Thyroid Function Affects the Risk of Stroke via Atrial Fibrillation: A Mendelian Randomization Study

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Context: Observational studies suggest that variations in normal range thyroid function are associated with cardiovascular diseases. However, it remains to be determined whether these associations are causal or not.

Objective: To test whether genetically determined variation in normal range thyroid function is causally associated with the risk of stroke and coronary artery disease (CAD) and investigate via which pathways these relations may be mediated.

Design, Setting, and Participants: Mendelian randomization analyses for stroke and CAD using genetic instruments associated with normal range thyrotropin (TSH) and free thyroxine levels or Hashimoto's thyroiditis and Graves' disease. The potential mediating role of known stroke and CAD risk factors was examined. Publicly available summary statistics data were used.

Main Outcome Measures: Stroke or CAD risk per genetically predicted increase in TSH or FT4 levels.

Results: A 1 standard deviation increase in TSH was associated with a 5% decrease in the risk of stroke (odds ratio [OR], 0.95; 95% confidence interval [CI], 0.91-0.99; $P = 0.008$). Multivariable MR analyses indicated that this effect is mainly mediated via atrial fibrillation. MR analyses did not show a causal association between normal range thyroid function and CAD. Secondary analyses showed a causal relationship between Hashimoto's thyroiditis and a 7% increased risk of CAD (OR, 1.07; 95% CI, 1.01-1.13; $P = 0.026$), which was mainly mediated via body mass index.

Conclusion: These results provide important new insights into the causal relationships and mediating pathways between thyroid function, stroke, and CAD. We identify variation in

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Abbreviations: AF, atrial fibrillation; BMA, Bayesian model averaging; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disorder; FT4, free thyroxine; GWAS, genome-wide association studies; IVW, inverse-variance-weighted; MR, Mendelian randomization; OR, odds ratio; SD, standard deviation; SNP, single nucleotide polymorphism; T2D, type diabetes; TSH, thyrotropin.

normal range thyroid function and Hashimoto's thyroiditis as risk factors for stroke and CAD, respectively. (*J Clin Endocrinol Metab* XX: 0–0, 2020)

Key Words: Mendelian randomization, thyroid function, TSH, stroke, coronary artery disease, mediation

Despite the undeniable progress in prevention and treatment in the past 2 decades, cardiovascular disorders (CVDs) remain the leading cause of mortality worldwide (1). Whereas smoking, hypertension, diabetes, obesity, and dyslipidaemia are the major modifiable cardiovascular risk factors (2), observational studies have demonstrated that also overt and subclinical thyroid dysfunction are associated with a higher risk of CVD (3-6). More recently, several population-based studies showed that even higher free thyroxine (FT_4) levels within the normal range are associated with an increased risk of CVD, including atherosclerotic disease and stroke (7-10). Bano et al estimated that this culminates in an increased risk of atherosclerotic cardiovascular mortality in euthyroid individuals, with a hazard ratio of 2.4 per 1 ng/dL increase in FT_4 levels and a hazard ratio of 0.92 per 1 log thyrotropin (TSH) increase in TSH levels (10).

Observational studies are typically prone to biases in study design, residual confounding, and reverse causality (11). It is therefore unclear if the observed associations between mild variations in thyroid function and atherosclerotic diseases are causal or not, which is a key question that needs to be resolved first. Mendelian randomization (MR) is an approach that can provide such information on causality (12). MR evaluates the effect of an exposure (eg, thyroid function) on an outcome (eg, CVD) using genetic variants associated with the exposure as instruments (13). MR draws from the fact that genetic variants segregate randomly from parents to offspring, which is comparable to the randomization used in clinical trials. As approximately 65% of the total variance in TSH and FT_4 levels is determined by genetic factors (14), there are good grounds for MR studies on thyroid function and various outcomes. A previous MR study suggested no causal association between thyroid function and the risk of ischemic heart disease (15). However, this study had limited statistical power as it used a small number of genetic variants as instruments, which only explained 5.6% of the variance for TSH and 2.3% of the variance for FT_4 levels (16). Other MR studies on thyroid function and CVD did not use the largest available genome-wide association studies (GWAS) for CVD (17) or any stroke (18). The ThyroidOmics Consortium performed the largest meta-analysis of GWAS on thyroid function in more than 72 000 participants, which more than doubled the number of genetic variants associated with thyroid function (19, 20). These findings have now paved the way to conduct well-powered MR studies to test the causality of the observed associations between thyroid function and CVD. In the current study, we performed 2-sample MR to investigate the effects of thyroid function on CAD and stroke, using the previously mentioned thyroid GWAS data, as well as data from the 2 largest GWAS on CAD and stroke (17, 18). Next to normal range thyroid function, MR studies on Hashimoto's thyroiditis and Graves' disease are presented as secondary analyses, thereby covering the entire spectrum of thyroid (dys)function. Finally, multivariable MR analyses were performed to investigate the pathophysiological mechanisms underlying the causal associations.

Materials and Methods

Primary analyses tested whether associations between variation in normal range thyroid function assessed via TSH and FT_4 levels and the risk of stroke or CAD are causal. Secondary analyses tested whether thyroid dysfunction, including Hashimoto's thyroiditis and Graves' disease, is causally associated with stroke or CAD. When we observed evidence for causality, we further assessed the role of potential mediators.

All *P* values are 2-sided, and statistical significance was defined as a *P* value of 0.0125, corresponding to a Bonferroni correction of 4 tests for the primary analyses (2 exposures [TSH and FT_4] and 2 outcomes [CAD and stroke]) as the exposures included in the secondary analyses are strongly related to TSH and FT_4 levels.

Genetic variants used as instruments

For normal range TSH and FT_4 levels, we used 55 and 29 single nucleotide polymorphisms (SNPs) associated at a genome-wide significant level ($P < 5 \times 10^{-8}$). One variant (rs8176645) in the *ABO* gene was excluded because of its pleiotropic effects on multiple traits. In order to cover the entire spectrum of thyroid (dys)function, we also assessed the effect of hypothyroidism and hyperthyroidism on stroke and CAD risk. For this reason, we also performed a literature search for genetic variants associated with Hashimoto's thyroiditis and Graves' disease, resulting in 20 variants and 49 variants, respectively. We included common genetic variants (ie, variants with minor allele frequency > 5%) associated with Hashimoto's thyroiditis or Graves' disease in case-control studies with a sample size of > 200 cases and controls each, at a significant level ($P < 0.05$) after a multiple testing correction for a number of variants tested in each study. Variants within the human leukocyte

antigen region associated in non-Caucasian populations were excluded and tagging SNPs for human leukocyte antigen alleles/haplotypes associated with Hashimoto's thyroiditis and Graves' disease in Caucasian populations were added, if available. Their associations with the corresponding thyroid disease were derived from UK Biobank, which recruited more than 500 000 individuals across Great Britain from 2006 through 2010 (21). Hashimoto's thyroiditis and Graves' disease were defined using hospital record data: International Classification of Diseases (ICD10) codes for Hashimoto's thyroiditis were E03.8, E03.9, and E06.3 and for Graves' disease were E05.0, E05.8, and E05.9.

Additional sensitivity analyses, as described below, were performed in order to address pleiotropy and heterogeneity.

Two-sample MR

We performed 2-sample MR analyses by using summary genetic data from the largest GWAS studies available for normal range thyroid function (19), CAD (17), and stroke (18). Genetic variants associated with TSH and FT_4 levels were derived from Teumer et al (19). In this study, TSH and FT_4 values were collected at a single time point in all cohorts, and a normal thyroid function was defined as a TSH level within the reference ranges, which are listed in Supplemental Table 1 of the respective manuscript (19). A 1 standard deviation (SD) increase in the genetically determined values of TSH corresponds to 0.8 mIU/L, and a 1 SD increase in genetically determined FT_4 corresponds to 0.2 ng/dL[16]. CAD data were derived from the largest GWAS meta-analysis by Van Harst et al, involving 122 733 CAD cases and 424 528 controls (17). Stroke data were derived from Malik et al, involving 67 162 cases and 454 450 controls (18).

Two-sample MR was performed using the inverse-variance-weighted (IVW) (22) method, which is the gold standard for MR analyses. To further elucidate the role of pleiotropic effects and heterogeneity, we performed additional sensitivity analyses that are presented in the supplementary material; All supplementary material and figures are located in a digital research materials repository (23).

Multivariable MR analyses

When MR showed a causal relationship between thyroid (dys)function and CAD or stroke, multivariable MR analyses were performed to evaluate the role of known risk factors for stroke or CAD. These risk factors included body mass index (BMI); heart rate; blood pressure (BP); systolic BP and diastolic BP; mean arterial pressure; pulse pressure; lipid traits including total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides as well as type 2 diabetes (T2D) and atrial fibrillation (AF). Educational attainment was assessed by age based on when full-time education was completed, which serves as an indicator of socioeconomic status and is inversely associated with CAD risk. In these analyses, the proportion of the effect mediated by the tested factor was evaluated by the change in the total effect of the genetically determined exposure on the outcomes (24). T2D data were derived from the DIAGRAM consortium (25); high-density lipoprotein, low-density lipoprotein, total cholesterol, and triglyceride levels were obtained from the GLGC (26) and ENGAGE consortia (27); and anthropometric traits including BMI from the GIANT consortium (28). BP traits were derived from International Consortium for Blood Pressure (29), AF data from Nielsen et al (30), and heart rate data from Verweij, et al (31). Finally, data on which age an individual completed full-time education were derived from publically available summary statistics (32).

No ethical approval was required as all data were extracted from publicly available summary data.

Power calculations

To estimate the power of our study, we calculated the minimally detectable odds ratio (OR) of the outcome variable (CAD and stroke) per SD of the exposure variables (TSH and FT_4 levels) using a noncentrality parameter-based approach (33), implemented in a publicly available mRnd web tool (power = 0.8, $\alpha = 0.05$) in our study. Proportion of total variance in TSH and FT_4 levels explained by the genetic variants used as instruments was 9.4% and 4.8%, respectively (19).

Statistical analyses were performed using R version 3.5.1.

Results

Normal range TSH and FT_4 levels

MR analyses showed a significant association between higher TSH levels within the normal range and a lower risk of stroke (OR, 0.95; 95% confidence interval [CI], 0.91-0.99; $P = 0.008$ per 1 SD increase in TSH levels). There was no evidence of directional pleiotropy (Egger intercept = 1.06e-5, standard error = 0.003) (Fig. 1A). As no single method controls for all statistical properties that may affect MR estimates, we applied additional MR approaches that yielded similar results. Direction and effect sizes remained similar when restricting the analyses to Europeans only.

There was no evidence for an association between normal range FT_4 levels and the risk of stroke (OR, 0.97; 95% CI, 0.89-1.06; $P = 0.50$ per 1 SD increase in FT_4 levels) (Fig. 1A).

For CAD, no causal associations were detected with normal range TSH (OR, 1.01; 95% CI, 0.96-1.05; $P = 0.80$ per 1 SD increase in TSH levels) or FT_4 levels (OR, 0.99; 95% CI, 0.94-1.04; $P = 0.66$ per 1SD in FT_4 levels) using IVW. Sensitivity analyses with other methods yielded similar results without signs of pleiotropy (Fig. 1B).

Secondary analyses

Hypothyroidism (Hashimoto's thyroiditis). MR analyses did not show a causal effect of Hashimoto's thyroiditis on the risk of stroke. Hashimoto's thyroiditis was causally associated with an increased risk of CAD (OR, 1.07; 95% CI, 1.01-1.13; $P = 0.026$). This was confirmed by other methods, while there was no evidence for pleiotropic effects; All supplementary material and figures are located in a digital research materials repository (23).

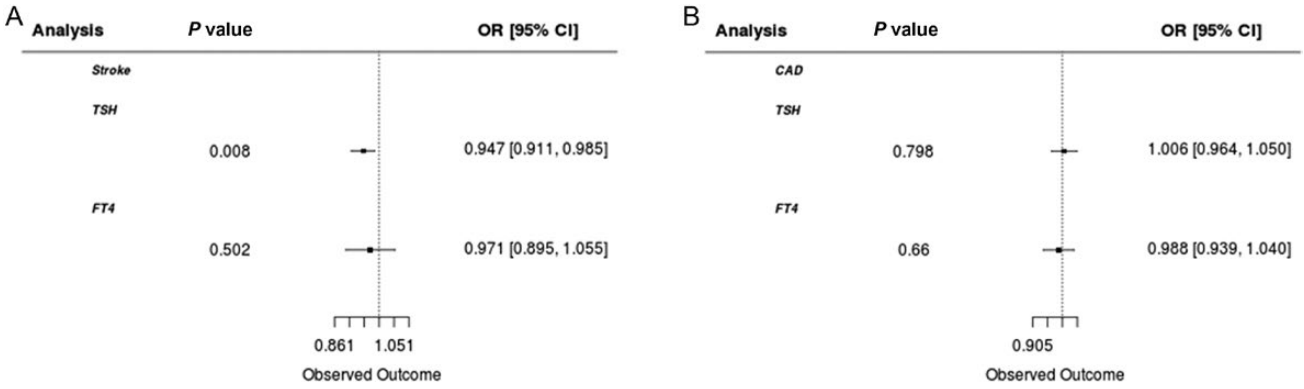

Figure 1. Two-sample Mendelian randomization analyses. Estimates of the effect of TSH and FT_4 levels on stroke (**A**) and coronary artery disease (**B**). Effect estimates represent the ORs (95% CI). CAD, coronary artery disease; CI, confidence interval; FT_4, free thyroxine; IVW, inverse-variance-weighted; OR, odds ratio, TSH, thyrotropin.

Hyperthyroidism (Graves' disease). No causal associations between Graves' disease and stroke (OR, 0.99; 95% CI, 0.96-1.02; $P = 0.37$) or CAD (OR, 1.01; 95% CI, 0.98-1.03; $P = 0.49$) were detected.

Multivariable MR analyses

TSH and stroke risk. We assessed the role of potential stroke risk factors that might mediate the observed association between normal range TSH levels and stroke. These analyses identified AF as a potential mediator as the association between TSH levels and the risk of stroke disappeared after adjustment for AF (OR, 1.00; 95% CI, 0.95-1.06; $P = 0.86$) (Fig. 2). Taking into account the genetic effect of lipid levels did not affect the effect of TSH on stroke risk and MR-Bayesian model averaging (BMA) did not rank lipids as an important mediator; All supplementary material and figures are located in a digital research materials repository (23).

Further multivariable MR analyses showed that the association between TSH and AF is not mediated by a number of known AF risk factors including BP, lipids, CAD, or T2D.

As the results presented above suggested that AF was a putative mediator in the association between TSH levels and stroke, we used the MR-BMA method to further corroborate this finding. MR-BMA can detect true causal risk factors even when the candidate risk factors are highly correlated (34). This analysis confirmed that AF was the top mediating factor. Further inspection of the models indicated 2 variants (rs74804879, rs17477923) as influential points. AF remained the top risk factor after exclusion of these variants, supporting the robustness of our findings; All supplementary material and figures are located in a digital research materials repository (23).

Hashimoto's thyroiditis and CAD risk. Multivariable MR analyses indicated that the effect of Hashimoto's thyroiditis on CAD may be mediated via BMI (Fig. 3). Additional analyses (MR-MBA method) were performed in order to get a more robust overview of the role of potential mediators after excluding potential pleiotropic variants. The top 2 risk factors identified were heart rate and BMI, as presented in Supplementary Table 8, All supplementary material and figures are located in a digital research materials repository (23).

Discussion

In this study, we performed the largest MR analyses of thyroid function on stroke and CAD risk to date. MR can provide important information on causality when randomized controlled trials are not feasible or unavailable. MR uses genotypes that are generally not susceptible to reverse causation or confounding. This provides a cost-effective approach to prioritize potential targets for disease prediction and/or prevention. Our results show that higher TSH levels within the normal range are associated with a lower risk of stroke and that this effect is mediated by a lower risk of AF. Furthermore, we show that Hashimoto's thyroiditis leads to a higher risk of CAD.

Association between high normal range TSH levels and lower risk of stroke is mediated by a decreased risk of AF

In 2016, a multicenter study including 43 598 participants investigated the association between variation in normal range thyroid function and stroke risk (9). This study showed that higher TSH levels within the normal range were associated with a decreased risk of stroke, while higher FT_4 levels within the normal range were associated with an increased risk of stroke. In an attempt to identify the responsible pathophysiological pathways, the authors tested various traditional cardiovascular risk factors, including systolic BP, total cholesterol levels, smoking status, and T2D (9) but could not identify a responsible mediator. More recently, an MR study also

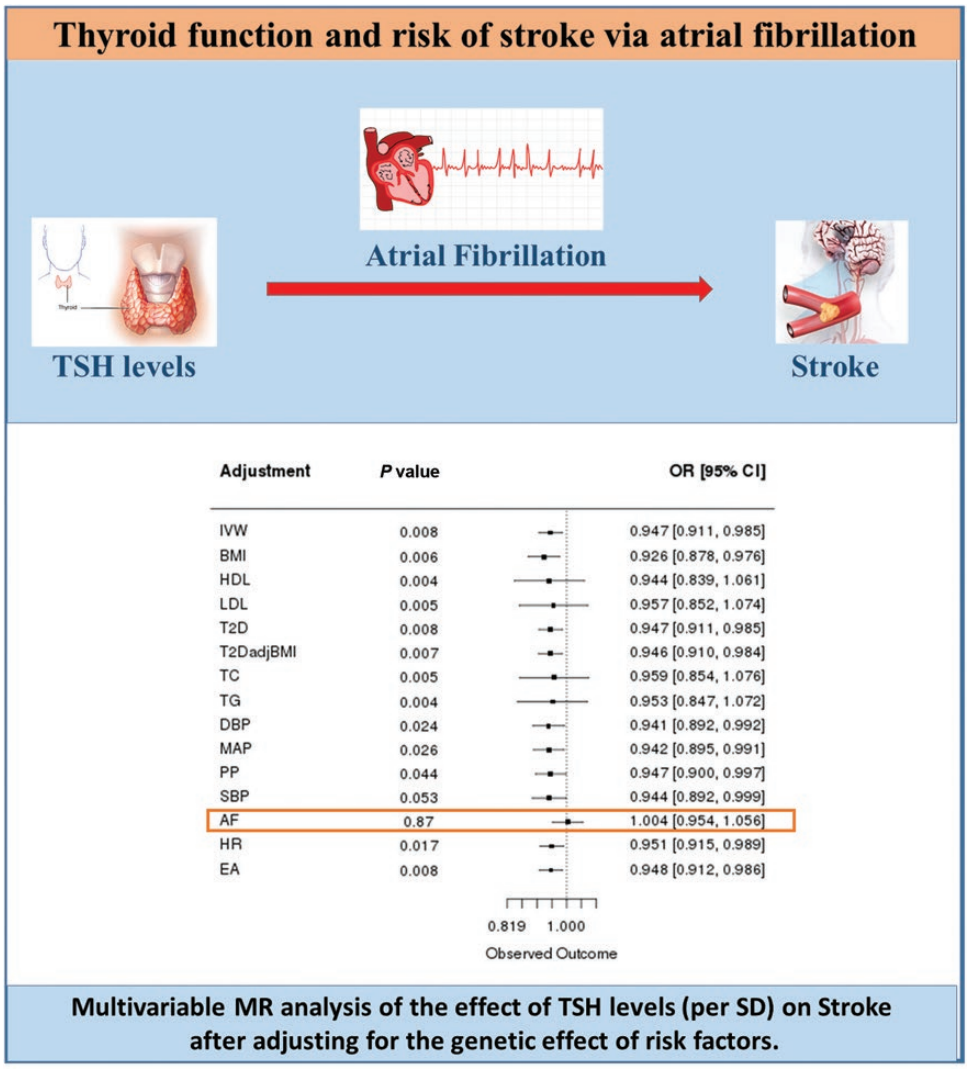

Figure 2. Multivariable MR analysis of the effect of TSH levels (per SD) on stroke after adjusting for the genetic effect of possible mediators. AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; EA, educational attainment; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; MAP, mean arterial pressure; MR-IVW, Mendelian randomization inverse-variance-weighted; OR, odds ratio; PP, pulse pressure; SBP, systolic blood pressure; SD, standard deviation; T2D, type 2 diabetes; T2DadjBMI, T2D adjusted for BMI; TC, total cholesterol; TG, triglycerides; TSH, thyrotropin.

reported a suggestive association of genetically decreased TSH levels with a higher risk of the cardiometabolic subtypes of stroke (35). In the current study, we also assessed causality in the observed associations between normal range thyroid function and stroke but used the largest available datasets for normal range thyroid function and any stroke type (18) to improve on power compared with the previous studies (35). We demonstrate that high-normal TSH levels are associated with a decreased risk of stroke, which we show to be mediated by a decreased risk of AF. This is both pathophysiologically plausible and in line with the results of observational studies showing that AF is a major risk factor for stroke, increasing stroke risk up to 5-fold (36). Our findings are also in line with various studies showing that a high-normal thyroid function is associated with an increased risk of AF (37) and that participants with a genetically predicted higher TSH level have a lower risk of AF (38). While multiple pathophysiological pathways could theoretically be responsible for the effect of variations in normal range thyroid function on stroke, we for the first time demonstrated that AF is the key mediator. In addition, this also showed that multivariable MR analyses can reveal pathophysiological mechanisms underlying the effects of variation in thyroid function.

The absence of causal associations between FT_4 levels and the risk of stroke in our study is not conclusive as it may be due to the fact that the genetic variants

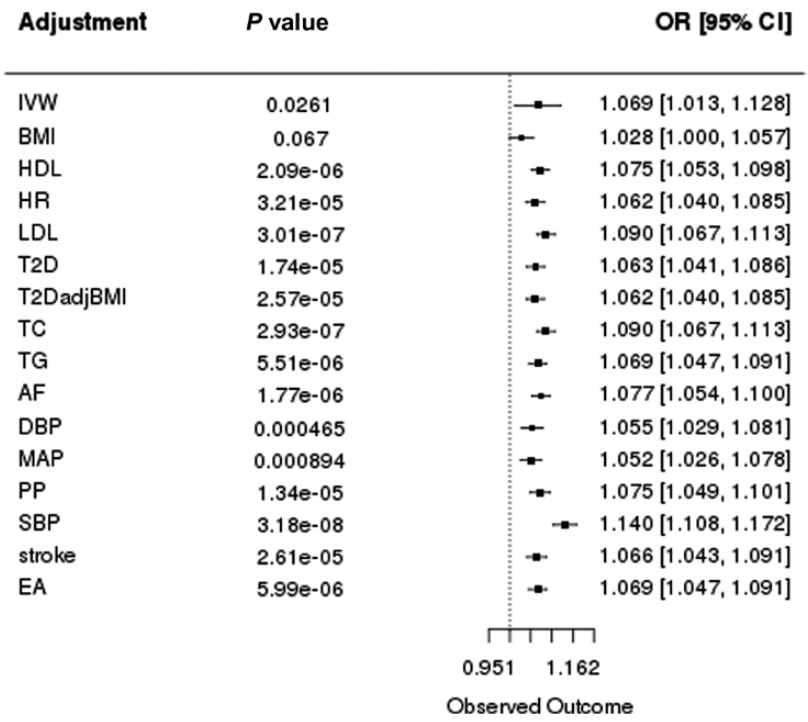

Figure 3. Multivariable MR analysis of the effect of Hashimoto disease on CAD after adjusting for the genetic effect of possible mediators. AF, atrial fibrillation; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; EA, educational attainment; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein; MAP, mean arterial pressure; MR-IVW, Mendelian randomization inverse-variance-weighted; OR, odds ratio; PP, pulse pressure; SBP, systolic blood pressure; SD, standard deviation; T2D, type 2 diabetes; T2DadjBMI, T2D adjusted for BMI; TC, total cholesterol; TG, triglycerides; TSH, thyrotropin.

used as instruments explained less variance in FT_4 than in TSH levels (ie, 4.8% and 9.4%, respectively) (19). Therefore, our study was better powered for TSH than for FT_4. Therefore, if there was a causal effect of normal range variation in FT_4 levels on stroke risk, it would be smaller than what our study is powered to detect.

MR analyses do not support a causal association between normal range thyroid function and the risk of CAD

Bano et al have shown that variation in thyroid hormone levels within the normal range are associated with subclinical atherosclerosis as assessed by a coronary artery calcification score and the overall risk of adverse atherosclerotic cardiovascular events, including fatal and nonfatal myocardial infarction, other CAD mortality, and stroke (10). While several other population-based studies have not found associations between normal range thyroid function and the risk of CAD only, most of these studies did show associations with increased CAD mortality (8, 39-41). While these studies suggest an important relation between thyroid function and CAD, observational studies are typically prone to various sources of bias, including biases in study design, reverse causality, and residual confounding (11). Previous MR studies did not find any causal association between thyroid function and CAD (35). In this study, we further increased power compared with previous efforts by using the biggest available dataset for CAD, and we did not find evidence for a causal association between normal range thyroid function and the risk of CAD either. However, we can obviously not exclude that thyroid function affects CAD risk, but the effect is smaller than what our study can detect. Furthermore, an effect of minor variation in thyroid function on CAD risk might be mediated by other factors such as hypertension and dyslipidemia. These cardiovascular risk factors are currently widely recognized and treated, which therefore might have diluted the potential cause-and-effect relationship between thyroid function and the CAD risk in the studied populations.

Hashimoto's thyroiditis and CAD risk

In observational studies, both overt and subclinical hypothyroidism have been associated with an increased risk of atherosclerosis and adverse cardiovascular events, including CAD (3, 4, 42). We focused our analyses on Hashimoto disease as this is the most common cause of hypothyroidism. While our study demonstrates for the first time a causal association between Hashimoto's thyroiditis and CAD, it is not clear whether this effect is attributed solely to a low thyroid function or to autoimmunity in general, since many observational studies suggest an increased risk of atherosclerosis and adverse

cardiovascular events in patients with other autoimmune disorders (43). Therefore, future studies are needed to further clarify the underlying pathophysiological relationship between Hashimoto's thyroiditis and CAD.

Strengths and limitations of the study

In order to increase the power of our study, we used summary data from the largest available GWAS on thyroid function recently published by the ThyroidOmics Consortium, which more than doubled the number of variants associated with TSH and FT_4 levels (19, 20). This significantly increased our statistical power compared with the previous published MR study on thyroid function and CVD by Zhao et al. (15) Furthermore, we used data from the 2 largest available GWAS meta-analyses on the tested cardiovascular outcomes, both including more than 500 000 participants (17, 18). This included data for any stroke type from the largest transethnic meta-analysis (21), which significantly increased the sample size of our study compared with an MR study previously performed by Larsson et al (35). Also, the sample size of Graves' disease cases in UK Biobank was limited. This might have led to less precise effect estimates, resulting in less power to detect a causal association in our secondary analyses on Graves' disease.

Given the relatively large number of variants with unclear physiological function included in the MR analyses, it is possible that some of them may confer pleiotropic effects. However, the use of multiple variants associated with TSH and FT_4 levels should reduce the impact of individual SNPs associated with the outcome through alternative pathways (13). Moreover, we performed sensitivity analyses excluding potentially pleiotropic variants, which did not change our results. Finally, the IVW method can lead to moderately biased estimates for binary outcomes (44). To address this issue, we applied different MR approaches, which in most cases led to consistent results.

In conclusion, these results show that variation in normal range thyroid function and Hashimoto's thyroiditis are causally associated with stroke and CAD and provide insights into the underlying pathophysiological pathways involved. The association between variation in normal range thyroid function and stroke suggests that the use of classical population-based reference ranges might not be optimal for optimizing an individual's disease risk. Future studies should investigate whether a disease risk-oriented range would be more beneficial, which should also take the risk of various other thyroid dysfunction-related complications into account.

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Additional Information

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Disclosures: The authors have no competing interest to declare.

Data Availability: All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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