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Genetically predicted hypothyroidism, thyroid hormone treatment, and the risk of cardiovascular diseases: a mendelian randomization study

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Abstract

Background In this study, we explored the impact of hypothyroidism and thyroid hormone replacement therapy on the risk of developing cardiovascular diseases, including myocardial infarction, heart failure, and cardiac death, via Mendelian randomization analysis.

Methods Genetic instrumental variables related to hypothyroidism, levothyroxine treatment (refer to Participants were taking the medication levothyroxine sodium) and adverse cardiovascular events were obtained from a large publicly available genome-wide association study. Two-sample Mendelian randomization analysis was performed via inverse-variance weighting as the primary method. To ensure the reliability of our findings, we performed MR–Egger regression, Cochran's Q statistic, and leave-one-out analysis. Additionally, multivariable Mendelian randomization was employed to regulate confounding factors, including systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), diabetes, cholesterol, low-density lipoprotein (LDL), triglycerides and metformin. A mediation analysis was conducted to assess the mediating effects on the association between exposure and outcome by treating atrial fibrillation and stroke as mediator variables of levothyroxine treatment and bradycardia as mediator variables of hypothyroidism.

Results Genetically predicted hypothyroidism and levothyroxine treatment were significantly associated with the risk of experiencing myocardial infarction [levothyroxine: odds ratio (OR) 3.75, 95% confidence interval (CI): 1.80–7.80; hypothyroidism: OR: 15.11, 95% CI: 2.93–77.88]. Levothyroxine treatment was also significantly related to the risk of experiencing heart failure (OR: 2.16, 95% CI: 1.21–3.88). However, no associations were detected between hypothyroidism and the risk of experiencing heart failure or between hypothyroidism or levothyroxine treatment and the risk of experiencing cardiac death. After adjusting for confounding factors, the results remained stable. Additionally, mediation analysis indicated that atrial fibrillation and stroke may serve as potential mediators in the relationships between levothyroxine treatment and the risk of experiencing heart failure or myocardial infarction.

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Conclusion The results of our study suggest a positive association between hypothyroidism and myocardial infarction and highlight the potential effects of levothyroxine treatment, the main thyroid hormone replacement therapy approach, on increasing the risk of experiencing myocardial infarction and heart failure.

Keywords Levothyroxine, Hypothyroidism, Mendelian randomization, Myocardial infarction, Heart failure

Introduction

Cardiovascular diseases, including heart failure (HF) and myocardial infarction (MI), accounting for approximately 19 million deaths globally, are considered major adverse cardiovascular events and continue to pose a significant public health problem worldwide, affecting millions of individuals and causing substantial health care costs [1, 2]. While several cardiovascular risk factors, such as hypertension, diabetes, and smoking, have already been recognized [3], a more comprehensive understanding of novel risk factors is still needed to address the ongoing impact of cardiovascular diseases on public health. Recent research has highlighted the associations between hormone levels and cardiovascular risk factors. Studies have shown that elevated aldosterone levels are associated with an increased risk of developing cardiovascular diseases such as hypertension and heart failure [4]. Chronic exposure to high cortisol levels can lead to hypertension and endothelial dysfunction [5]. Moreover, a higher plasma aldosterone concentration (PAC) has been linked to an elevated risk of developing cardiovascular diseases [6]. Similarly, thyroid hormones play critical roles in myocardial contraction, heart rate, diastolic function, and systemic vascular resistance [7]. Hypothyroidism is defined as increased thyrotropin (TSH) levels, with free thyroxine or triiodothyronine levels below the reference range [8]. Given the essential role of thyroid hormones in maintaining cardiovascular health, thoroughly investigating the potential impacts of hypothyroidism and its primary treatment, levothyroxine (L-T4), on cardiovascular diseases is essential.

Hypothyroidism caused by thyroid hormone deficiency has been demonstrated to be related to an increased incidence of HF, MI, and cardiovascular mortality in observational studies [7, 9, 10], but the exact causality has not been revealed. The current standard treatment approach for hypothyroidism primarily involves replacement therapy with L-T4. Typically, patients with hypothyroidism need to take L-T4 on a long-term basis [11]. Although long-term L-T4 therapy provides significant benefits for millions of hypothyroidism patients [12], a large retrospective cohort study including 705,307 adults aged 18 years or older, among whom 701,929 initiated thyroid hormone treatment with at least 2 thyrotropin measurements, revealed that patients with hypothyroidism treated with L-T4 had a higher cardiovascular mortality rate due to exogenous hypothyroidism [13]. Another cohort study revealed that the risk of experiencing cardiac death and MI increases when hypothyroidism patients with HF are treated with L-T4 [14]. In contrast, in a small retrospective study of 64 patients admitted due to acute myocardial infarction with concomitant hypothyroidism, patients who did not undergo L-T4 therapy presented an increased 30-day mortality rate and an increased incidence of new or exacerbated HF [15]. A previous observational study also revealed that among all adults with atrial fibrillation, compared with not receiving ($n=11,094$) levothyroxine treatment, receiving levothyroxine treatment ($n=12,283$) was not associated with the risk of experiencing myocardial infarction, ischemic stroke, or congestive heart failure [16]. Nevertheless, these observational studies may be limited by sample size and potential confounding factors, and the exploration of this association is also hindered by challenges in conducting large-scale randomized clinical trials. To address these limitations and gain more insight into the potential causal relationships of hypothyroidism and L-T4 treatment with the risk of developing cardiovascular diseases, we conducted a Mendelian randomization study.

Mendelian randomization is a novel epidemiological approach that uses genetic variations as instrumental variables to analyze the relationship between exposures and outcomes, providing a more robust approach to comprehensively investigating the impact of exposures on outcomes than conventional observational epidemiological techniques do [17]. More importantly, Mendelian randomization can be used to overcome the limitations of confounding factors and reverse causality commonly encountered in observational studies [18, 19], making it a valuable technique for our study. Therefore, we employed Mendelian randomization analysis to explore the correlations between several exposures (hypothyroidism and L-T4 use) and outcomes (major adverse cardiovascular events, including HF, MI, and cardiac death). In addition, multivariable Mendelian randomization (MVMR) analysis was applied to adjust for confounding factors. We acknowledge that multiple causal pathways may exist between the genetic instrumental variables and the outcome, necessitating the use of multivariable analysis to eliminate potential confounding. We selected systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), diabetes, cholesterol, low-density

lipoprotein (LDL), triglycerides, and metformin as confounders based on their impact on both exposure and outcome variables. To control for potential confounding, summary statistics of these variables were utilized in the MVMR analysis. Additionally, we conducted a mediation analysis to examine the potential role of atrial fibrillation (AF) and stroke as mediators of L-T4 treatment, and bradycardia as a mediator of hypothyroidism, as suggested by previous research.

The purpose of this study was to analyze the relationships of hypothyroidism and L-T4 treatment with the risk of experiencing major adverse cardiovascular events, such as HF, MI, and cardiac death. The findings of this study may have significant clinical implications, guiding the development of targeted interventions to improve cardiovascular outcomes in patients with hypothyroidism.

Methods

Study design

In our research, we implemented a two-sample Mendelian randomization approach using summary statistics from various genome-wide association studies (GWASs) for exposure and outcome, enhancing statistical power and reducing bias. We further employed multivariable Mendelian randomization to control for potential pleiotropic effects, allowing us to estimate the causal effect of the exposure on the outcome. Additionally, we conducted mediation analysis to assess the direct and indirect effects of our exposure on the outcome, enabling us to dissect the pathways through which our exposure influences the outcome. First, two-sample Mendelian randomization analysis was employed to assess the relationships of hypothyroidism and L-T4 treatment with the risk of experiencing major adverse cardiovascular events. This Mendelian randomization approach was based on three key assumptions: First, the genetic variants were closely related to exposure, ensuring that there was a systematic difference between the subgroups. If the genetic variant is not strongly associated with the exposure, it is referred to as a weak instrument variable, which reduces the statistical power to detect causal effects. Second, the instrumental variables had no association with any confounding factor, ensuring that the comparison between the genetic subgroups was fair; that is, all other variables were distributed equally between the subgroups. Third, instrumental variables affected the results only through exposure, not through other pathways. This means that the genetic variant is not directly associated with the outcome, nor is there any alternative pathway by which the variant is associated with the outcome other than that through the exposure. One of the fundamental assumptions of MR is the "no horizontal pleiotropy" assumption, which requires that the IVs used for MR analysis affect only the target outcome through the exposure of interest. Horizontal pleiotropy occurs when the variation affects other traits apart from the target exposure pathway and has an impact on the target outcome, leading to inaccurate causal estimates. However, we may not be able to completely avoid horizontal pleiotropy. The inverse-variance weighting method requires that each SNP fully complies with the three principles of MR studies to obtain a correct causal estimate. However, if there are invalid instrumental variables, this method may produce bias. Sensitivity analyses were performed to mitigate this issue, as well as an MVMR analysis. MVMR analysis was subsequently performed to regulate confounding factors to eliminate the influence of confounders and achieve more accurate causality estimates. Finally, we conducted mediation analysis, which is a method of decomposing the direct effect of an exposure on an outcome or its effect through intermediate variables [18]. The mediating effects of AF, stroke, and bradycardia on outcomes were analyzed as potential intermediate factors in the causal pathway. An overview of the research design is presented in Fig. 1.

Data sources

Data on cardiac deaths are available from the FinnGen database, and all other data are available on the IEU website.

Data for exposures and outcomes

In this study, hypothyroidism and L-T4 treatment were selected as the exposures, and the outcomes were major adverse cardiovascular events, including HF, MI, and cardiac death. The genome-wide association study (GWAS) data for both hypothyroidism [20] and L-T4 [20] treatment were derived from the MRC-IEU Consortium, including 9,674/18,947 patients and 453,336/443,986 control participants, which can be searched from the IEU website (hypothyroidismtps://gwas.mrcieu.ac.uk/). The phenotypes for L-T4 treatment is refer to "participants were taking the medication levothyroxine sodium". The phenotypes for hypothyroidism is ICD10: E03.9 refer to "Hypothyroidism, unspecified" indicates that the participant has been diagnosed with "unspecified hypothyroidism".

In addition, genetic variants associated with MI were extracted from the IEU website and included 14,825 patients and 2,680 control participants [21]. Data for HF were derived from the Heart Failure Molecular Epidemiology for Therapeutic Targets [22], including 47,309 patients and 930,014 control participants. Data on cardiac death were obtained from the FinnGen database

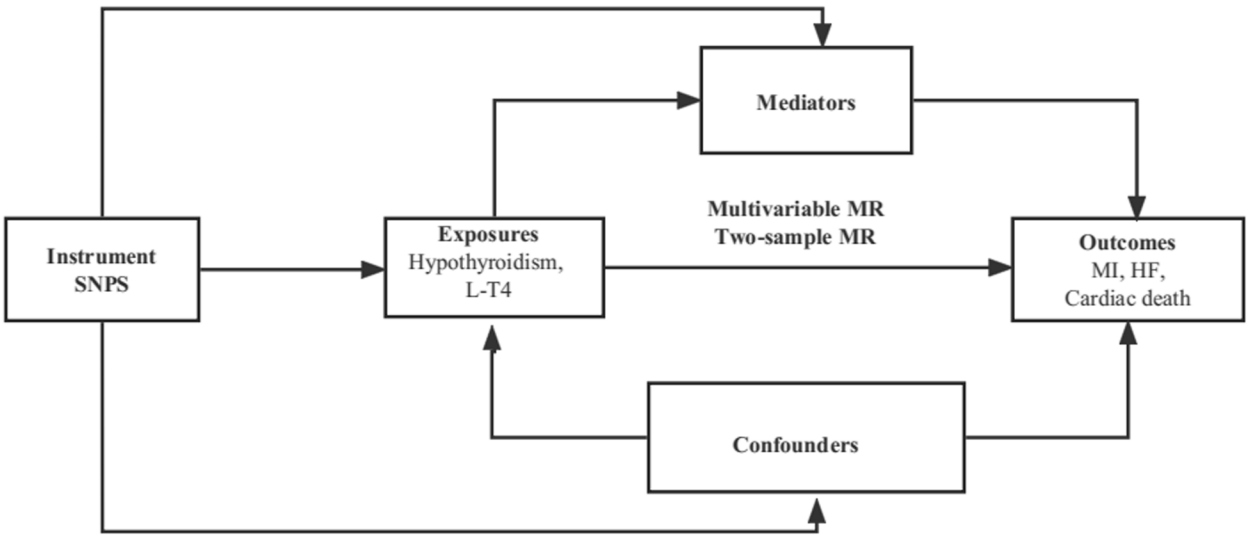

Fig. 1 Study design flowchart of the Mendelian randomization study. This Mendelian randomization approach was based on three assumptions: the genetic variants were closely related to exposure; the instrumental variables had no association with any confounding factor; and the instrumental variables affected the results only by the exposure, not by other pathways. HF, heart failure; L-T4, levothyroxine; MI, myocardial infarction; SNP, single-nucleotide polymorphism

(https://www.finngen.fi/datasets). The details of the data are provided in Table 1.

Data for confounders and mediators

Genetic variants associated with SBP, DBP, metformin, diabetes, triglycerides, LDL and BMI were identified from publicly available genome-wide association studies. The data for SBP and DBP were obtained from the International Consortium of Blood Pressure [23]. The data for metformin [20] were derived from MRC-IEU. The summary data for BMI [20], triglycerides [24] and LDL [25] were obtained from the UK Biobank. The data for cholesterol [26] and diabetes [27] were derived from the IEU website (hypothyroidismtps://gwas.mrcieu.ac.uk/). Our GWAS summary statistics for AF, stroke were obtained from the FinnGen project, which can be accessed at https://gwas.mrcieu.ac,uk/datasets .The bradycardia [20] data were obtained from the MRC-IEU Consortium. Details on these data sources are presented in Table 1.

Instrumental variable selection

The appropriate SNPs were selected as instrumental variables through the following steps. We selected SNPs strongly associated with hypothyroidism and L-T4 treatment. The genetic instrumental variable selection process was as follows. First, we identified SNPs that were significantly associated ($p < 5 \times 10^{-8}$) with both hypothyroidism and L-T4 treatment. Second, linkage disequilibrium analysis was performed to retain SNPs with the strongest associations. These SNPs were independent by pruning SNPs within a 10,000 kb window with a threshold of $r^2 < 0.001$. We then harmonized the exposure and outcome datasets to ensure that the β values corresponded to the same alleles and removed palindromic SNPs. Following the above steps, we identified a total of 36 SNPs that exhibited a strong association with hypothyroidism. Similarly, we selected 79 SNPs that were associated with L-T4. Finally, MR–PRESSO and RadialMR were used to remove outliers. After removing outliers, there were 27 remaining SNPs associated with hypothyroidism. For the MR analysis between L-T4 and MI, 70 SNPs associated with L-T4 remained. When conducting the MR analysis between L-T4 and HF, 77 SNPs associated with L-T4 remained (See Supplementary Material 1 for details). For the screened SNPs, we used F statistics to evaluate the strength of the IVs to avoid weak-tool bias.

Two-sample mendelian randomization

Mendelian randomization is based on the principle that genetic variants utilize genetic variants as instrumental variables to investigate the causal relationship between an exposure and an outcome, which are less likely to be influenced by confounding factors or reverse causation than traditional observational studies are. In this study, we used three Mendelian randomization analysis methods, including inverse-variance weighting, weighted median, and MR–Egger, to assess the causal effects of hypothyroidism and LT4 treatment on the risk of experiencing major adverse cardiovascular events via two-sample data from genome-wide association studies. Among

Table 1 Data source of exposures, outcomes, confounding factors and mediators

Trait	Consortium	Sample Size	Population	Participants
Exposures				
hypothyroidism [20]	MRC-IEU	463,010	European	9,674 cases 453,336 ncontrols
L-T4 [20]	MRC-IEU	462,933		18,947 cases 443,986 ncontrols
Outcomes				
MI [21]	IEU-Website	395,795	European	14,825 cases 2,680 ncontrols
HF [22]	HERMES	977,323		47,309 cases 930,014 ncontrols
cardiac death	Finngen	--		17,793 cases 324,706 controls
Confunders				
SBP [23]	ICBP	757,601	European	--
DBP [23]	ICBP	757,601		--
Metformin [20]	MRC-IEU	462,933		11,552 ncases
BMI [20]	MRC-IEU	461,460		451,381 ncontrols --
diabetes [24]	IEU	655,666		61,714 ncases 1,178 ncontrols
cholesterol [25]	UK Biobank	437,878		
LDL [26]	IEU-Website	343,621		
triglycerides [27]	UK Biobank	441,016		
Mediators				
AF	FinnGen	---	European	10,516 ncases 116,926 ncontrols
Stroke	FinnGen	--		18,661 ncases 162,201 ncontrols
Bradycardia [20]	MRC-IEU	463,010		1,254 ncases 46,1756 ncontrols

them, inverse-variance weighting was used as the primary method. By employing a meta-analytic approach to combine Wald estimates for each instrumental variable, the inverse-variance weighting method could be used to provide a relatively stable and accurate causal evaluation [28, 29]. When there were more than 3 genetic instrumental variables, a random-effects model was used for analysis; otherwise, a fixed-effects model was adopted. The inverse-variance weighting method requires that the SNP fully complies with the three principles of MR studies to obtain a correct causal estimate [30]. However, if there are invalid instrumental variables, this method may produce bias. Additional analyses, including the weighted median and MR–Egger methods, were conducted as supplemental analyses. When instrumental variables (IVs) account for more than 50% of the weight, the weighted median method is still able to generate robust results. In the presence of horizontal pleiotropy, the weighted median method can be used to provide a more precise evaluation of causality [31]. MR–Egger regression analysis was used to identify and correct for directional pleiotropy. The MR–Egger regression model takes into account the presence of an intercept. The intercept of the model reveals the presence or absence of horizontal pleiotropy (a P value < 0.05 was considered to indicate the presence of horizontal pleiotropy) [29]. Together, this multimethod approach facilitated a comprehensive evaluation of the causal relationships of hypothyroidism and L-T4 treatment with the risk of experiencing major adverse cardiovascular events.

Sensitivity analyses

In this study, we conducted sensitivity analyses through multiple methods. First, Cochran's Q statistic was used to evaluate the heterogeneity among single-nucleotide polymorphisms (SNPs). If the p value exceeded 0.05, indicating the absence of heterogeneity, the fixed-effects inverse-variance weighting method was chosen as the primary approach. In contrast, a random-effects model was used when heterogeneity was detected. Second,

MR–Egger was employed as a crucial sensitivity analysis tool in Mendelian randomization to examine pleiotropy [32]. If the *p* value was less than 0.05, the inverse-variance weighting estimate would be suggested to be biased. A leave-one-out sensitivity analysis was subsequently applied to examine whether the results were driven by individual SNPs. Funnel plots were generated to assess the presence of heterogeneity directly. In addition, the MR–PRESSO global test can be used to evaluate horizontal pleiotropy and correct for outliers [33]. Through the incorporation of multiple complementary sensitivity analyses, a comprehensive assessment of the robustness of the results and reliability of the conclusions was performed. Finally, we conducted colocalization analysis via the commonly applied Bayesian model to investigate whether levothyroxine treatment, hypothyroidism and cardiovascular disease share a common causal variant in a given region. The colocalization analyses tests the following 4 hypotheses [34]: H0: Phenotype 1 (GWAS) and Phenotype 2 (e.g., eQTL) are not significantly associated with any SNP variants in a particular genomic region. H1/H2: Phenotype 1 (GWAS) or Phenotype 2 (e.g., eQTL) is significantly associated with SNP variants in a particular genomic region. H3: Phenotype 1 (GWAS) and Phenotype 2 (e.g., eQTL) are significantly associated with SNP variants in a particular genomic region, but driven by different causal variants. H4: Phenotype 1 (GWAS) and Phenotype 2 (e.g., eQTL) are significantly associated with SNP variants in a particular genomic region, and the association is driven by the same causal variant. For exposure gene loci in which there was evidence to support a causal relationship with cardiovascular disease onset ($P<0.05$), variables within 10 kilobases of the corresponding instrumental single-nucleotide polymorphisms were extracted and used to calculate the posterior probability (posterior probability). As a convention, a posterior probability H4 of 0.80 or higher was considered evidence of colocalization [35, 36]. However, If the disease GWAS and another GWAS trait share an associated variant, it suggests pleiotropy, where the shared variant is responsible for the association of the two traits. While most papers focus on H4, we can also use H1 to demonstrate the point. This is not about the co-localization of genes, but rather the co-localization between the two GWAS. Therefore, in the colocalization analyses between GWAS traits, the focus is on whether the estimate for H4 is close to 0, as this would indicate the two traits do not share a causal variant and are not influenced by pleiotropy. The results of the colocalization analysis are shown in Table 2. The funnel plot and leave-one-out analysis diagram can be found in Figs. 2 and 3, respectively.

MVMR and mediation analysis

In this study, we further performed MVMR and mediation analysis, as shown in Fig. 2. Although MR analysis can help overcome confounding issues in traditional observational studies, multivariable analysis is still needed to eliminate such confounding when there are

Table 2 The results of colocalization

Trait 1	Trait 2	H_0	H_1	H_2	H_3	H_4
L-T4	MI	< 0.01	0.88	< 0.01	0.12	< 0.01
L-T4	HF	< 0.01	0.99	< 0.01	< 0.01	< 0.01
Hypothyroidism	MI	< 0.01	0.97	< 0.01	0.02	0.01

L-T4, levothyroxine

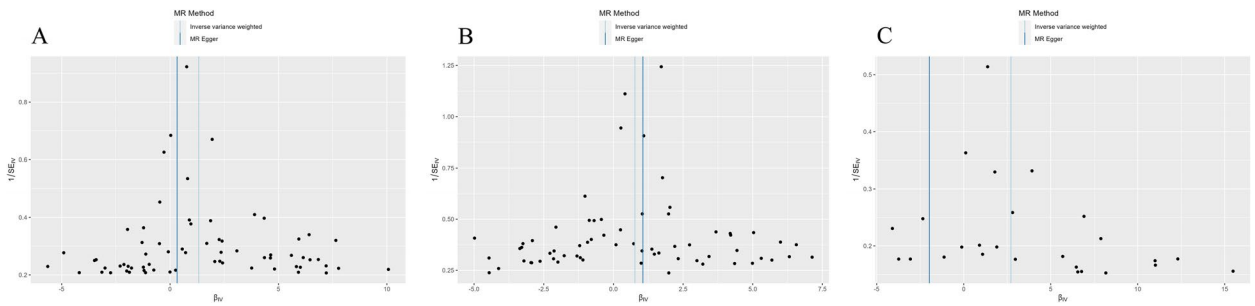

Fig. 2 **A** Funnel plot of the association between levothyroxine treatment and the risk of experiencing myocardial infarction; **B**, funnel plot of the association between levothyroxine treatment and the risk of experiencing heart failure; **C**, funnel plot of the association between hypothyroidism and the risk of experiencing myocardial infarction

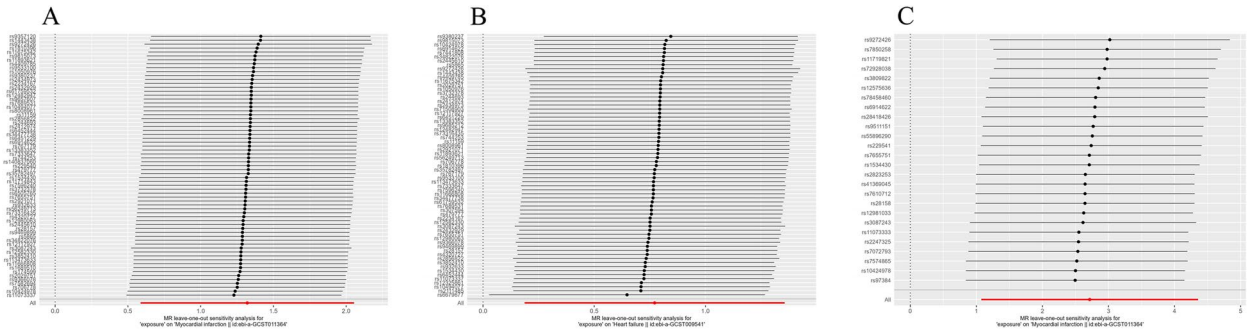

Fig. 3 **A**, Leave-one-out analysis of the association between levothyroxine treatment and the risk of experiencing myocardial infarction; **B**, leave-one-out analysis of the association between levothyroxine treatment and the risk of experiencing heart failure; **C**, leave-one-out analysis of the association between hypothyroidism and the risk of experiencing myocardial infarction

multiple causal pathways between the genetic instrumental variables and the outcome. We chose systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), diabetes, cholesterol, low-density lipoprotein (LDL), and triglycerides and metformin as confounders on the basis of their impact on both exposure and outcome. According to previous observational research, hypertension and body mass index (BMI) are associated with an increased risk of developing cardiovascular diseases. Both diastolic blood pressure (DBP) and SBP) exhibit a graded independent relationship with mortality and incidence rates [37–39], while metformin has been shown to reduce the occurrence of cardiovascular diseases [40, 41]. Previous studies have also demonstrated that SBP, DBP, and BMI are clearly associated with hypothyroidism and L-T4 treatment [42–44]. Moreover, a meta-analysis revealed that metformin treatment can lower TSH levels in patients with hypothyroidism, indicating that metformin has a direct effect on thyroid function [45]. In addition, some studies have shown that diabetes and dyslipidemia may also have an impact on hypothyroidism and treatment outcomes [46–48]. To control for potential confounding factors, summary statistics of BMI, DBP, SBP, diabetes, cholesterol, LDL, triglyceride and metformin treatment were utilized in the MVMR analysis. Moreover, previous studies have shown that the intensity of thyroid hormone replacement therapy is a modifiable risk factor for atrial fibrillation (AF) and stroke [49]. Hypothyroidism is often associated with bradycardia [50]. Consequently, we further conducted a mediation analysis to examine the potential role of mediator variables on the final outcomes by treating AF and stroke as mediators of L-T4 treatment and bradycardia as a mediator of thyroid hormone. The mediation percentage was obtained by calculating the ratio of the indirect effect to the total effect, thereby quantifying the magnitude of the mediation effect. For the mediation analysis, we first estimated the causal effect of the genetically determined exposure on the mediator (β1), followed by estimating the causal effect of the mediator (β2) on the outcome. The proportion of the total effect of exposure on the outcome mediated by the mediators was then calculated by taking the indirect effect, obtained by multiplying the results from the two previous steps (β1 × β2 pooled) and dividing it by the total effect [51]. This approach allowed us to estimate the proportion of the total effect of the exposure on the outcome that was mediated through AF, stroke, and bradycardia.

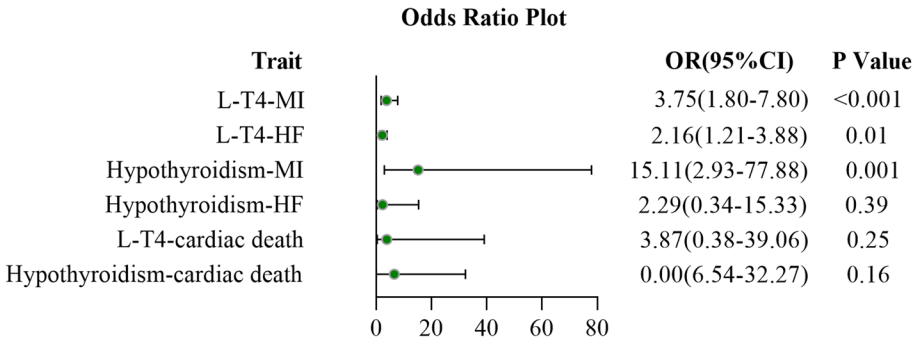

Fig. 4 Mendelian randomization analyses of the associations of L-T4 treatment and hypothyroidism with the risk of experiencing MI and HF. CI, confidence interval; HF, heart failure; L-T4, levothyroxine; MI, myocardial infarction; OR, odds ratio; SNPs, single-nucleotide polymorphisms

Mendelian randomization analyses were performed via R software (version 4.2.2, R) with the "TwoSampleMR" (version 0.5.6), "MR–PRESSO" (version 1.0.0) and "RadialMR" (version 1.0) packages.

Results

Associations of hypothyroidism and L-T4 treatment with the risk of experiencing major adverse cardiovascular events

By performing a series of instrumental variable selection procedures, we identified SNPs as instrument variables for hypothyroidism and L-T4 treatment. These genetic instruments were utilized to perform a two-sample Mendelian randomization analysis. Our study revealed that genetically predicted hypothyroidism and L-T4 may increase the risk of experiencing MI. Specifically, inverse-variance weighting analyses suggested that a one-standard-deviation increase in the genetically predicted risk of hypothyroidism and L-T4 treatment, there was an increased risk of experiencing MI [hypothyroidism: odds ratio (OR) = 15.11, 95% confidence interval (CI): 2.93–77.88; L-T4: OR = 3.75, 95% CI: 1.80–7.80]. In addition, genetically predicted L-T4 treatment was significantly associated with an increased risk of experiencing HF (OR = 2.16, 95% CI: 1.21–3.88). However, there was no significant association between hypothyroidism ($OR = 1.45 \times 10^{-4}$, 95% CI: 6.54×10^{-10}-32.27) or L-T4 treatment (OR = 3.87, 95% CI: 0.38–39.06) and the risk of experiencing cardiac death. Similarly, genetically predicted hypothyroidism had no causal association with the risk of experiencing HF (OR = 2.29, 95% CI: 0.34–15.33). The results are shown in Fig. 4.

Sensitivity analyses of mendelian randomization

First, Cochran's Q statistic was employed in the heterogeneity test to monitor variations within the data. The results indicated no heterogeneity between hypothyroidism, L-T4, and the outcomes ($p > 0.05$). Second, the MR–Egger regression intercept suggested the absence of pleiotropy. Finally, we carried out MR–PRESSO and Radial methods to test for horizontal pleiotropy and identify several outlier SNPs in the instrumental variables associated with hypothyroidism and L-T4 treatment. After removing outlier SNPs, we re-performed MR analysis (The detailed results were shown in Fig. 4). Furthermore, we used the leave-one-out method to examine the influence of individual SNPs, which suggested that the causal relationship between exposure and outcome was not dependent on any single SNP. Diverse sensitivity analyses supported the robustness and validity of this study. The details of

Table 4 The associations of genetically predicted L-T4 and hypothyroidism with MI or HF

Exposure	Trait of adjustment	OR	*P* value
L-T4-MI	BMI	7.32 (2.90-18.44)	<0.01
	DBP	17.09 (4.69-62.25)	<0.01
	SBP	13.53 (4.12-44.46)	<0.01
	Metformin	7.14 (2.70-18.89)	<0.01
	Diabetes	13.47 (3.15-57.73)	<0.01
	Cholesterol	12.31 (3.91-38.74)	<0.01
	LDL	9.55 (1.85-49.33)	<0.01
	triglycerides	8.94 (3.17-25.24)	<0.01
Hypothyroidism-MI	BMI	7.32 (2.90-18.44)	<0.01
	DBP	1.07 (1.00-1.15)	0.046
	SBP	1.15 (1.08-1.22)	<0.01
	Metformin	1.10 (1.04-1.17)	0.002
L-T4-HF	BMI	2.20 (1.11-4.39)	0.024
	Diabetes	62.64 (2.59-1512.67)	0.011
	Cholesterol	41.00 (2.17-774.38))	0.01
	LDL	75.13 (1.90-2973.40)	0.02
	triglycerides	64.02 (7.65-535.69)	<0.01
	DBP	2.74 (1.19-6.28)	0.017
	SBP	3.26 (1.43-7.43)	<0.01
	Metformin	2.64 (1.29-5.39)	0.007
	Diabetes	3.91 (1.69-9.06)	<0.01
	Cholesterol	3.91 (38.74-12.31)	<0.01
	LDL	2.85 (1.15-7.03))	0.02
	triglycerides	2.55 (1.17-5.56)	0.02

BMI Body mass index, *DBP* Diastolic blood pressure, *L-T4* Levothyroxine, *OR* Odds ratio, *SBP* Systolic blood pressure, *LDL* Low-density lipoprotein

Table 3 Pleiotropy and heterogeneity test

Outcomes/Exposures	Pleiotropy test			Heterogeneity test					
	MR-Egger			MR-Egger			Inverse variance weighting		
	Intercept	SE	*P*	Cochran'sQ	Q_df	Q_*pval*	Q	Q_df	Q_*pval*
MI/L-T4	0.004	0.004	0.27	57.16	65	0.75	58.38	66	0.74
MI/hypothyroidism	0.014	0.007	0.05	20.10	24	0.70	24.29	25	0.50
HF/L-T4	-0.001	0.003	0.65	75.61	63	0.13	75.86	64	0.15

df Degree of freedom, *HF* Heart failure, *L-T4* Levothyroxine, *MI* Myocardial infarction, *Q* Heterogeneity statistic Q

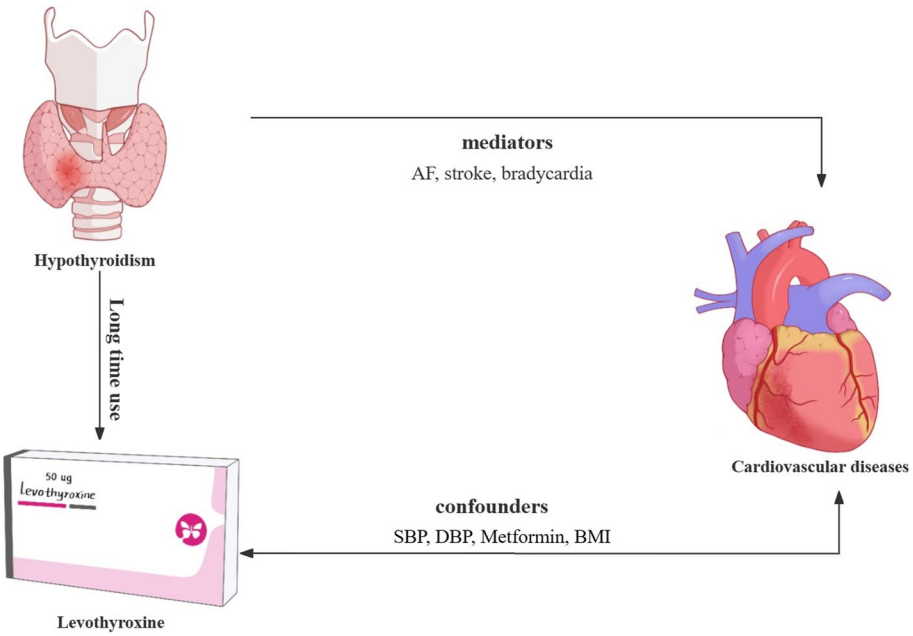

Fig. 5 Flow chart of MVMR and mediation analysis. AF, atrial fibrillation; BMI, body mass index; DBP, diastolic blood pressure; MI, myocardial infarction; SBP, systolic blood pressure

the pleiotropy and heterogeneity tests are provided in Table 3.

Adjusting for confounders and the mediation analysis results

The MVMR method was used to regulate confounding factors. After adjustment for confounders, L-T4 treatment remained significantly associated with the risk of experiencing HF and MI. Additionally, hypothyroidism remained significantly associated with the risk of experiencing MI. The MVMR results are shown in Table 4. First, we evaluated the mediating effects of AF and stroke on the relationships of L-T4 treatment with the risk of experiencing MI and HF. The mediating effect of AF on the relationship between L-T4 treatment and the risk of experiencing MI accounted for 3.8%, whereas the mediating effects of AF and stroke on the association between L-T4 treatment and the risk of experiencing HF accounted for 20.3% and 20.1%, respectively. Notably, stroke was demonstrated to have no mediating effect on the relationship between L-T4 treatment and the risk of experiencing MI. A mediation analysis was subsequently conducted to evaluate the potential mediating effect of bradycardia on the association between hypothyroidism and the risk of experiencing MI. However, the results did not indicate a mediating effect of bradycardia on the relationship between hypothyroidism and the risk of experiencing MI. Details regarding the mediating effects data are shown in Figs. 5, 6 and 7.

Discussion

In this study, we assessed the causal associations of hypothyroidism and L-T4 treatment with the risk of experiencing HF, MI, and cardiac death. Both hypothyroidism and the use of L-T4 were revealed to increase the risk of experiencing MI. In addition, L-T4 treatment was also suggested to be positively associated with the risk of experiencing HF, whereas no significant correlation was found between hypothyroidism and the risk of experiencing HF. For cardiac death, no substantiated evidence was observed to indicate a connection between L-T4 usage or hypothyroidism and the risk of experiencing cardiac death. The robustness of our Mendelian randomization results was maintained even after adjusting for confounding factors via MVMR, suggesting their reliability. Furthermore, mediation analysis indicated that the impact of L-T4 treatment on the risk of experiencing HF or MI may be mediated through AF or stroke.

A meta-analysis of 55 cohort studies involving 1,898,314 participants revealed that compared with euthyroidism, hypothyroidism was significantly associated with an increased risk of developing ischemic heart disease, MI, and cardiac mortality [10]. However, our Mendelian randomization study demonstrated a causal relationship between hypothyroidism and an increased risk of experiencing MI, but we did not detect significant associations between hypothyroidism and the risk of experiencing HF or cardiac death. Owing to the intrinsic susceptibility of traditional

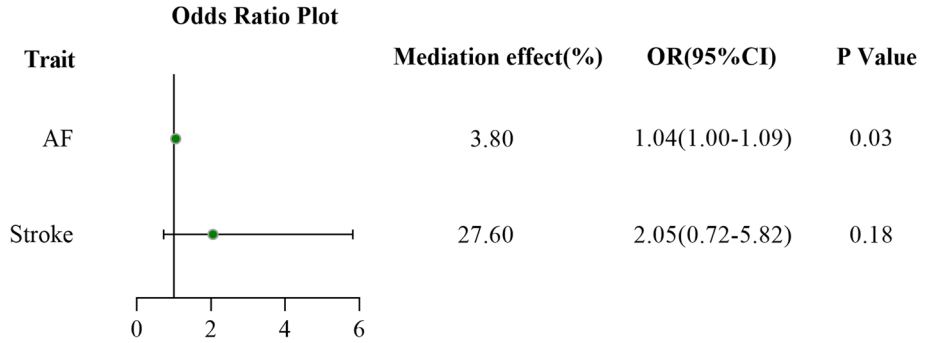

Fig. 6 Mediating effects on the association between L-T4 treatment and the risk of experiencing MI. AF, atrial fibrillation; L-T4, levothyroxine; MI, myocardial infarction

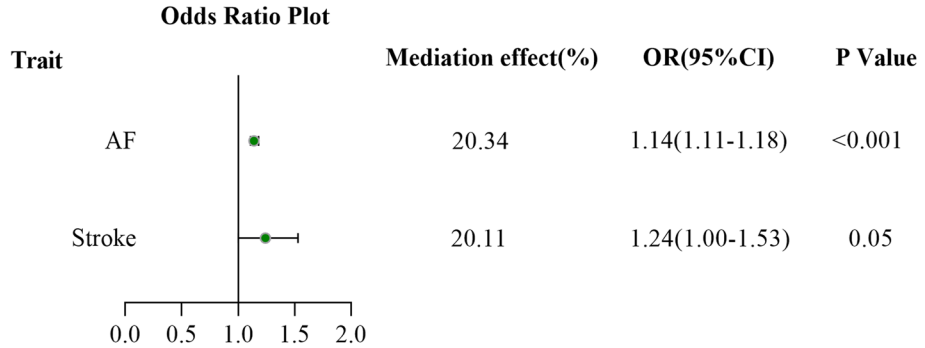

Fig. 7 Mediating effects on the association between L-T4 treatment and the risk of experiencing HF. AF, atrial fibrillation; HF, heart failure; L-T4, levothyroxine

studies to the effects of confounding factors, the causal relationship between hypothyroidism and the risk of experiencing HF and cardiac death merits further investigation in large, well-designed studies. A previous observational study also revealed an increased risk of experiencing MI during continuous L-T4 treatment [14], which was consistent with our Mendelian randomization result. However, other studies reported a lower mortality rate within 30 days and a decreased rate of onset or exacerbation of HF, which was not statistically significant in patients receiving L-T4 therapy compared with those who did not receive L-T4 therapy [15], possibly due to the limitations of the retrospective design and small sample size of the study. Additionally, only 44% of patients admitted with acute myocardial infarction underwent thyroid evaluation, leading to a highly selective population. This provided unstable estimates and reduced our ability to detect differences between groups (and subgroups) and to adequately adjust for confounding factors. In another study, among patients with atrial fibrillation, those receiving L-T4 treatment had no associated risk of experiencing MI, ischemic stroke, or congestive heart failure compared with those not receiving treatment [16]. However, the limited number of patients included in that study, along with the inherent biases of observational studies, restricts the conclusions that can be drawn. Furthermore, it was not possible to ascertain whether L-T4 treatment was used on a long-term basis, although in most cases, such treatment is lifelong. Conversely, a study focusing on women with primary hypothyroidism indicated that levothyroxine replacement therapy led to improvements in insulin resistance, endothelial dysfunction, and markers of atherosclerosis risk [52]. These findings suggest that the relationships of hypothyroidism and levothyroxine treatment with cardiovascular risk may be complex. However, that study was limited by its relatively small sample size and its focus on female patients, and due to ethical considerations, it is not possible to conduct a placebo-controlled prospective study on newly diagnosed and untreated hypothyroid patients. By addressing the limitations of existing studies and investigating the underlying mechanisms, Mendelian randomization analysis could complement the results observed in previous studies and may help explain inconsistent findings in prior observational research. However, Mendelian randomization also has inevitable

limitations and potential biases. Further validation of our results through randomized controlled trials and other studies is still needed.

Understanding the specific functions and underlying mechanisms of hypothyroidism and L-T4 in the pathogenesis of cardiovascular diseases may offer novel perspectives for the prevention and management of cardiovascular conditions. Through a literature review and our findings, we identified several potential mechanisms. Hypothyroidism can have various effects on myocardial infarction through multiple mechanisms. Primarily, thyroid dysfunction elicits alterations in cardiac output, myocardial contractility, blood pressure, and vascular resistance, consequently augmenting susceptibility to MI. Furthermore, it induces conditions of oxidative stress while reducing antioxidant capacity, resulting in endothelial dysfunction and promoting atherosclerosis. Additionally, it also provokes elevated total cholesterol and low-density lipoprotein cholesterol levels, which exacerbates the risk of experiencing myocardial infarction [53, 54]. However, some previous observational studies have also demonstrated that clinically overt hypothyroidism leads to a hypercoagulable state and increased bleeding risk [55, 56], which seems contradictory to the mechanism associated with hypothyroidism and increased myocardial infarction risk. Apart from possible confounding factors and other biases existing in observational studies, this contradiction may also be attributed to hypothyroidism increasing cardiovascular risk through alternate mechanisms, such as its effects on lipid metabolism and cardiac output, rather than solely through coagulation.

In previous studies, approximately 50% of hypothyroid patients failed to monitor their TSH levels in real time and receive appropriate L-T4 therapy compatible with current health conditions, thus developing exogenous hyperthyroidism or hypothyroidism during L-T4 therapy and subsequently leading to an increased incidence of cardiovascular adverse events [13, 53, 54]. A study revealed that thyroid cancer patients had an elevated risk for CHD among those who took a relatively high dosage of levothyroxine [57]. Therefore, unstable TSH levels arising from improper doses of L-T4 therapy could contribute to the increased risk of experiencing MI and HF in patients receiving L-T4 therapy. In particular, individuals with congenital hypothyroidism who receive long-term L-T4 replacement therapy experience early vascular alterations, specifically endothelial dysfunction and impaired arterial vasodilation. Inadequate L-T4 replacement therapy leading to recurrent elevations in TSH levels has been identified as a predictive factor influencing vascular endothelial function [58]. Studies have shown that in patients with Hashimoto's thyroiditis receiving levothyroxine treatment, the pulsatility index of their carotid arteries is increased, leading to decreased vascular compliance and increased arterial tension. This is an early manifestation of atherosclerosis, indicating an elevated risk of developing cardiovascular disease [59]. Moreover, during L-T4 therapy, excessive utilization of L-T4 beyond the physiological dosage may increase coagulation factor levels, inhibit fibrinolysis, and increase the risk of thrombosis through various mechanisms [60, 61]. Moreover, our mediation analysis suggested that the impact of L-T4 treatment on the risk of experiencing HF or MI may be mediated through AF or stroke. The findings of our study provide valuable insights into the plausible underlying mechanisms linking thyroid function, L-T4 use, and cardiovascular diseases. On the one hand, when dealing with patients with hypothyroidism in the clinic, it is imperative to emphasize cardiovascular function, in addition to thyroid function. On the other hand, real-time monitoring of thyroid hormone levels and cardiovascular is highly valuable when L-T4 is administered for routine replacement therapy in patients with hypothyroidism.

Our study has several strengths. First, this Mendelian randomization analysis was conducted using a large and publicly available genome-wide association study dataset to comprehensively investigate the potential relationships of hypothyroidism and L-T4 treatment with the risk of developing cardiovascular disease, thereby partially avoiding traditional confounding factors, an insufficient sample size, and inverse causality. Second, MVMR methods were used to adjust for confounding factors, demonstrating the reliability of our results. Finally, we applied mediation analysis and conducted sensitivity and heterogeneity analyses to confirm that the results were unaffected by bias.

First, regarding potential biases, although we conducted multiple sensitivity analyses, it is unlikely that there are substantial biases in our causal estimates. However, caution should be exercised when interpreting causal relationships. We recognize that although we have tried our best to minimize bias, it was impossible to completely avoid bias in all statistical models, as some assumptions of the method itself are unstable and cannot entirely rule out the influence of confounding factors. Second, in our study, we only demonstrated the long-term effects of L-T4 treatment on the risk of experiencing cardiovascular events, and we did not investigate the short-term treatment effects. Therefore, in the future, we will conduct further experiments to fill these research gaps as much as possible. Third, although this study involved multivariable Mendelian and mediation analyses, we did not include all possible confounding factors and did not cover all potential mediation pathways. We will make efforts to identify and incorporate as many

relevant confounding factors as possible on the basis of previous studies and clinical knowledge. The population in this study included only individuals of European ancestry, which may limit the generalizability of our findings to other populations. Therefore, we should further extend the study subjects to other regions. Fourth, we choosed only E03.9 for hypothyroidism may introduce selection bias and limit the generalizability of our results. Additionally, in our study, the exposure variables associated with hypothyroidism and L-T4 treatment were not stratified by age, which may lead to different impacts of thyroid hormone replacement therapy on the risk of experiencing major adverse cardiovascular events in hypothyroid patients of different ages. What is more, Large observational studies have shown that among participants taking L-T4, exogenous hypothyroidism and hyperthyroidism are associated with cardiovascular disease (CVD). Therefore, it is important to consider exogenous hypothyroidism and hyperthyroidism as potential mediators for L-T4's effect on CVD. However, due to the lack of available databases on exogenous hypothyroidism and hyperthyroidism, we were unable to assess these conditions as mediators in our study. Fifth, our colocalization analysis yielded negative results, suggesting an absence of pleiotropy. However, it was important to note that the inability to detect colocalization could result from several factors, such as a lack of statistical power, which often occured when the sample size was too small or the effect size was too weak, the presence of real LD bias among genetic variants, or the methodological requirement for a strongly associated genetic variant for each trait within the given genetic region. Finally, one of the fundamental assumptions of MR is the "no horizontal pleiotropy" assumption, which requires that the IVs used for MR analysis affect only the target outcome through the exposure of interest. However, we may not be able to completely avoid horizontal pleiotropy. If invalid instrumental variables are present, the use of the IVW method may result in bias. Therefore, we conducted a sensitivity analysis to ensure the robustness of the results as much as possible. For these reasons, the general applicability and external validity of the results of our study need further improvement, and caution should be taken in interpreting the results.

In conclusion, this study suggests a positive association between hypothyroidism and the risk of experiencing MI and highlights the potential risks of experiencing MI and HF associated with the use of L-T4. These findings suggest that the management of hypothyroid patients may involve a comprehensive consideration of the impact of both the disease and its treatment on cardiovascular health. When hypothyroid patients are treated with L-T4, close monitoring of cardiovascular adverse events is essential. Additionally, regular monitoring of thyroid hormone levels is crucial to prevent the occurrence of exogenous hyperthyroidism or hypothyroidism caused by L-T4 treatment, which may lead to HF or MI. Furthermore, our mediation analysis indicates that the risk of HF or MI associated with L-T4 treatment is partially mediated by AF and stroke. Therefore, it is important to actively manage risk factors for AF and stroke in hypothyroid patients. Moreover, comprehending the mechanisms involved in the relationships of hypothyroidism and L-T4 usage with the risk of developing cardiovascular diseases may lead to the development of personalized treatment approaches for individuals with hypothyroidism and related cardiovascular risks. Further research should be aimed at reducing the incidence of major adverse cardiovascular events in hypothyroid patients undergoing L-T4 therapy.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
All authors made a significant contribution to this study, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas. LJC and XB conceived and designed the experiments. YHT, ZY, GAW, CSC, GCJ and ZSD performed and analysed the experiments. ZSD wrote the manuscript. LJC and all other authors read and approved the final manuscript.

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Availability of data and materials
The datasets are available on the IEU website (hypothyroidismtps://gwas.mrcieu.ac.uk/).

Data availability
The datasets are available in the IEU website (hypothyroidismtps://gwas.mrcieu.ac.uk/) or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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