Accepted manuscript

Autoimmune thyroid diseases, Celiac disease and Gluten-free diet: a Mendelian randomization study

Yue-Heng Pu, Cai-Yi Long, Ren-Song Yue*, Bo-Xun Zhang, Ya-Yi Jiang, Zi-Han Li

Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China

***Corresponding Author:** Ren-Song Yue; songrenyue@cdutcm.edu.cnand Nutrition Science

Running title: Thyroid Autoimmunity and Gluten-free Diet

Abbreviations: Autoimmune thyroid disease (AITD); Celiac disease (CeD) ; Gluten-free diet (GFD) ; GD (Graves' disease) ; Hashimoto's disease (HT) ; Instrumental variables (IVs) ; Inverse variance weighted (IVW) ; Mendelian Randomization (MR) ; Single nucleotide polymorphisms (SNPs) ; UK Biobank (UKB) ; Weighted median estimator (WME)

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114525103917
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

Abstract

Previous studies have reported comorbidities of autoimmune thyroid disorders (AITD), including Hashimoto's disease (HT) and Graves' disease (GD), and celiac disease (CeD), as well as the possible beneficial effects of a gluten-free diet (GFD) on AITD. Nonetheless, it remains uncertain whether there is a genetic causal relationship between AITD and CeD, while the beneficial effects on a GFD are controversial. This study aim to explore the causal relationship between CeD and AITD, particularly with HT, and to determine whether a GFD is beneficial for AITD. We performed a two-sample Mendelian Randomization (MR) analysis on data from the largest meta-analysis summary statistics of AITD, CeD and GFD. Genetic instrumental variables were established by pinpointing single nucleotide polymorphisms (SNPs) that relate to corresponding factors. In assessing sensitivity and heterogeneity, we conducted examinations of MR Egger, weighted median, simple mode, weighted mode, and MR Egger intercept tests. HT was found to play a pathogenic role in increasing the risk of CeD (OR_{IVW} = 1.544 [95%CI 1.153-2.068], p = 0.00355), and our Mendelian randomization study does not support genetic liability related to CeD with GD (Graves' disease) and GFD with AITD. This study supports the positive correlation between HT risk and CeD risk, while GFD has no protective effect on AITD and may exert its effect through other mechanisms. These findings provide valuable insights into potential targets for disease intervention and treatment at the genetic level.

Key words: Autoimmune thyroid disease; Celiac disease; Gluten-free diet; Hashimoto's disease; Graves' disease; Mendelian randomization

1 Introduction

Autoimmune thyroid diseases (AITD), mainly comprising autoimmune thyroiditis (AIT)/Hashimoto's disease (HT), and Graves' disease (GD), are complex polygenic disorders with similarities and differences, primarily programmed by genetic factors [1,2]. AITD is one of the most common pathologies in endocrine diseases and is the most common cause of hypothyroidism and hyperthyroidism. AITD affects 2-5% of the population, with a majority being women [3]. AITD patients are often accompanied by other autoimmune disorders, one of which is celiac disease (CeD). CeD is an immune-mediated disease caused by ingestion of gluten, affecting 1% of the total population[4,5], yet many patients with CeD go undiagnosed[6-9]. Its pathogenesis involves the immune response of gluten protein, the activation of immune cells and the damage of intestinal mucosa. A lifelong strict gluten-free diet (GFD) is the key to treatment[6] .

In recent years, AITD comorbid with CeD has attracted attention. A systematic review and meta-analysis of 27 studies calculated the median prevalence of CeD in AITD to be 1.4%, with a higher prevalence of CeD in hyperthyroidism/Graves' patients (2.6%) [10,11]; the incidence of AITD was also found to be increased in CeD patients, especially HT [12,13]. This co-morbid phenomenon opens up new possibilities for the care of AITD: is it possible to migrate the GFD treatment to patients with AITD? It seems feasible, as it has been hypothesized that gluten may contribute to the deterioration of immune-mediated disorders [14], and GFD treatment exhibits a general anti-inflammatory effect in extraintestinal autoimmune inflammatory diseases [15,16]. Therefore, GFD is very popular in AITD, especially HT, despite the lack of favorable evidence to suggest its benefit in AITD[17,18].

Mendelian randomization analysis serves as a viable substitute for Randomized Controlled Trials in determining causality. This methodology, grounded in the principles of Gregor Mendel's laws of segregation and independent assortment, is employed to emulate a trial that is randomized on a genetic level. Researchers utilize genetic variants, which have a strong association with the exposure under investigation, as instrumental variables (IVs). This enables the estimation of the causal effect of a particular exposure or intervention on a specific outcome, while reducing the impact of confounding variables. Moreover, the

application of Mendelian randomization allows to effectively bypass the problem of reverse causation bias, considering that genetic variations are determined.

In this study, we aim to explore the causal relationship between CeD and AITD, particularly with HT, using the method of Mendelian randomization. Additionally, we attempt to provide genetic evidence that GFD is beneficial for AITD.

2 Methods

2.1 Data source

Genetic instruments were retrieved from the MRC IEU OpenGWAS database (https://gwas.mrcieu.ac.uk) and the NHGRI-EBI GWAS catalog (https://www.ebi.ac.uk/gwas/), which are the two largest databases of GWAS analysis summary statistics, as well as from previously published GWAS studies. GWAS we used were corrected for age, age2, sex, age*sex, age2*sex, and first 20 principal components of genotype data. All the data we used were selected from publicly available databases and does not require ethical approval. GWAS data source for each exposure are provided in Table 1.

2.1.1 Autoimmune thyroid diseases

Summary genome-wide association study (GWAS) data for HT and GD were sourced from a study by Sakaue et al.[19] , which investigated 220 human phenotypes, including AITD. This study integrated UK Biobank (UKB) and FinnGen data and included 395,640 Europeans. The UKB project recruited approximately 500,000 people aged between 40-69 years across the UK between 2006 and 2010. This study retrieved clinical information and then defined HT and GD using phecodes. The FINNGEN project had the participation of Six regional and three country-wide Finnish biobanks to track the health status of participants from birth to death information by linking to the national health registries national health registry (1969-2016), the study used pooled data from FinnGen version 3 data and defined diseases with ICD.10 standards. We retained the GWAS data from the European population.

2.1.2 Celiac disease and Gluten-free diet

CeD and GFD data are from the study by Jiang, L. et al[20], where the original data were derived from the UKB project. For CeD, the researcher used UKB's unsorted text data (UKB datafield 41202). UKB defines CeD using the ICD-10 code K90.0, and the researcher then grouped the ICD-10 code into Phecode based on the ICD-10 to Phecode v.1.2 map20. The final CeD data presented meets the PheCode 557.1 standards definitions. For GFD, the UKB project collected data from 58,913 volunteers from 2009-2012 who responded to a 24-hour recall diet questionnaire, "Types of Special Diets Followed," via online follow-up (More specific information is on *https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=20086*).

2.2 Assumptions

This study adheres to the three assumptions of Mendelian Randomization (MR) research: 1) Genetic variations are closely associated with the risk factor (relevance); 2) Genetic variations are independent of confounding factors (independence); 3) Genetic variations influence the outcome only through the risk factor (exclusivity) [21]. A schematic diagram is shown in Figure 1.

2.3 Method for selecting instrumental variables

First, meaningful SNPs were selected from the GWAS summary data used as exposure (selection criteria: for AITD, p < 5E-08, linkage disequilibrium r^2=0.001, and the width of the linkage disequilibrium region is 10,000 kb, to ensure that each SNP is independent and to exclude the impact of gene pleiotropy on the results; for CeD and GFD, to obtain sufficient data for analysis, we expanded the selection criteria: p < 5E-06, linkage disequilibrium r^2=0.01, and the width of the linkage disequilibrium region is 5000 kb). We calculated the F-statistics to quantify the strength of genetic variation, with F<10 being a weak instrumental variable that needs to be excluded. The formula for calculating the F-statistics is: $F=(N-K-1)/K \times R^2/(1-R^2)$, where N is the sample size of the exposure database, K is the

number of SNPs, and R^2 is the proportion of variation explained by the SNP in the exposure database. The formula for calculating R^2 is: $R^2 = 2 \times MAF \times (1 - MAF) \times \beta^2$, where MAF is the minor allele frequency, and β is the allele effect value. Then, the exposure-related SNPs screened out from the GWAS summary data of the study outcome were extracted; missing SNPs were directly excluded without using alternative methods. SNPs directly related to the study outcome were excluded (p < 5E-08). Before the MR analysis, we performed an MR-PRESSO analysis to exclude any outliers with potential pleiotropy to ensure the reliability of our MR estimates [22]. According to the above steps, the remaining SNPs were finally used as genetic instruments.

2.4 Methods of MR analysis

The primary analysis method is the inverse variance weighted (IVW) method [23], which combines Wald ratio estimates to obtain a consistent estimate of the causal impact of exposure on the outcome, characterized by not considering the existence of an intercept term during regression. We also used other MR analysis methods, including MR-Egger regression [24], weighted median estimator (WME) [25], simple mode [26], and weighted mode [26] to verify the causal relationship between exposure and outcome. The main difference between the MR-Egger regression method and the IVW analysis method is the addition of an intercept term, mainly used to judge whether there is horizontal pleiotropy. WME assumes that at least half of the IVs in the analysis are valid, and then obtains a consistent estimate of the causal relationship. Simple mode classifies SNPs according to causal effects and divides similar values into a cluster, using the cluster with the most SNPs to evaluate the estimated causal effect. Weighted mode weights the causal effect value of SNPs in each cluster with the number of SNPs in the module, and the returned result is a temporary estimate with the maximum SNP number weight. The use of a consistent causal effect estimate of the weighted mode requires the “ZEMPA assumption” (zero modal pleiotropy assumption) [26].

2.5 Sensitivity Analysis

In this study, Cochran's Q test was used to judge the heterogeneity of the IVW model; when $p < 0.05$, heterogeneity is considered to exist, and at this time, the random effects model of IVW is used for causal inference. MR-Egger regression analysis was used to detect whether genetic pleiotropy exists; when the intercept term differs significantly from 0, it indicates the presence of horizontal pleiotropy. Leave-one-out sensitivity test was performed to check deeper.

2.6 Software and package

Statistical analysis was conducted using the Two Sample MR package (version 0.5.7) in R software (version 4.3.0).

3 RESULT

3.1 Instrumental variables

The instrumental variables were selected according to the method previously described. Details of instrumental variables for exposures are shown in Supplementary Table S1.

3.2 Mendelian randomization with GFD as exposure

We first explored the GFD as exposure in relation to CeD and AITD. Complete primary and sensitivity analyses of MR are presented in Figure 2 and Table 2.

There was no evidence to support a genetic liability related to a GFD with CeD (OR_{IVW} = 1.178 [95%CI: 0.983-1.412], p = 0.077), HT (OR_{IVW} = 0.996 [95%CI: 0.936-1.059], p = 0.895) and GD (OR_{IVW} = 1.007 [95%CI 0.976-1.039], p = 0.648) (Figure 2). There was no indications of heterogeneity in the MR Egger and IVW regression tests, with no evidence of any horizontal pleiotropy in the MR Egger intercept (Table 2).

3.3 Mendelian randomization with CeD as exposure

Complete primary and sensitivity analyses of MR for each exposure in relation to AITD are presented in Figure 3 and Table 3.

In the aforementioned MR Analysis, there was no statistically significant effect of CeD (OR_{IVW} = 1.007 [95%CI: 0.976-1.039], p = 0.648) and genetic susceptibility associated with HT (Figure 3). Under the MR Egger intercept, the results were also robust, but they did not meet the criteria of the heterogeneity test (Table 3). Given that we used random-effects IVW as the primary MR analysis, heterogeneity in this context is not unacceptable [27].

Our results support a genetic liability related to CeD (OR_{IVW} = 1.060 [95%CI 1.013-1.109], p = 0.012) with GD (Figure 3), and this result was robust under the MR Egger intercept, but they did not meet the criteria of the MR Egger and IVW heterogeneity test. Therefore, we repeated the MR analysis with a more stringent selection of CeD-related instrumental variables (selection criteria: p < 5E-06, LD clumping window=10000kb, LD clumping r^2=0.001, effective instrumental variables are shown in the Supplementary Table S1), and obtained negative results. These results passed the MR Egger, IVW, and MR Egger intercept tests (Table 4).

3.4 Mendelian randomization with AITD as exposures

Complete primary and sensitivity analyses of MR for reverse causation between CeD and AITD are presented in Figure 4 and Table 5.

In the reverse MR, strong evidence suggests that HT plays a pathogenic role in increasing the risk of CeD (OR_{IVW} = 1.544 [95%CI: 1.153-2.068], p = 0.004) (Figure 5 shows the scatter plot). There is no evidence of heterogeneity in our MR Egger (p = 0.064) and IVW regression tests (p = 0.097), nor is there any evidence of horizontal pleiotropy in the MR Egger intercept (p = 0.729) (Table 5). The results remain robust under further leave-one-out analysis (Figure 6).

However, there is no evidence to suggest that GD has a pathogenic or protective effect (OR_{IVW} = 1.189 [95%CI: 0.988-1.428], p = 0.066) on CeD (Figure 4), and under the MR Egger intercept, this result is robust (Table 5).

4 DISCUSSION

Our study provides the following evidence: 1) An increased risk of HT is associated with an increased risk of CeD, and this causal relationship is unidirectional, that is, suffering from HT increases the risk of CeD , whereas there is no adequate evidence suggesting that developing CeD increases the risk of HT; 2) An increased risk of GD is not associated with an increased risk of CeD; 3) There is no evidence to support that a GFD is beneficial in reducing the risk of AITD.

Both celiac disease and AITD have dysregulated cellular immunity, and the leaky gut state triggered by celiac disease permits the passage of non-self epitopes and antigens which may trigger autoimmunity [(28)]. It is in light of this significant correlation that GFD to improve AITD have become increasingly popular, but whether or not patients benefit is controversial. Previous studies have found potential beneficial effects of GFD in AITD: In female HT patients with IgA-tTG seropositivity but asymptomatic CeD, GFD could decrease the gradient of thyroid TPO TG antibodies [(29)]; thyroid function abnormalities were reversed in some newly diagnosed CeD patients after one year of GFD [(30)]. The results of a meta-analysis also support the beneficial effects of a GFD [(31)].However, some studies have provided contrary evidence, with no effect on the levels of TPO antibodies in 10 cases (37%) of newly diagnosed CeD patients after one year of GFD treatment, and even with the establishment of GFD in patients without CeD, the thyroid volume still significantly decreased [(32)]. These conflicting opinions drew our attention. In clinical practice, the strict implementation of GFD can be brutal for clinical patients, especially Asian patients, and the lack of evidence can again shake clinical patients' resolve to implement GFD, therefore, obtaining firm evidence is critical. Considering conducting clinical trials of gluten-free diets is difficult, especially with low patient compliance and results confounded by multiple factors, we used a Mendelian

randomization method, “nature’s randomized trial,” trying to answer the question: Is there any relationship between gluten-free diets and celiac disease and AITD? Our hypothesis was that “GFD improves AITD by improving CeD”, but surprisingly, our results did not support this idea, while supporting the conclusions of the clinical study by Metso S et al [32].

However, the negative result is still instructive: although CeD and AITD share cellular immune dysregulation, they may not interact, but rather, more likely, there is a more sophisticated network of regulatory mechanisms, i.e., pathogenic and protective roles in parallel, resulting in a failure to show a clear pathogenic or protective role at the disease phenotype level. This suggests that further studies at the cellular as well as molecular level are necessary and will be the next step in our in-depth research, and the positive finding of an increased risk of CeD due to HT will be an important entry point. Besides, HT and GD, as two subtypes of AITD, are potentially coexisting or transforming into each other. In the clinic, it is able to detect positivity of relevant antibodies and changes in different antibody titers before and after transformation, indicating that the immune mechanisms that play a major role in the disease network also change during the transformation process. Although both belong to AITD, our results only clarify the possible pathogenic role of HT in CeD, this result suggests that the abnormal cellular immune state in HT is more relevant to CeD. There is a greater possibility to uncover the core pathways and targets from this positive result.

Our study possesses multiple strengths. Given that lifestyle elements and socioeconomic status are susceptible to real-world bias, it becomes challenging to accurately determine their actual impact through observational studies. The utilization of MR analysis minimizes the potential influence of residual confounding factors. In addition, considering the heterogeneity of HT and GD, our MR analysis independently analyzes these two AITD subtypes, making it one of the few known MR analyses that independently analyze HT. The data we used is based on the currently available largest GWAS summary statistics for HT. To enhance the robustness of the results, thorough sensitivity analyses were performed using multiple MR methods, and no violations of any assumptions were found. Practically, our study results help provide more dietary strategy information for AITD patients.

This investigation also presents certain limitations. Initially, we confined our research participants to individuals of European ancestry in an effort to mitigate population stratification bias, and thus, the findings are not yet generalizable to other ethnic populations. We attempted to extrapolate the study results to the Asian population, but failed due to the inability to find GWAS data related to CeD and GFD. We look forward to more GWAS studies on the Asian population. Second, in the choice of dietary exposure, we also tried other GWAS data, but it was difficult to continue the analysis due to the lack of sufficient valid instrumental variables. Third, similar to all MR studies, we are unable to tackle the issue of unobserved horizontal pleiotropy. Fourth, because Mendelian randomization analyses, the 'natural RCT', use genetic variants strongly associated with exposure factors as instrumental variables to assess the causal relationship between exposure factors and outcomes, thus avoiding interference from common confounding factors such as acquired environmental and social factors and excluding reverse causal effects. Therefore, the order of diagnosis does not influence our results, nor does age or gender, but we cannot exclude that age and gender play some mediating role. Fifthly, the original data for CeD were obtained from the UKB project, where the majority of cases were identified by serology and endoscopy, both of which are widely recognized as reliable diagnostic criteria for CeD, although a proportion of cases were based on symptomatic presentation alone, which may lack the specificity and accuracy of serological or histological confirmation (More specific information is on *https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=21069*). Differences in diagnostic methods, such as relying on symptoms alone versus strict serologic or histologic confirmation, can lead to heterogeneity in case definition, which creates limitations when interpreting and comparing results. Sixth, the inclusion of GFD was largely based on a single self-reported piece of data, which may be subject to recall bias, social desirability bias, or individual differences in understanding. And the questionnaire only addresses the past 24 hours of diet, making it difficult to assess whether individuals have adhered to a GFD over time. It is difficult to carry out a strict assessment on a large scale, which is precisely why we chose Mendelian randomization analyses to assess the causal relationship between exposure factors and outcomes, by using genetic variants closely related to the exposure factors as instrumental variables, thus avoiding the interference of common confounding factors.

However, we also expect more objective and standardized methods, such as biomarker-based assessments or validated dietary questionnaires, to provide a more rigorous raw database for analysis.

The findings of this study support that an increased risk of HT is associated with an increased risk of CeD, while do not support a protective effect of a GFD on HT. This suggests that the "effect" of GFD in HT may be due to the improvement of CeD-related symptoms in patients with HT. However, some studies have shown that adhering to a GFD may positively impact the absorption of selenium and vitamin D elements necessary for thyroid function and health [33–35]. We cannot rule out that GFD may be beneficial to patients with HT through these effective intermediary factors but is masked by other non-beneficial factors. In the future, continuous large-scale GWAS summary statistics of dietary pattern genetic variations are needed to more accurately assess the effective dietary strategies for AITD.

Acknowledgements

We thank the associate editor and the reviewers for their useful feedback that improved this paper.

Funding Statement

This study was supported by the National Natural Science Foundation of China (82274486).

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authorship

P-YH: Data curation, Writing – original draft. L-CY: Data curation, Writing – review & editing. J-YY: Data curation, Writing – review & editing. L-ZH: Data curation, Writing – review & editing. Z-BX: Writing – review & editing, Supervision. Y-RS: Supervision, Writing – review & editing.

Reference

1. Antonelli A, Ferrari SM, Ragusa F, et al. Graves' disease: Epidemiology, genetic and environmental risk factors and viruses. *Best Pract Res Clin Endocrinol Metab*. 2020;34(1):101387. doi:10.1016/j.beem.2020.101387

2. Zhang QY, Liu W, Li L, et al. Genetic Study in a Large Cohort Supported Different Pathogenesis of Graves' Disease and Hashimoto's Hypothyroidism. *J Clin Endocrinol Metab*. 2020;105(7):dgaa170. doi:10.1210/clinem/dgaa170

3. McLeod DSA, Cooper DS. The incidence and prevalence of thyroid autoimmunity. *Endocrine*. 2012;42(2):252-265. doi:10.1007/s12020-012-9703-2

4. Ludvigsson JF, Bai JC, Biagi F, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. *Gut*. 2014;63(8):1210-1228. doi:10.1136/gutjnl-2013-306578

5. Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, et al. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK. *Lancet (London, England)*. 2023;401(10391):1878-90.doi: 10.1016/S0140-6736(23)00457-9

6. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. *Lancet Lond Engl*. 2018;391(10115):70-81. doi:10.1016/S0140-6736(17)31796-8

7. Lebwohl B, Ludvigsson JF, Green PHR. Celiac disease and non-celiac gluten sensitivity. *BMJ*. 2015;351:h4347. doi:10.1136/bmj.h4347

8. Catassi C, Kryszak D, Bhatti B, et al. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. *Ann Med*. 2010;42(7):530-538. doi:10.3109/07853890.2010.514285

9. Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther*. 2007;26(9):1217-1225. doi:10.1111/j.1365-2036.2007.03502.x

10. Roy A, Laszkowska M, Sundström J, et al. Prevalence of Celiac Disease in Patients with Autoimmune Thyroid Disease: A Meta-Analysis. *Thyroid Off J Am Thyroid Assoc*. 2016;26(7):880-890. doi:10.1089/thy.2016.0108

11. Ferrari SM, Fallahi P, Ruffilli I, et al. The association of other autoimmune diseases in patients with Graves' disease (with or without ophthalmopathy): Review of the literature and report of a large series. *Autoimmun Rev*. 2019;18(3):287-292. doi:10.1016/j.autrev.2018.10.001

12. Dore MP, Fanciulli G, Rouatbi M, Mereu S, Pes GM. Autoimmune Thyroid Disorders Are More Prevalent in Patients with Celiac Disease: A Retrospective Case-Control Study. *J Clin Med*. 2022;11(20):6027. doi:10.3390/jcm11206027

13. Bibbò S, Pes GM, Usai-Satta P, et al. Chronic autoimmune disorders are increased in coeliac disease: A case-control study. *Medicine (Baltimore)*. 2017;96(47):e8562. doi:10.1097/MD.0000000000008562

14. Lerner A, Shoenfeld Y, Matthias T. Adverse effects of gluten ingestion and advantages of gluten withdrawal in nonceliac autoimmune disease. *Nutr Rev*. 2017;75(12):1046-1058. doi:10.1093/nutrit/nux054

15. Bruzzese V, Scolieri P, Pepe J. Efficacy of gluten-free diet in patients with rheumatoid arthritis. *Reumatismo*. 2021;72(4):213-217. doi:10.4081/reumatismo.2020.1296

16. Passali M, Josefsen K, Frederiksen JL, Antvorskov JC. Current Evidence on the Efficacy of Gluten-Free Diets in Multiple Sclerosis, Psoriasis, Type 1 Diabetes and Autoimmune Thyroid Diseases. *Nutrients*. 2020;12(8):2316. doi:10.3390/nu12082316

17. Pobłocki J, Pańka T, Szczuko M, Telesiński A, Syrenicz A. Whether a Gluten-Free Diet Should Be Recommended in Chronic Autoimmune Thyroiditis or Not?-A 12-Month Follow-Up. *Journal of clinical medicine*. 2021;10(15). doi: 10.3390/jcm10153240.

18. Malandrini S, Trimboli P, Guzzaloni G, Virili C, Lucchini B. What about TSH and Anti-Thyroid Antibodies in Patients with Autoimmune Thyroiditis and Celiac Disease Using a Gluten-Free Diet? A Systematic Review. *Nutrients*. 2022;14(8).doi: 10.3390/nu14081681.

19. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet*. 2021;53(10):1415-1424. doi:10.1038/s41588-021-00931-x

20. Jiang L, Zheng Z, Fang H, Yang J. A generalized linear mixed model association tool for biobank-scale data. *Nat Genet*. 2021;53(11):1616-1621. doi:10.1038/s41588-021-00954-4

21. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. *JAMA*. 2017;318(19):1925-1926. doi:10.1001/jama.2017.17219

22. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50(5):693-698. doi:10.1038/s41588-018-0099-7

23. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity Analyses for Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic Variants. *Epidemiol Camb Mass*. 2017;28(1):30-42. doi:10.1097/EDE.0000000000000559

24. Slob EAW, Groenen PJF, Thurik AR, Rietveld CA. A note on the use of Egger regression in Mendelian randomization studies. *Int J Epidemiol*. 2017;46(6):2094-2097. doi:10.1093/ije/dyx191

25. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol*. 2016;40(4):304-314. doi:10.1002/gepi.21965

26. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol*. 2017;46(6):1985-1998. doi:10.1093/ije/dyx102

27. Burgess S, Davey Smith G, Davies NM, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome Open Res*. 2019;4:186. doi:10.12688/wellcomeopenres.15555.3

28. Lerner A, Jeremias P, Matthias T. Gut-thyroid axis and celiac disease. *Endocr Connect*. 2017;6(4):R52-R58. doi:10.1530/EC-17-0021

29. Krysiak R, Szkróbka W, Okopień B. The Effect of Gluten-Free Diet on Thyroid Autoimmunity in Drug-Naïve Women with Hashimoto's Thyroiditis: A Pilot Study. *Exp Clin Endocrinol Diabetes Off J Ger Soc Endocrinol Ger Diabetes Assoc*. 2019;127(7):417-422. doi:10.1055/a-0653-7108

30. Valentino R, Savastano S, Tommaselli AP, et al. Prevalence of coeliac disease in patients with thyroid autoimmunity. *Horm Res*. 1999;51(3):124-127. doi:10.1159/000023344

31. Piticchio T, Frasca F, Malandrino P, et al. Effect of gluten-free diet on autoimmune thyroiditis progression in patients with no symptoms or histology of celiac disease: a meta-analysis. *Front Endocrinol*. 2023;14:1200372. doi:10.3389/fendo.2023.1200372

32. Metso S, Hyytiä-Ilmonen H, Kaukinen K, et al. Gluten-free diet and autoimmune thyroiditis in patients with celiac disease. A prospective controlled study. *Scand J Gastroenterol*. 2012;47(1):43-48. doi:10.3109/00365521.2011.639084

33. Stazi AV, Trinti B. Selenium status and over-expression of interleukin-15 in celiac disease and autoimmune thyroid diseases. *Ann Ist Super Sanita*. 2010;46(4):389-399. doi:10.4415/ANN_10_04_06

34. Hinks LJ, Inwards KD, Lloyd B, Clayton BE. Body content of selenium in coeliac disease. *Br Med J Clin Res Ed*. 1984;288(6434):1862-1863. doi:10.1136/bmj.288.6434.1862

35. Verma A, Lata K, Khanna A, et al. Study of effect of gluten-free diet on vitamin D levels and bone mineral density in celiac disease patients. *J Fam Med Prim Care*. 2022;11(2):603-607. doi:10.4103/jfmpc.jfmpc_1190_21

TABLE 1. Overview of the data sources

Trait	Year	case/control	Population	Study accession	Pubmed ID
Hashimoto's disease	2021	15,654/379,986	Europe	GCST90018855	34594039
Graves disease	2021	1,678/456,942	Europe	GCST90018847	34594039
Celiac	2021	1,036/455,312	Europe	GCST90044158	34737426
Glutenfree diet	2018	1,376/63,573	Europe	GCST90042610	34737426

Table 2. Sensitivity analyses of MR for each outcome in relation to GFD

Exposure	Outcome	Heterogeneity test (IVW)		Heterogeneity test (MR Egger)		Directional horizontal pleiotropy (MR Egger intercept)	
		Q	P-value	Q	P-value	Intercept	P-value
Gluten-free diet	Celiac disease	2.362	0.797	2.032	0.730	-0.049	0.596
	Hashimoto's disease	4.691	0.584	3.895	0.565	-0.027	0.413
	Graves' disease	4.433	0.618	4.283	0.509	-0.016	0.715

Table 3. Sensitivity analyses of MR for CeD as exposure

Exposure	Outcome	Heterogeneity test (IVW)		Heterogeneity test (MR Egger)		Directional horizontal pleiotropy (MR Egger intercept)	
		Q	P-value	Q	P-value	Intercept	P-value
Celiac disease	Hashimoto's disease	72.651	0.0004	71.316	0.0004	-0.013	0.417
	Graves' disease	52.852	0.0085	52.377	0.0069	-0.014	0.606

Table 4. MR and Sensitivity analyses of MR for CeD in relation to GD with a more stringent selection

Celiac - Graves' disease (p<5e-06, LD clumping window=10000kb, LD clumping r^2=0.001)					
Methods		nSNP	OR	95%CI	P-value
MR Egger		22	1.101	0.984-1.232	0.109
Weighted median		22	1.119	1.048-1.195	0.001
Inverse variance weighted		22	1.049	0.998-1.103	0.060
Simple mode		22	0.984	0.857-1.130	0.825
Weighted mode		22	1.094	1.029-1.163	0.009
Sensitivity analyses					
Heterogeneity test (IVW)		Heterogeneity test (MR Egger)		Directional horizontal pleiotropy (MR Egger intercept)	
Q	P-value	Q	P-value	Intercept	P-value
26.158	0.200	25.064	0.199	-0.024	0.361

Table 5. Sensitivity analyses of MR for reverse causation between CeD and AITD

Exposure	Outcome	Heterogeneity test (IVW)		Heterogeneity test (MR Egger)		Directional horizontal pleiotropy (MR Egger intercept)	
		Q	P-value	Q	P-value	Intercept	P-value
Hashimoto's disease	Celiac disease	10.723	0.097	10.442	0.064	0.028	0.729
Graves' disease		31.119	0.008	29.334	0.009	-0.052	0.372

Figure 1. A schematic diagram of the three main assumptions in this MR analysis

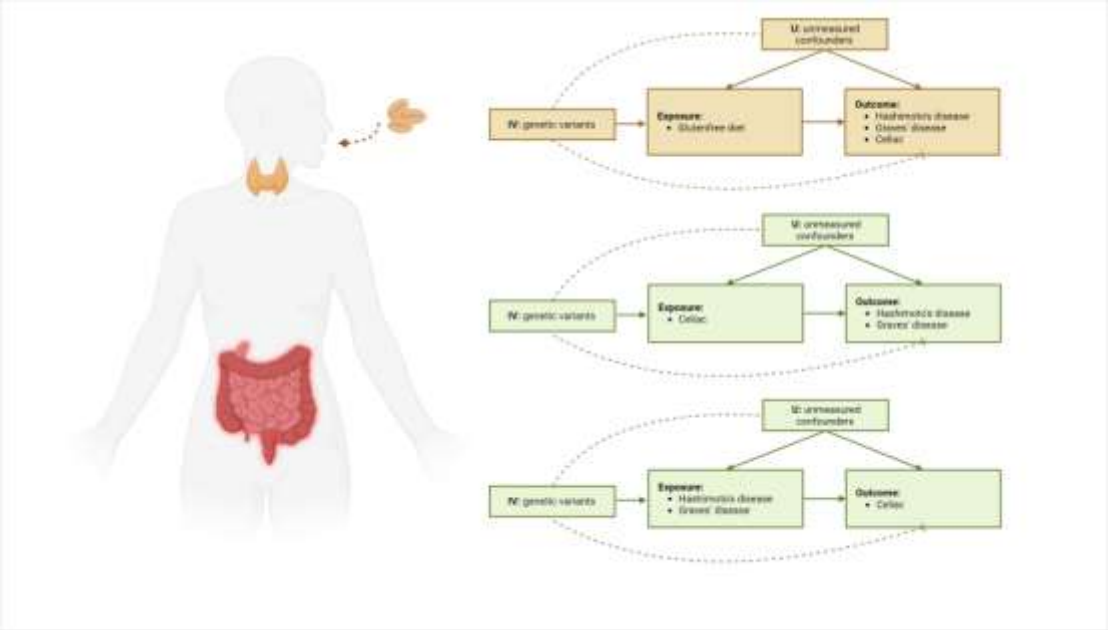

FIGURE 2. MR for Estimating the association between GFD, CeD and AITD using IVW method, weighted median estimator, MR Egger regression, simple mode and weighted mode

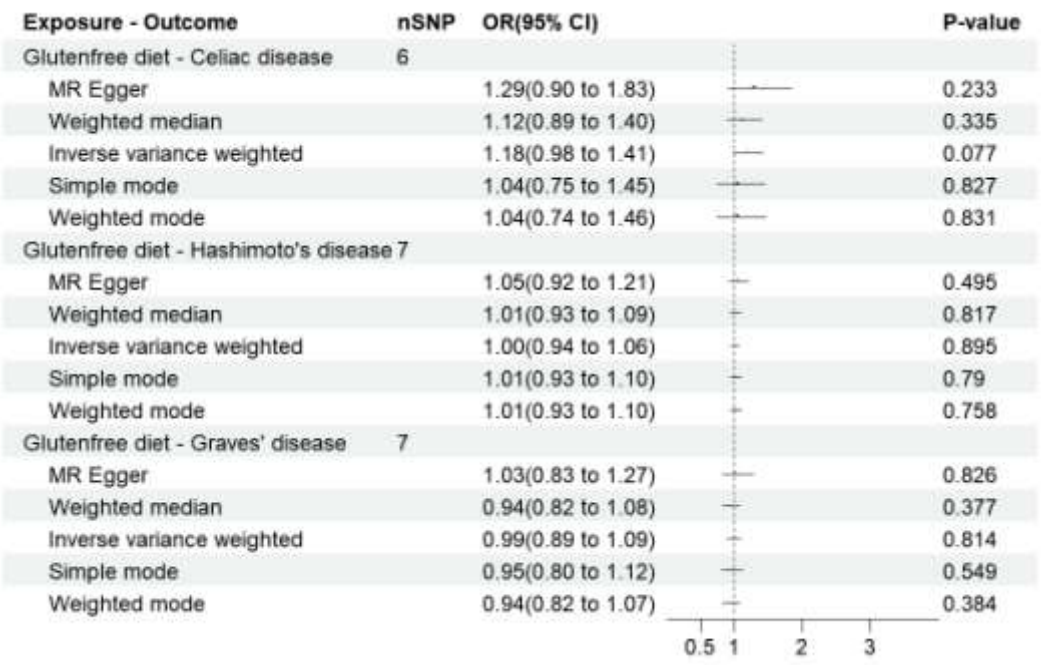

FIGURE 3. MR for Estimating the association between CeD and AITD using IVW method, weighted median estimator, MR Egger regression, simple mode and weighted mode

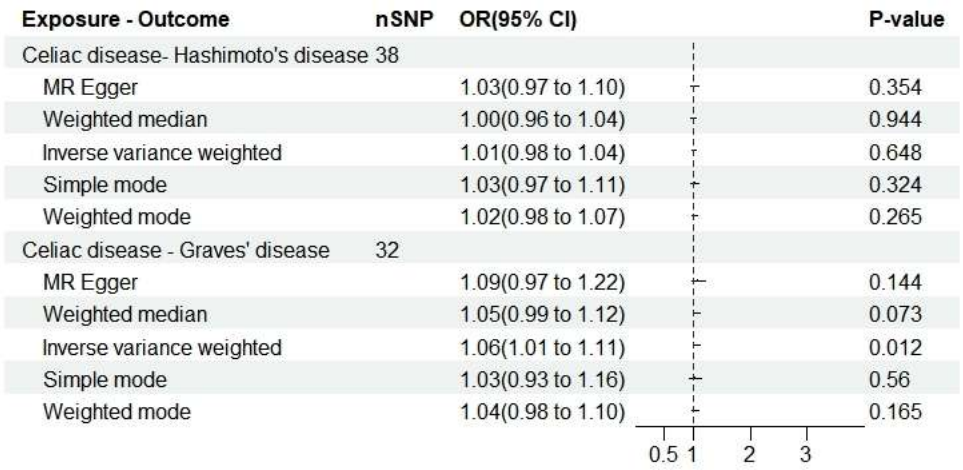

FIGURE 4. MR for Estimating the association reverse causation between CeD and AITD using IVW method, weighted median estimator, MR Egger regression, simple mode and weighted mode

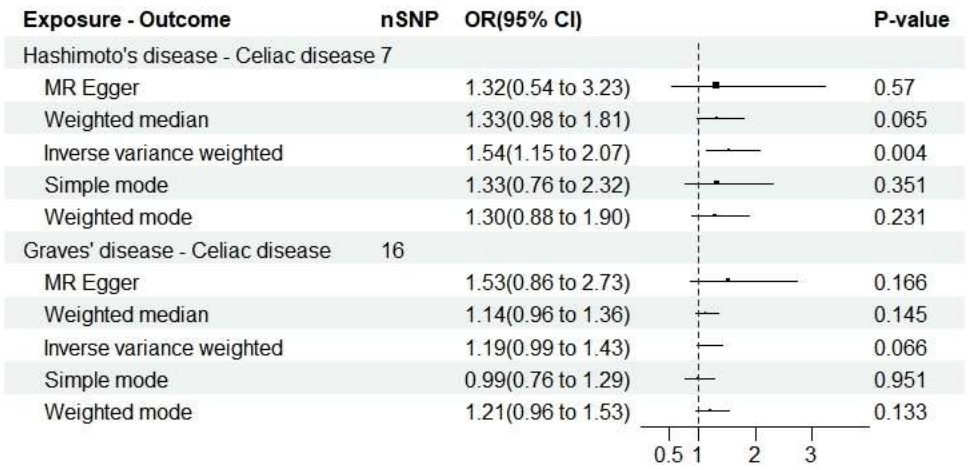

FIGURE 5. Scatter plot for HT on CeD

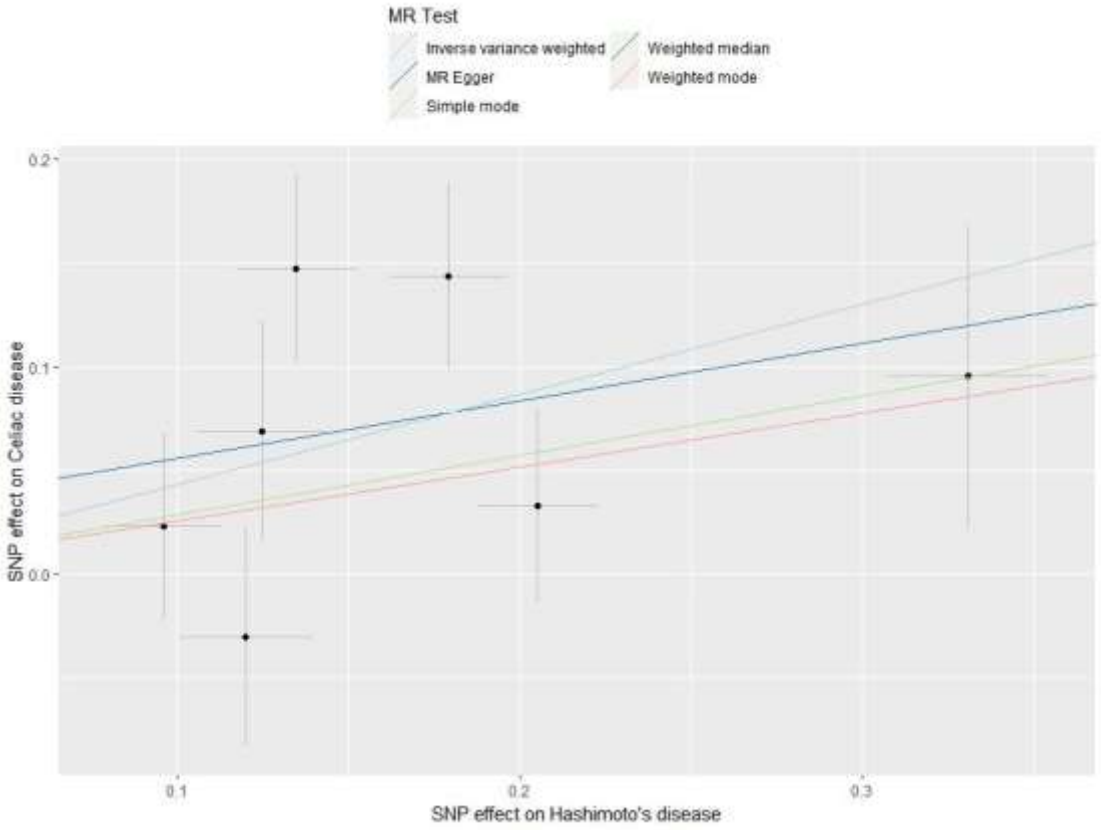

FIGURE 6. Leave-one-out for HT on CeD