COVID-19 and thyroid function: A bi-directional two-sample Mendelian randomization study

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Abstract

Background: Thyroid dysfunction has been observed among some patients with COVID-19. It is unclear whether SARS-CoV-2 infection (or its severity) leads to the development of thyroid dysfunction, or vice versa. In this study, we examined the bi-directional causal relationship between host genetic liability to three COVID-19 phenotypes (including SARS-CoV-2 infection, hospitalized and severe COVID-19) and three thyroid dysfunction traits [including hyperthyroidism, hypothyroidism, and autoimmune thyroid disease (AITD)] and three continuous traits of thyroid hormones [including thyroid-stimulating hormone (TSH) and free thyroxine (FT4) within reference range, and TSH in full range].

Methods: Summary statistics from the largest available meta-analyses of human genome-wide association studies were retrieved for the following variables: SARS-CoV-2 infection (n=1,348,701), COVID-19 hospitalization (n=1,557,411), severe COVID-19 (n=1,059,456), hyperthyroidism (n=51,823), hypothyroidism (n=53,423), AITD (n=755,406), TSH within reference range (n=54,288), FT4 within reference range (n=49,269), and TSH in full range (n=119,715). Using a two-sample Mendelian randomization (MR) approach, the inverse-variance weighted (IVW) method was adopted as the main MR analysis. Weighted median, contamination mixture, MR-Egger, and MR-PRESSO methods were applied as sensitivity analyses.

Results: Host genetic susceptibility to SARS-CoV-2 infection was causally associated with hypothyroidism in the main IVW analysis (per doubling in prevalence of SARS-CoV-2 infection, Odds Ratio (OR)=1.335; 95% CI: 1.167-1.526; $p=2.4x10^{-5}$, surpassing the Bonferroni multiple-testing threshold). Similar causal estimates were observed in the sensitivity analyses (weighted median: OR=1.296; 95% CI: 1.066-1.575; $p=9x10^{-3}$; contamination mixture: OR=1.356; 95% CI: 1.095-1.818; p=0.013; MR-Egger: OR=1.712; 95% CI: 1.202-2.439; $p=2.92x10^{-3}$, and MR-PRESSO: OR=1.335; 95% CI: 1.156-1.542;

p=5.73x10^{-4}). Host genetic liability to hospitalized or severe COVID-19 was not associated with thyroid dysfunction or thyroid hormone levels. In the reverse direction, there was no evidence to suggest that genetic predisposition to thyroid dysfunction or genetically determined thyroid hormone levels altered the risk of the COVID-19 outcomes.

Conclusions: This bi-directional MR study supports that host response to SARS-CoV-2 viral infection plays a role in the causal association with increased risk of hypothyroidism. Long-term follow-up studies are needed to confirm the expected increased hypothyroidism risk.

Key words: SARS-CoV-2, COVID-19, thyroid, Mendelian randomization

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified to cause clusters of fatal pneumonia in Wuhan, China, in December 2019 (1). Two years after announcement of the coronavirus disease (COVID-19) outbreak as a pandemic by the World Health Organization (WHO) on 11 March 2020, there have been more than 500 million confirmed cases of COVID-19 and over 6 million deaths globally as of April 2022. Since SARS-CoV-2 enters human cells via the angiotensin converting enzyme 2 (ACE2) receptor (2) which is highly expressed in the thyroid tissue (3), the thyroid has been considered a potential direct target of the viral infection (4). Alternatively, SARS-CoV-2 might indirectly induce thyroid gland inflammation through triggering abnormal immune-inflammatory responses and cytokine storm (4).

Subacute thyroiditis (SAT) has been reported in COVID-19 patients (5-8). SAT is defined as an inflammatory disorder of the thyroid gland, likely originating from a viral infection, characterized by self-limiting thyrotoxicosis followed by hypothyroidism with variable duration, before resumption to normal thyroid function (9). In general, permanent hypothyroidism has been observed in 15% patients with SAT after 28-year follow-up (10). Moreover, autoimmune thyroid disease (AITD), including Graves' disease (11) and Hashimoto thyroiditis (12), have been reported after a diagnosis of SAT. It remains unclear if SARS-CoV-2 induced SAT would induce long-term thyroid dysfunction.

Case reports and cohort studies revealed that patients with COVID-19 experienced thyroid dysfunction, including AITD (7,13-16), thyrotoxicosis (8,15,17,18), hypothyroidism (5,8,15,17-19), and nonthyroidal illness syndrome (14,15,19). Nevertheless, the cohort studies to-date were limited by small sample sizes with short follow-up periods, and they could not

infer causality. Whether SARS-CoV-2 infection or COVID-19 severity would cause long-term thyroid dysfunction warrants further investigation. In the reverse direction, a meta-analysis (20) and three retrospective studies (21-23) provided contradictory evidence for the association of pre-existing thyroid disease with risk of COVID-19-related outcomes. It remains unknown if thyroid dysfunction alters the susceptibility to SARS-CoV-2 infection and COVID-19 severity.

Mendelian randomization (MR) analysis makes use of genetic variants as instruments to represent the exposure, aiming to examine the causal association of the exposure with the lifelong risk of the outcome (24). In this two-sample MR study, we evaluate the bi-directional causal relationship of host genetic liability to three COVID-19 phenotypes (including SARS-CoV-2 infection, hospitalized COVID-19, and severe COVID-19) with three binary thyroid dysfunction traits (including hyperthyroidism, hypothyroidism and AITD), and three continuous thyroid hormone level traits [including thyroid-stimulating hormone (TSH) and free thyroxine (FT4) within reference range, and TSH in full range].

Materials and Methods

Study design

This is a two-sample bi-directional MR study assessing the causal relationship of host genetic liability to COVID-19 phenotypes with thyroid-related traits. The study design and assumptions of MR analysis are illustrated in Figure 1. In the forward direction with COVID-19 phenotypes as exposure and thyroid-related traits as outcome, we determined if host genetic liability to COVID-19 phenotypes had causal effects on the thyroid traits. In the reverse direction, we assessed if genetically determined thyroid traits are causally associated with COVID-19 phenotypes.

Data sources and genetic instruments

The largest possible meta-analyses of genome-wide association studies (GWAS), which were conducted in Europeans, were selected as the data sources (see Supplementary Methods 1). The details of each data source, including the eligibility criteria of study participants, sample size, ancestry and analytical procedures, are presented in Table 1. Ethics approval of all the relevant GWAS was obtained from the respective institutional review boards and additional ethics review was not required for this MR study using previously reported data. The protocol for selection of genetic instruments is detailed in Supplementary Methods 2. In the forward direction, independent SNPs with suggestive significance ($p<5x10^{-6}$) were selected as the initial genetic instruments for the three COVID-19 phenotypes in the primary MR analysis. If significant causal relationship was revealed in the primary analysis, sensitivity analysis using instruments with a more stringent threshold at genome-wide significance ($p<5x10^{-8}$) was performed. The F-statistics, a measure of the strength of genetic instruments, were computed for each MR analysis. Together with the number of genetic instruments adopted in each MR analysis, these figures are listed in Table 2. Methods of calculating power (Supplementary Figures 1 and 2) and bias (Supplementary Table 1) due to sample overlap were detailed in Supplementary Methods 3.

Mendelian randomization

The main MR analysis was the conventional inverse-variance weighted (IVW) method (25), with random effects model selected to address heterogeneity which was assessed using the Cochran's Q test. Sensitivity analyses included weighted median (26), MR-Egger regression (27), contamination mixture (28) methods, and the outlier test of MR pleiotropy residual sum and outlier (MR-PRESSO) (29) method. To check for the presence of directional pleiotropy, we applied the MR-Egger intercept (27) and MR-PRESSO global (29) tests. If pleiotropic

outliers were identified by MR-PRESSO, the main and sensitivity analyses were repeated upon exclusion of the outliers. Details of these MR methods are described in Supplementary Methods 4. Conservative Bonferroni correction for 36 tests ($\alpha=0.05/36=1.39\times10^{-3}$) was used to account for multiple testing.

Results

MR in evaluating the causal effects of host genetic liability to COVID-19 phenotypes on thyroid-related traits (Forward direction)

In the primary analyses, we identified 19, 28 and 40 genetic instruments at $p<5\times10^{-6}$ for SARS-CoV-2 infection (Supplementary Table 2a), hospitalized COVID-19 (Supplementary Table 2b) and severe COVID-19 (Supplementary Table 2c) respectively. Genetic liability to SARS-CoV-2 infection was associated with an increased risk of hypothyroidism in the main IVW analysis after correction for multiple testing [per doubling in prevalence of SARS-CoV-2 infection, OR=1.335; 95% Confidence Interval (CI):1.167-1.526; Figure 2a]. Similar causal estimates were obtained from sensitivity analyses of weighted median method (OR=1.296; 95% CI: 1.066-1.575), contamination mixture method (OR=1.356; 95% CI: 1.095-1.818), MR-Egger regression (OR=1.712; 95% CI: 1.202-2.439), and MR-PRESSO (OR=1.335; 95% CI: 1.156-1.542; Figure 2a). Leave-one-out analysis confirmed that the causal association was not driven by any individual instrument (Supplementary Figure 3). In the sensitivity analysis using five independent instruments at $p<5\times10^{-8}$ (Supplementary Table 2d), a similar causal association was observed for the IVW (OR=1.468; 95% CI: 1.098-1.963) and weighted median methods (OR=1.437; 95% CI: 1.091-1.895), while wider CIs crossing the null were observed for other sensitivity analyses (Figure 2b). No association was observed for genetic liability to SARS-CoV-2 infection with hyperthyroidism and AITD (Figure 2a), as well as the three thyroid

158 hormone level traits (Supplementary Figure 4). MR-Egger intercept and MR-PRESSO global
159 tests were not statistically significant (Figures 2a-b, Supplementary Figure 4).
160
161 We observed null causal effects of genetic liability to COVID-19 hospitalization (Figure 3,
162 Supplementary Figure 5) and severe COVID-19 infection (Figure 4, Supplementary Figure 6)
163 on all thyroid traits examined, as reflected by all the main IVW analyses. Although nominal
164 significance was obtained in MR-Egger regression and/or contamination mixture methods for
165 the causal association of genetic liability to hospitalized COVID-19 with increased risk of
166 hyperthyroidism and hypothyroidism (Figure 3), and genetic liability to severe COVID-19 with
167 increased risk of hypothyroidism (Figure 4), lower TSH level (within reference range) and
168 higher TSH level (in full range) (Supplementary Figure 6), the MR-Egger intercept tests were
169 significant. Despite the insignificant MR-PRESSO global tests (Figures 3-4, Supplementary
170 Figure 6), the possibility of horizontal pleiotropy could not be ruled out unequivocally.
171
172 *MR analyses in evaluating the causal association of genetically determined thyroid-related*
173 *traits with COVID-19 phenotypes (Reverse direction)*
174 In the main IVW analyses, there was no evidence to support that genetic predisposition to
175 hyperthyroidism (Figure 5), hypothyroidism (Figure 6) and AITD (Figure 7) had causal effects
176 on any COVID-19 phenotypes. Although nominal significance was obtained for genetically
177 susceptibility to hyperthyroidism with reduced risk of SARS-CoV-2 infection, the multiple
178 testing threshold was not surpassed (Figure 5). Genetically determined TSH and FT4 levels
179 within reference range (Supplementary Figures 7-8), and TSH level in full range
180 (Supplementary Figure 9), also had null association with the COVID-19 phenotypes in the
181 main IVW analyses. Majority of the MR analyses were not subjected to directional pleiotropy,
182 with insignificant MR-Egger intercept and MR-PRESSO global tests. Exceptions were seen

for the MR analysis of AITD with COVID-19 hospitalization (Figure 7), TSH level within reference range with SARS-CoV-2 infection and COVID-19 hospitalization (Supplementary Figure 7), and TSH in full range with hospitalization (Supplementary Figure 9) that the MR-Egger intercept and/or MR-PRESSO global tests were significant, even though no significant pleiotropic outliers were identified. We also observed heterogeneity in these four analyses (Cochrane's Q test heterogeneity $p<0.05$), but not for others (Supplementary Table 3).

Discussion

To our knowledge, this is the first MR study evaluating the casual relationship between host genetic liability to COVID-19 phenotypes and thyroid-related traits. In this bi-directional two-sample MR study, we observed that genetic susceptibility to SARS-CoV-2 infection was associated with increased risk of hypothyroidism. In the reverse direction, we found no evidence that genetic predisposition to thyroid-related traits would alter the susceptibility to SARS-CoV-2 infection, COVID-19 hospitalization, or severity.

Our primary analysis using genetic instruments at $p<5x10^{-6}$ provided strong evidence supporting that genetic susceptibility to SARS-CoV-2 infection, or host response to the viral infection, increased the risk of hypothyroidism. Assuming the incidence rate of hypothyroidism among individuals without SARS-CoV-2 infection was similar to that in the European population before the pandemic [226.2 per 100,000 person-year (30)], the absolute risk difference of hypothyroidism between individuals with and without infection is estimated to be 75.8 incident cases per 100,000 person-year per doubling the prevalence of SARS-CoV-2 infection. Consistent and robust causal estimates were attained in the sensitivity analyses using instruments with $p<5x10^{-8}$. Notably, in the original GWAS meta-analysis, hypothyroidism was defined as TSH level above the reference range such that both overt and mild subclinical

hypothyroidism were included (31). Therefore, our MR findings implied that the risk of both overt and subclinical hypothyroidism might be elevated in individuals who were genetically susceptible to SARS-CoV-2 infection, which was consistent with previous studies that SARS-CoV-2-infected patients experienced subclinical (5,15,19) or overt hypothyroidism (17) up to 108 days after hospital admission (19). Given that most of the participants (>80%) in these studies had a mild course of COVID-19 infection treated outside the intensive care unit (ICU) (5,15,17,19), these observations aligned with our findings in SARS-CoV-2 infected individuals. One possible explanation for the development of hypothyroidism in individuals with SARS-CoV-2 infection is the development of SAT, which is prevalent among COVID-19 patients (5-8). Permanent hypothyroidism was observed in approximately 15% of individuals with SAT at 28-year follow-up (10). Nevertheless, only low prevalence rates of hypothyroidism (≤5.2%) have been observed among prospective studies of COVID-19 patients to-date (17,18), which may be attributed to a short follow-up period (≤108 days). Notably, SARS-CoV-2 infection might cause symptoms even after the infection was resolved, which is known as long COVID. Symptoms may include fatigue (32), cognitive problems (i.e. memory and concentration) (33), and mood problems (e.g. depression and anxiety) (34). Furthermore, fatigue is commonly observed among hypothyroid patients, and hypothyroidism is associated with increased risk of cognitive impairment (35) and depression (36,37). Taking together our MR findings suggesting that genetic liability to SARS-CoV-2 infection might increase the risk of hypothyroidism, future investigation in long-term cohort studies examining thyroid function and long covid are warranted.

Genetic susceptibility to SARS-CoV-2 infection was causally associated with increased risk of hypothyroidism, but not other thyroid-related traits. Although AITD cases included a substantial proportion of individuals with subclinical and overt hypothyroidism, null

association was observed for host genetic liability to SARS-CoV-2 infection with AITD in our MR study. One plausible reason was the different selection criteria of cases. While the GWAS meta-analysis of hypothyroidism (31) selected cases as those with TSH level above the reference range irrespective of the causes, the GWAS meta-analysis of AITD specifically excluded hypothyroidism cases with non-autoimmune causes (38). Another reason might be due to the dilution effects by including individuals with Graves' disease and Hashimoto thyroiditis as AITD (38). We also found that genetic liability to SARS-CoV-2 infection had null causal effects on the continuous traits of TSH levels. TSH within reference range included euthyroid individuals only (31), while TSH in full range included patients with euthyroidism, hyperthyroidism or hypothyroidism. Notably, our MR study suggested a link of genetic liability to SARS-CoV-2 infection with risk of hypothyroidism, but not hyperthyroidism. The different study participants included in the MR analysis of hypothyroidism and TSH might explain why discrepant results were observed.

There was insufficient evidence to suggest any causal association of genetic liability to hospitalized and severe COVID-19 with hypothyroidism. One plausible explanation is that different host response mechanisms exist in affecting the susceptibility to SARS-CoV-2 infection and progression to more severe COVID-19. Based on the GWAS meta-analysis conducted by COVID-19 HGI, only four out of 13 genome-wide significant loci identified for the three COVID-19 phenotypes were shared between SARS-CoV-2 infection and hospitalized COVID-19 (39). In addition, these four loci had stronger links to SARS-CoV-2 infection than progression to severe COVID-19 (39). The presence of causal association with hypothyroidism for individuals genetically susceptible to SARS-CoV-2 infection does not necessarily imply that the same causal association exists for those liable to hospitalized or severe COVID-19. Even such causal association exists, the genuine causal effect on hypothyroidism might be too

small to be detected (Supplementary Figures 1a-c). To detect a causal association with small effect, the MR analyses might be re-visited when summary statistics from better-powered GWAS of hypothyroidism, hospitalized and severe COVID-19 become available.

There was insufficient evidence to suggest a causal association of SARS-CoV-2 infection with hyperthyroidism, but this should be interpreted with caution. The statistical power in evaluating this association was the lowest among all other analyses in the current study, as the proportion of variance explained by the genetic instruments on SARS-CoV-2 infection was small (<1%) and outcome dataset was of medium sample size only. We had 80% power to detect a genuine causal association if hyperthyroidism had an OR≥2.4 per standard deviation increase in the exposure (Supplementary Figure 1a). Therefore, the MR analysis investigating the causal relationship of SARS-CoV-2 infection with hyperthyroidism should be re-visited when genetic instruments explaining higher proportion of variance on SARS-CoV-2 infection, or a higher-powered GWAS of hyperthyroidism, become available.

We saw no significant causal association of genetically determined thyroid-related traits with COVID-19 phenotypes. This finding is consistent with three retrospective studies which reported that pre-existing hyperthyroidism (21) and hypothyroidism (21-23) were not associated with increased risk of SARS-CoV-2 infection, COVID-19-associated hospitalization, ICU admission and mortality. Although a meta-analysis suggested that individuals with pre-existing thyroid abnormalities and hypothyroidism had poorer COVID-19-related outcomes in terms of severity, ICU admission, hospitalization and mortality (20), the discrepancy might be explained by the different study designs, sample size, and unavailability of covariates in some of the included studies. Importantly, our findings support

the current assumption that patients with AITD are unlikely more susceptible to SARS-CoV-2 infection or more severe COVID-19 (40).

This study has several clinical implications. It has been suggested that SARS-CoV-2 might directly infect the thyroid gland, indirectly cause inflammation of the thyroid via triggering abnormal immune-inflammatory responses, or both (4,19). Our study findings support that host response to SARS-CoV-2 infection might increase the risk of overt and subclinical hypothyroidism. It may be beneficial if clinicians are aware of the possible occurrence of overt or subclinical hypothyroidism among individuals who are genetically susceptible to SARS-CoV-2 infection. Appropriate prevention, monitoring the thyroid function for individuals with previous infection of SARS-CoV-2, and thus timely treatment, might mitigate the risk of undiagnosed symptomatic hypothyroidism. However, whether physiological changes caused by SARS-CoV-2 infection altered the risk of hypothyroidism requires future investigation.

Our study has several strengths. We investigated the causal relationship between two diseases using the MR approach, which is infeasible to be assessed by the randomized clinical trials. Moreover, MR approach enables the evaluation of lifelong effect of the exposure on the outcome. In our study, relatively high F-statistics were observed for the instruments (Table 2), indicating weak instrument bias is unlikely. We adopted different MR methods which are based on different assumptions. Yet, similar causal estimates were attained for the causal association of host genetic liability to SARS-CoV-2 infection with hypothyroidism, demonstrating the robustness of the finding.

There are also limitations. First, bias might arise due to participant overlap in two-sample MR analysis (41), which was present in the exposure-outcome pairs of AITD-COVID19-

hospitalization (17.62%), hyperthyroidism-SARS-CoV-2-infection (0.331%), hypothyroidism-SARS-CoV-2-infection (0.331%) , TSH within reference range-SARS-CoV-2 infection (0.636%), FT4-SARS-CoV-2 infection (0.331%), and TSH in full range-SARS-CoV-2 infection (1.4%). The bias and Type I error were 0 and 0.05 respectively for all the overlapping pairs (Supplementary Table 1), which is considered minimal. Second, SARS-CoV-2 infection can be asymptomatic, contributing to under-diagnosis of COVID-19, subsequently leading to misclassification of the SARS-CoV-2 infection status in the GWAS meta-analysis of SARS-CoV-2 infection conducted by COVID-19 HGI. Hence, the power of the GWAS meta-analysis and our MR study were reduced. As aforementioned, the statistical power for the MR analysis assessing the causal association of genetic susceptibility to SARS-CoV-2 infection with hyperthyroidism was particularly low. Nevertheless, the current study had sufficient power for the remaining MR analyses that suggested null association, indicating that even if a causal association exists, the causal effect on the outcome may not be clinically meaningful. Third, the GWAS meta-analysis of hyperthyroidism and hypothyroidism conducted by ThyroidOmics Consortium (31) did not differentiate the overt and subclinical cases. Thus, we cannot provide the risk estimates and absolute risks that genetic susceptibility to SARS-CoV-2 infection had on overt and subclinical hypothyroidism respectively. Fourth, this MR study utilized summary statistics derived from GWAS meta-analyses conducted in Europeans. Whether the findings could be generalized to other ethnicities warrant future investigations. Fifth, genetic instruments might act on the outcome via pathways other than the exposure, violating the third MR assumption (Figure 1). Yet, we have adopted the MR-Egger intercept and MR-PRESSO global tests to detect for the presence of directional pleiotropy. For the association of SARS-CoV-2 infection on hypothyroidism, both tests were insignificant, implying horizontal pleiotropy was unlikely, although the possibility cannot be completely excluded.

In conclusion, this study revealed that genetic susceptibility to SARS-CoV-2 infection is causally associated with increased risk of hypothyroidism. The MR approach using instruments of host genetics supports that host response to viral infection plays a role in this causal relationship.

Authors' Contributions

G.H.L. and C.L.C. conceptualized and designed the study. G.H.L. and C.M.T. conducted the statistical analysis. G.H.L. drafted the manuscript. All authors were involved in interpreting the data and revising the manuscript for final submission.

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Author Disclosure Statement

The authors have nothing to disclose.

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Data and data sharing

The present MR study uses publicly available summary statistics from GWAS/GWAS meta-analysis that can be downloaded from the websites of the corresponding consortiums. The references of these GWAS/GWAS meta-analysis are provided in Table 1.

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458 **Table 1.** Data Sources used in Mendelian Randomization Analysis.

Traits	Description	Ancestry	Sample size	Data analysis and selection of independent genome-wide significant variants
COVID-19 phenotypes				
SARS-CoV-2 infection (39)	A case-control GWAS meta-analysis comprising all cases of reported SARS-CoV-2 infection irrespective of symptoms, and controls without known SARS-CoV-2 infection.	Summary statistics from the GWAS meta-analysis of European population were retrieved, excluding the UK Biobank participants	Total: 1,348,701 Cases: 32,494 Controls: 1,316,207	Scalable and Accurate Implementation of Generalized mixed model and PLINK were recommended for each contributing study. Suggested covariates included age, age-squared, sex, age-sex, principal components, and other study-specific covariates. Summary statistics of variants present in not less than two-thirds of the studies were meta-analyzed using the inverse-variance weighted method. A total of 13 independent loci passed the genome-wide significance threshold corrected for multiple testing ($p<1.67x10^{-8}$) in at least one of the three COVID-19 phenotypes. Yet, the methodology and threshold in defining "independent" was not described.
COVID-19 hospitalization (39)	A case-control GWAS meta-analysis comprising cases of moderate or severe COVID-19, who were hospitalized because of SARS-CoV-2 infection associated symptoms. Controls had no known SARS-CoV-2 infection.		Total: 1,557,411 Cases: 8,316 Controls: 1,549,095	
COVID-19 severity (39)	A case-control GWAS meta-analysis comprising critically ill COVID-19 cases who required respiratory support at hospitals, or who died because of COVID-19. Controls had no known SARS-CoV-2 infection.		Total: 1,059,456 Cases: 4,792 Controls: 1,054,664	

Traits	Description	Ancestry	Sample size	Data analysis and selection of independent genome-wide significant variants
Thyroid dysfunction traits				
Hyperthyroidism (31)	A case-control GWAS meta-analysis comprising cases with reduced level of thyroid-stimulating hormone (TSH) below the reference range, and controls with TSH level within reference range. Individuals on thyroid medication or with history of thyroid surgery were excluded.	100% European	Total: 51,823 Cases: 1,840 Controls: 49,983	Association of the SNPs with hyper- and hypothyroidism were analyzed in individual studies using logistic regression, with adjustment for age, age-squared, principal components, study center, and family structure. Fixed-effect meta-analysis was conducted using inverse-variance weighting. Within each genome-wide significant locus associated with hyper- or hypothyroidism, the SNP with the lowest p-value was firstly defined as the index SNP. SNPs with $r^2>0.01$ within 1Mb of the index SNP were pruned out. The genome-wide significant index SNPs ($p<5x10^{-8}$) remained in the pruning process were identified as independent SNPs. Eight independent SNPs were associated with hyper- and hypothyroidism respectively.
Hypothyroidism (31)	A case-control GWAS meta-analysis including cases with increased TSH level above the reference range, and controls with TSH level within reference range. Individuals on thyroid medication or with history of thyroid surgery were excluded.	100% European	Total: 53,423 Cases: 3,440 Controls: 49,983	
Autoimmune thyroid disease (38)	A case-control GWAS meta-analysis comprising individuals from Iceland and the UK Biobank. Cases of autoimmune thyroid disease were defined as those diagnosed with Graves' disease,	100% European, comprising 54.1% British (Caucasian) and 45.9% Icelandic	Total: 755,406 Cases: 30,234 Controls: 725,172	Meta-analysis was performed using logistic regression with adjustment for year of birth, sex and origin (Icelandic participants) and principal components (UK Biobank participants) in individual studies. At each genomic locus, the

Traits	Description	Ancestry	Sample size	Data analysis and selection of independent genome-wide significant variants
	Hashimoto thyroiditis, other hypothyroidism, and/or received thyroxine treatment. Individuals with hypothyroidism due to non-autoimmune causes were excluded.			genetic variant with the lowest p-value was the index variant ($p<1x10^{-8}$). Secondary signals within 500kb of the index variant were identified by conditional analysis with reference to linkage disequilibrium information of Icelandic individuals. A total of 93 independent loci were identified to be associated with autoimmune thyroid disease.
Thyroid hormone level traits				
TSH (within reference range) (31)	A meta-analysis of the ThyroidOmics consortium comprising individuals having TSH level within the cohort-specific reference range. Upon inverse normal transformation, TSH level was analyzed as a continuous variable. Individuals on thyroid medication or with history of thyroid surgery were excluded.	100% European	54,288 (discovery stage) Up to 72,167 subjects in meta-analysis	Association of the SNPs with TSH and FT4 levels (within reference range) were analyzed in individual studies using linear regression, with adjustment for age, age-squared, principal components, study center, and family structure. Fixed-effect meta-analysis was conducted using inverse-variance weighting. Within each genome-wide significant locus associated with TSH or FT4, the SNP with the lowest p-value was firstly defined as the index SNP. SNPs with $r^2>0.01$ within 1Mb of the index SNP were pruned out. The genome-wide significant index SNPs ($p<5x10^{-8}$) remained in the pruning process were
Free thyroxine (within reference range) (31)	A meta-analysis of the ThyroidOmics consortium comprising individuals having free thyroxine (FT4) level within the	100% European	49,269 (discovery stage)	

Traits	Description	Ancestry	Sample size	Data analysis and selection of independent genome-wide significant variants
	cohort-specific reference range. Upon inverse normal transformation, FT4 level was analyzed as a continuous variable. Individuals on thyroid medication or with history of thyroid surgery were excluded.		Up to 72,167 subjects in meta-analysis	identified as independent SNPs. 61 and 31 independent SNPs were associated with TSH and FT4 (within reference range) respectively.
TSH (full range) (42)	A GWAS meta-analysis of TSH level of the HUNT study, the Michigan Genomics Initiative (MGI) biobank, and the ThyroidOmics consortium. In the HUNT study, individuals with thyroid disorders (based on self-report / blood tests / cancer registry data) were excluded. In the MGI biobank study, individuals with diagnosis of thyroid disorders in the electronic health records were excluded. For the studies in the ThyroidOmics consortium, individuals on thyroid medication or with history of thyroid surgery were excluded.	100% European	119,715	In the HUNT study, GWAS was conducted for the inverse-normalized TSH using linear mixed model, with adjustment for batch, first 15 principal components, age at TSH measurement and sex. In the MGI study, GWAS was conducted for the inverse-normalized TSH using linear regression model, with adjustment for the first four principal components, age and sex. The meta-analysis of the ThyroidOmics consortium was described above. Fixed-effect meta-analysis was subsequently conducted using METAL. LD-clumping using r^2 threshold of 0.2 and 500Kb window with reference to the European reference panel of the 1000 Genomes Project was performed. 99 independent genome-wide significant SNPs were identified.

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460 **Table 2.** Summary of genetic instruments adopted in the MR analyses.

	Exposure	Outcome	Number of genetic instruments included in MR analysis *	Variance of liability explained by the genetic instruments on exposure (%)	F-statistics (per instrument)
1	SARS-CoV-2 infection	Hyperthyroidism	17 (19-2-0)	0.6	478.88
2		Hypothyroidism	18 (19-1-0)	0.62	467.44
			Sensitivity analysis: 5 (5-0-0)	Sensitivity analysis: 0.29	Sensitivity analysis: 784.52
3		Autoimmune thyroid disease	19 (19-0-0)	0.65	464.41
4		TSH (within reference range)	16 (19-2-1)	0.53	449.13
5		FT4 (within reference range)	17 (19-2-0)	0.6	478.88
6		TSH (full range)	16 (19-1-2)	0.49	415.07
7	COVID-19 hospitalization	Hyperthyroidism	22 (28-6-0)	3.2	2,340.18
8		Hypothyroidism	20 (28-6-2)	2.98	2,391.79
9		Autoimmune thyroid disease	22 (28-6-0)	3.2	2,340.18
10		TSH (within reference range)	21 (28-6-1)	3.11	2,380.45
11		FT4 (within reference range)	22 (28-6-0)	3.2	2,340.18
12		TSH (full range)	21 (28-5-2)	3.14	2,404.16
13	COVID-19 severity	Hyperthyroidism	32 (40-8-0)	7.32	2,614.84
14		Hypothyroidism	32 (40-8-0)	7.32	2,614.84
15		Autoimmune thyroid disease	31 (40-7-2)	6.52	2,383.62
16		TSH (within reference range)	31 (40-8-1)	7.07	2,599.99
17		FT4 (within reference range)	32 (40-8-0)	7.32	2,614.84
18		TSH (full range)	36 (40-3-1)	8.25	2,646.14
19	Hyperthyroidism	SARS-CoV-2 infection	7 (8-1-0)	2.43	184.35
20		COVID-19 hospitalization	7 (8-1-0)	2.43	184.35
21		COVID-19 severity	8 (8-0-0)	2.69	179.04
22	Hypothyroidism	SARS-CoV-2 infection	8 (8-0-0)	1.85	125.85
23		COVID-19 hospitalization	8 (8-0-0)	1.85	125.85
24		COVID-19 severity	8 (8-0-0)	1.85	125.85

	Exposure	Outcome	Number of genetic instruments included in MR analysis *	Variance of liability explained by the genetic instruments on exposure (%)	F-statistics (per instrument)
25	Autoimmune thyroid disease	SARS-CoV-2 infection	88 (93-5-0)	4.66	419.52
26		COVID-19 hospitalization	87 (93-6-0)	4.58	416.71
27		COVID-19 severity	87 (93-6-0)	4.58	416.71
28	TSH (within reference range)	SARS-CoV-2 infection	57 (61-3-1)	8.98	93.87
29		COVID-19 hospitalization	57 (61-3-1)	9.02	94.32
30		COVID-19 severity	57 (61-3-1)	9.11	95.36
31	FT4 (within reference range)	SARS-CoV-2 infection	31 (31-0-0)	4.87	81.31
32		COVID-19 hospitalization	31 (31-0-0)	4.87	81.31
33		COVID-19 severity	31 (31-0-0)	4.87	81.31
34	TSH (full range)	SARS-CoV-2 infection	87 (99-10-2)	11.06	170.99
35		COVID-19 hospitalization	86 (99-12-1)	10.95	171.05
36		COVID-19 severity	89 (99-9-1)	11.59	176.2

*Number of genetic instruments adopted in MR analysis = Total number of independent genetic variants identified in GWAS of exposure – number of genetic variants excluded due to lack of proxies – number of outliers identified by MR-PRESSO