Introduction

Graves’ disease (GD) and Hashimoto’s thyroiditis (HT) are the most common autoimmune thyroid disease (AITD) types (Fallahi et al. 2019; Rayman 2019; Knezevic et al. 2020). AITD is a set of organ-specific autoimmune diseases with similar genetic and immunological features. The pathogenesis of AITD depends on multiple factors, but the exact mechanism is still unclear. It is generally believed that genetic susceptibility, environmental and survival factors (gender difference), stress, and other factors have important roles in the pathogenesis. However, recent evidence has suggested that the gut microbiota is closely associated with some immune-related diseases, including type 1 diabetes mellitus (T1DM) (Kugelberg 2017), rheumatoid arthritis (RA) (Lynch and Pedersen 2016; Horta-Baas et al. 2017), multiple sclerosis, Graves’ ophthalmopathy (Covelli and Ludgate 2017; Shi et al. 2019), HT, and inflammatory bowel disease (Masetti et al. 2018; Zhao et al. 2018; Kozhieva et al. 2019). The gut microbiota has a crucial role in the metabolism, absorption, immune function, and defense mechanism against pathogens.
(Pickard et al. 2017; Azad et al. 2018; Reddel et al. 2019). Nevertheless, the exact effect of gut microbiota on AITD, particularly HT and GD, is still not well defined. It has been suggested that gut microbiota targets the TSH receptor (Knezevic 2020; Yao et al. 2020). The combination of microbe and thyroid autoantibody suggests that it may have a role in AITD (Kristensen 2016). Therefore, a deep understanding of the exact mechanism behind these changes and their relationship with AITD may help develop new prevention and treatment strategies. This study explored the alterations and putative activity of gut microbiota in GD and HT. Fecal samples from the GD, HT patients, and healthy people were collected and analyzed using 16s rRNA sequencing.

**Experimental**

**Materials and Methods**

**Sample collection.** All subjects were of Han nationality, born in northeast China. The participants in the experiment were divided into three groups: GD group, HT group, and control group (healthy subjects). Twenty-seven samples from the GD group and 27 samples from the HT group were collected from the Department of Endocrinology, Daqing Oilfield General Hospital; 16 samples were from the healthy people recruited from Daqing Campus Harbin Medical University.

The inclusion criteria for patients with AITD (GD and HT group) were: (1) age 18–70 years; (2) GD group had the clinical hypermetabolic symptoms and signs, the FT3 of the thyroid function test was > 6.8 pmol/l, FT4 was > 22 pmol/l, TSH was < 0.27 mIU/l, TRAb was > 1.22 IU/l, and thyroid ultrasound indicated a diffuse thymegaly; in the HT group, FT4 was < 12 pmol/l, TSH was > 4.2 MIU/l, TPOAb was > 34 IU/ml, thyroid ultrasound indicated that it was consistent with Hashimoto’s disease; (3) the patients did not receive anti-thyroid or replacement therapy. In the control group, all thyroid function, TGAb, TPOAb and TRAb, and thyroid ultrasound were within the normal range. The reference range is defined as follows: FT3: 3.1–6.8 pmol/l, FT4: 12–22 pmol/l, TSH: 0.27–4.2 mIU/l, TPOAb: 0–34 IU/ml, TGAb: 0–115 IU/ml, TRAb: 0–1.22 IU/l.

The exclusion criteria were: (1) hypertension, diabetes, lipid disorders, pregnancy, lactation, smoking, alcohol addiction, use of antibiotics in recent three months; use of probiotics, prebiotics, symbiosis, hormone drugs, laxatives, proton pump inhibitors, insulin sensitization agent, and Chinese herbal medicine; (2) other autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, irritable bowel syndrome, and malignant tumor; (3) previous onset of gastrointestinal surgery (e.g., gastrectomy, bariatric surgery, colon resection, resection of the ileum, cholecystectomy, or appendectomy).

All subjects were examined in the morning after overnight fasting (≥ 8 hours). Peripheral blood (6 ml) was collected from all subjects and stored at the temperature of 4°C in EDTA tubes; then, thyroid function and thyroid antibody levels were analyzed. In addition, all subjects were provided with a toilet specimen collection kit to collect feces. Each fecal sample was divided into equal samples, frozen with dry ice, and stored at –80°C.

**Thyroid function and thyroid autoantibodies tests.** Serum levels of thyroid-stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3), anti-thyroid peroxidase antibody (TPOAb) and anti-thyroglobulin antibody (TGAb), and thyrotropin receptor antibody (TRAb) were measured by chemiluminescence immunoassay (Roche E602, Germany) according to manufacturer’s instructions.

**DNA extraction.** Fecal microbes’ DNA was extracted from fecal samples according to the fecal genomic DNA extraction kit (Beijing D2700, Solebo, China). DNA concentration and purity were detected by NanoDrop2000, and DNA extraction quality was detected by 1% agar-gel electrophoresis.

**Amplicon generation and purification.** The V3-V4 variable region of the 16S rRNA gene was amplified using PCR with 338F (5’-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) primers. The amplification conditions were as follows: pre-denaturation at 95°C for 3 min, 27 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s), and final extension at 72°C for 10 min (PCR instrument: ABI GeneAmp® type 9700, Applied Biosystems, USA). PCR products were recovered using 2% agarose Gel, purified by AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA), eluted by TRIS-HCl, and detected by 2% agarose electrophoresis. QuantiFluor™-ST (Promega, USA) was used for quantitative measurement.

**Illumina MiSeq sequencing.** Illumina’s Miseq PE300 platform was used for sequencing (Shanghai Maggi Bio-Pharmaceutical Technology Co., Ltd., China).

**Microarray chip analysis.** Gene microarrays (GSE10001, GSE32445) and the GEO2R software (https://www.ncbi.nlm.nih.gov/geo/geo2r/) microarray data analysis were used in this study.

**Functional enrichment analysis.** The analysis of gut microbiota’s biological functions and metabolic pathways was performed using the KEGG and COG.

**Statistical analyses.** The analysis of clinical parameters was performed using IBM SPSS Statistics for Windows v19.0 (IBM Corp., USA). The original sequence
was controlled by the Trimmomatic software and spliced by the FLASH software. UPARSE software (version 7.1; http://drive5.com/uparse/) with a similarity of 97% to OTU sequence clustering, a single sequence in the process of clustering and chimeras was obtained. The classifier (http://rdp.cme.msu.edu/) was employed to annotate the species classification of each sequence, and it was compared to the Silva database (SSU123) with the comparison threshold of 70%. A \( p \)-value < 0.05 was statistically significant.

**Results**

**Study population.** Twenty-seven GD patients, 27 HT patients, and 16 healthy people were included in the study. The demographic and clinical parameters of the subjects are summarized in Table I.

The gut microbiota abundance and diversity in the GD and HT groups were similar to those in the healthy groups, but the overall structure was different. To identify whether the GD and HT were associated with changes in microbiota diversity, we sequenced and analyzed fecal samples. Thirteen phyla, 23 classes, 43 orders, 75 families, 221 genera, 422 species, and 595 operational taxa (OTU) were found in the GD group; 12 phyla, 21 classes, 33 orders, 61 families, 201 genera, 394 species and 585 out in the HT group; and 13 phyla, 22 classes, 35 orders, 64 families, 180 genera, 322 species, and 436 OTU were in the control group, all of which had 97% similarity. According to OTU analysis results, the grade-abundance curves of the GD and HT patients and the healthy control group presented similar patterns (Fig. 1A and 1B). The results showed that the richness and diversity of gut microbiota in the healthy control group tended to be lower than those in the GD and HT, but the differences were not significant. According to the Sobs and Simpson index in PAN/Core species analysis, alpha diversity analysis, and a Shannon index and dilution curve where both

|          | GD (n = 27) | HT (n = 27) | Controls (n = 16) |
|----------|------------|------------|------------------|
| Age (years) | 49.20 ± 8.68 | 56.77 ± 12.44 | 49.31 ± 13.36 |
| Sex (M/F) | 8/19 | 11/16 | 7/9 |
| FT3 (pmol/l) | 14.74 ± 8.65** | 3.93 ± 1.22 | 5.13 ± 0.76 |
| FT4 (pmol/l) | 52.19 ± 24.83** | 7.73 ± 2.99* | 17.91 ± 1.88 |
| TSH (mU/l) | 0.005 ± 0.000** | 38.798 ± 32.452** | 3.030 ± 0.806 |
| ATG (IU/ml) | 371.84 ± 320.30** | 1248.39 ± 2623.73*** | 56.72 ± 26.04 |
| ATPO (IU/ml) | 352.04 ± 148.07** | 519.40 ± 833.86** | 12.27 ± 8.43 |
| TRAb (IU/ml) | 8.69 ± 2.90** | 1.21 ± 0.66 | 0.68 ± 0.2 |

Compared with the control group *\( p < 0.05\), **\( p < 0.01\).

Fig. 1. The gut microbiota of GD and HT patients were different from that of the healthy control group. A) The rank-abundance curve of the GD group, B) the rank-abundance curve of the HT group.
species richness and uniformity are considered, the species abundance, total species, and core species obtained by sequencing were sufficient. Consequently, the sample sequencing quantity was considered satisfactory, indicating the results were convincing.

The dilution curve analysis showed that the gut microbiota of the GD and HT patients had a similar species richness compared to the healthy group. A total of 686 OTUs were detected in all the samples, among which 389 were commonly shared among groups. Sixty-three, 61, and 21 unique OTUs were identified in the GD, HT, and healthy control samples.

Next, taxon-dependent analysis was performed using the Ribosome Database Project (RDP) classifier to describe gut microbiota composition in different groups. The HT group had the highest content of Proteobacteria and Actinomycetes, followed by the GD group and the healthy control group. Notably, the HT group contained a small number of Verrucomicrobiae. At the level of "family" and "genus", the community composition of the GD and HT group were different compared with that of the healthy control group (Fig. 1C and 1D). Moreover, PLS-DA analysis using a binary Jaccard similarity algorithm showed that the overall microbial composition of the GD group and HT group was somewhat different from that of the healthy control group, but there was no significant difference between the GD group and HT group (Fig. 1E). In addition, ANOSIM showed that gut microbiota composition was significantly different between the GD group, HT group, and healthy control group, with an R-value of 0.2519 (Fig. 1F).

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**Fig. 1.** The gut microbiota of GD and HT patients were different from that of the healthy control group. C) histogram of horizontal flora composition of “family”; D) histogram of horizontal flora composition of “genus”; E) PLS-DA analysis with group supervision.
These results indicated that the levels of bacterial abundance and diversity in the gut microbiota of the GD and HT patients were similar to those of the healthy controls. In contrast, the overall structure of the gut microbiota of both patients and healthy controls were significantly different.

**The abundance of gut microbiota in the GD and HT groups.** The linear discriminant effect size (LEfSe) method was used to identify specific bacteria associated with GD and HT. The branching diagram showed fecal microflora and major bacterial structures in the healthy controls and the GD and HT patients and compared the most considerable taxonomic group differences between the two communities (Fig. 2A). The LEfSe analysis revealed 24 discriminant features of class \( (n = 3) \), order \( (n = 3) \), family \( (n = 4) \), and genus \( (n = 14) \).

**Fig. 1.** The gut microbiota of GD and HT patients were different from that of the healthy control group. F) ANOSIM analysis.

**Fig. 2.** Bacterial flora classification map obtained by LEfSe analysis. A) LEfSe shows the greatest difference in abundance (taxa) between the three groups (LDA threshold > 3).
Zhao H. et al.

(Linear discriminant analysis LDA > 3, p < 0.05) when comparing the GD group and the control group, and 13 discriminant features of class (n = 2), order (n = 3), family (n = 3) and genus (n = 5) when comparing the HT group and the control group (linear discriminant analysis LDA > 3, p < 0.05).

The abundance of Negativicutes in healthy control samples and Proteobacteria and Erysipelotrichia in GD patient samples increased. Coriobacteriaceae and Erysipelotrichia were more abundant in HT patient samples than in other samples (Fig. 2A).

At the “phylum” level, the proportions of Cyanobacteria in the GD samples were higher than those in the healthy control samples, while the proportions of abnormal cocci and Cyanobacteria were lower (Fig. 2B). Moreover, the proportions of Cyanobacteria in the samples of the HT patients were higher than that of the healthy control group, while the proportions of abnormal Coccinobacteria and Cyanobacteria were lower (Fig. 2C).

At the level of “family”, Lachnospiraceae, Alcaligenaceae, Christensenellaceae, and Erysipelotrichaceae were prevalent in the GD patient samples (Fig. 2D); Enterococcaceae, Erysipelotrichaceae, and Bacillaceaceae were abundant in the HT patient samples, while Peptostreptococcaceae, Baccillaceae, and Matophyaceae were high in the healthy control samples (Fig. 2E).

At the level of “genera”, Prevotella_9, Ruminococcus_2, and Lachnospiraceae_NK4A136_group were higher in GD patient samples, while the proportion of

**Fig. 2.** Bacterial flora classification map obtained by LEfSe analysis.

B–G) the difference in microbiota between the GD group or HT groups and the healthy control group at the phylum level (B, C), at the family level (D, E), and at the genus level (F, G). *p < 0.05; **p < 0.01; ***p < 0.001.
Gut microbiota in GD and HT

Fig. 2. E, F, G.
Megamonas genus was abundant in the healthy controls (Fig. 2F). The contents of Ruminococcus_2 and Enterococcus in the HT patient samples were relatively high (Fig. 2G).

These data suggest a difference in the microbiome of GD and HT patients compared to the healthy control group. Although there was no significant change in bacterial diversity, the abnormal composition of fecal microflora indicated gut microbiota imbalance in the GD and HT patients.

**Bacillus, Blautia, and Ornithinimicrobiium** can be used as potential markers to distinguish GD and HT patients from the healthy people. Next, a random forest analysis was performed to compare the GD group (Fig. 3A) or HT group (Fig. 3B) with a healthy control group. The results of random forest analysis showed that the areas under the verification information curve of the top three strains of Bacillus, Blautia, and Ornithinimicrobiium in the GD patients and the top two strains of Bacillus and Ornithinimicrobiium in the HT patients.

![Fig. 3. Random forest analysis and validation information. A) Random forest analysis between the GD and healthy control groups, and B) between the HT group and control groups.](image-url)
were 0.98842 and 1, respectively (Fig. 3C and 3D). It suggested that these bacteria may be valuable markers for distinguishing healthy patients from GD and HT patients and could be used as potential diagnostic markers of GD and HT.

Functional categories according to the COG and KEGG in different groups. We also predicted the functional categories according to the COG and KEGG in different groups; the results are shown in Fig. 4. According to the COG distribution in Fig. 4A and 4B, the GD and HT groups were highly enriched in carbohydrate transport and metabolism (function G) compared to the control group, while the amino acid transport and metabolism (function E) was lower than that of the healthy control group. The function of “translation, ribosome structure, and biogenesis” (function J) was highest in the GD group, followed by the healthy control group, being lowest in the HT group. As this revealed a significant difference between the disease group and the healthy control group, it should be a focus of future
research. Interestingly, the “transcription” (function K) of GD and HT groups was significantly stronger than the healthy control group.

According to the KEGG distribution in Fig. 4C, there were significant differences in purine metabolism, aminoacyl-tRNA biosynthesis, cysteine, and methionine metabolism between the GD group and the healthy control group (all \( p < 0.05 \)). Moreover, there were significant differences in ribosome and pyrimidine metabolism between the HT and healthy control groups (Fig. 4D). According to the KEGG-based results in Fig. 4C and 4D, the ABC transporter, responsible for the ATP transport pathway, was significantly more abundant in the disease group than in the healthy control group.

According to the COG database, the enzyme “glycosyltransferase” was a specific enzyme in the GD group (Fig. 4E). Also, the resolving enzyme has been suggested as a specific enzyme for the HT group (Fig. 4F).
According to the KEGG database, the “ATP-dependent RNA helicase DHX58” (EC 3.6.3.14) was the highest in the GD group, followed by the healthy control group, while it was the lowest in the HT group (Fig. 4G and 4H). “Glutamine synthase” (EC 6.3.1.2) (Fig. 4G) and “DNA-directed RNA polymerase B subunit” (EC 2.7.7.6) (Fig. 4H) were the specific enzymes for the GD group and HT group, respectively.

Ten different strains of the two groups were divided into three categories, as shown in Fig. 5. Table II shows the top ten predictions using the KEGG database for the abundance of these three categories. The metabolic
Fig. 4. Prediction Results using the COG and KEGG databases.

E, F) the difference in the COG abundance prediction between the disease and control groups.

* \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \).

ko02010 – ABC transporters, ko00230 – purine metabolism, ko00520 – amino sugar and nucleotide sugar metabolism, ko02020 – two-component system, ko00330 – arginine and proline metabolism, ko00970 – aminoacyl-tRNA biosynthesis, ko00500 – starch and sucrose metabolism, ko00680 – methane metabolism, ko00250 – alanine, aspartate and glutamate metabolism, ko00010 – glycolysis/gluconeogenesis, ko00190 – oxidative phosphorylation, ko00860 – porphyrin and chlorophyll metabolism, ko00270 – cysteine and methionine metabolism, ko00720 – carbon fixation pathways in prokaryotes, ko00620 – pyruvate metabolism, ko03010 – ribosome, ko00240 – pyrimidine metabolism, ko03440 – homologous recombination.
pathway of the "ABC transporter" (responsible for ATP transport) existed in the prediction results of the three different strains, indicating that this metabolic pathway is highly correlated with the occurrence of GD and HT.

Next, we conducted a Venn diagram analysis based on the differential strains in Fig. 3A and 3B. Ten differential strains were further divided into two groups and three categories, as shown in Fig. 5. Table II shows the prediction results according to the KEGG database of the top ten strains different in abundance. The "ABC transporter" pathway (responsible for ATP transport) was found in the predicted results of three different

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**Fig. 4.** Prediction Results using the COG and KEGG databases.

G, H) the difference in the KEGG enzyme prediction between the disease and the control groups.

\*p < 0.05; \**p < 0.01, \***p < 0.001.
strains, suggesting that this pathway was strongly associated with the development of the GD and HT.

**GEO database screening for the HT differential genes according to the KEGG database.** We first cross-referenced the differentially expressed genes in the two chips and then annotated them with the KEGG database. Next, the annotation results were compared to the predicted results for different strains using KEGG. The four metabolic pathways common in the two predicted results were glutathione metabolism, arachidonic acid metabolism, purine metabolism, and pyrimidine metabolism. Therefore, we suggest that four strains unique to the HT (Ruminococcus_1, Flavonifractor, Moryella, and Anaerotruncus) may affect the occurrence and development of HT by regulating glutathione metabolism and arachidonic acid metabolism. The six common bacteria (Bacillus, Corynebacterium, Ornithimicrobium, Brachybacterium, Nocardioides, and Ruminococcus_gnavus_group) can participate in the occurrence and development of the HT by regulating purine metabolism and pyrimidine metabolism.

**Discussion**

GD and HT are two major representative diseases of AITD. A previous study suggested an association between gut microbiota imbalance and HT or GD (Virili et al. 2018; Yao 2020). However, thus far, no studies have reported a common imbalance of gut microbiota in GD and HT patients. Our study found multiple bacteria with similar change direction and shared metabolic pathways involved in the GD and HT patients.

In this study, genomic DNA was extracted from the GD, HT, and healthy subjects feces and analyzed using the 16S rRNA gene sequencing. We found that the abundance and diversity of gut microbiota in the GD and HT patients were similar to the healthy control group. However, we also discovered that Actinobacteria and Proteobacteria contents were the highest in the HT group, followed by the GD group, while they were the lowest in the control group. A previous retrospective study showed the highest alteration in the abundance of Bacteroidetes, Proteobacteria, and Firmicutes between the systemic inflammatory disease group and the healthy group (Nam et al. 2013; Clemente et al. 2018; Faucher et al. 2020). Zhao et al. (2018) found a higher gut microbiota richness and diversity in HT patients with normal thyroid function. Firmicutes were the most abundant, while Bacteroides were less common in HT patients, consistent with our findings. Nevertheless, in this study, HT patients were all hypothyroidism patients, that are different from the study reported by Zhao et al. (2018). Furthermore, Zhou et al. (2014) showed the gut microbiota diversity in the GD patients; Bifidobacteria and Lactobacillus were significantly reduced, but Clostridium and Enterococcus were increased compared to the healthy groups. Other studies reported on *Helicobacter pylori* and *Yersinia enterocolitica*, mainly focusing on the relationship between *H. pylori* CagA and AITD (Köhling et al. 2017; Figura et al. 2019; Cuan-Baltazar and Soto-Vega 2020). Bassi et al. (2012) and Soveid et al. (2012) suggested that *H. pylori* is associated with GD, but not with HT (Bassi et al. 2014), while Wenzel et al. (1988) found that IgA and IgG anti-*Yersinia* antibodies were significantly increased in GD and HT. Moreover, Takuno et al. (1990) found that *Y. enterocolitica* was significantly correlated with GD, but not with HT. Effraimidis et al. (2011) reported no causal relationship between *Y. enterocolitica* infection and the AITD. Another study showed an increased number of Bacteroidetes and Proteobacteria and decreased levels of Firmicutes in GD patients, which is consistent with our data (Ebert 2010; Köhling et al. 2017). The same study suggested a higher abundance of *Pasteurellaceae* and *Prevotella* in GD patients compared to healthy people (Ebert 2010; Köhling et al. 2017); yet, this was not observed in our study. ANOSIM analysis showed

![Diagram of random forest differential strains.](image)
significant differences in gut microbiota composition between the HT, GD, and the healthy group, which further indicated that the intestinal microecology of the HT and GD patients was unbalanced.

The LEfSe analysis showed similar trends in bacteria in the GD and HT groups, where the most apparent changes included an increased abundance of Erysipelotrichia, Cyanobacteria, Ruminococcus_2, and decreased abundance of Bacillaceae and Megamonas. These data suggest that there may be a common gut microbiota disorder in GD and HT patients. A previous study (Kozhieva et al. 2019) demonstrated the increased abundance levels of Blautia, Roseburia, Ruminococcus_torques_group, Romboutsia, Dorea, Fusobacterium, and Eubacterium_hallii_group, while the Fecalibacterium, Bacteroides, Prevotella_9, and Lachnoclostridium decreased in HT patients. Other studies (Liu et al. 2020) suggested that Lachnospiraceae_incertae_sedis, Lactomurisfactor, Alistipes, and Subdoligranulum were more enriched in HT patients with euthyroidism, while Phascolarctobacterium was more abundant in those with hypothyroidism. Yan et al. (2020) found that the number of Bacilli, Lactobacillales, Prevotella, Megamonas, and Veillonella strains in GD patients increased, while Ruminococcus, Rikenellaceae, and Alistipes decreased compared with the healthy people. Our data are consistent with the above results, suggesting that the gut microbiota of GD and HT patients is in an unbalanced state. However, our study confirmed that there are species with the similar trend change in HT and GD patients, confirming that there may be a common imbalance of flora involved in the occurrence of GD and HT.

The results of random forest analysis indicated that the areas under the verification information curve of Bacillus, Blautia, and Ornithinimicrobium in the GD patients and Bacillus and Ornithinimicrobium in the HT patients were equal to 0.98842 and 1, respectively, suggesting that these strains may be used as biomarkers to distinguish healthy individuals from the GD and HT patients. In order to analyze the differences in microbial composition between the disease group and the healthy control group, we used the PIS-DA analysis based on a binary Jaccard to replace the traditional PCoA analysis, as the PIS-DA analysis adds grouping information. Through group supervision, this method can ignore the random differences within the groups and highlight the systematic differences between the groups, which is more illustrative than PCoA and other methods.

In this study, the COG database was used to predict the function of the HT, GD, and healthy control groups. We found that HT and GD patients were enriched in carbohydrate transport and metabolism compared to the control group but had lower amino acid transport and metabolism activity. According to the COG prediction of the function of different strains, four unique strains of the HT group and six common strains of the HT and GD groups were identified as “S” (Function unknown). It may indicate that there is still an unknown metabolic pathway in the development of such diseases, and it might be the focus of subsequent research. In this study, KEGG was used to predict the functions of differential bacteria unique to the GD and HT groups and the bacteria common to these two groups. Venn diagram analysis was simultaneously performed. We found that the “ABC transporter” metabolic pathway existed in the predicted results of three different strains, indicating that this metabolic pathway was highly correlated with the occurrence of GD and HT. Studies have shown that the levels of L-arginine, L-ornithine, lysine, and guanabutamine in the GD and HT patients are higher than those in the healthy group, while the levels of putrescine, 1,3-diaminopropylene, spermine, and N-acetylputrescine are lower than those in the healthy group (Song et al. 2019). Some polyamine metabolites were only different in the GD or HT patients compared with the healthy group. Spermidine proportions were significantly reduced in all the patients. This study confirmed that most metabolites of the GD and HT had similar patterns compared with the healthy group, suggesting a common pathophysiological basis or metabolic pathway.

This study has a few limitations. First, it was a single-center study with a relatively small sample size, which may lead to bias. Second, the specific mechanism of abnormal gut microbiota involved in GD and HT was not examined. Third, all the people were selected from inland North China in this study. Considering the differences in dietary structure and ethnicity, the higher iodine intake in coastal areas, thyroid function, and gut microbiota may differ. Thus, further in-depth multicenter studies with a large sample size should be carried out to confirm these findings.

**Conclusion**

Our study demonstrated that Bacillus, Blautia, and Ornithinimicrobium could be used as potential markers to distinguish the GD and HT from the healthy population and the “ABC transporter” metabolic pathway may be involved in the pathogenesis of the GD and HT. In our future work, we plan to construct an animal model of the GD and HT, after which a fecal bacteria transplantation or intervention of differential metabolites will be conducted. It aims to clarify further the regulatory role of gut microbiota in the occurrence and development of GD and HT, and to explore the specific mechanism of abnormal gut microbiota involved in GD and HT. Our study suggested that GD and HT patients...
have similar changes in gut microbiota and the same metabolic pathway usage, suggesting that there may be common changes in gut microbiota inAITD patients, which needs to be further studied.

Ethical statement
The study was carried out in accordance with the Helsinki Declaration. The protocol used in this study was approved by the Ethics Committee of Harbin Medical University (China, No. HMUDQ2019102801). All subjects were informed of the nature of the study. All participants provided informed consent.

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Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Ajan RA, Weetman AP. The pathogenesis of Hashimoto's thyroiditis: Further developments in our understanding. Horm Metab Res. 2015 Sep;47(10):702–710. https://doi.org/10.1055/s-0035-1548832

Azad MA, Sarker M, Li T, Yin J. Probiotic species in the modulation of gut microbiota: An overview. Biomed Res Int. 2018 May;2018:9478630. https://doi.org/10.1155/2018/9478630

Banga JP, Schott M. Autoimmune thyroid diseases. Horm Metab Res. 2018 Dec;50(12):837–839. https://doi.org/10.1055/a-0799-5068

Bassi V, Fattoruso O, Polistina MT, Santinelli C. Graves' disease shows a significant increase in the Helicobacter pylori recurrence. Clin Endocrinol (Oxf). 2014 Nov;81(5):784–785. https://doi.org/10.1111/cen.12410

Bassi V, Marino G, Iengo A, Fattoruso O, Santinelli C. Autoimmune thyroid diseases and Helicobacter pylori: The correlation is present only in Graves's disease. World J Gastroenterol. 2012 Mar 14;18(10):1093–1097. https://doi.org/10.3748/wjg.v18.i10.1093

Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. BMJ. 2018 Jan 8;360:j1545. https://doi.org/10.1136/bmj.j1545

Covelli D, Ludgate M. The thyroid, the eyes and the gut: a possible connection. Endocrinol Invest. 2017 Jun;40(6):567–576. https://doi.org/10.1007/s40418-016-0594-6

Cuan-Baltazar Y, Soto-Vega E. Microorganisms associated to thyroid autoimmunity. Autoimmun Rev. 2020 Sep;19(9):102614. https://doi.org/10.1016/j.autrev.2020.102614

Ebert EC. The thyroid and the gut. J Clin Gastroenterol. 2010 Jul;44(6):402–406. https://doi.org/10.1097/MCG.0b013e3181d6bc3e

Effraimidis G, Tijsjen JS, Strieder TG, Wiersinga WM. No causal relationship between Yersinia enterocolitica infection and autoimmune thyroid disease: evidence from a prospective study. Clin Exp Immunol. 2011 Jul;165(1):38–43. https://doi.org/10.1111/j.1365-2249.2011.04399.x

Fallahe P, Elia G, Ragusa F, Ruffilli I, Camastra S, Giusti C, Paparo SR, Donnella D, Shoenfeld Y, Ferrari SM, et al. The aggregation betweenAITD with rheumatologic, or dermatologic, autoimmune diseases. Best Pract Res Clin Endocrinol Metab. 2019 Dec;33(6):101372. https://doi.org/10.1016/j.beem.2019.101372

Faucher MA, Greathouse KL, Hastings-Tolsma M, Padgett RN, Sakovich K, Choudhury A, Sheikh A, Ajami NJ, Petrosino JF. Exploration of the vaginal and gut microbiome in African American women by body mass index, class of obesity, and gestational weight gain: A pilot study. Am J Perinatol. 2020 Sep;37(11):1160–1172. https://doi.org/10.1056/s-0039-1692715

Figura N, Di Cairano G, Moretti E, Iacoponi F, Santucci A, Bernardini G, Donnella S, Giordano N, Ponzetto A. Helicobacter pylori infection and autoimmune thyroid diseases: The role of virulent strains. Antibiotics (Basel). 2019 Dec 30;9(1):12. https://doi.org/10.3390/antibiotics9010012

Horta-Baas G, Romero-Figueroa MDS, Montiel-Jarquin AJ, Pizano-Zarate ML, Garcia-Mena J, Ramirez-Duran N. Intestinal dysbiosis and rheumatoid arthritis: A link between gut microbiota and the pathogenesis of rheumatoid arthritis. J Immunol Res. 2017;2017:4835189. https://doi.org/10.1155/2017/4835189

Knezevic J, Starcli C, Tmava Berisha A, Amrein K. Thyroid-gut-axis: How does the microbiota influence thyroid function? Nutrients. 2020 Jun 12;12(6):1769. https://doi.org/10.3390/nu12061769

Köhling HL, Plummer SF, Marchesi JR, Davidge KS, Ludgate M. The microbiota and autoimmunity: Their role in thyroid autoimmune diseases. Clin Immunol. 2017 Oct;183:63–74. https://doi.org/10.1016/j.clim.2017.07.001

Kozhiyeva M, Naumova N, Alinka T, Boyko A, Vlassov V, Kabilov MR. Primary progressive multiple sclerosis in a Russian cohort: relationship with gut bacterial diversity. BMC Microbiol. 2019 Dec 30;19(1):309. https://doi.org/10.1186/s12866-019-1685-2

Kristensen B. Regulatory B and T cell responses in patients with autoimmune thyroid disease and healthy controls. Dan Med J. 2016 Feb;63(2):B5177.

Kugelberg E. Microbiota: Diet can protect against type 1 diabetes. Nat Rev Immunol. 2017 May;17(5):279. https://doi.org/10.1038/nri.2017.40

Liu S, An Y, Cao B, Sun R, Ke J, Zhao D. The composition of gut microbiota in patients bearing Hashimoto's thyroiditis with euthyroidism and hypothyroidism. Int J Endocrinol. 2020 Nov 10; 2020:5036959. https://doi.org/10.1155/2020/5036959

Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. N Engl J Med. 2016 Dec 15;375(24):2369–2379. https://doi.org/10.1056/NEJMr1600266

Masetti G, Moskhelogsha S, Köhling HL, Covelli D, Banga JP, Berchner-Pfannschmidt U, Horstmann M, Diaz-Cano S, Masetti G, Moshkelgosha S, Köhling HL, Goertz GE, Plummer SF, et al.; INDIGO consortium. Primary progressive multiple sclerosis: Further developments in our understanding. Biomed Res Int. 2018 May 24;2018:4835189. https://doi.org/10.1155/2018/4835189

Mukamel E, Rozen O, Aharoni O, Sadeh A, Silberstein A, Pizano-Zarate ML, Garcia-Mena J, Ramirez-Duran N. Autoimmune thyroid disease: evidence from a prospective study. Clin Exp Immunol. 2011 Jul;165(1):38–43. https://doi.org/10.1111/j.1365-2249.2011.04399.x

Paparo SR, Donnella D, Shoenfeld Y, Ferrari SM, et al. The aggregation betweenAITD with rheumatologic, or dermatologic, autoimmune diseases. Best Pract Res Clin Endocrinol Metab. 2019 Dec;33(6):101372. https://doi.org/10.1016/j.beem.2019.101372

Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. Immunol Res. 2017 Sep;72(1):70–89. https://doi.org/10.1007/s12026-017-9404-2

Zhao H. et al.
Rayman MP. Multiple nutritional factors and thyroid disease, with particular reference to autoimmune thyroid disease. Proc Nutr Soc. 2019 Feb;78(1):34–44. https://doi.org/10.1017/s0029665118001192

Reddel S, Putignani L, Del Chierico F. The impact of low-FODMAPs, gluten-free, and ketogenic diets on gut microbiota modulation in pathological conditions. Nutrients. 2019 Feb 12;11(2):373. https://doi.org/10.3390/nu11020373

Shi TT, Hua L, Wang H, Xin Z. The potential link between gut microbiota and serum TRAb in Chinese patients with severe and active Graves’ orbitopathy. Int J Endocrinol. 2019 Dec 18;2019: 9736968. https://doi.org/10.1155/2019/9736968

Song J, Shan Z, Mao J, Teng W. Serum polyamine metabolic profile in autoimmune thyroid disease patients. Clin Endocrinol (Oxf). 2019 May;90(5):727–736. https://doi.org/10.1111/cen.13946

Soveid M, Hosseini Asl K, Omrani GR. Infection by Cag A positive strains of Helicobacter pylori is associated with autoimmune thyroid disease in Iranian patients. Iran J Immunol. 2012 Mar;9(1):48–52.

Takuno H, Sakata S, Miura K. Antibodies to Yersinia enterocolitica serotype 3 in autoimmune thyroid diseases. Endocrinol Jpn. 1990 Aug;37(4):489–500. https://doi.org/10.1507/endocrj1954.37.489

Virili C, Fallahi P, Antonelli A, Bennenga S, Centanni M. Gut microbiota and Hashimoto’s thyroiditis. Rev Endocr Metab Disord. 2018 Dec;19(4):293–300. https://doi.org/10.1007/s11154-018-9467-y

Wenzel BE, Heesemann J, Wenzel KW, Scriba PC. Patients with autoimmune thyroid diseases have antibodies to plasmid encoded proteins of enteropathogenic Yersinia. J Endocrinol Invest. 1988 Feb;11(2):139–140. https://doi.org/10.1007/bf03350122

Yan HX, An WC, Chen F, An B, Pan Y, Jin J, Xia XP, Cui ZJ, Jiang L, Zhou SJ, et al. Intestinal microbiota changes in Graves’ disease: a prospective clinical study. Biosci Rep. 2020 Sep 30;40(9): BS20191242. https://doi.org/10.1042/BSR20191242

Yao Z, Zhao M, Gong Y, Chen W, Wang Q, Fu Y, Guo T, Zhao J, Gao L, Bo T. Relation of gut microbes and L-thyroxine through altered thyroxine metabolism in subclinical hypothyroidism subjects. Front Cell Infect Microbiol. 2020 Sep 18;10:495. https://doi.org/10.3389/fcimb.2020.00495

Yoo WS, Chung HK. Recent advances in autoimmune thyroid diseases. Endocrinol Metab (Seoul). 2016 Sep;31(3):379–385. https://doi.org/10.3803/EnM.2016.31.3.379

Zhao F, Feng J, Li J, Zhao L, Liu Y, Chen H, Jin Y, Zhu B, Wei Y. Alterations of the gut microbiota in Hashimoto’s thyroiditis patients. Thyroid. 2018 Feb;28(2):175–186. https://doi.org/10.1089/thy.2017.0395

Zhou L, Li X, Ahmed A, Wu D, Liu L, Qiu J, Yan Y, Jin M, Xin Y. Gut microbiome analysis between hyperthyroid and healthy individuals. Curr Microbiol. 2014 Nov;69(5):675–680. https://doi.org/10.1007/s00284-014-0640-6