode	86	12.46	2.35-65.94	0.004		

SNP: single nucleotide polymorphism, ALD: alcoholic liver disease, NAFLD: non-alcoholic fatty liver disease.

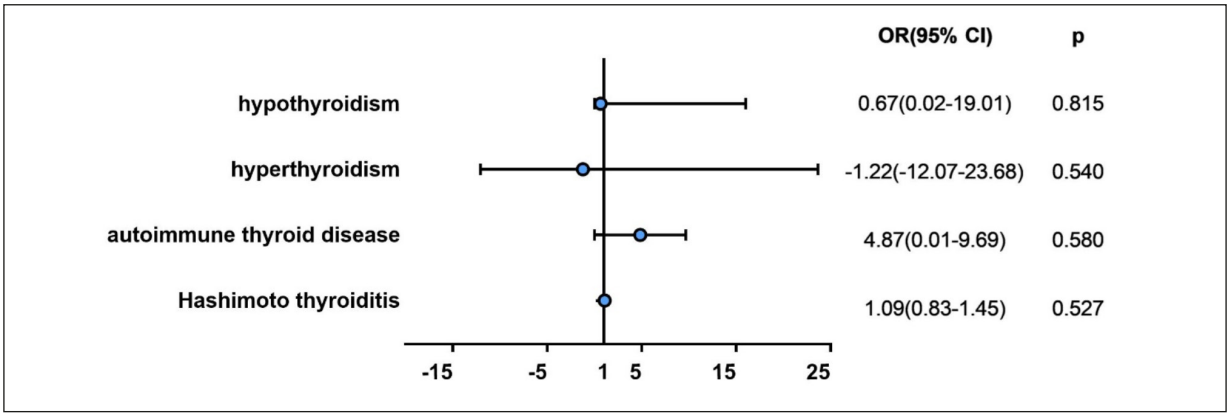

Figure 2. Mendelian randomization analysis of thyroid disease with fatty liver disease risk.

ALD

We observed no significant association between thyroid disease and ALD using IVW (Figure 2). Hypothyroidism: OR=0.67, 95% CI: 0.02-19.04, p=0.815; hyperthyroidism, OR= -1.22, 95% CI: -12.07-23.68, p=0.540; autoimmune thyroid disease, OR=4.87, 95% CI: 0.01-9.69, p=0.580; Hashimoto's thyroiditis, OR=1.09, 95% CI: 0.83-1.45, p=0.527. We also used MR Egger, weighted median, simple mode, and weighted mode methods for verification, yielding similar results (Table III). The scatter plot of the SNP-ALD association against the SNP-thyroid disease is shown in **Supplementary Figure 1**. Meanwhile, leave-one-out analysis is shown in **Supplementary Figure 2**.

NAFLD

We observed that abnormal thyroid hormone levels could increase the risk of NAFLD. Hypothyroidism significantly increased the risk of NAFLD using the IVW method (OR=7.62, 95% CI: 2.61-22.25, p<0.001), and the other four methods yielded consistent results (Figure 3). Simultaneously, MR-Egger regression did not suggest potential evidence of directional pleiotropy (intercept p=0.698, Table III). Figure 4 shows the scatter plot of the SNP-NAFLD association against the SNP-hyperthyroidism and SNP-hypothyroidism association. The scatter plot of the SNP-NAFLD association against the SNP-autoimmune thyroid disease and SNP-Hashimoto's thyroiditis are shown in **Supplementary Figure 3**. Hyperthyroidism also significantly increased the risk of NAFLD when using the IVW method (OR=11.83, 95% CI: 2.9-22.54, p=0.026, Figure 3). The MR-Egger regression did not suggest potential directional pleiotropy (intercept p=0.295), scatter plots also suggested low heterogeneity (**Supplementary Figure 4**). We further performed a leave-one-out analysis, which revealed a con-

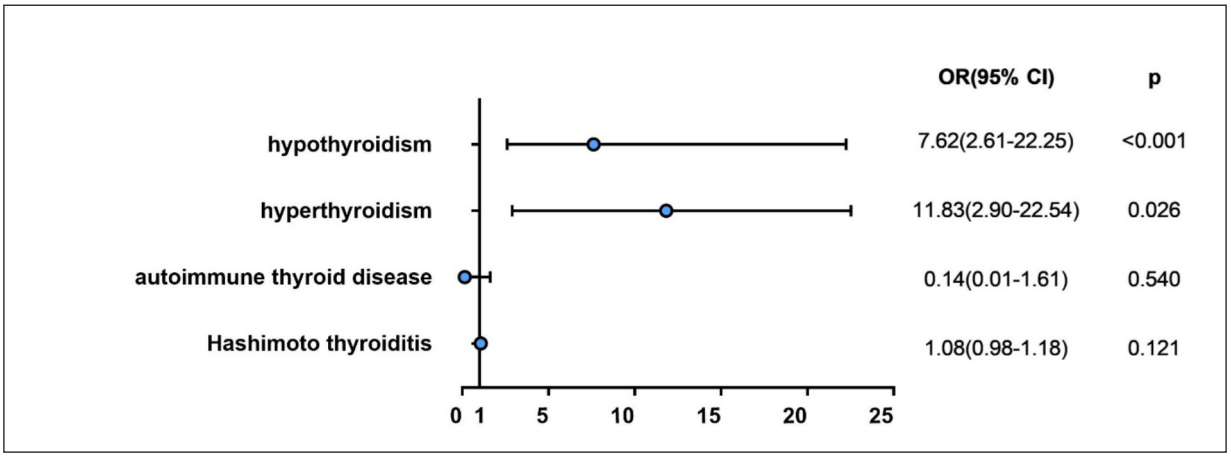

Figure 3. Mendelian randomization analysis of thyroid disease with nonalcoholic fatty liver disease.

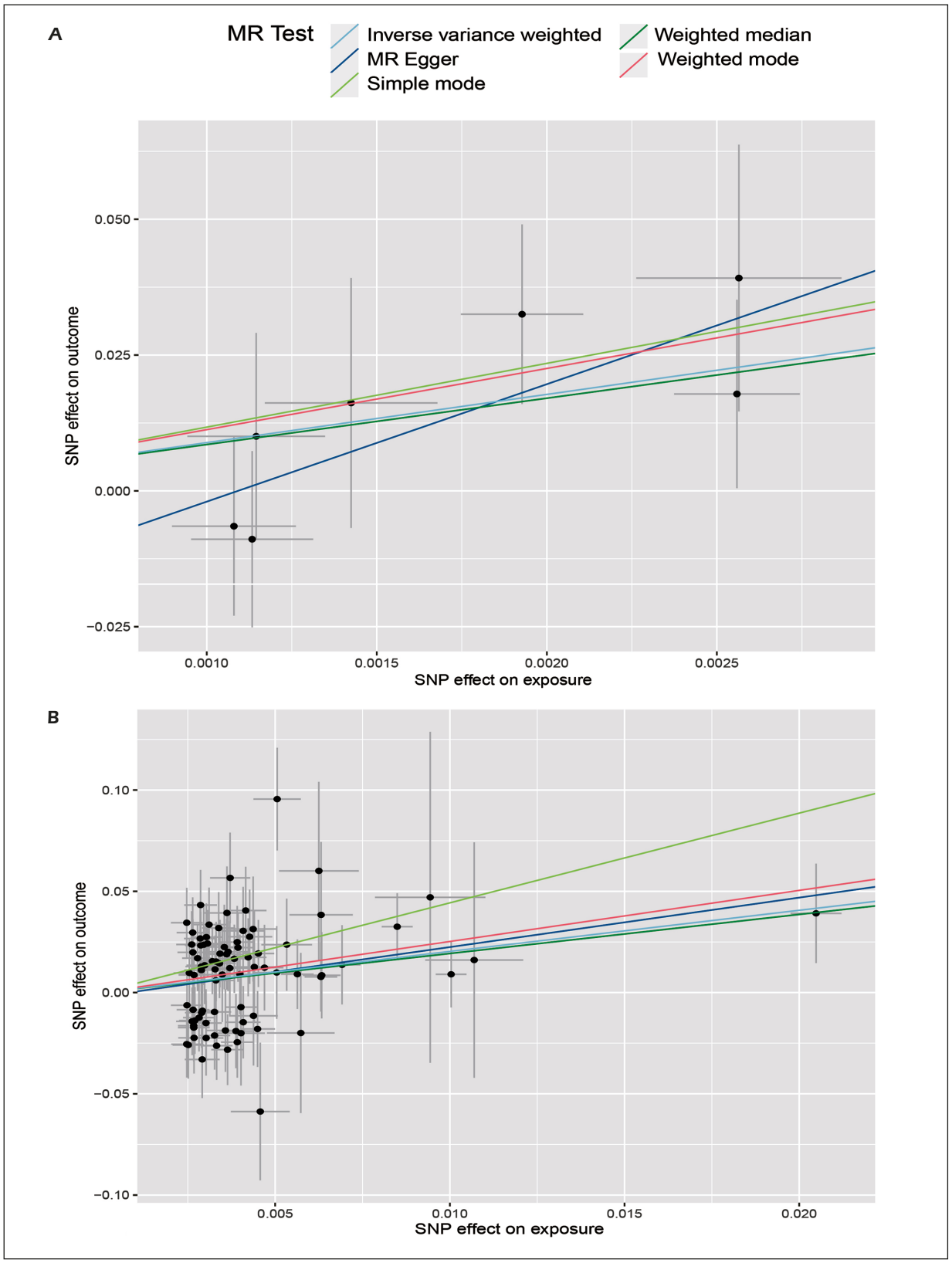

Figure 4. Scatter plot results from Mendelian randomization analysis of hyperthyroidism and hypothyroidism with non-alcoholic fatty liver disease. **A**, Hyperthyroidism; **B**, Hypothyroidism.

sistent positive association between genetically predicted NAFLD and risk of hyperthyroidism, hypothyroidism (**Supplementary Figure 5**). Autoimmune thyroiditis and Hashimoto's thyroiditis were not strongly correlated with outcome indicators (autoimmune thyroiditis: OR=0.14, p=0.540; Hashimoto's thyroiditis: OR=1.08, p=0.121, Figure 3).

Discussion

In this study, we applied a two-sample MR Analysis using data from the GWAS Catalog and demonstrated that hyperthyroidism and hypothyroidism could increase the risk of NAFLD. However, thyroid disease had no strong causal relationship with alcoholic liver disease.

NAFLD has become a serious problem worldwide; however, its complex pathophysiology remains elusive[28]. Metabolic syndromes, such as insulin resistance, dysglycemia, obesity, and dyslipidemia, are associated with NALFD, and thyroid hormones are important regulators of various energy metabolic processes[29,30]. T3 is an important active form of thyroid hormone, and its receptor, thyroid hormone receptor β2 (TRβ2), is enriched in the liver, which is also an important target organ of the thyroid hormone[31]. Thyroid hormones affect hepatic lipid homeostasis *via* various pathways, including stimulating the transport of free fatty acids to the liver, re-esterifying triglycerides, and increasing fatty acid β-oxidation, thereby affecting hepatic fat accumulation[32].

Approximately one-third of NALFD patients have hypothyroidism, which can drive NAFLD occurrence *via* extrahepatic mechanisms[33]. Multivariate regression analysis revealed that hypothyroidism was an independent risk factor for NALFD (OR=1.38, 95% CI: 1.17-1.62), with a dose-response relationship between hypothyroidism and NALFD degree[34]. Low thyroid hormone levels decrease the body's inhibitory effect on lipolysis, induce hepatic insulin resistance, and increase the flow and accumulation of free fatty acids from the adipose tissue to the liver, thereby promoting NAFLD progression[35]. Therefore, we examined the involvement of multiple metabolism-related SNPs. Rs2476601[36] and rs2111485[37] are associated with the occurrence of autoimmune diabetes, while rs3087243[38] can independently reduce the postprandial plasma glucagon-like peptide 1 (GLP-1) concentration, leading to a decreased insulin response. Elevated thyroid-stimulating hormone (TSH) levels stimulate hepatic lipogenesis by stimulating the peroxisome proliferator-activated receptor-α (PPARα) pathway and sterol regulatory element binding transcription factor 1 (SREBP) directly *via* the liver surface TSH receptor[39]. Hypothyroidism also leads to increased circulating inflammatory factor levels, which can increase hepatic inflammatory response and fibrosis progression[40].

Although hypothyroidism is a common risk factor for NAFLD, its prevalence in hyperthyroid patients ranged from 11.97 to 21.5%[41,42]. T3 stimulates lipolysis in adipose tissue, increases serum-free fatty acid levels, and is subsequently used as a substrate for triglyceride synthesis in the liver[43]. Free thyroid hormone levels are also an independent risk factor for central obesity[44]. Additionally, T3 stimulates *de novo* lipogenesis in the liver because an increase in liver fat content is frequently observed in hyperthyroid subjects[45]. In central hyperthyroidism patients, increased levels of TSH also promote the differentiation of preadipocytes into adipocytes, an important step in adipose tissue formation[46]. Hyperthyroidism can lead to oxidative stress *via* β-oxidation and promote liver inflammation and fibrosis progression[47].

Innate and adaptive immunity play important roles in NAFLD progression, and abnormal autoantibody levels are strongly associated with histological damage to the liver[48]. Similarly, thyroid autoantibodies can act on the thyroid gland to cause thyroid damage and extrathyroidal tissues, such as the liver, to cause liver fibrosis progression in autoimmune thyroid diseases[49]. For instance, cross-sectional studies[50] have demonstrated that Thyroid peroxidase antibodies (TPOAb) may be an independent risk factor for progressive liver fibrosis in NALFD patients. The present study did not find an association between autoimmune thyroid disease/Hashimoto's thyroiditis and the risk of NAFLD.

First, this is the first attempt to explore the causal relationship between thyroid disease and fatty liver disease using a two-sample MR analysis with pooled GWAS-level statistics, minimizing potential confounding and reverse causality by aggregating a large amount of genetic data. Second, a sensitivity analysis was performed by applying multiple MR methods with different model assumptions and comprehensively evaluating the effects of outliers and pleiotropy. However, this study had some limitations. First, we used

independent SNPs ($p<5\times10^{8}$) that reached the genome-wide significance level, and F statistics satisfied >10 to avoid weak IVs, thus reducing the validity of the MR Study. The number of SNPs that ultimately lead to autoimmune thyroiditis is small, and future GWAS with larger sample sizes are still needed to find more SNPs. Second, we could not perform a detailed analysis based on the available GWAS data because of a lack of data on subclinical thyroid disease. Third, the results may not apply to the entire population because the study population was limited to Europeans, and the conclusions of the study should be extrapolated with caution.

Conclusions

In conclusion, this MR study provides genetic evidence of a causal relationship between thyroid dysfunction and NAFLD. Our results suggest that hypothyroidism and hyperthyroidism may be the risk factors for NAFLD; however, additional studies are required to support this finding. Therefore, patients with abnormal thyroid function should be aware of the occurrence of NAFLD.

Conflict of Interest
The authors declare that they have no conflict of interests.

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Data Availability
All data used in the analysis are presented in the manuscript. Further inquiries can be directed to the corresponding author.

Ethics Approval
All data were derived from the Genetic Alliance's publicly available compilation of statistical data from genome-wide association studies (GWAS). All original studies received a specific ethical review and informed consent. Mendelian randomization involved analyzing previously published data and did not involve any direct interaction with human subjects or animals. Therefore, no ethical clearance was required.

Informed Consent
Mendelian randomization involved analyzing previously published data and did not involve any direct interaction with human subjects or animals. Therefore, informed consent is not applicable.

ORCID ID
Haifu Zhang: 0000-0003-3654-2226
Qinxia Zhang: 0000-0001-7128-2568
Yonghang Feng: 0009-0005-8077-4771
Shuojun Li: 0009-0008-5557-7786
Biyun Xie: 0009-0001-5508-0306

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Authors' Contribution
Haifu Zhang and Biyun Xie conceived and designed the study, Qinxia Zhang and Yonghang Feng collected data and performed data analysis. Qinxia Zhang and Shuojun Li wrote the draft of this manuscript. Haifu Zhang and Yonghang Feng edited the manuscript.

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