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Relationship between serum vitamin D levels and thyroid- and parathyroid-related diseases: a Mendelian randomisation study

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Abstract

Previous studies have indicated an association between vitamin D and thyroid- and parathyroid-related diseases. However, it remains unclear whether it is a cause of the disease, a side effect of treatment or a consequence of the disease. The Mendelian randomisation (MR) study strengthens the causal inference by controlling for non-heritable environmental confounders and reverse causation. In this study, a two-sample bidirectional MR analysis was conducted to investigate the causal relationship between serum vitamin D levels and thyroid- and parathyroid-related diseases. Inverse variance weighted, weighted median and MR-Egger methods were performed, the Cochran *Q* test was used to evaluate the heterogeneity and the MR-PRESSO and MR-Egger intercepts were utilised to assess the possibility of pleiotropy. The Bonferroni-corrected significance threshold was 0·0038. At the Bonferroni-corrected significance level, we found that vitamin D levels suggestively decreased the risk of benign parathyroid adenoma (OR = 0·244; 95 % CI 0·074, 0·802; $P = 0·0202$) in the MR analyses. In the reverse MR study, a genetically predicted risk of thyroid cancer suggestively increased the risk of elevated vitamin D (OR = 1·007; 95 % CI 1·010, 1·013; $P = 0·0284$), chronic thyroiditis significantly increased the risk of elevated vitamin D (OR = 1·007; 95 % CI 1·002, 1·011; $P = 0·0030$) and thyroid nodules was significantly decreased the vitamin D levels (OR = 0·991; 95 % CI 0·985, 0·997; $P = 0·0034$). The findings might be less susceptible to horizontal pleiotropy and heterogeneity ($P > 0·05$). This study from a gene perspective indicated that chronic thyroiditis and thyroid nodules may impact vitamin D levels, but the underlying mechanisms require further investigation.

Keywords: **Thyroid gland: Parathyroid glands: Thyroid cancer: Vitamin D: Mendelian randomisation study**

Vitamin D is a steroid hormone that regulates the metabolism of Ca, P and bone and promotes gene expression through endocrine and autocrine mechanisms[1]. 25-Hydroxyvitamin D is produced by the metabolism of vitamin D in the liver, and it is the main circulating form of vitamin D. Therefore, 25-hydroxyvitamin D can be used as a clinical indicator of vitamin D levels[2]. There is growing evidence that vitamin D may play a role in the development of a number of diseases, including cancer, diabetes, autoimmune diseases and infectious diseases[3–5]. The results of these studies indicated that vitamin D plays a significant role in Ca homeostasis, immunomodulation and anti-inflammation.

Some publicly available studies have reported an association between vitamin D and the development of autoimmune thyroid disease, but the results have been inconclusive[6–10]. For example, a study conducted by Kivity *et al.* revealed that the prevalence of vitamin D deficiency was considerably greater among 50 patients with autoimmune thyroid disease than among 98 healthy individuals (72 % *v.* 30·6 %; $P < 0·001$)[6]. In a recent 5-year randomised controlled trial conducted in the USA, 25 871 participants were followed, and a 22 % reduction in autoimmune disease incidence was observed after vitamin D supplementation after 5 years[10]. The association between vitamin D and autoimmune diseases may be related to variations in the vitamin D receptor gene, which is involved in vitamin D function[11,12]. Nevertheless, a study conducted by D'Aurizio *et al.* revealed that there was no statistically significant difference in vitamin D levels between patients with autoimmune thyroid disease and the general population[13]. Furthermore, vitamin D has been reported to reduce the incidence of thyroid cancer. Vitamin D can play a role through the following mechanisms: increasing apoptosis, arresting the cell cycle, inhibiting proliferation and differentiation, decreasing the inflammatory response and decreasing aggressiveness[14–16]. The parathyroid gland is a

Abbreviations: GWAS, genome-wide association study; IVW, inverse variance weighted; SNP, IVW; inverse variance weighted; MR, Mendelian randomisation.

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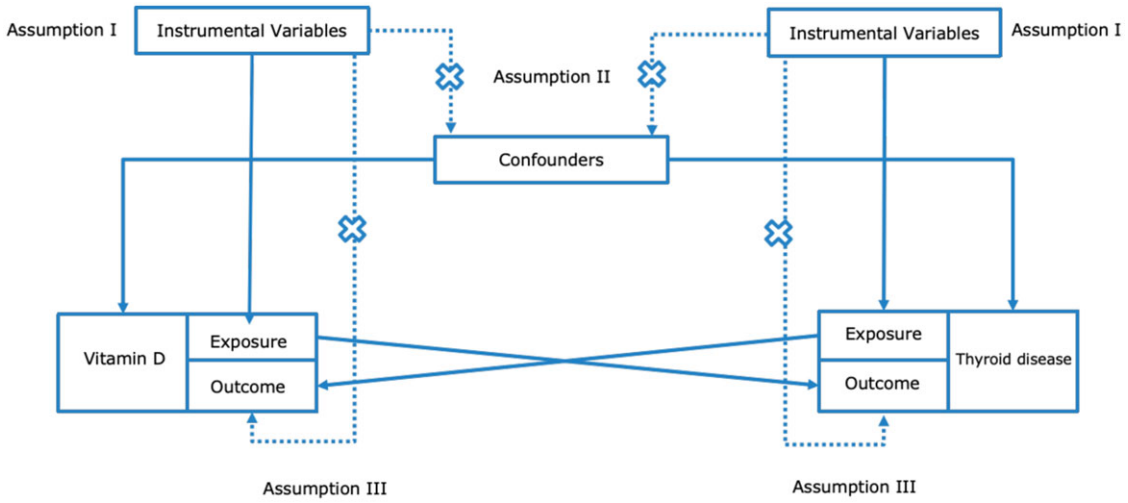

Fig. 1. An overview of the Mendelian randomisation study design.

direct target of vitamin D. Parathyroid cells express both the vitamin D receptor and 1-α-hydroxylase. The existing literature indicates that vitamin D metabolites exert an influence on parathyroid hormone secretion and might act to prevent parathyroid cell proliferation[17]. The majority of published studies have focused on the impact of vitamin D on autoimmune thyroid disease and thyroid cancer. Few studies have explored the effects of vitamin D on other thyroid- and parathyroid-related diseases.

Data on vitamin D and thyroid- and parathyroid-related diseases derived from observational studies will inevitably be affected by factors such as sample size, ethnicity and other confounding variables, making causal inference challenging. Mendelian randomisation (MR) is a highly effective methodology for investigating the causal relationship between exposure and disease since disease status typically does not alter the germline DNA sequences[18]. This is accomplished by utilising genetic variation as an instrumental variable (IV)[19]. Genetic variants are randomly allocated during meiosis, much like a random assignment in a randomised controlled trial; additionally, the genetic variants undergo minimal changes throughout an individual's lifetime[20], which minimises unmeasured confounding factors and biases caused by reverse causation[21]. Previous MR studies have demonstrated that there is no causal relationship between serum vitamin D levels and the development of Graves' disease or thyroid cancer[22,23]. The causal relationships between vitamin D and other thyroid- and parathyroid-related diseases are unclear. It is not clear what role vitamin D plays in the development of thyroid- and parathyroid-related diseases, whether it is a cause of the disease, a side effect of treatment or a consequence of the disease[24]. The MR studies can clarify the relationship between vitamin D and thyroid- and parathyroid-related diseases from a gene- and bidirectional perspective, which can facilitate the optimisation of disease prevention and management strategies.

Therefore, we performed a two-sample bidirectional MR to comprehensively evaluate the causal relationships between vitamin D levels and diseases related to the thyroid and parathyroid glands using an extensive genome-wide association study (GWAS, which is a strategy that is used in the analysis of complex traits. GWAS involves the scanning of the genome for millions of SNP molecular markers, with the aim of identifying genotypic and phenotypic correlations that affect these traits[25]).

Methods

Data availability and ethics statement

This study involved a secondary examination of publicly available data, and it did not involve new human or animal research. All utilised GWAS datasets that were used are openly accessible, negating the need for ethical approval or informed consent. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomisation reporting guidelines[26].

Study design

To examine the possible causal relationship between vitamin D levels and thyroid- and parathyroid-related diseases, we conducted a two-way, bidirectional MR analysis. The use of MR necessitates satisfying three fundamental assumptions[27]: (i) association, where the chosen IV must be highly connected to the exposure; (ii) exclusivity, where the IV can solely influence the outcome via the exposure; and (iii) independence, where confounding factors cannot impact the effect of exposure on the outcome. Fulfilling these three criteria allows us to estimate the exposure-outcome relationship, utilising the obtained IV[20]. The framework is presented in Fig. 1.

Exposure and outcome data sources

Summary statistics on vitamin D levels were extracted from the UK Biobank[28], comprising phenotypic, genotypic and clinical data for 417 580 individuals of European descent (age range 40–69 years), with ID number 'ebi-a-GCST90000614'. Since vitamin D from any source is quickly transformed into 25(OH)D, which

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Table 1. Detailed description of the genome-wide association study database in this study

Trait	Database	Number of cases	Number of controls	Populations
Serum vitamin D levels	UK Biobank	Sample size: 417 580		European
Graves' disease	FinnGen Biobank	1828	279 855	European
Hashimoto's thyroiditis	FinnGen Biobank	40 926	274 069	European
Thyroid cancer	FinnGen Biobank	1783	287 137	European
Papillary adenocarcinoma of the thyroid gland	FinnGen Biobank	1386	287 137	European
Follicular adenocarcinoma of the thyroid gland	FinnGen Biobank	149	369 104	European
Acute thyroiditis	FinnGen Biobank	109	320 703	European
Subacute thyroiditis	FinnGen Biobank	742	320 703	European
Chronic thyroiditis	FinnGen Biobank	108	320 703	European
Thyroid nodule	FinnGen Biobank	2027	320 703	European
Benign thyroid adenomas	FinnGen Biobank	875	376 402	European
Benign parathyroid adenoma	FinnGen Biobank	274	377 003	European
Hyperparathyroidism	FinnGen Biobank	5590	361 988	European
Hypoparathyroidism	FinnGen Biobank	899	361 988	European

has a long half-life of approximately 2 weeks and is a crucial precursor of active hormones, it is generally accepted that the serum 25(OH)D levels represent the levels of vitamin D that are stored in the body and the serum 25(OH)D levels are the optimal indicator for estimating vitamin D nutritional intake[2]. In this study, the serum 25(OH)D concentration (nmol/l) was measured using the LIAISON XL 25(OH)D assay (DiaSorin). The median, mean and interquartile range of serum 25(OH)D levels were 47·9, 49·6 and 33·5–63·2 nmol/l, respectively. Serum 25(OH)D concentrations below 25 nmol/l were considered to indicate vitamin D deficiency[29].

To eliminate potential bias caused by overlapping samples in terms of both exposure and outcomes, we collected summary statistics on conditions involving the thyroid (such as autoimmune diseases, thyroid cancer, thyroiditis and thyroid nodules) and parathyroid glands from the FinnGen Biobank. FinnGen Biobank conducted a genomics and personalised medicine research initiative that involved by examining genomic and health data taken from 500 000 Finnish biobanks to understand the genetic basis of disease[30]. Specific GWAS summary data are provided in Table 1. To prevent differences in ethnicity from impacting pleiotropy, the biobank included only study participants of European origin. Using the summary data from GWAS, an independent study employing two-sample bidirectional MR was conducted to investigate the relationship between serum vitamin D levels and thyroid- and parathyroid-related diseases.

Mendelian randomisation analysis

Selection of instrumental variables. Instrumental variables were determined through various processes. First, to meet the association assumption, a threshold of $P < 5 \times 10^{-8}$ was established, and the threshold was relaxed to 5×10^{-6} or 1×10^{-5} when adequate SNP, which primarily DNA sequence polymorphisms that result from variation in a single nucleotide at the genomic level[31], were not available for analysis at the $P < 5 \times 10^{-8}$ threshold. Second, to minimise the occurrence of multiple results due to linkage disequilibrium, linkage disequilibrium analyses were conducted ($r^2 < 0{\cdot}001$, kb = 10 000) in accordance with the required independence assumptions. The linkage disequilibrium levels were then obtained from the European samples of the 1000 Genomes Project[32]. To ensure that the impact of selected SNP on both exposure and outcome aligned with the corresponding alleles (an allele is one of two or more versions of the DNA sequence at a given genomic location[33]), we eliminated the palindromic structure and utilised surrogate SNP when relevant SNP were absent from the GWAS dataset for outcome. Confounders not associated with SNP (including other types of thyroid disease, other vitamin levels, smoking, alcohol consumption, obesity, etc.) were manually removed by PhenoScanner[34]. The direction of causality between exposure and outcome was evaluated using Steiger filtering. If the IV met the criteria, the instrument's direction was 'TRUE', and SNP with a direction of 'FALSE' were excluded[35]. To assess the strength of the IV, we calculated the *F*-statistic (*F* reflects the bias of an IV or a set of IV[36]), using the formula[37] $\left(\frac{n-k-1}{k}\right)\left(\frac{R^2}{1-R^2}\right)$, with R^2 representing the proportion of variance explained by the IV, n representing sample size and k representing the number of SNP[38]. An *F*-statistic greater than 10 indicates that the IV may be resistant to the effects of weak instrumental bias. This analysis adheres to the conventional academic structure and employs clear, objective language with precise technical terms[39].

Statistical analysis. All the MR analyses were conducted using R version 4.3.1 (The R Foundation for Statistical Computing). The estimation of causal relationships was conducted using the 'TwoSampleMR' (version 0.5.7), 'MR-PRESSO' (version 1.0), 'ggplot2' (version 3.4.3), 'plyr' (version 1.8.8) and 'phenoscanner' (version 1.0) packages. The MR methods applied included the inverse variance weighted (IVW), weighted median and MR-Egger methods. The IVW method assumed that all IV met the validity criteria to obtain unbiased estimates[40]; specifically, it assumed that all SNP were not related to the pleiotropic effect of exposure (known as the InSIDE hypothesis)[41], which had the highest test efficacy. When the weighted median method assumed that only more than half of the IV were unbiased[42], the IVW method was the primary reference for obtaining results. The weighted median and MR-Egger methods were used to

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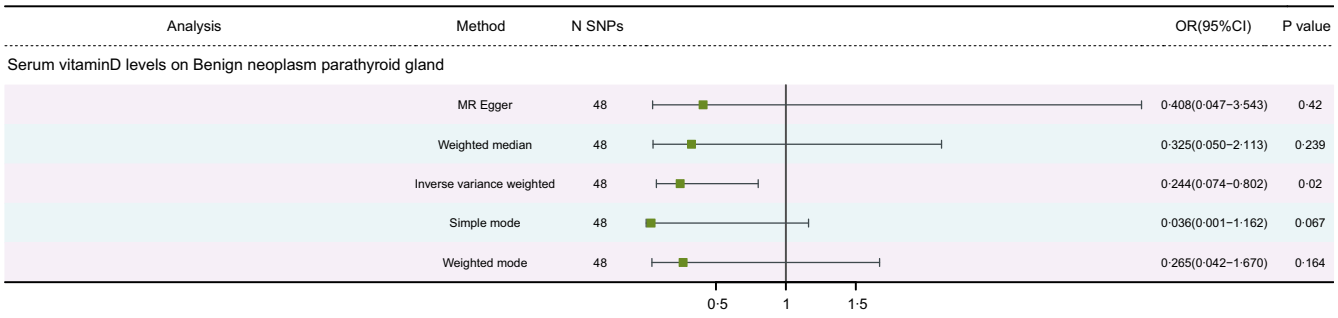

Fig. 2. The Mendelian randomisation findings of serum vitamin D levels and benign parathyroid adenoma.

complement the IVW analysis. Causal effects were assessed as OR, indicating an increased risk of outcome for each increase in the exposure log ratio. Our sensitivity analyses used four main methods, including Cochran's *Q* test, MR-Egger intercept analysis, MR-PRESSO and the leave-one-out sensitivity test. Horizontal pleiotropy was evaluated through the MR-Egger test's intercept, with a significance threshold of $P < 0{\cdot}05$ denoting the existence of horizontal pleiotropy and the intercept term's value distance from 0 indicating the magnitude of horizontal pleiotropy[43]. Heterogeneity was evaluated using the Cochran *Q* statistic, whereby a *P* value of less than 0·05 indicated the existence of heterogeneity[44]. When heterogeneity was detected, we utilised MR-PRESSO to identify potential outliers and then removed them before re-evaluating causality with the remaining SNP[45]. The 'leave-one-out' test was utilised to evaluate the impact of a single SNP on the analysis, and this test enhanced the robustness of the outcomes[20]. We performed RadialMR analysis using modified second-order weights to identify outliers[46]. Radial plots offered advantages in identifying peripheral studies, detecting small study biases and identifying outliers more directly than traditional scatter plots.

The outcomes of the MR analyses are presented as scatter plots, forest plots, 'leave-one-out' plots and funnel plots. Each point in the scatter plots represents an SNP, thereby demonstrating the association of that SNP with exposure and outcome. The forest plot comprised horizontal lines and points, with each line representing the effect size of an SNP and its 95 % CI. Given the lack of robustness of the results for individual SNP, it was necessary to combine them, and this is represented by the bottom red line (All − IVW). The leave-one-out forest plot was used to calculate the meta-effect of the remaining SNP after removing each SNP one by one. If all the error lines were consistent to the right or left of 0, the results were deemed reliable. The funnel plot was generated to determine whether the points situated on either side of the IVW line were approximately symmetrical. The presence of any outlying points indicated the potential for outliers, which could be removed, and the analysis process was repeated.

To consider multiple testing, we used a conservative approach and applied a Bonferroni-corrected significance level of 0·05 divided by 13 (1 exposure × 13 outcomes, i.e. 0·0038)[47]. $P < 0{\cdot}0038$ was considered to indicate a significant association. A $P < 0{\cdot}05$ but above the Bonferroni-corrected significance threshold was considered to indicate a potential association.

Results

Causal association of serum vitamin D levels on thyroid- and parathyroid-related diseases

Mendelian randomisation analysis of serum vitamin D levels on thyroid- and parathyroid-related diseases. Forty-eight vitamin D alleles were linked to benign parathyroid adenoma. The *F*-statistic for assessing the relationship of serum vitamin D levels to benign parathyroid adenoma ranged from 25 to 1220, indicating the absence of weak IV (online Supplementary Table S1). After applying the Bonferroni correction, MR estimation through the IVW method revealed that serum vitamin D levels was suggestively decreased the risk of benign parathyroid adenoma (OR = 0·244; 95 % CI 0·074, 0·802; $P = 0{\cdot}0202$). The MR-Egger and weighted median OR were also less than 1, and the *P* value was less than 0·05, further validating our findings (Fig. 2).

Based on the IVW method, the MR estimation did not reveal a causal association of vitamin D with other thyroid- and parathyroid-related diseases (all *P* values were greater than 0·05) (online Supplementary Table S2). The *F*-statistics of the serum vitamin D levels for various thyroid- and parathyroid-related diseases were all greater than 10.

Sensitivity analysis and visualisation of results of serum vitamin D levels on thyroid- and parathyroid-related diseases. The selected SNP indicated no heterogeneity or pleiotropy (Cochran's Q *P* value, MR-Egger intercept *P* value and MR-PRESSO test *P* value were all greater than 0·05) (Table 2). The leave-one-out analysis indicated that none of the SNP exerted a dominant influence on the effect of serum vitamin D levels on benign parathyroid adenomas (Fig. 3(c)). RadialMR detected two outliers (online Supplementary Fig. S1) After excluding the outliers that were detected by the RadialMR analysis, the MR estimates did not change significantly (OR = 0·254; 95 % CI 0·076, 0·847; $P = 0{\cdot}026$). The funnel plot displayed an even distribution of points, indicating that causal associations were less susceptible to potential bias (Fig. 3(d)).

Causal association of thyroid- and parathyroid-related diseases on serum vitamin D levels

Mendelian randomisation analysis of thyroid- and parathyroid-related diseases on serum vitamin D levels.

Table 2. Variability and diversity of findings exist regarding the relationship between serum vitamin D levels and benign parathyroid adenoma, thyroid cancer, chronic thyroiditis and thyroid nodule

Exposure	Outcome	MR-Egger *P*	Cochran *Q*-value	MR-PRESSO *P*
Serum vitamin D levels	Benign parathyroid adenoma	0·58	0·65	0·67
Thyroid cancer	Serum vitamin D levels	0·23	0·42	0·40
Chronic thyroiditis	Serum vitamin D levels	0·91	0·98	Not enough SNP
Thyroid nodule	Serum vitamin D levels	0·91	0·31	0·33

Five thyroid cancer alleles, three chronic thyroiditis alleles and nineteen thyroid nodule alleles were associated with serum vitamin D levels. The *F*-statistics were greater than 10, indicating that there were strong associations of thyroid cancer, chronic thyroiditis and thyroid nodules with serum levels of vitamin D. Additionally, the results indicated a low probability of being influenced by weak IV (online Supplementary Table S3–5) After applying the Bonferroni correction, MR estimation using the IVW approach suggested genetically predicted risk of thyroid cancer was suggestively increased the risk of elevated vitamin D (OR = 1·007; 95 % CI 1·010, 1·013; *P* = 0·0284), and chronic thyroiditis was significantly increased the risk of elevated vitamin D (OR = 1·007; 95 % CI 1·002, 1·011; *P* = 0. 0030); thyroid nodules were significantly decreased the vitamin D levels (OR = 0·991; 95 % CI 0·985, 0·997; *P* = 0·0034). Both the MR-Egger and weighted median OR and *P* values were consistent with the results of IVW results, further confirming our findings (Fig. 4).

Based on the IVW method, the MR estimation did not reveal a causal effect of other thyroid and parathyroid diseases on serum vitamin D levels (all *P* values greater than 0·05) (online Supplementary Table S2). The *F*-statistics of various thyroid- and parathyroid-related diseases were all greater than 10.

Sensitivity analysis and visualisation of results of thyroid- and parathyroid-related diseases on serum vitamin D levels.

No heterogeneity or pleiotropy was observed among the chosen SNP (Cochran's Q *P* values, MR-Egger intercept *P* values and MR-PRESSO test *P* values, except for the SNP linked to chronic thyroiditis, which was inadequate for MR-PRESSO) (Table 2). Furthermore, the leave-one-out method indicated that none of the SNP played a dominant role in the causal associations of thyroid cancer, chronic thyroiditis or thyroid nodules on serum vitamin D levels (Fig. 5–7(c)). RadialMR did not detect an outlier (online Supplementary Fig. S2–4). The funnel plot displayed an even distribution of points, indicating that causal associations are less susceptible to potential bias (Fig. 5–7(d)).

Discussion

To the best of our knowledge, this is the first comprehensive study using a bidirectional MR approach to investigate the relationship between serum vitamin D levels and thyroid- and parathyroid-related diseases. Our genetic analysis revealed an inverse suggestive association of vitamin D levels on parathyroid tumour development. However, our reverse MR analysis revealed a genetically suggestive causal association of thyroid cancer on serum vitamin D levels and significant causal associations of chronic thyroiditis and thyroid nodules on serum vitamin D levels. Additionally, we did not observe the causal effect between serum vitamin D levels and other thyroid and parathyroid diseases. These findings highlighted the role of vitamin D levels in potential disease intervention, prognosis and monitoring goals.

Our research indicated that vitamin D has a potential role in benign parathyroid adenoma development. Active vitamin D has been demonstrated to hinder parathyroid cell growth both *in vitro* and *in vivo*[48,49]. Furthermore, it can impede cell cycle progression and contribute to multiple cellular pathways that are involved in tumour formation[50,51]. A meta-analysis revealed a negative correlation between the 25(OH)D concentration and total cancer incidence and mortality[52]. Additionally, the results of a recent animal study indicated that the growth of parathyroid tumours was accelerated by vitamin D deficiency[53]. These studies were consistent with our findings. Our research also indicated that thyroid cancer had a suggestive association on vitamin D levels. Most previous studies have focused on the low serum concentration of vitamin D in patients with thyroid cancer[54]. However, as multiple recent studies have suggested the anti-tumour effects of vitamin D[50,51,55], further studies are necessary to validate these findings.

The results of our study indicated that thyroid nodules and chronic thyroiditis might have a causal association on vitamin D. Patients with thyroid nodules had serum vitamin D levels that were lower than the normal range[56,57]. Kim *et al.* discovered that patients with larger nodules had lower vitamin D levels and that there was a negative correlation between vitamin D levels and nodule diameter[58]. There are several potential explanations for the causal effect of thyroid nodules and chronic thyroiditis on vitamin D levels. A review of clinical data indicated that vitamin D deficiency was a common occurrence in patients with thyroid disease. Research has indicated that ethnicity, geography and seasonal factors might be associated with vitamin D deficiency, but the precise effect of each factor remains unclear[59,60]. Furthermore, thyroid-stimulating hormone levels have been proposed to be a risk factor for thyroid nodules[61]. Additionally, vitamin D deficiency has been linked to an elevated risk of impaired thyroid hormone sensitivity[62], which suggests that there might be an association between thyroid-stimulating hormone, thyroid nodules and vitamin D. However, the underlying mechanisms of the effects of thyroid nodules and chronic thyroiditis on vitamin D remain unclear and require further investigation.

In conclusion, the complex mechanisms that link vitamin D to thyroid- and parathyroid-related diseases necessitate further

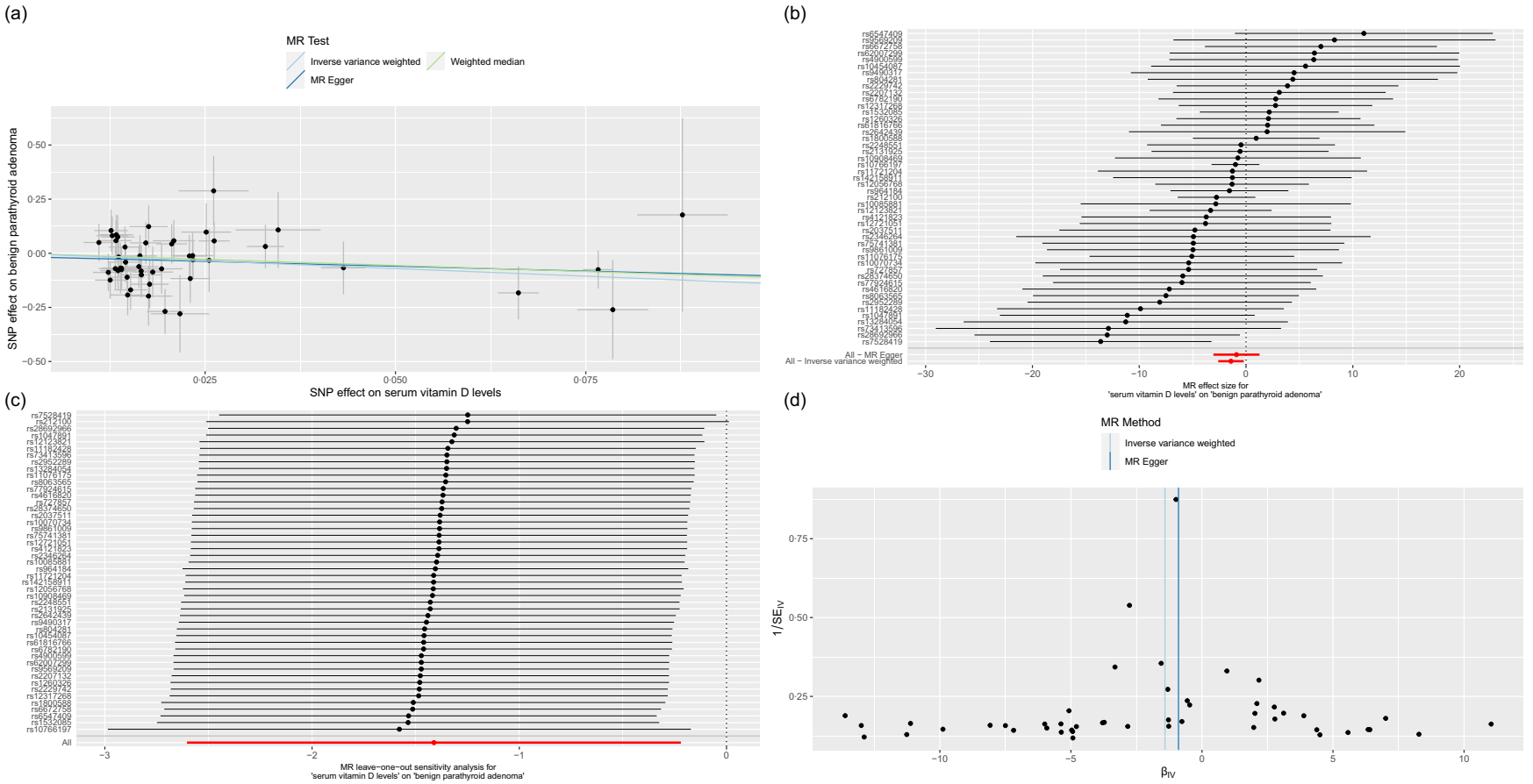

Fig. 3. The relevant plot displaying the relationship between serum vitamin D levels and benign parathyroid adenoma. Scatter plots (a) showing the Mendelian randomisation (MR) effect of each exposure on benign parathyroid adenoma. Individual inverse variance (IV) associations with serum vitamin D levels risk are displayed *v.* individual IV associations with benign parathyroid adenoma in black dots. The 95 % CI of OR for each IV is shown by vertical and horizontal lines. The slopes of the lines represent the estimated causal effects of the MR methods. Forest plots (b) show the susceptibility to the risk of benign parathyroid adenoma; the red points show the combined causal estimate using all SNP together in a single instrument, using two different methods (MR-Egger and inverse variance weighted). Leave-one-out plots (c) for serum vitamin D levels on benign parathyroid adenoma. If all the error lines were consistent to the right or left of 0, the results were deemed reliable. Funnel plots (d) showing the inverse variance weighted MR estimates of serum vitamin D levels and SNP in patients with benign parathyroid adenoma *v.* 1/SE ($1/SE_{IV}$).

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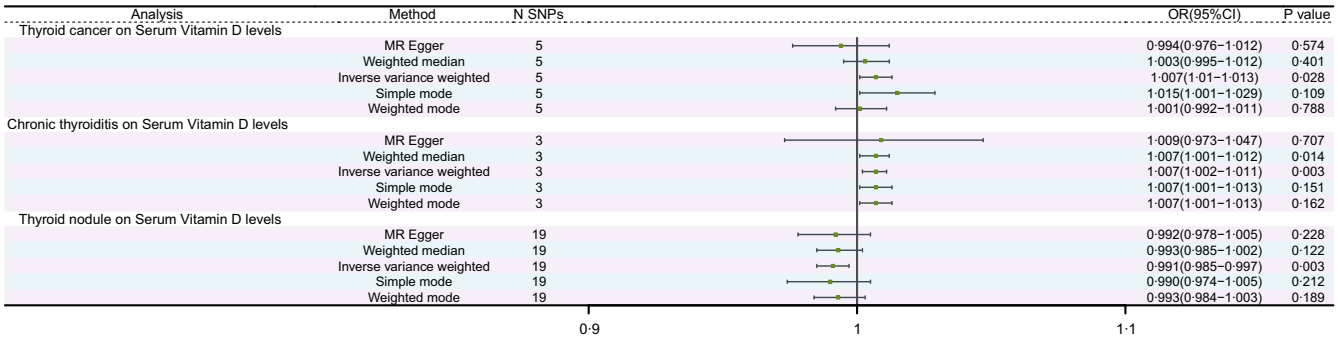

Fig. 4. The Mendelian randomisation results of thyroid cancer, chronic thyroiditis and thyroid nodules with serum vitamin D levels.

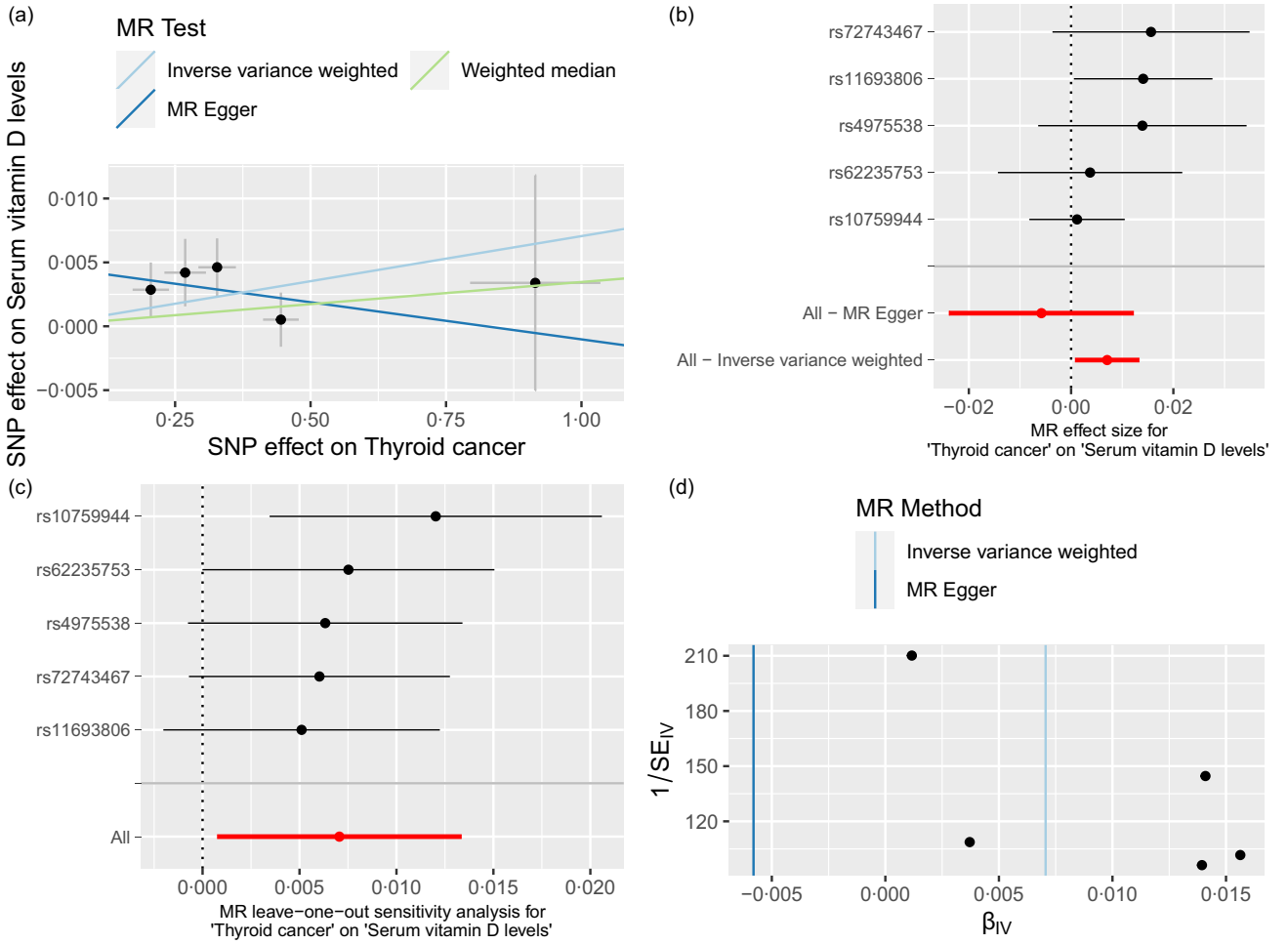

Fig. 5. The relevant plot displaying the relationship between thyroid cancer and serum vitamin D levels. Scatter plots (a) showing the Mendelian randomisation (MR) effect of each exposure on serum vitamin D levels. Individual inverse variance (IV) associations with thyroid cancer risk are displayed *v.* individual IV associations with serum vitamin D levels in black dots. The 95 % CI of OR for each IV is shown by vertical and horizontal lines. The slopes of the lines represent the estimated causal effects of the MR methods. Forest plots (b) showing the susceptibility to the risk of serum vitamin D levels; the red points show the combined causal estimate using all SNP together in a single instrument, using two different methods (MR-Egger and inverse variance weighted). Leave-one-out plots (c) for thyroid cancer on serum vitamin D levels. If all the error lines were consistent to the right or left of 0, the results are deemed reliable. Funnel plots (d) showing the inverse variance weighted MR estimates of thyroid cancer SNP with serum vitamin D levels *v.* $1/\mathrm{SE}$ ($1/\mathrm{SE}_{\mathrm{IV}}$).

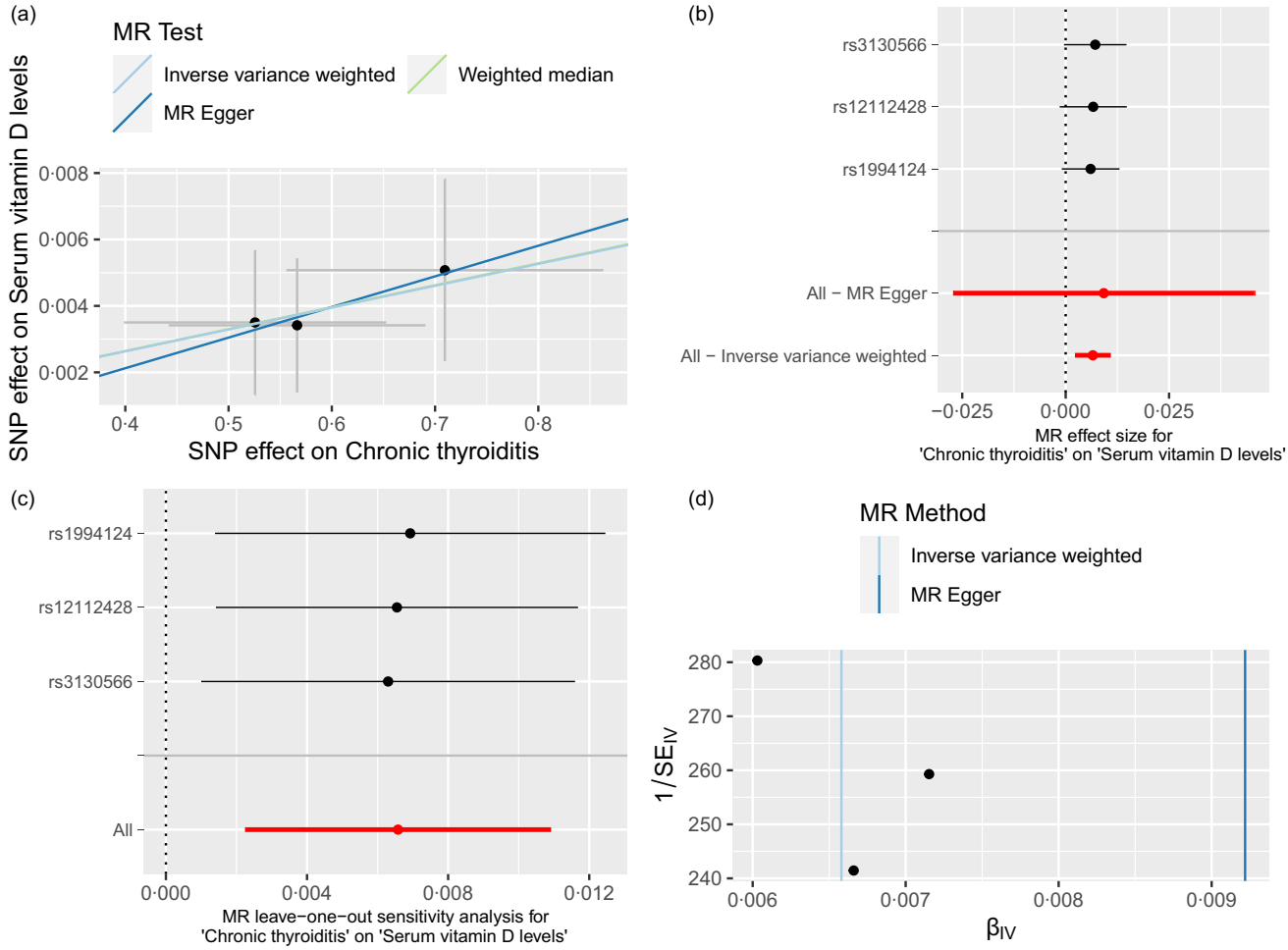

Fig. 6. The relevant plot displaying the relationship between chronic thyroiditis and serum vitamin D levels. Scatter plots (a) showing the Mendelian randomisation (MR) effect of each exposure on serum vitamin D levels. Individual inverse variance (IV) associations with thyroid cancer risk are displayed *v.* individual IV associations with serum vitamin D levels in black dots. The 95 % CI of OR for each IV is shown by vertical and horizontal lines. The slopes of the lines represent the estimated causal effects of the MR methods. Forest plots (b) showing the susceptibility to the risk of serum vitamin D levels; the red points show the combined causal estimate using all SNP together in a single instrument, using two different methods (MR-Egger and inverse variance weighted). Leave-one-out plots (c) for chronic thyroiditis on serum vitamin D levels. If all the error lines were consistent to the right or left of 0, the results are deemed reliable. Funnel plots (d) showing the inverse variance weighted MR estimates of chronic thyroiditis SNP with serum vitamin D levels *v.* $1/\text{SE}$ ($1/\text{SE}_{\text{IV}}$).

investigation. Observational studies suffer from reverse causality and are limited by confounding factors, thereby limiting their ability to identify aetiologic explanations[63]. The present study used MR to minimise confounding factors and establish a causal association. The strengths of the study included the use of MR, which overcomes the limitations of observational studies in terms of confounders and causality associations. Additionally, all *F*-statistics were greater than 10, indicating a reduced susceptibility to weak instrument bias.

However, our study had several limitations. First, the data from our study were obtained from summary GWAS data, and specific information necessary for further analysing age, sex and time of blood collection among the study population is lacking. Second, the data in the FinnGen database were derived from the primary diagnosis of the study population. However, patients might have additional comorbidities, which could lead to biased results. Third, because the number of SNP included in some diseases was insufficient for MR analysis, the thresholds for selecting SNP were appropriately lowered. Additionally, some of the available SNP for the exposure-disease associations were low, which may have affected the study conclusions. Fourth, while we have employed various approaches to minimise pleiotropy, potential unidentified pathways and confounders between exposure and outcome might still lead to inaccuracies in our findings. Fifth, the study subjects were primarily of European origin (the study's participants were primarily from FinnGen and the UK database), which limits the generalisability of the study findings to the broader European population, and there may be genetic variations between different races. Whether the findings from this research can be extended to other racial groups remains uncertain, and additional corroboration of the outcomes is necessary in the future.

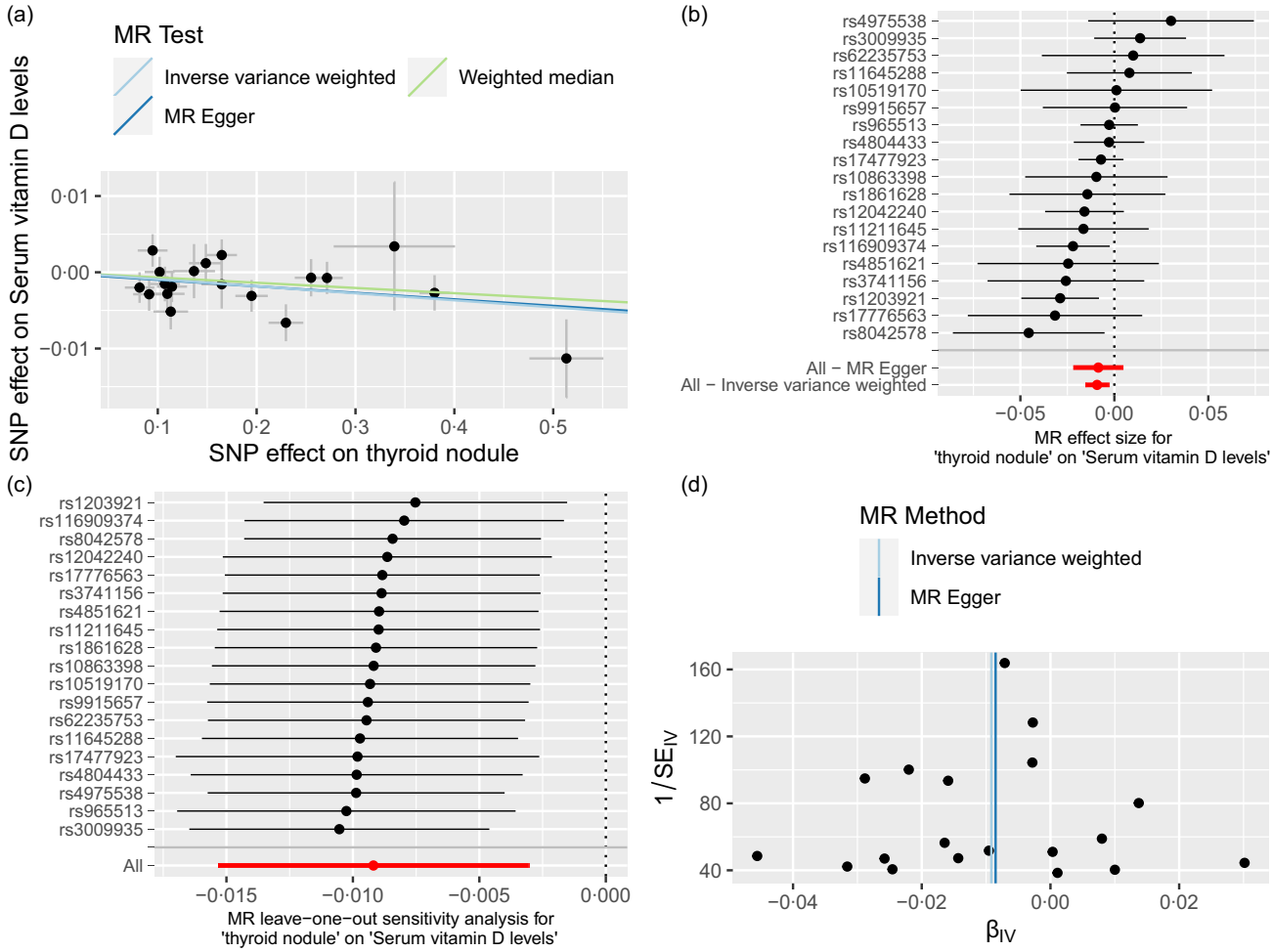

Fig. 7. The relevant plot displaying the relationship between thyroid nodules and Serum vitamin D levels. Scatter plots (a) showing the Mendelian randomisation (MR) effect of each exposure on serum vitamin D levels. Individual inverse variance (IV) associations with thyroid cancer risk are displayed *v.* individual IV associations serum vitamin D levels in black dots. The 95 % CI of OR for each IV is shown by vertical and horizontal lines. The slopes of the lines represent the estimated causal effects of the MR methods. Forest plots (b) showing the susceptibility to the risk of serum vitamin D levels; the red points showed the combined causal estimate using all SNP together in a single instrument, using two different methods (MR-Egger and inverse variance weighted). Leave-one-out plots (c) for thyroid nodules on serum vitamin D levels. If all the error lines were consistent to the right or left of 0, the results are deemed reliable. Funnel plots (d) showing the inverse variance weighted MR estimates of thyroid nodules SNP with serum vitamin D levels *v.* 1/SE ($1/SE_{IV}$).

Conclusion

Our study confirmed that chronic thyroiditis and thyroid nodules impact the vitamin D levels, although the underlying mechanisms require further investigation. However, the relationships of serum vitamin D levels on benign parathyroid adenoma and the relationship of thyroid cancer on vitamin D levels still need to be examined in larger multicentre GWAS that include more SNP to validate or reassess our conclusions. Moreover, our study did not reveal a causal relationship between vitamin D and other thyroid- and parathyroid-related diseases.

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Information on the datasets covered in this manuscript can be found in https://gwas.mrcieu.ac./datasets/ebi-a-GCST90000614/uk and r9.risteys.finngen.fi.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524001843.

References

1. Heaney RP (2008) Vitamin D in health and disease. *Clin J Am Soc Nephrol* **3**, 1535–1541.
2. Bouillon R, Antonio L & Olarte OR (2022) Calcifediol (25OH Vitamin D(3)) deficiency: a risk factor from early to old age. *Nutrients* **14**, 1168.
3. Manson JE, Cook NR, Lee IM, *et al.* (2019) Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med* **380**, 33–44.
4. Grammatiki M, Rapti E, Karras S, *et al.* (2017) Vitamin D and diabetes mellitus: causal or casual association? *Rev Endocr Metab Disord* **18**, 227–241.
5. Sîrbe C, Rednic S, Grama A, *et al.* (2022) An update on the effects of vitamin D on the immune system and autoimmune diseases. *Int J Mol Sci* **23**, 9784.
6. Kivity S, Agmon-Levin N, Zisappl M, *et al.* (2011) Vitamin D and autoimmune thyroid diseases. *Cell Mol Immunol* **8**, 243–247.
7. Unal AD, Tarcin O, Parildar H, *et al.* (2014) Vitamin D deficiency is related to thyroid antibodies in autoimmune thyroiditis. *Cent Eur J Immunol* **39**, 493–497.
8. Wang J, Lv S, Chen G, *et al.* (2015) Meta-analysis of the association between vitamin D and autoimmune thyroid disease. *Nutrients* **7**, 2485–2498.
9. Bolat H & Erdoğan A (2022) Benign nodules of the thyroid gland and 25-hydroxy-vitamin D levels in Euthyroid patients. *Ann Saudi Med* **42**, 83–88.
10. Hahn J, Cook NR, Alexander EK, *et al.* (2022) Vitamin D and marine *n*-3 fatty acid supplementation and incident autoimmune disease: VITAL randomized controlled trial. *BMJ* **376**, e066452.
11. Feng M, Li H, Chen SF, *et al.* (2013) Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. *Endocrine* **43**, 318–326.
12. Inoue N, Watanabe M, Ishido N, *et al.* (2014) The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. *Clin Exp Immunol* **178**, 262–269.
13. D'Aurizio F, Villalta D, Metus P, *et al.* (2015) Is vitamin D a player or not in the pathophysiology of autoimmune thyroid diseases? *Autoimmun Rev* **14**, 363–369.
14. Hansen CM, Binderup L, Hamberg KJ, *et al.* (2001) Vitamin D and cancer: effects of 1,25(OH)2D3 and its analogs on growth control and tumorigenesis. *Front Biosci* **6**, D820–D848.
15. Clinckspoor I, Verlinden L, Mathieu C, *et al.* (2013) Vitamin D in thyroid tumorigenesis and development. *Prog Histochem Cytochem* **48**, 65–98.
16. Liu W, Zhang L, Xu HJ, *et al.* (2018) The anti-inflammatory effects of vitamin D in tumorigenesis. *Int J Mol Sci* **19**, 2736.
17. Bienaimé F, Prié D, Friedlander G, *et al.* (2011) Vitamin D metabolism and activity in the parathyroid gland. *Mol Cell Endocrinol* **347**, 30–41.
18. Nattel S (2013) Canadian Journal of Cardiology January 2013: genetics and more. *Can J Cardiol* **29**, 1–2.
19. O'Donnell CJ & Sabatine MS (2018) Opportunities and challenges in Mendelian randomization studies to guide trial design. *JAMA Cardiol* **3**, 967.
20. Zheng J, Baird D, Borges MC, *et al.* (2017) Recent developments in Mendelian randomization studies. *Curr Epidemiol Rep* **4**, 330–345.
21. Sanderson E, Glymour MM, Holmes MV, *et al.* (2022) Mendelian randomization. *Nat Rev Methods Primers* **2**, 6.
22. Yu Y, Yang X, Wu J, *et al.* (2023) A Mendelian randomization study of the effect of serum 25-hydroxyvitamin D levels on autoimmune thyroid disease. *Front Immunol* **14**, 1298708.
23. Shen J, Zhang H, Jiang H, *et al.* (2024) The effect of micronutrient on thyroid cancer risk: a Mendelian randomization study. *Front Nutr* **11**, 1331172.
24. Vieira IH, Rodrigues D & Paiva I (2020) Vitamin D and autoimmune thyroid disease-cause, consequence, or a vicious cycle? *Nutrients* **12**, 2791.
25. Flint J (2013) Genome-wide association study. *Curr Biol* **23**, R265–R266.
26. Skrivankova VW, Richmond RC, Woolf BAR, *et al.* (2021) Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA* **326**, 1614–1621.
27. König IR & Greco FMD (2018) Mendelian randomization: progressing towards understanding causality. *Ann Neurol* **84**, 176–177.
28. Collins R (2012) What makes UK Biobank special? *Lancet* **379**, 1173–1174.
29. Revez JA, Lin T, Qiao Z, *et al.* (2020) Genome-wide association study identifies 143 loci associated with 25 hydroxyvitamin D concentration. *Nat Commun* **11**, 1647.
30. FINNGEN. (2015) https://www.finngen.fi/en/node/17 (accessed May 2024).
31. Wikipedia Single-Nucleotide Polymorphism. (2015) https://en.wikipedia.org/wiki/Single-nucleotide_polymorphism (accessed May 2024).
32. Abecasis GR, Altshuler D, Auton A, *et al.* (2010) A map of human genome variation from population-scale sequencing. *Nature* **467**, 1061–1073.
33. National Human Genome Research Institute Allele. (2021). https://www.genome.gov/genetics-glossary/Allele (accessed May 2024).
34. Kamat MA, Blackshaw JA, Young R, *et al.* (2019) PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. *Bioinformatics* **35**, 4851–4853.
35. Hemani G, Tilling K & Davey Smith G (2017) Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet* **13**, e1007081.
36. Pierce BL, Ahsan H & Vanderweele TJ (2011) Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol* **40**, 740–752.
37. Burgess S & Thompson SG (2011) Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* **40**, 755–764.
38. Papadimitriou N, Dimou N, Tsilidis KK, *et al.* (2020) Physical activity and risks of breast and colorectal cancer: a Mendelian randomisation analysis. *Nat Commun* **11**, 597.
39. Burgess S & Thompson SG (2012) Improving bias and coverage in instrumental variable analysis with weak instruments for continuous and binary outcomes. *Stat Med* **31**, 1582–1600.
40. Burgess S, Butterworth A & Thompson SG (2013) Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* **37**, 658–665.
41. Burgess S & Thompson SG (2017) Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol* **32**, 377–389.
42. Bowden J, Davey Smith G, Haycock PC, *et al.* (2016) Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* **40**, 304–314.

43. Bowden J, Davey Smith G & Burgess S (2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* **44**, 512–525.
44. Hemani G, Zheng J, Elsworth B, *et al.* (2018) The MR-Base platform supports systematic causal inference across the human phenome. *Elife* **7**, e34408.
45. Ong JS & MacGregor S (2019) Implementing MR-PRESSO and GCTA-GSMR for pleiotropy assessment in Mendelian randomization studies from a practitioner's perspective. *Genet Epidemiol* **43**, 609–616.
46. Bowden J, Spiller W, Del Greco MF, *et al.* (2018) Improving the visualization, interpretation and analysis of two-sample summary data Mendelian randomization via the Radial plot and Radial regression. *Int J Epidemiol* **47**, 1264–1278.
47. Curtin F & Schulz P (1998) Multiple correlations and Bonferroni's correction. *Biol Psychiatry* **44**, 775–777.
48. Nygren P, Larsson R, Johansson H, *et al.* (1988) 1,25(OH)2D3 inhibits hormone secretion and proliferation but not functional dedifferentiation of cultured bovine parathyroid cells. *Calcif Tissue Int* **43**, 213–218.
49. Cantley LK, Russell J, Lettieri D, *et al.* (1985) 1,25-Dihydroxyvitamin D3 suppresses parathyroid hormone secretion from bovine parathyroid cells in tissue culture. *Endocrinology* **117**, 2114–2119.
50. Buchwald PC, Westin G & Akerström G (2005) Vitamin D in normal and pathological parathyroid glands: new prospects for treating hyperparathyroidism (review). *Int J Mol Med* **15**, 701–706.
51. Christakos S, Dhawan P, Verstuyf A, *et al.* (2016) Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* **96**, 365–408.
52. Yin L, Ordóñez-Mena JM, Chen T, *et al.* (2013) Circulating 25-hydroxyvitamin D serum concentration and total cancer incidence and mortality: a systematic review and meta-analysis. *Prev Med* **57**, 753–764.
53. Costa-Guda J, Corrado K, Bellizzi J, *et al.* (2023) Influence of vitamin D deficiency on cyclin D1-induced parathyroid tumorigenesis. *Endocrinology* **164**, bqad137.
54. Zhao J, Wang H, Zhang Z, *et al.* (2019) Vitamin D deficiency as a risk factor for thyroid cancer: a meta-analysis of case-control studies. *Nutrition* **57**, 5–11.
55. Carlberg C & Muñoz A (2022) An update on vitamin D signaling and cancer. *Semin Cancer Biol* **79**, 217–230.
56. Aboelnaga MM, Elshafei MM & Elsayed E (2016) Vitamin D status in Egyptian Euthyroid multinodular non-toxic goiter patients and its correlation with TSH levels. *Endocrinol Nutr* **63**, 380–386.
57. Du X, Liu Y, Zhao C, *et al.* (2019) Changes of serum 25(OH) D3 and IGF-1 levels in patients with thyroid nodules. *BMC Endocr Disord* **19**, 48.
58. Kim JR, Kim BH, Kim SM, *et al.* (2014) Low serum 25 hydroxyvitamin D is associated with poor clinicopathologic characteristics in female patients with papillary thyroid cancer. *Thyroid* **24**, 1618–1624.
59. Diffey BL (2010) Modelling the seasonal variation of vitamin D due to sun exposure. *Br J Dermatol* **162**, 1342–1348.
60. Datta S, Pal M & De A (2014) The dependency of vitamin d status on anthropometric data. *Malays J Med Sci* **21**, 54–61.
61. Fiore E & Vitti P (2012) Serum TSH and risk of papillary thyroid cancer in nodular thyroid disease. *J Clin Endocrinol Metab* **97**, 1134–1145.
62. Zhou L, Wang Y, Su J, *et al.* (2023) Vitamin D deficiency is associated with impaired sensitivity to thyroid hormones in Euthyroid adults. *Nutrients* **15**, 3697.
63. Lawlor DA, Davey Smith G, Kundu D, *et al.* (2004) Those confounded vitamins: what can we learn from the differences between observational *v.* randomised trial evidence? *Lancet* **363**, 1724–1727.

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