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Relationship Between Gut Microbiota and Hashimoto's Thyroiditis: A Two-Sample Bidirectional Mendelian Randomization Study

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ABSTRACT

Background: Hashimoto's thyroiditis（HT）is one of the most common autoimmune diseases. Observational studies have proved that the gut microbiome is related to the occurrence of HT, but the causal-effect relationship between HT and gut microbiome is not clear. Therefore, the causal-effect relationship between the gut microbiome and HT remains to be determined.

Method: In this study, bidirectional Mendelian randomization (MR) was used to explore the relationship between HT and gut microbiome. Genome wide association studies (GWAS) data of gut microbiota were obtained from the MiBioGen database, containing 18340 samples. HT data were from the IEU open GWAS database, which contains 568833 samples. The methods used for MR analysis in this paper include inverse variance weighting (IVW), weighted median, weighted mode, Mr egger, and simple mode. F-statistics and sensitivity analysis were used to measure bias and reliability. Single Nucleotide Polymorphisms (SNPs) were used as Instrumental Variables (IVs) in MR studies.

Results: After false discovery rate (FDR) correction, we found a total of 9 bidirectional causal-effect relationships between gut microbiome and HT. Five of them demonstrated the influence of gut microbiome on HT, respectively were genus Anaerostipes （OR=1.239296,95%CI 1.03378-1.485669,p=0.020389 , family Alcaligenaceae (OR=0.742608, 95%CI 0.61164-0.901619, p=0.002645)、genus Ruminococcaceae（OR=0.89581, 95%CI 0.810137-0.990542,p=0.03193）、genus Prevotella7 (OR=0.895026,95%CI 0.810775-0.988031,p=0.027898)、phylum Verrucomicrobia (OR=0.838726,95%CI 0.722321-0.973889,p=0.021051). Four described the effect of HT on the gut microbiome as follows, phylum Verrucomicrobia（OR=0.968114, 95%CI 0.938525-0.998637,p=0.040743）, class Deltaproteobacteria（OR=0.970233, 95%CI 0.942334-0.998958,p=0.042353）, family Verrucomicrobiaceae（OR=0.963272, 95%CI 0.933234-0.994277,p=0.020607）, family Christensenellaceae（OR=1.044625, 95%CI 1.003784-1.087127,p=0.031903）.No obvious horizontal pleiotrophy was found by MR-Egger intercept test and MR-PRESSO global test.

Conclusions: This MR study revealed the relationship between gut microbiome and autoimmune disease ——HT, which may provide a new direction for the future research on the interaction mechanism between the two, and provide a new reference for the study of risk factors of HT. In turn, we can use the intestinal flora as a biomarker for the early diagnosis of HT.

INTRODUCTION

HT, also known as chronic lymphocytic or autoimmune thyroiditis, is an autoimmune thyroid disease characterized by increased thyroid volume, infiltration of parenchymal lymphocytes, and the presence of thyroid antigen-specific antibodies Yao et al. (2023). HT, together with Grave's disease (GD), is considered an autoimmune thyroid disease (AITD) Osowiecka et al. (2023). Its main manifestation is hypothyroidism, which is the result of thyroid immune attack caused by T cell-mediated immune system dysregulation. The mechanisms that trigger autoimmune attack on the thyroid are still under investigation. About the occurrence of Hashimoto disease, genetic factors account for 70-80% and environmental factors account for 20-30%. At present, environmental factors, such as radiation, iodine, smoking, infection, stress and drugs, and genetic susceptibility play a key role Weetman et al. (2021). At present, HT is one of the most common thyroid diseases, and its incidence is 0.3-1.5 cases per

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1000 people. More than 10% of women have positive antibodies, and about 2% of women have clinical symptoms, which is only 1 / 10 in men Ralli et al. (2020). GWAS confirmed that several genes, such as gdcg4p14h, Bach2, were significantly associated with the presence of HT and thyroid antibodies Antonelli et al. (2015).

The gut microbiota is a complex biological community that includes bacteria, viruses, and eukaryotes that colonize the digestive tract after birth Passos et al. (2017). Intestinal bacterial disorders are considered to be risk factor of diseases in many systems, such as: gastrointestinal diseases Vitale et al. (2021), respiratory diseases Jiang et al. (2022), cardiovascular diseases Karlsson et al. (2012), immune diseases Chu et al. (2017). They play an important role in regulating the digestion and absorption of food, the immune response of the body and the occurrence of mental diseases.

In recent years, the relationship between the composition of microbiota and thyroid autoimmunity has received extensive attention. Although there is no experimental evidence to clarify the mechanism of microbiota and thyroid autoimmunity, many observational studies have shown that. These studies showed that the intestinal flora plays an important role in the pathogenesis of HT. They can affect the occurrence and development of HT by affecting celiac disease and non-celiac wheat sensitivity, and may regulate thyroid function by ingesting thyroid related micronutrients, such as iodine, iron, zinc, selenium, vitamin D Knezevic et al. (2020), Metabolic enzymes derived from the microbiota may regulate iodothyronine metabolism to affect thyroid hormone homeostasis Virili et al. (2017), or may interact with host immune cells and cytokines to regulate thyroid immunity Shao et al. (2018). Previous studies have suggested that there may be an important thyroid-gut axis regulating HT, an autoimmune disease Virili et al. (2018).

An observational study in 2021 showed that Bacteroidetes were significantly increased and Firmicutes were significantly decreased in AITD patients compared with the healthy control group. No statistical difference was found when comparing the GD and HT subgroups with the control group. Therefore, gut microbiota can be used as a biomarker to distinguish AITD patients El-Zawawy et al. (2021).An observational study on European populations in 2020 showed that victoraceae significantly increased in HT patients compared with healthy people, alistipes, anaerostipes or dorea, which can also be regarded as pathogenic organisms, can show the ability to damage the host in the disease environment Cornejo-Pareja et al. (2020). An observational study in 2017 showed that the genera Bacteroides, Escherichia Shigella and parasutterella increased in abundance in the HT group compared with the control group, while Prevotella_ 9 and decreased abundance of dialister. genera Ishaq et al. (2017). However, some studies believe that Bacteroidetes are enriched in fecal samples of healthy people, while members of Firmicutes and synergistetes are enriched in HT patients, which contradicts the above studies. This may be due to the confounding caused by different study populations and different detection technologies. This observational study also showed that Prevotella_ 9 decreased in abundance in the HT population, consistent with the findings mentioned above Zhao et al. (2018). HT patients are often accompanied by hypothyroidism and other thyroid function changes, and thyroid function changes will affect the distribution of flora. In order to eliminate this confounding factor, HT patients were classified according to different thyroid function status. The study found that HT patients with normal thyroid function had more Lachnospiraceae_certae_sedis, Lactonifactor, Alistipes, and Suboligranlum, while hypothyroid HT patients have more phascolarctobacterium, which may be involved in the progression of HT Liu et al. (2020). The differences in the results of the above observational studies may be due to the interference of environmental factors such as detection technology, subjects' diet and lifestyle, and the use of antibiotics on the intestinal micro ecosystem. And these studies only analyzed the correlation between thyroid and intestinal bacteria, without analyzing the causal relationship between them.

This study used MR as a new method to analyze the bidirectional causal-effect relationship between HT and gut microbiome. It uses SNPs selected from GWAS data that are strongly associated with exposure as IVs to explore the relationship between exposure and outcome Greenland et al. (2018). Because SNPs are randomly assigned from parent to offspring before exposure and outcome, many confounding factors can be excluded when using SNPs as IVs, thus avoiding the impact on the results Davey Smith et al. (2014). MR has been widely used to explore the causal relationship between gut microbiome and thyroid problems, including GD Cao et al. (2023) and thyroid function Xie et al. (2023). The GWAS data used in this study were from MiBioGen and IEU open GWAS project. Two sample bidirectional MR analysis was used to measure the causal-effect relationship between gut microbiome and HT.

METHODS

Data sources

GWAS have tested hundreds of thousands to millions of genetic variants in many individual genomes to determine genotype phenotype associations. The data used in the MR study in this study were all from the publicly available GWAS database, which are related to the gut microbiome or HT, respectively, so no additional ethical statement or informed consent is required. GWAS data related to gut microbiota were obtained from 24 cohorts including

African Americans in Europe, the Middle East, Central Asia, and Latin America, with a total of 18340 individuals. A large-scale, multifunctional meta-analysis of 16S ribosomal RNA gene sequencing profiles and genotyping data from participants' stool samples was conducted to explore the relationship between human autosomal variation and gut microbiota Tam et al. (2019). A total of 211 taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) Kurilshikov et al. (2021). The GWAS summary data of HT were obtained from the IEU open GWAS project, containing 568833 samples (16191 HT individuals and 552642 non HT individuals), and the dataset contained 24146037 SNPs Sakaue et al. (2021).

Instrumental variable

MR rests on 3 assumptions: (1) the genetic variant is associated with the risk factor; (2) the genetic variant is not associated with confounders; and (3) the genetic variant influences the outcome only through the risk factor Emdin et al. (2021). The linkage disequilibrium threshold was set to r2=0.001 and the clustering distance was set to 10000 KB, thus ensuring that each IV was independent and there was no linkage disequilibrium. We determine whether IV is weak by calculating the F-statistic. IVs with F-statistic less than 10 would be excluded, as was the case when reverse MR analysis was performed with HT as exposure and gut microbiota as outcome. The IVs used in this study are listed in Supplement 1.The study design based on the 3 assumptions is show in the Figure.1.

Figure 1: MR Conceptual framework for the bidirectional association of gut microbiota and HT.

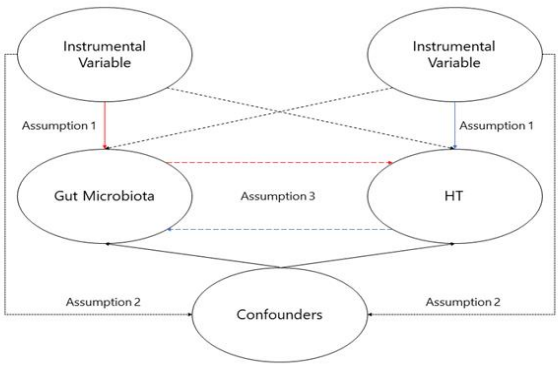

Figure 1. MR conceptual framework for the bidirectional association of gut microbiota and HT

MR Analysis

We used five commonly used statistical methods for MR analysis, including the IVW test: the simplest and reliable method for MR analysis is multiple SNPs is to perform an IVW meta analysis of each Wald ratio. when horizontal pleiotropy is balanced, IVW will get an unbiased analysis result Hemani et al. (2018), Hartwig et al. (2017).

Weighted mode: The Weighted mode introduces additional variables similar to IVW and weighted median, weighting the contribution of each SNP to the clustering by the inverse variance of its outcome effect Hemani et al. (2018), Hartwig et al. (2017). MR-Egger: Mr egger adjusted the IVW analysis by allowing a non-zero intercept, thereby allowing the net level pleiotropic effects of all SNPs to be unbalanced or oriented.MR-Egger adapts the IVW analysis by allowing a non-zero intercept, allowing the net horizontal pleiotropic effect across all SNPs to be unbalanced, or directional Hemani et al. (2018), Bowden et al. (2015).

Weighted median estimator:This approach can provide a consistent estimate of causal effects, even though up to 50% of the analyzed information comes from genetic variants with null IV Bowden et al. (2016). Simple mode: Although simple mode is not as effective as IVW, it can provide robust pleiotropy tests Xu et al. (2022). Compared with other methods, IVW method is considered to be more reliable, so we mainly use the results of IVW method as a supplement Bowden et al. (2016).

Sensitivity analyses

In MR, horizontal pleiotropy arises when the effect of variable on disease exceeds its effect on exposure. Violating the hypothesis of "no horizontal pleiotropy" will lead to serious bias of MR. To detect horizontal pleiotropy, we used the MR egger intercept test Bowden et al. (2016) and the MR-PRESSO global test Verbanck et al. (2018). Outliers from the analysis will be removed. IVs Q-test is a useful tool to explore the existence of heterogeneity due to pleiotropy or other reasons.

Its power increases with the increase of pleiotropy and sample size. When $p<0.05$, it means that there is obvious heterogeneity Greco et al. (2015). The leave-one-out method is used for sensitivity analysis.

It can gradually eliminate each SNP and observe whether the result changes. The ideal result is that the result does not change much after eliminating a SNP Burgess et al. (2017). In addition, to prevent the occurrence of a class of errors, we used the FDR method to correct the p-value obtained from MR analysis Sesia et al. (2021).

Reverse MR Analysis

In order to investigate whether HT has a causal effect on gut microbiota, this study used the method of reverse MR, that is, taking HT as exposure, gut microbiota as outcome, and SNPs as instrumental variables to conduct MR analysis. The specific method is the same as above.

The implementation of this study mainly performed and displayed by package TwoSampleMR, MR PRESSO and Mendelian Randomization in R (Version 4.3.1).

RESULTS

Causal effects of the gut microbiome on patients with HT

At least the causal-effect relationships of family Alcaligenaceae、genus Ruminococcaceae、genus Prevotella7、phylum Verrucomicrobia、genus Anaerostipes with HT can be proved by IVW method, and the p-value is still significant after FDR method correction.

IVW method proved that genus Anaerostipes （OR=1.239296,95%CI 1.03378-1.485669,p=0.020389）played a protective role in the occurrence of HT. Family Alcaligenaceae (OR=0.742608, 95%CI 0.61164-0.901619,p=0.002645)、genus Ruminococcaceae（OR=0.89581,95%CI 0.810137-0.990542,p=0.03193）、genus Prevotella7 (OR=0.895026,95%CI 0.810775-0.988031,p=0.027898)、phylum Verrucomicrobia (OR=0.838726,95%CI 0.722321-0.973889,p=0.021051) are risk factors in the development of HT.

Table 1: Causal effects of the gut microbiome on HT

Exposure	Method	nsnp	b	se	pval	or	or_lci95	or_Uci95	AdjustP
Phylum Verrucomicrobia	Inverse Variance Weighted	12	-0.18	0.08	0.02	0.84	0.72	0.97	0.03
	MR Egger	12	-0.04	0.20	0.83	0.96	0.64	1.43	0.95
	Simple mode	12	-0.21	0.17	0.24	0.81	0.59	1.13	0.38
	Weighted median	12	-0.16	0.11	0.12	0.85	0.69	1.05	0.14
	Weighted mode	12	-0.17	0.14	0.24	0.84	0.64	1.10	0.47
Family Alcaligenaceae	Inverse Variance Weighted	12	-0.30	0.10	0.00	0.74	0.61	0.9	0.02
	MR Egger	12	-0.72	0.46	0.15	0.49	0.20	1.19	1.00
	Simple mode	12	-0.40	0.20	0.07	0.67	0.45	1.00	0.59
	Weighted median	12	-0.30	0.14	0.02	0.74	0.57	0.96	0.19
	Weighted mode	12	-0.33	0.19	0.11	0.72	0.49	1.04	0.89
Genus Prevotella_7	Inverse Variance Weighted	11	-0.11	0.05	0.03	0.90	0.81	0.99	0.03
	MR Egger	11	-0.17	0.31	0.60	0.85	0.46	1.56	0.97
	Simple mode	11	-0.23	0.12	0.10	0.80	0.63	1.02	0.26
	Weighted median	11	-0.11	0.07	0.09	0.89	0.78	1.02	0.19
	Weighted mode	11	-0.20	0.13	0.15	0.82	0.64	1.05	0.41
Genus Ruminococcaceae	Inverse Variance Weighted	8	-0.11	0.06	0.03	0.90	0.81	0.99	0.03
	MR Egger	8	-0.08	0.26	0.75	0.92	0.56	1.52	1.00
	Simple mode	8	-0.12	0.10	0.27	0.89	0.73	1.08	0.36
	Weighted median	8	-0.11	0.07	0.09	0.89	0.79	1.02	0.25
	Weighted mode	8	-0.11	0.09	0.24	0.89	0.75	1.06	0.38
Genus Anaerostipes	Inverse Variance Weighted	13	0.21	0.09	0.02	1.24	1.03	1.49	0.03
	MR Egger	13	0.23	0.34	0.50	1.26	0.65	2.44	1.00
	Simple mode	13	0.25	0.22	0.29	1.28	0.83	1.99	0.33
	Weighted median	13	0.20	0.13	0.12	1.22	0.95	1.56	0.16
	Weighted mode	13	0.26	0.21	0.25	1.29	0.85	1.95	0.34

Family Alcaligenaceae (OR=0.742608, 95%CI 0.61164-0.901619,p=0.002645)、genus Ruminococcaceae（OR=0.89581,95%CI 0.810137-0.990542,p=0.03193）、genus Prevotella7 (OR=0.895026,95%CI 0.810775-0.988031,p=0.027898)、phylum Verrucomicrobia (OR=0.838726,95%CI 0.722321-0.973889,p=0.021051) are risk factors in the development of HT. The results of other MR analysis and FDR are also written in the table 1. Cochran's Q method was used for heterogeneity test, and no heterogeneity was found. The horizontal pleiotropy was analyzed by MR Egger method, and it was found that there was no horizontal pleiotropy. No outliers were found in the leave-one-out method and MR Presso. Reasults are show in Supplement 2 and Supplement 3. Leave-one-out diagram and scatter diagram are as follows Figure.2 and Figure.3 . Leave-one-out analysis revealed that no SNP was significantly associated with the outcome.

Figure 2: Leave-one-out analysis for gut microbiome on HT

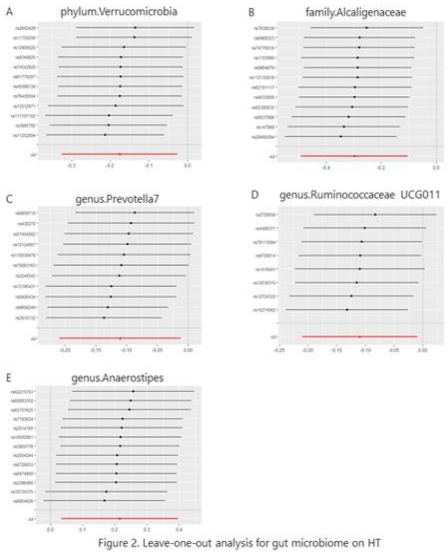

Figure 2. Leave-one-out analysis for gut microbiome on HT

Figure 3: Scatterplot of the effect of the gut microbiome on HT

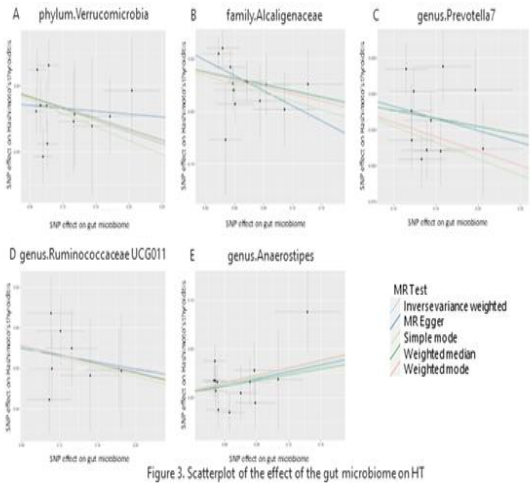

Figure 3. Scatterplot of the effect of the gut microbiome on HT

HT can be proved to be related to family Verrucomicrobiaceae、phylum Verrucomicrobia、class Deltaproteobacteria、class Verrucomicrobiae 、genus Flavonifractor、genus Eubacterium ruminantium group 、family Christensenellaceae at least by IVW method. Only class Deltaproteobacteria, phylum Verrucomicrobia , family Verrucomicrobiaceae, family Christensenellaceae was significant after FDR correction. HT is a risk factor of phylum Verrucomicrobia（OR=0.968114, 95%CI 0.938525-0.998637,p=0.040743）, class Deltaproteobacteria（OR=0.970233, 95%CI 0.942334-0.998958,p=0.042353）, family Verrucomicrobiaceae（OR=0.963272, 95%CI 0.933234-0.994277,p=0.020607）and protective factor of family Christensenellaceae（OR=1.044625, 95%CI 1.003784-1.087127,p=0.031903）The results of other MR analysis and FDR are also written in the Table.2. Cochran's Q method was used for heterogeneity test, and no heterogeneity was found. The horizontal pleiotropy was analyzed by Mr egger method, and it was found that there was no horizontal pleiotropy. No outliers were found in the leave-one-out method and MR Presso. Details are show in Supplement 2 and Supplement 3. Leave-one-out diagram and scatter diagram are as follows Figure.4 and Figure.5.

Figure 4: Leave-one-out analysis for HT on gut microbiome

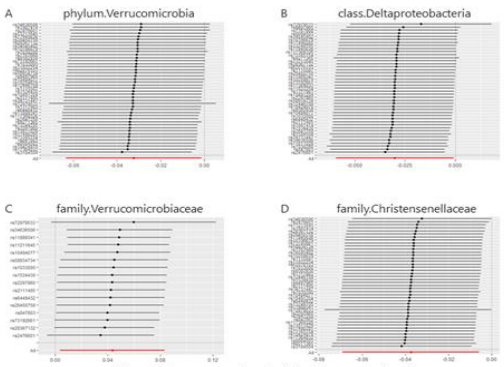

Figure 4. Leave-one-out analysis for HT on gut microbiome

Figure 5: Scatterplot of the effect of the gut microbiome on HT

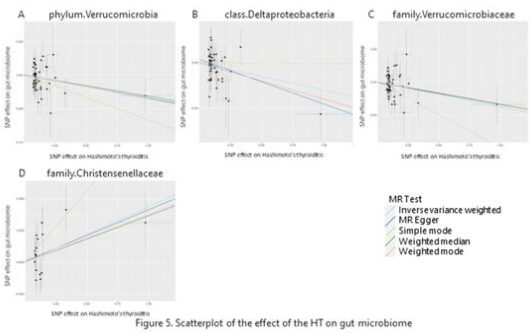

Figure 5. Scatterplot of the effect of the HT on gut microbiome

Table 2: Causal effects of HT on the gut microbiome

outcome	method	nsnp	b	se	pval	or	or_lci95	or_Uci95	adjustP
family Verrucomicrobiaceae	MR Egger	43	-0.03	0.03	0.22	0.97	0.92	1.02	0.37
	Weighted median	43	-0.03	0.03	0.21	0.97	0.92	1.02	0.52
	Inverse variance weighted	43	-0.04	0.02	0.02	0.96	0.93	0.99	0.04
	Simple mode	43	-0.10	0.05	0.05	0.91	0.82	1.00	0.48
	Weighted mode	43	-0.03	0.02	0.16	0.97	0.93	1.01	0.54
phylum verrucomicrobia	MR Egger	43	-0.04	0.03	0.20	0.97	0.92	1.02	0.66
	Weighted median	43	-0.03	0.03	0.25	0.97	0.92	1.02	0.31
	Inverse variance weighted	43	-0.03	0.02	0.04	0.97	0.94	1.00	0.05
	Simple mode	43	-0.07	0.05	0.18	0.94	0.85	1.03	0.29
	Weighted mode	43	-0.03	0.02	0.13	0.97	0.92	1.01	0.67
class Deltaproteobacteria	MR Egger	43	-0.05	0.03	0.06	0.95	0.91	1.00	0.58
	Weighted median	43	-0.04	0.02	0.07	0.96	0.92	1.00	0.72
	Inverse variance weighted	43	-0.03	0.01	0.04	0.97	0.94	1.00	0.04
	Simple mode	43	0.00	0.04	0.99	1.00	0.92	1.09	0.99
	Weighted mode	43	-0.04	0.02	0.09	0.96	0.92	1.00	0.86
family Christensenellaceae	MR Egger	16	0.04	0.03	0.19	1.04	0.98	1.10	0.95
	Weighted median	16	0.04	0.03	0.17	1.04	0.98	1.09	0.57
	Inverse variance weighted	16	0.04	0.02	0.03	1.04	1.00	1.09	0.05
	Simple mode	16	0.11	0.07	0.13	1.12	0.98	1.28	0.25
	Weighted mode	16	0.04	0.03	0.17	1.04	0.99	1.09	0.34

DISCUSSION

This study analyzed the interaction between 211 intestinal bacteria and HT. Finally, we identified Phylum Verrucomicrobia、Family Alcaligenaceae、Genus Prevotella7、Genus Ruminococcaceae、Genus Anaerostipes is causally related to the occurrence of HT.HT has an effect on the abundance of family Verrucomicrobiaceae、phylum Verrucomicrobia 、class Deltaproteobacteria、family Christensenellaceae.

AITD is the most common group of autoimmune diseases, which mainly includes HT and GD. Studies have shown that gut microbiota and its metabolites may directly or indirectly affect thyroid immunity, leading to the occurrence of AITD. These regulatory mechanisms may include inducing a shift in T helper cell responses from type 1 to type 2. T cell responses, activation of Toll like receptor 4 by lipopolysaccharide, and changes that induce transcriptional, proteomic, and metabolic changes Xie et al. (2023). It may also be through antigen cross immune responses between the gut microbiota and the thyroid gland, including cross immune responses induced by Yersinia enterocolitica via thyrotropin receptor like substances Kiseleva et al. (2011). Examination under transmission electron microscopy can observe the changes in the thickness of microvilli in the duodenum of HT patients and the increased gap between adjacent microvilli, and allow toxin antigens or bacterial metabolites to enter the bloodstream from the intestine Sasso et al. (2004). Therefore, the microbiota and its metabolites breaking the intestinal barrier into the systemic circulation and promoting the release of inflammatory factors may be one of the mechanisms of AITD Jiang et al. (2022). The dietary habits of HT patients are significantly different from those of non HT patients. The study found that the intake of animal protein was negatively correlated with the amount of Bacteroides gene. This indicates that dietary habits can affect the gut microbiome, so they may act as a mediator between diet and the occurrence of HT Cayres et al. (2021). The microbiota affects the intake of thyroid related minerals, including iodine, selenium, zinc, and iron. Iodine and selenium content are risk factors for HT. Although the selenium content required for deiodinase

activity is very low, selenium deficiency reduces thyroid hormone synthesis and appears to have an effect on thyroid function. For example, lactobacilli and bifidobacteria are negatively correlated with dietary iron, and positively correlated with selenium and zinc. Gut microbes may affect HT through the regulation of minerals Knezevic et al. (2020). This study took the lead in using the two sample MR method to explore the causal-effect relationship between HT and gut microbiome. And not only the effect of gut microbiota on HT, but also the effect of HT on gut microbiota was studied. MR is an analysis method based on GWAS data. It uses SNPs strongly associated with traits for analysis, so as to avoid the influence of confounding factors on the results as much as possible.

Genus Anaerostipes is the risk factor of HT, its OR is the largest as 1.24.Existing observational studies have found that anaerostipes can be used as a characteristic flora of HT and considered as a pathogenic factor by extracting DNA from fecal samples for analysis Cornejo-Pareja et al. (2020). In this MR study, genus Prevotella7 is a risk factor for HT,it has been confirmed by previous studies that Prevotella has relationship with the decrease of proinflammatory Th17 polarization, anti-inflammatory Treg differentiation, and the production of anti-inflammatory metabolites in the gut.Therefore,Th17/Treg homeostasis regulation may lead to Prevotella Li et al. (2016). In this study,we found that there is a bidirectional regulation between phylum Verrucomicrobia and HT. That is, phylum Verrucomicrobia not only has a protective effect on the occurrence of HT, but also HT is a risk factor of phylum Verrucomicrobia. At the same time,HT is a confounding factor to phylum Verrucomicrobia、family Verrucomicrobiaceae、class Verrucomicrobiae, How HT affects Verrucomicrobiae deserves further study. In order to carry out sensitivity analysis and horizontal pleiotropy detection, more genetic variants need to be used as instrumental variables.Therefore, the SNPs used in the analysis did not meet the traditional GWAS significance threshold ($P < 5 \times 10 - 8$). To this end, this study uses FDR correction to limit the possibility of errors. Previous studies mostly focused on the impact of gut microbiota on HT. This study also analyzed the impact of HT on gut microbiota and found that before FDR correction, HT played a protective role of genus Eubacterium ruminantium group、family Christensenellaceae. And it exists as a risk factor for family Verrucomicrobiaceae、phylum Verrucomicrobia、family Verrucomicrobiaceae 、class Verrucomicrobiae、class Deltaproteobacteria、genus Flavonifractor. After FDR correction,HT is only meaningful for class Deltaproteobacteria, phylum Verrucomicrobia, family Verrucomicrobiaceae, family Christensenellaceae. The abundance of intestinal flora is affected by dietary habits, age, host genotype, exercise, smoking, antibiotics and other factors Gomaa et al. (2020). As mentioned above, there are significant differences in age, gender and dietary habits between HT patients and normal people. And smoking is a risk factor for the incidence of HT, and the incidence of HT in smokers has greatly increased. Therefore, HT may affect the intestinal flora through these factors, and the value of this effect may be further analyzed using the two-step MR method.

The incidence of HT is high in female population. The GWAS data used in this study did not consider the possible impact of gender ratio on the gut microbiome. Although variants related to sex chromosomes have been excluded, it is still possible to generate bias due to gender. On the other hand, studies have shown that the status of thyroid function may have an impact on the type and number of gut microbiome in HT patients. Because the analysis uses summary statistical data rather than original data, it is impossible to analyze HT patients in different status of thyroid function in groups. For patients in different status of thyroid function, the relationship between HT and gut microbiome needs further exploration.

CONCLUSION

With the progress of intestinal microecology research in thyroid disease, more and more evidence shows that the intestinal microbiota is an important environmental factor that directly or indirectly affects the progression of HT, and HT also affects the intestinal microbiota. This study used MR method to explore the bidirectional causal-effect relationship between the gut microbiome and the occurrence of HT, and finally determined that there was a positive causal-effect relationship between the occurrence of HT and the incidence of genus anaerostipes, family Alcaligenaceae、genus Ruminococcaceae、genus Prevotella7、phylum Verrucomicrobia has a negative causal-effect relationship with the occurrence of HT.HT has cause-effect relationship with family Verrucomicrobiaceae、phylum Verrucomicrobia 、class Deltaproteobacteria、family Christensenellaceae. However, how the gut microbiome regulates thyroid autoimmunity and how HT affects the gut microbiome need further exploration. Perhaps there is a thyroid-gut-axis that plays a regulatory role.

DECLARATIONS

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Conflict of Interest

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately for this work.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author Contributions

Shao Xinran: study conception, design, data analyses, and draft preparation. Jiang Jiuzhi、Fei xiang、Zhang Ying: literature search, and translation of paper. Cui Jianchun、Zhang Dongxiao: supervision of the study. All authors contributed to the article and approved the submitted version.

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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.

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