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REVIEW

Gut Microbiota Changes and Its Potential Relations with Thyroid Disorders: From Composition to Therapeutic Targets

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Abstract: Composed of over 1200 species of anaerobes and aerobes bacteria along with bacteriophages, viruses, and fungal species, the human gut microbiota (GM) is vital to health, including digestive equilibrium, immunologic, hormonal, and metabolic homeostasis. Micronutrients, usually refer to trace elements (copper, iodine, iron, selenium, zinc) and vitamins (A, C, D, E), interact with the GM to influence host immune metabolism. So far, microbiome studies have revealed an association between disturbances in the microbiota and various pathological disorders, such as anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, anxiety, depression, early-onset cancers, type 1 diabetes (T1D) and type 2 diabetes (T2D). As common conditions, thyroid diseases, encompassing Graves' disease (GD), Graves' orbitopathy (GO), Hashimoto's thyroiditis (HT), benign nodules, and papillary thyroid cancer (TC), have negative impacts on the health of all populations. Following recent studies, GM might play an integral role in triggering diseases of the thyroid gland. Not only do environmental triggers and genetic predisposing background lead to auto-aggressive damage, involving cellular and humoral networks of the immune system, but the intestinal microbiota interacts with distant organs by signals that may be part of the bacteria themselves or their metabolites. The review aims to describe the current knowledge about the GM in the metabolism of thyroid hormones and the pathogenesis of thyroid diseases and its involvement in the appearance of benign nodules and papillary TC. We further focused on the reciprocal interaction between GM composition and the most used treatment drugs for thyroid disorders. However, the exact etiology has not yet been known. To elucidate more precisely the mechanism for GM involvement in the development of thyroid diseases, future work is needed.

Keywords: gut microbiota, Graves' disease, Hashimoto's thyroiditis, Graves' orbitopathy, thyroid nodules, thyroid cancer

Introduction

Consisting of billions of bacteria, viruses, and fungi, the human intestinal microbiota has recently come to be recognized as a "hidden" organ system playing the trophic, metabolic, and immune functions within the human body.[1] The combined and tightly controlled interaction between intestinal bacteria and the immune system associated with the intestinal mucosa [namely the gut-associated lymphoid tissue (GALT)] serves as a crucial part in maintaining efficiency in immune tolerance to commensals and food antigens.[2] On one side, gut microbiota (GM) is important for the development, differentiation, and maturation of the host immune system, and the metabolic products of GM can mediate the cross-talking between epithelial and immune cells; while on the other side, the host immunity regulates the homeostasis of GM through the defensive role of intestinal mucosal barrier, separating the host immune cells from microbiota.[3] Including intestinal bacteria, intestinal epithelium, blood, lymph, and the nervous and GALT systems in the lamina propria, the intestinal barrier is a physical and functional structure within the gut, the integrity of which is defined as selective permeability to molecules of a certain size and molecular charge. However, the integrity of this barrier may be overcome when the ability of the intestinal barrier to control the transport of antigens to the blood vessels altered by chronic variations in diets, or lifestyle patterns, or environmental exposures, causing subclinical inflammation in situ[4] and thus persistent inflammation migrating to specific tissues and organs,[5]

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known as "leaky gut".[6] This alteration of tight junctions is typical of auto-aggressive phenomena, allowing toxins, antigens, and bacterial fragments to enter the systemic circulation. Figure 1 summarizes the relationship between GM dysbiosis and thyroid dysfunction.

The microbiota, which mainly refers to bacteria, viruses, and fungi resident in the human body, plays an essential role in both the maintenance of normal physiology and the occurrence of clinical outcomes.[7] Consisting of almost 1200 bacterial species with at least 160 such species in each individual,[8] the GM includes Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia.[9] A gut-endocrine–homeostasis-disease axis has been shown in recent studies, which reported a close relationship of the GM in patients with anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis,[10] anxiety,[11] depression,[12] early-onset cancers (generally used for adulthood cancer diagnosed under 50 years of age),[13] type 1 diabetes (T1D) or type 2 diabetes (T2D),[14] respectively, further suggesting that microbiota analysis could provide an alternative non-invasive diagnostic methodology for various diseases. Similarly, there are studies revealing the association between the GM and thyroid hormone (TH) metabolism and thyroid disorders. A schematic overview of the main mechanisms of GM affecting thyroid disorders is summarized in Figure 2.

Herein, this article is aimed at the interaction of intestinal microbiota with thyroid-related micronutrients. Additionally, we will characterize the associations between GM and thyroid diseases or the most used treatments for diseases to evaluate their potential implications in the pathophysiology and open up scientific avenues for future precision studies of the thyroid-gut axis (TGA).

Micronutrients Impact the GM

Micronutrients, including trace elements (copper, iodine, iron, selenium, zinc) and vitamins (vitamin A, vitamin C, vitamin D, vitamin E),[15] not only interact with the GM to influence host immune metabolism but are positioned to alter host-microbe symbiosis to influence micronutrient bioavailability. On the one hand, micronutrient elements can change the composition of the intestinal microbiota, gut barrier function, compartmentalized metabolic inflammation, and endocrine control of TH.[16] On the other hand, host microorganisms can alter immunity, dietary components (carbohydrates,[17] fats,[18] fibers,[19] proteins,[20] or cell fate) and function of pancreatic beta cells to the aim of regulating vitamins. In the bidirectional relationship between bacterial composition and dietary components, diet components continuously contribute to shaping a diverse GM[21] that supports the immune response (for example, plant-derived fibers and nondigestible polysaccharides, prebiotic oligosaccharides) by the biotransformation of gut microbes to food.[22] Indeed, on a microscopic level, an altered diet can result in the

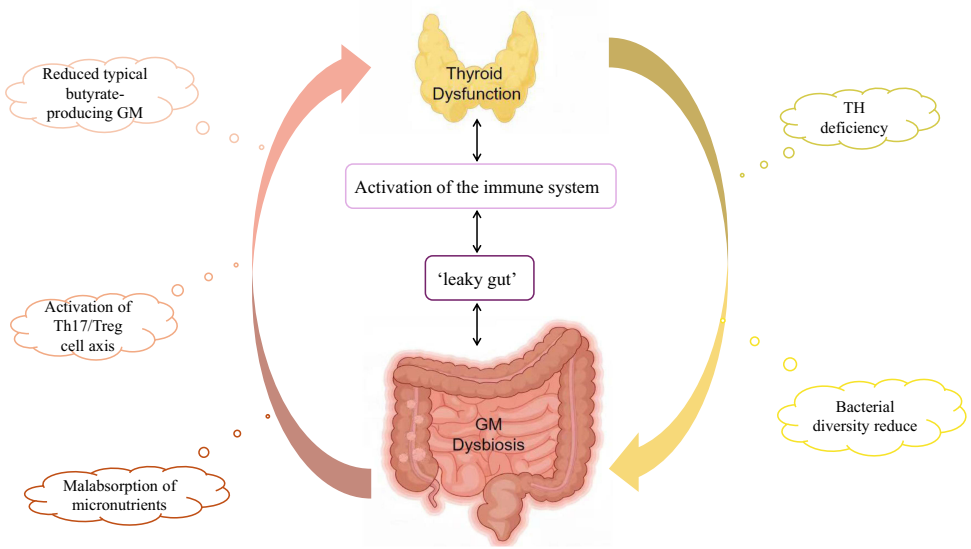

Figure 1 The interrelationship between gut microbiota dysbiosis and thyroid dysfunction. Under physiological conditions, the thyroid gland and GM have a mutually beneficial association. When thyroid function or GM homeostasis changes, the other party would be influenced. In other words, the differences in GM can lead to the development of thyroid dysfunction, and the development of the thyroid gland can cause a series of endocrine changes, leading to the differences in GM.
Abbreviations: GM, gut microbiota; TH, thyroid hormones.

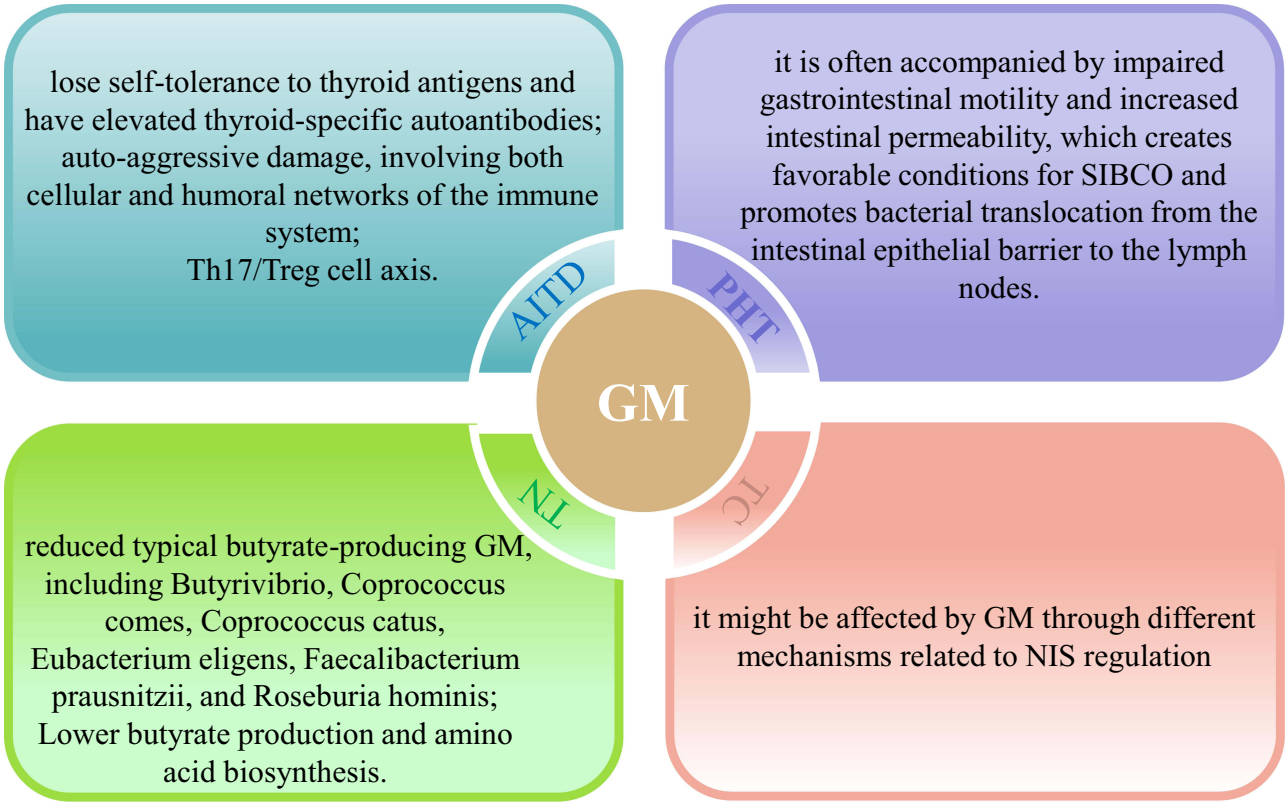

Figure 2 A schematic overview of main mechanisms of gut microbiota influencing thyroid diseases, including AITD, PHT, TN and TC.
Abbreviations: GM, gut microbiota; AITD, autoimmune thyroid disease; PTH, primary hypothyroidism; TN, thyroid nodules; TC, thyroid cancers; SIBCO, small intestinal bacterial colonization and overgrowth; NIS, sodium/iodide symporter.

reduction of the relative abundance of Bacteroidetes and Firmicutes (dominant two key phyla in human GM), and the increase of the less abundant phyla to impact host immunity and metabolism.[21] Furthermore, it seems that micronutrient and microbiota-derived factors also participate in host-microbe responses and balance.

Although reports of micronutrients in maintaining homeostasis of host-microbe metabolism are still emerging, the mechanisms linking the dietary composition of specific micronutrients to changes in gut microbes directly linked to metabolic disease risk are not yet clear. Dietary micronutrients should be considered in pre-clinical and clinical studies investigating host-microbe factors in metabolic diseases, thyroid dysfunction included. A summary outlining the effects of micronutrients on gut bacteria is provided in Table 1.

Copper

Data on copper deficiency and intestinal microbiota composition are limited. The influences of copper on GM, microbial metabolites, and host metabolism were initially found in rats and pufferfish. Wei et al demonstrated that dietary copper alters the abundance of over 20 metabolites including fatty acids and amino acids in the feces of rats.[23] Wang et al also reported that the composition of the GM was altered by elevated copper exposure in pufferfish.[24] The rats fed with copper complexed to chitosan nanoparticles showed an increased abundance of bacteria with probiotic potential within the genus Lactobacillus and decreased potentially pathogenic cecal bacteria (Salmonella, Clostridium),[25] while excess dietary copper exposure in mice increased the relative abundance of Corynebacterium associated with pathological intestinal lesions.[42] Overall, these results suggest that dietary copper can alter GM, inflammation, and barrier function, which may provide a link between this micronutrient and gut bacteria.

Iodine

The iodine is an essential micronutrient involved in TH synthesis and GM is crucial to the intestinal absorption of iodine. To study the effect of the intestinal microbiota on the degree of iodine absorption, Vought et al conducted a study; the

Table 1 The Main Effects of Micronutrients on the Gut Microbiota

Micronutrients	Effects on GM	Reference(s)
Copper (Cu)	Dietary Cu alters the abundance of over 20 metabolites; in pufferfish, the composition of the GM was altered by elevated Cu exposure; the Cu fed rats: increased abundance of bacteria with probiotic potential within the genus Lactobacillus and decreased potentially pathogenic cecal bacteria (Salmonella, Clostridium).	[23–25]
Iodine (I)	Administration of kanamycin rats group vs control group: lower uptake of radioactive I; in mice, oral I supplementation altered the gut flora, increasing the relative abundance of Allobaculum and Oscillibacter, and decreasing Blautia; there are vast disproportions in the GM between the patients with short gut syndrome receiving parenteral nutrition and the control group, but similar I excretion levels.	[26–28]
Iron (Fe)	In mice with heme-rich conditions, microbial diversity decreases and the Proteobacteria, namely Clostridiales and Lactobacillales, grow well; oral Fe supplements increase colonic Fe, leading to adverse effects, decreasing the beneficial barrier of commensal gut bacteria, increasing the abundance of enterobacteria such as enteropathogenic Escherichia coli; GM also can increase the bioavailability of dietary Fe by converting EA to UA, which remains active without having to bind Fe^{3+}.	[29–31]
Selenium (Se)	In chickens, organic Se-enriched feeding increased cecal Lactobacilli spp., while suppressing pathogenic E. coli and Salmonella spp.; in mice, Se-enriched diets increase gut bacterial diversity, which have an increased relative abundance of bacteria such as Bacteroides, Lactobacillus, Prevotella, and Roseburia; the gut microbes can regulate the biotransformation of Se from inorganic to organic states to enhance its bioavailability.	[32–34]
Zinc (Zn)	In mice, Zn deficiency enhanced the bacterial persistence of the pathogen Shigella flexneri, while Zn supplementation reversed inflammatory impairments induced by the pathogen; in chicks, the gut microbe with an increase in the microbial communities of Proteobacteria and a decline in Firmicutes was found to have a response to Zn therapy; in mice, excess Zn can promote Clostridium difficile susceptibility and toxin activity, which is associated with lower α-diversity of Turicibacter genera, bloom in Enterococcus and Clostridium genera; in Crohn's disease patients, Zn supplementation can resolve permeability alterations and improve gut barrier function.	[35–38]
Vitamins	Mice fed with a vitamin A-deficient diet showed a reduction of intestinal Th17 cells, which correlates to worsened metabolic outcomes; changes in the GM composition determine our requirements of dietary B vitamins; the mucosal barriers of mice with vitamin D receptor knockout were damaged, implicating a protective effect of vitamin D on the intestinal mucosa.	[39–41]

Abbreviations: GM, gut microbiota; EA, ellagic acid; UA, urolithin A.

administration of kanamycin in rats showed lower uptake of radioactive iodine compared to the control group,[26] suggesting that GM truly affects the intestinal absorption of iodine. In 2019, Shen and others found that oral iodine supplementation altered the gut flora in mice, increasing the relative abundance of Allobaculum and Oscillibacter, as well as decreasing Blautia in mice.[27] This study concluded that iodine supplementation can alter host metabolism and the composition of the GM. This is consistent with previous conclusion. However, there are vast disproportions in the GM between the patients with short gut syndrome receiving parenteral nutrition and the control group, but similar iodine excretion levels.[28] These findings in mice as well as in humans were controversial. Given the above research findings, drawing any definitive conclusions is difficult.

Iron

Iron is an essential micronutrient for the thyroid gland to synthesize TH and is needed for effective iodine utilization by the iron-dependent enzyme thyroid peroxidase (TPO). Heme iron Fe^{2+}, a necessary constituent of the intestinal

microbiota, is directly absorbed by heme/folate carrier protein 1 (HCP1) in the host and by siderophores like enterobactin in bacteria.[43] Constante et al demonstrated that microbial diversity decreases and the Proteobacteria, namely Clostridiales and Lactobacillales, grow well in mice with heme-rich conditions,[29] which illustrated that iron is essential for bacterial growth. Oral iron supplements can increase colonic iron, leading to adverse effects, decreasing the beneficial barrier of commensal gut bacteria, and increasing the abundance of enterobacteria such as enteropathogenic Escherichia coli.[30] Meanwhile, microbiota have the ability to enhance iron bioavailability in the colon and lower intestinal pH via the production of short-chain fatty acids (SCFAs).[44] As a part of the human microbiota, Lactobacillus fermentum exhibits ferric-reducing activity due to the excretion of p-hydroxyphenyllactic acid, consequently facilitating iron absorption. Moreover, GM also can increase the bioavailability of dietary iron by converting ellagic acid (EA) to urolithin A (UA), which remains active without having to bind Fe^{3+}.[31] Iron is also vital for efficient iodine utilization and TH synthesis. On the one hand, iron deficiency (ID) is a common clinical finding in hypothyroidism;[45] on the other hand, ID is frequently diagnosed in up to 60% of hypothyroidism patients like Graves' disease (GD) and Hashimoto's thyroiditis (HT).[46]

Selenium

As a trace element, selenium is involved mainly in antioxidant defenses and in regulating thyroid function, immunity, and reproduction.[47] Observational studies demonstrate that it also plays a crucial role in improving gut microbe homeostasis.[48] In chickens, organic selenium-enriched feeding increased cecal Lactobacilli spp. while suppressing pathogenic E. coli and Salmonella spp.[32] Based on findings from animal studies, selenium-enriched diets increase gut bacterial diversity in mice, which have an increased relative abundance of bacteria such as Bacteroides, Lactobacillus, Prevotella, and Roseburia.[33] In addition, the biotransformation of selenium from inorganic to organic states can be regulated by gut microbes to enhance its bioavailability.[34] Altogether, these results demonstrate a bidirectional relationship between host selenium status and the GM.

Zinc

Zinc is the second most abundant trace element in the mammalian body after iron, whose absorption is affected by the presence of other minerals in the intestinal lumen.[49] In a study, zinc deficiency enhanced the bacterial persistence of the pathogen Shigella flexneri in mice, while zinc supplementation reversed inflammatory impairments induced by the pathogen.[35] In another study, the gut microbe with an increase in the microbial communities of Proteobacteria and a decline in Firmicutes was found to have a response to zinc therapy in chicks, characterizing distinct intestinal microbiome shifts were induced by chronic dietary zinc depletion rather than acute zinc deficiency.[36] Although zinc supplementation may limit some pathogens by modulating protective immune responses and the GM,[50] excess zinc can promote Clostridium difficile susceptibility and toxin activity in mice, which is associated with lower α-diversity of Turicibacter genera, bloom in Enterococcus and Clostridium genera,[37] increased intestinal permeability, and systemic inflammation.[51] Similarly, zinc supplementation can resolve permeability alterations and improve gut barrier function in Crohn's disease patients.[38] Thus, these results demonstrate that dietary zinc deficiency can impair the intestinal barrier, alter GM, and may set up gut inflammation and changes in host metabolism.

Vitamins (Vitamin A, Vitamin B, Vitamin C, Vitamin D, Vitamin E, Vitamin K)

Intestinal bacteria play a role in vitamin synthesis[52] and regulation of the immune response.[53] As an important component of innate immunity, gut dysbiosis-driven perturbations to the gut barrier homeostasis are linked to poor vitamin absorption.[54] Specifically, mice fed with a vitamin A-deficient diet showed a reduction of intestinal Th17 cells, which correlates to worsened metabolic outcomes.[39] In the study of Magnúsdóttir et al, they proposed that GM is the second most important source of B vitamins in addition to diet supplements. In other words, changes in the GM composition determine our requirements of dietary B vitamins.[40] Kong et al found that the mucosal barriers of mice with vitamin D receptor knockout were damaged, implicating a protective effect of vitamin D on the intestinal mucosa.[41] Taking into account the existing shreds of evidence, the composition of the microbiota and vitamins suggests a reciprocal relationship.

Association Between GM and Autoimmune Thyroid Disease (AITD)

Altered GM and AITD like GD, Graves' orbitopathy (GO) and HT frequently coexist, entailing numerous potential impacts on diagnostic and therapeutic approaches.[55] AITD patients lose self-tolerance to thyroid antigens and have elevated thyroid-specific autoantibodies, such as thyroid peroxidase antibodies (TPOAb), thyroglobulin (TGAb), and thyroid-stimulating hormone receptor antibodies (TRAb).[56] Possible correlations might exist through the immune system and inflammatory responses caused by changed GM, promoting autoimmune diseases, as well as shared cytokines in pathogenesis pathways, cross-reacting antibodies, or malabsorption of micronutrients that are essential for the thyroid. Recent studies have shown that the intestinal flora is related to the occurrence and development of AITD, and the normal operation of the thyroid gland is closely related to iodine and selenium. When the intestinal microecology destroyed, the microbiota is out of balance, and the enzymes and transporters on the intestinal surface are both affected, affecting the absorption and utilization of iodine and selenium. At the same time, selenium is an essential element for intestinal microbes to participate in the regulation and repairment of inflammatory response, and its deficiency will cause the loss of microbiota regulation and induce host immune inflammation.[57] Cuan-Baltazar et al concluded that Helicobacter pylori can exacerbate the production of thyroid autoantibodies, which is considered to be a trigger for thyroid autoimmunity.[58] Intestinal dysbiosis is a common coexisting finding in patients with AITD but might protect from autoimmunity by wielding immunoregulatory and tolerogenic impacts.[59] As far as the current perception is concerned, thyroid function is assumed to be involved in the onset and progression of the gut barrier, presumably plays a substantial protective role for intestinal mucosa, and affects the thyroid via its immunomodulatory effects.

An association between GD and GM has been identified, but the causal effect between them still needs to be elucidated. Fecal samples collected from 55 GD patients and 48 healthy controls (HCs) were analyzed using 16S rRNA (ribosomal RNA) gene amplification and sequencing, showing 18 increased taxa and 4 decreased taxa ($P < 0.0001$).[60] Changes in Bifidobacterium abundance in patients with newly diagnosed GD were positively correlated with TRAb, TGAb, and TPOAb, which are thought to be related to the immune mechanism of GD.[61] Clinical research has identified direct changes in the GM of GD. In addition to clinical research, basic medical research has also provided evidence of the association. The higher levels of Firmicutes in the GM of the GO murine model, the richer orbital adipogenesis, showing a significant positive correlation between.[62] When the composition of the intestinal microbiota is unbalanced, there will be a decrease in Treg lymphocytes and an increase in the activity of TH17 lymphocytes, resulting in the production of a large number of cellular inflammatory factors, and thus promoting apoptosis and necrosis of thyroid cells. The combined effect of environmental triggers and GM dysbiosis background leads to auto-aggressive damage, involving both cellular and humoral networks of the immune system.

Meanwhile, Th17 and Treg cells (Th17/Treg cell axis) are involved in the pathogenesis of HT.[63] In the experiments of GONG et al,[64] it was found that the AITD group had fewer beneficial bacteria such as lactic acid bacteria and bifidobacteria, and the harmful microbiota such as Bacteroides fragilis increased significantly, compared with the control group. This indicates that dysbiosis and changes in structure and function are one of the reasons affecting the occurrence of HT. It has been found that the immune imbalance of the Th17/Treg cell axis runs through different stages of HT.[65] The GM composition was compared between patients with HT and HCs and concluded meaningful results. This study suggested that the GM differed significantly between the two groups, with enriched Akkermansia, Lachnospiraceae, Bifidobacterium, Shuttleia, and Clostriworthdia in those with HT, and enriched Lachnoclostridium, Bilophila, and Klebsiella in the healthy people.[66]

Thus, the GM composition between AITD patients and HCs is significantly different, indicating that GM may play a role in the pathogenesis of AITD. Further studies are needed to fully elucidate the role of GM in the development of AITD.

Gut Dysbiosis is Associated with Primary Hypothyroidism with Interaction on TGA

Prior studies have shown a link between the composition of GM and thyroid diseases, including GD,[67,68] HT [69] and GO.[70] Nevertheless, the precise relationship between intestinal flora and primary hypothyroidism remains elusive. It is widely

accepted that hypothyroidism is often accompanied by impaired gastrointestinal motility and increased intestinal permeability, which creates favorable conditions for small intestinal bacterial colonization and overgrowth (SIBCO) and promotes bacterial translocation from the intestinal epithelial barrier to the lymph nodes.[71] However, the bad side is overgrown bacteria increase the risk for the impairment of neuromuscular function of the gastrointestinal tract, which is a major cause and exacerbating factor of chronic gastrointestinal symptoms occurred in hypothyroid patients.[72] Recent studies have found that antibiotic therapy can have an inhibitory effect on the excessive growth of tiny intestinal bacteria occurring in hypothyroid patients, further improving gastrointestinal symptoms.[73] Taken together, these findings portend a reciprocal relationship between hypothyroidism and GM and emphasize the constructive meaning of assessing the growth of tiny intestinal bacteria in managing hypothyroid patients.

A literature research was conducted to characterize GM in primary hypothyroidism patients. The GM between 52 primary hypothyroidism patients and 40 healthy controls was analyzed by 16S rRNA sequencing technology. It has been observed that significant differences exist in α and β diversities of GM between these two groups. The four intestinal bacteria, Veillonella, Paraprevotella, Neisseria, and Rheinheimera, have the highest accuracy, distinguishing untreated primary hypothyroidism patients from healthy individuals. Furthermore, fecal microbiota transplantation (FMT) was performed using flora from both the two groups, and thyroid function was then assessed in the mice. This result showed that mice receiving the gut bacterial transplant from primary hypothyroidism patients displayed decreased total thyroxine levels.[74] Their study confirmed the reciprocal interaction between GM composition and primary hypothyroidism. In a two-sample Mendelian randomization (MR) study, Xie et al utilized summary data from a genome-wide association study of GM composition in 18,340 participants from 24 cohorts to investigate the causal relationships between GM and FT4, TSH, and hypothyroidism. This study has suggested a diminished prevalence of Bifidobacteriaceae within individuals afflicted by hypothyroidism. The need of hypothyroid patients for LT-4 amounts can be reduced by the administration of a mixture of Lactobacillus and Bifidobacterium, as well as by keeping more stable TH levels.[75]

Overall, these findings could explain the fact that primary hypothyroidism could change the GM, and an altered flora can affect thyroid function in mice in turn, which could help understand the development of primary hypothyroidism and might be further used to develop potential probiotics to facilitate the adjuvant treatment of this disease. However, further research is needed to explore the specific effects of GM on thyroid function.

GM Composition, Oral Preparations of TH, and Antithyroid Drugs

Both endogenous and exogenous TH at practically every level were impacted by the GM, which affects the enterohepatic cycling of TH, the bioavailability of levothyroxine, and the metabolism of antithyroid medication used in the treatment of hyperthyroidism.[76]

Only cross the intestinal barrier can oral preparations of TH get into systemic circulation. Intestinal microbiota can modulate the expression of tight junctions and then affect intestinal permeability as well as the shape of enterocytes and the composition of the mucus layer of the barrier.[77] In a 2020 observational study, Yao et al investigated the relationships between intestinal microbiota and L-thyroxine in subclinical hypothyroidism patients.[78],117 patients were categorized into two separate subgroups: patients receiving oral L-thyroxine replacement therapy and patients with no treatment. Firstly, they observed that the abundance level of bacteria belonging to the genus Ruminococcus in human GM was elevated in the group with no treatment, compared to the patient group receiving oral TH. Secondly, there were discrepancies in the relative abundance of the genera Odoribacter and Enterococcus depending on the dosage of L-thyroxine, with the lowest abundance shown in those receiving a high dose. The most used antithyroid drugs methimazole (MMI) and propylthiouracil (PTU)[79] have been analyzed for their effects on GM composition in GD patients. Comparing the reciprocal differences in gut microbial population among a group treated with MMI, another one treated with PTU, and 50 healthy people. The analysis of community diversity indicated a significant difference between the MMI and PTU groups, and the former has a significantly higher Ace index.[80]

As demonstrated, both the L-thyroxine therapy and antithyroid drugs have the potential to impact the intestinal health microbiota composition and thus also affect its function. There is conclusive evidence on the role of the microbiome in oral thyroid-relevant medicine, while these aspects of pharmacomicrobiomics should be further explored.

GM Alterations in Patients with Thyroid Nodules (TN)

The incidence of TN is increasing worldwide, such that they are now present in nearly 50% of the adult population.[81] Being evident of risk factors for the development of TN includes age, sex, metabolic syndrome, iodine intake,[82,83] and several human genome loci (eg, TRPM3, EPB41L3, and AP005059),[84] yet the role of GM in TN remains to be fully elucidated.

Evidence suggests that TN is associated with the composition of GM. In the study of Zhang et al, the GM composition of 18 TN patients, and 36 matched healthy controls were analyzed by 16S rRNA gene-based sequencing protocol. The results inferred that both the relative abundance of Neisseria and Streptococcus were significantly higher ($P < 0.001$) for TN, and TN displayed a notably lower relative abundance both of Butyricimonas and Lactobacillus ($P < 0.001$) than the healthy controls.[85] Whilst the present study described the characteristics of the GM in patients with TN and the relationship between the GM and thyroid functions, its significance was restricted by the small number of patients studied and the insufficient resolution based on 16S rRNA gene amplicon sequencing. A recent study showed that the number of observed species (OBS) was significantly lower in the patients with TN compared to the controls ($P < 0.05$). The numbers of OBS and observed gene families (OBG) were also lower in the Thyroid Imaging Reporting and Data System (TI-RADS) level 2 (TN2) group compared with the TI-RADS level 3 (TN3) and TI-RADS level 4 (TN4) groups ($P < 0.05$),[86] indicating the presence of TN and the TI-RADS was associated with lower level gut microbial species and gene richness. They also found reduced typical butyrate-producing GM, including Butyrivibrio, Coprococcus comes, Coprococcus catus, Eubacterium eligens, Faecalibacterium prausnitzii, and Roseburia hominis, lower butyrate production, and amino acid biosynthesis in patients with HTN (classify TN3 or TN4 as having advanced or high-grade TIRADS) as compared to LTN (classify the control and TN2 groups as having no or low-grade TIRADS).

Collectively, these studies provided novel and compelling evidence that key GM-driven metabolic pathways, such as butyrate formation, were associated with the presence of TN. Although microbial nutrition metabolism-mediated gut-thyroid link has been demonstrated, more work is needed to conclusively determine the influences on and effects of thyroid of GM composition and dysregulation.

Interactive Association Between GM and Thyroid Cancer (TC)

Currently, the endocrine malignancy TC has plagued the world, and the high morbidity of TC is an increasing healthcare burden worldwide, leading to a wider concern.[87] Although the causative factors of TC remain partly unclear, particularly that poorly differentiated, medullary, and anaplastic TC,[88] recent discoveries on the existence of TC microenvironment provide further evidence for the essential role of tumor microorganisms in TC etiology and severity, affecting thyroid homeostasis, exerting critical effects on TC development as well as progression, and promoting immune escape in cancer.[89] Therefore, we aimed to investigate the relationship among the GM community, metabolites, and the development of TC and elucidate the mechanisms involved, which may act as prognostic markers and as a potential target of adjuvant care in the prevention, treatment, and management of TC patients.

Originally, the thyroid was thought to be free of bacterial colonization; however, after the development of genetic sequencing technologies, it was proved to be colonized by a large microbiota.[90] In addition, a study identified the higher microbial richness and significant compositional differences in the GM of TC patients compared to those in HCs, further indicating an interrelationship between GM and TC.[85] In TC patients, the relative abundance of Clostridiaceae, Neisseria, and Streptococcus significantly increased. Feng et al used 16S rRNA gene sequencing to characterize the GM community of fecal samples in 30 TC patients and 35 HCs and observed differential microbiota compositions, with significant enrichment of 19 and depletion of 8 genera in TC samples ($Q < 0.05$).[91] Although 16S rRNA gene sequencing technology can accurately distinguish and quantitatively analyze various bacteria,[92] its resolution is insufficient.[93] Based on this reason, another literature research was performed to examine the interplay between GM and TC that should be considered when treating patients suffering from TC. Lu et al showed that not only the diversity and richness of the GM in the TC patients were markedly decreased but the composition of the GM was significantly altered (the dominant enterotype in TC patients was Bacteroides enterotype).[94] Hou et al performed a bidirectional MR to investigate the causality from microbiota taxa (from the MiBioGen study, N = 18,340) and metabolism pathways (from the Dutch

Microbiome Project, N = 7738) to TC (from the Global Biobank Meta-analysis Initiative, N cases = 6699, N participants = 1620 354). They performed a systematic review of previous observational studies and compared these results with MR findings. Both systematic review and MR showed that the Holdemanella genus, Proteobacteria phylum, Ruminococcus genus, and Streptococcaceae family were significantly associated with TC.[95]

GM might be associated with TC through different mechanisms related to sodium/iodide (Na/I) symporter (NIS) regulation.[96] However, it is unknown whether the differences in GM lead to the development of TC or whether the development of TC causes a series of endocrine changes, leading to the differences in GM. In the future, well-powered human studies are warranted to evaluate the impact of alterations in GM on thyroid function and diseases, thus establishing multifactorial therapeutic and preventive management strategies more specifically adjusted to patients, depending on their gut bacteria composition.

Conclusion

The human intestinal microbiota, consisting of billions of bacteria, viruses, and fungi resident in the human body, not only plays an essential role in the maintenance of normal physiology (TH metabolism included) but is closely linked with patients suffering from NMDAR encephalitis, anxiety, depression, early-onset cancers, T1D or T2D, respectively. Similarly, the interaction between GM and thyroid diseases also has been reported. For example, the GM composition between AITD patients and HCs is significantly different, indicating that GM may play a role in the pathogenesis of AITD. The primary hypothyroidism could change the GM, and an altered flora can affect thyroid function in turn. Both endogenous and exogenous TH at practically every level were impacted by the GM. The key GM-driven metabolic pathways, such as butyrate formation, were associated with the presence of TN. Currently, discoveries on the existence of TC microenvironment provide further evidence for the essential role of tumor microorganisms in TC etiology and severity. GM might be associated with TC through different mechanisms related to NIS regulation. In other words, a gut-endocrine–homeostasis-disease axis has been shown in these studies, further suggesting that microbiota analysis could provide an alternative non-invasive diagnostic methodology for various diseases (thyroid disorders included). Micronutrients, including trace elements (copper, iodine, iron, selenium, zinc) and vitamins (A, C, D, E), are proved to interact with the GM. On the one hand, micronutrient elements can change the composition of the intestinal microbiota and endocrine control of TH. On the other hand, host microorganisms can regulate vitamins. Although microbial nutrition metabolism-mediated gut-thyroid link has been demonstrated, more work is needed to conclusively determine the influences on and effects of thyroid of GM composition and dysregulation.

In summary, both the GM and colonized bacteria play a role in maintaining endocrine homeostasis in the thyroid gland and the development of relevant diseases. As the interaction between GM and thyroid gland diseases is gradually being revealed, the thyroid gland, as an important endocrine organ, constitutes the gut-thyroid axis. Similar to other diseases, the relationship between thyroid disorders and intestinal microbiota is interactive and balancing. On one side, breaking this balance and forming positive loops between each other through multiple metabolites and pathways can promote the development and progression of various conditions. On the other side, dysbiosis of the GM leads to the accumulation of metabolites that, through specific mechanisms, lead to instability and susceptibility in the thyroid, also ultimately leading to the development and progression of various conditions. Besides, owing to the presence of these metabolites, autoimmune responses occurred, resulting in an imbalance of endocrine homeostasis and the development of AITD.

Collectively, this narrative review provided novel and compelling evidence that the two condition entities GM and thyroid gland have a strong relevant correlation, and is expected to provide new ideas for describing their reciprocal impact and developing treatment therapeutic options that should be further explored by future studies. Our extra aim is to provide some possible clinical applications of GM markers for early diagnosis, treatment, and postoperative management of TC. Our findings provide a solid foundation for addressing individual differences in TC patients and could elicit effects on clinical management not only for TC prevention management strategy but also for multi-disciplinary treatment concerning their GM composition and pathological characteristics.

Abbreviation

GALT, gut-associated lymphoid tissue; GM, gut microbiota; NMDAR, anti-N-methyl-D-aspartate receptor; T1D, type 1 diabetes; T2D, type 2 diabetes; TH, thyroid hormones; TGA, thyroid-gut axis; TPO, thyroid peroxidase; HCP1, heme/folate carrier protein 1; SCFAs, short-chain fatty acids; EA, ellagic acid; UA, urolithin A; ID, iron deficiency; GD, Graves' disease; HT, Hashimoto's thyroiditis; AITD, autoimmune thyroid disease; GO, Graves' orbitopathy; TPOAb, TPO antibodies; TGAb, thyroglobulin; TRAb, thyroid-stimulating hormone receptor antibodies; HCs, healthy controls; rRNA, ribosomal RNA; FMT, fecal microbiota transplantation; MR, Mendelian randomization; MMI, methimazole; PTU, propylthiouracil; TN, thyroid nodules; OBS, observed species; OBG, observed gene families; TI-RADS, Thyroid Imaging Reporting and Data System; TNn, TI-RADS level n; HTN, classify TN3 or TN4 as having advanced or high-grade TIRADS; LTN, classify the control and TN2 groups as having no or low-grade TIRADS; TC, thyroid cancer; Na/I, sodium/iodide; NIS, sodium/iodide symporter.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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