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Review

The gut microbiota and its role in Graves' Disease: a systematic review and meta-analysis

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Emerging research indicates the potential involvement of gut bacteria in the etiology of Graves' Disease (GD). However, the evidence regarding this matter is still conflicting. The primary objective of this investigation was to examine the correlation between gut microbiota and GD. A comprehensive search was conducted of the Cochrane Library, Scopus, Europe PMC, and Medline databases up until August 1, 2023, utilizing a combination of relevant keywords. This review incorporates literature that examined the composition of gut microbiota in patients with GD. We employed random-effect models to analyze the standardized mean difference (SMD) and present the outcomes together with their corresponding 95% confidence intervals (CIs). A total of ten studies were incorporated. The results of our meta-analysis indicated that patients with GD have a reduced alpha diversity of gut microbiota as evidence by a significant reduction of Chao1 (std. mean difference −0.58; 95% CI −0.90, −0.26, p=0.0004; I^2=61%), ACE (std. mean difference −0.64; 95% CI −1.09, −0.18, p=0.006; I^2=77%), and Shannon index (std. mean difference −0.71; 95% CI −1.25, −0.17, p=0.01; I^2=90%) when compared with healthy controls. At the phylum level, the abundance of Firmicutes was reduced in GD patients, while that of Bacteroidetes was increased. This study suggests a notable decrease in the richness and variety of gut microbiota among people diagnosed with GD in comparison with healthy controls.

Key words: Graves' Disease, autoimmune thyroid disease, gut microbiome, microbiology, endocrinology

INTRODUCTION

Graves'-Basedow's disease, also known as Graves' Disease (GD), is an autoimmune disorder characterized by the erroneous recognition of thyroid gland receptors by the immune cells within the body [1]. The consequence of this phenomenon is overstimulation of the thyroid gland (hyperthyroidism) and subsequent thyrotoxicosis characterized by excessive production of thyroid hormone [1]. Graves' disease is a prevalent etiology of thyrotoxicosis, constituting a substantial proportion, around 60–80%, of all instances of thyrotoxicosis [1]. Updated global data regarding the prevalence of GD is still scarce, so the exact number is not known. According to data from 2016, the annual incidence of GD is predicted to be between 20 and 50 individuals per 100,000 population [2]. The lifetime probability of having GD is reported to be 3% in women and 0.5% in men [2, 3]. In a similar vein, the most recent statistics from the year 2022 pertaining to Asian countries revealed an age-adjusted incidence rate of GD of 26.57 cases per 100,000 individuals annually [4]. Despite its relatively low frequency, GD can lead to a significantly elevated death rate, surpassing that of the general population by 23% [5].

Until now, the pathophysiology of the emergence of GD has not been fully known [6, 7]. Recent evidence suggests the possibility of the thyroid-gut axis exerting an influence in the pathogenesis of this disease [6, 7]. From an embryological perspective, it can be observed that the thyroid and gut have a shared origin during development [6, 7]. This common origin accounts for certain similarities in both the structure and function of the gut and thyroid follicular cells [6, 7]. Furthermore, it has been postulated that the gut microbiota, consisting of numerous microorganisms, on the order of trillions, may have a role in the development of several autoimmune disorders, including GD [6, 7]. Unfortunately, existing evidence still shows conflicting results concerning the relationship between the gut microbiota and GD [8, 9]. For example, a 2018 study by Ishaq *et al.* [8] showed that the alpha diversity of the gut microbiota, demonstrated by the ACE and

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Chao1 index, was significantly decreased in individuals with GD compared with healthy controls. On the other hand, a study by Chang *et al.* [9] found no statistically significant differences in either the ACE or Chao1 index values between patients diagnosed with GD and a control group consisting of healthy persons. In light of this incongruity, the utilization of a systematic review and meta-analysis methodology may prove beneficial. The primary objective of this study was to conduct a comprehensive analysis of the correlation between the gut microbiome and GD.

MATERIALS AND METHODS

Eligibility criteria

This systematic review and meta-analysis were conducted in accordance with the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [10]. The protocol of the present study was filed in the PROSPERO database under the registration number CRD42023451903. The inclusion criteria for the study were established utilizing the PECOS method, incorporating the following particulars:

(1) Population = adult patients aged over 18 years.

(2) Exposure = individuals with a diagnosis of GD.

(3) Control = healthy individuals without a history of thyroid disease or medications.

(4) Outcome = have data on the:

- Primary Outcome = alpha diversity index shown through the ACE, Chao1, and Shannon index;

- Secondary Outcome = relative abundances at the phyla (Firmicutes and Bacteroidetes), family (*Prevotellaceae* and *Lachnospiraceae)*, and genus (*Bacteroides*, *Faecalibacterium*, and *Bifidobacterium*) levels in both study groups. The selection of these taxa was based on an initial screening conducted by the authors in earlier investigations, wherein the data for the majority of studies were limited to these taxa.

(5) Study Design = observational study (cohort, case-control, or cross-sectional).

Meanwhile, the exclusion criteria were as follows: (1) investigation focused on the pediatric population, specifically persons under the age of 18; (2) animal studies; (3) studies with incomplete data on the gut microbial diversity; (4) studies with no comparison group; (5) non-primary investigations; (6) research articles that were not accessible in their entirety or studies that had not undergone the process of publication.

Search strategy and study selection

A comprehensive review of the literature was performed, focusing specifically on papers written in the English language. The search encompassed the time frame up to August 1, 2023, and was undertaken in four prominent worldwide databases: Medline, Scopus, Europe PMC, and the Cochrane Library. The search term utilized for the literature review was as follows: "(Graves' Disease OR Graves' hyperthyroidism OR Graves'-Basedow disease OR Morbus Basedow OR Basedow's Disease OR GD OR GBD OR exophthalmic goiter OR toxic diffuse goiter) AND (gut microbiota OR gut microbiome OR gut flora OR gut microorganisms OR gastrointestinal microbiota)". Supplementary Table 1 provides additional information pertaining to the search approach employed for each database. The first step was to initiate the screening process by assessing the compatibility of the titles and/or abstracts with our eligibility criteria. Any primary research articles that were referenced in the systematic reviews or meta-analyses but not initially identified during the search process were also be incorporated into the study if they met the inclusion criteria and did not meet the exclusion criteria. Redundant articles were eliminated. Subsequently, a thorough evaluation of complete-text articles was conducted. Both reviewers conducted all of these steps independently. In the event of disagreement during the screening process, the disagreement was resolved through the solicitation of a third reviewer's perspective.

Data extraction

The process of extracting data was conducted autonomously by two reviewers. The following data that was extracted: the study's authors, year of publication, study design, country of origin, sample size, mean age, sex distribution, body mass index (BMI), thyroid function test results, sample for analysis, microbiological assessment, and outcome of interest.

Risk of bias assessment

The evaluation of potential bias in each study was conducted by two independent reviewers using standardized assessment tools. The Newcastle-Ottawa Scale (NOS) was employed to assess the quality of each observational study [11]. This scale incorporates evaluations about the selection of study participants, comparability between groups, and the outcomes of the studies [11]. The potential range of scores achievable when utilizing this instrument encompassed values from 0 to 9 [11]. In the context of this research, studies attaining a total score of 7 or more were deemed to possess a commendable level of quality (good quality) [11].

Statistical analysis

The alpha diversity outcomes (continuous variable) were calculated by using the Mantel-Haenszel formula to obtain the standardized mean difference (SMD) along with the 95% confidence interval (95% CI). The prevalence rates of gut microbiota were calculated by using the generic inverse-variance formula to obtain the relative abundance in percentage value along with the 95% CI. The presence of diverse participant characteristics and microbiological evaluation methodologies necessitated the consideration of a substantial amount of variability. To address this, random-effect models were employed. The I-squared (I2; Inconsistency) statistic was employed to quantify the heterogeneity among research, with values exceeding 50% indicating a substantial or noteworthy level of heterogeneity [12]. If the number of papers included in the meta-analysis exceeded 10, a funnel plot was employed to evaluate the presence of publication bias. All analyses in this investigation were conducted using Review Manager 5.4, a software tool developed by the Cochrane Collaboration.

RESULTS

Study selection and characteristics

A comprehensive search of the databases resulted in the identification of 702 studies. Following screening of the titles and abstracts and subsequent removal of duplicate articles, a total of 24 full-text publications were evaluated based on the eligibility criteria for this study. Among the 24 full-text papers considered

for this investigation, 14 articles were deemed ineligible due to various reasons. Specifically, six articles were omitted because they were review articles, four articles lacked data pertaining to the primary outcomes, two articles were based on animal studies, and two articles did not include healthy controls. Ultimately, the remaining 10 articles [8, 9, 13–20] with a total of 358 GD patients and 303 healthy controls were included in the final analysis (Fig. 1). All of the included studies had case-control design. Out of the 10 studies, 8 studies came from China, with the remaining two studies coming from Taiwan and Spain, respectively. All studies used high-throughput sequencing from fecal samples for the microbiological assessment. Tables 1 and 2 provide a comprehensive overview of the characteristics of each study included in this analysis.

Quality of study assessment

All the case-control studies included in this study demonstrated high quality as assessed by the NOS, with values ranging from 7 to 9 (Table 3). All studies were considered suitable for inclusion in the meta-analysis.

Alpha diversity of the gut microbiota (primary outcomes)

Chao1 index

There were 7 studies which reported the alpha diversity of the gut microbiota based on the Chao1 index. The meta-analysis revealed that these studies showed a significant reduction in the Chao1 index in individuals with GD when compared with healthy

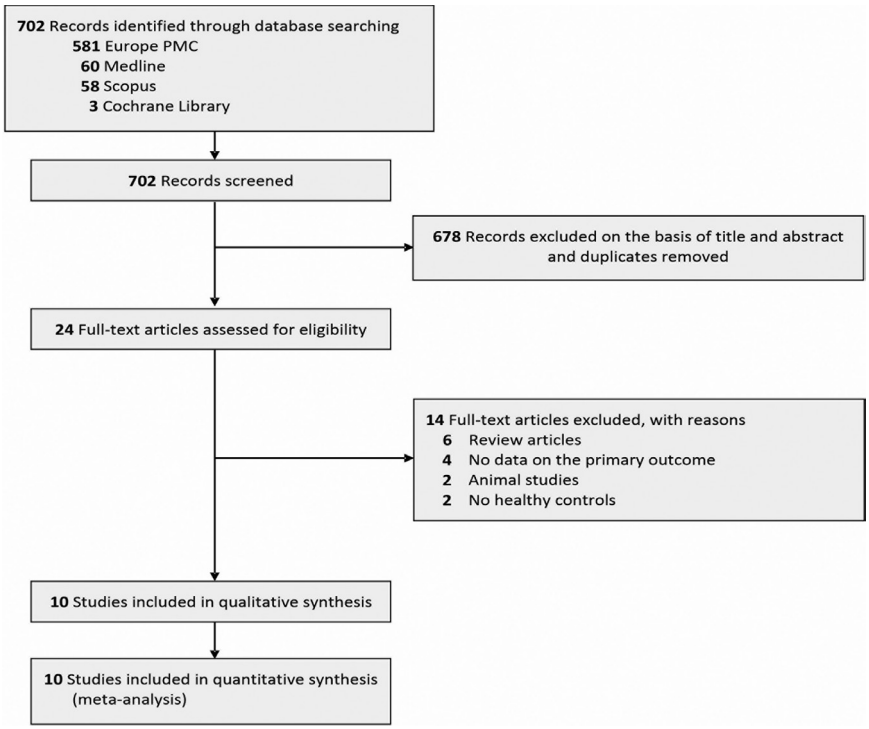

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram of the detailed process of selection of studies for inclusion in the systematic review and meta-analysis.

Table 1. Characteristics of the included studies

Study ID			Cases				Control			
Authors	Country	Sample size	Age (mean ± SD)	Male (%)	BMI kg/m^2	Sample size	Age (mean ± SD)	Male (%)	BMI kg/m^2
Chang *et al.* [9] 2021	Taiwan	55	45.1 ± 12.1	36.3%	23.8 ± 4.2	48	42.6 ± 9.8	37.5%	23.2 ± 3.4
Chen *et al.* [13] 2021	China	15	28.8 ± 6.8	46.6%	20.8 ± 1.9	14	27.3 ± 5.7	42.8%	22.3 ± 3.4
Cornejo-Pareja *et al.* [14] 2020	Spain	9	46.2 ± 8.6	22.2%	25.2 ± 4.7	11	48.8 ± 6.2	36.3%	25 ± 2
Deng *et al.* [15] 2023	China	65	31.9 ± 11.7	38.3%	20.8 ± 2.9	33	27.3 ± 2.3	30.3%	20.4 ± 2.1
Ishaq *et al.* [8] 2018	China	27	35–50	37%	NR	11	35–50	36.3%	NR
Jiang *et al.* [16] 2021	China	45	37.6 ± 11.1	26.6%	NR	59	43.5 ± 10.6	37.2%	NR
Shi *et al.* [17] 2020	China	30	45 ± 12.8	33.3%	NR	32	43.4 ± 9.7	50%	NR
Su *et al.* [18] 2020	China	58	42.1 ± 10.2	39.6%	22.5 ± 2.5	63	43.8 ± 9.2	44.4%	22.5 ± 2.2
Yang *et al.* [19] 2019	China	15	46–55	NR	NR	15	46–55	NR	NR
Yan *et al.* [20] 2020	China	39	37.5 ± 12.9	28.2%	NR	17	33.4 ± 9.1	35.2%	NR

BMI: body mass index; NAFLD: non-alcoholic fatty liver disease; USA: United States of America; SD: standard deviation; NR: not reported.

individuals as the comparison group (SMD −0.58; 95% CI −0.90, −0.26, p=0.0004, I^2=61%, random-effect model; Fig. 2A).

ACE index

There were 5 studies which reported the alpha diversity of the gut microbiota based on the ACE index. The meta-analysis revealed that these studies showed a significant reduction in the ACE index in individuals with GD when compared with healthy individuals as the comparison group (SMD −0.64; 95% CI −1.09, −0.18, p=0.006, I^2=77%, random-effect model; Fig. 2B).

Table 2. Additional characteristics of the included studies

Study ID Authors	Thyroid parameters		Sample	Microbiology assessment
	Graves' Disease (GD)	Control group		
Chang *et al.* [9] 2021	- TSH: 0.40 ± 0.83 mIU/L - FT4: 2.25 ± 1.58 pmol/L	- TSH: 1.48 ± 0.60 mIU/L - FT4: 1.22 ± 0.14 pmol/L	Fecal	High-throughput sequencing
Chen *et al.* [13] 2021	- TSH: 0.05 ± 0.13 mIU/L - FT3: 22.18 ± 10.44 pmol/L - FT4: 51.45 ± 18.63 pmol/L - TG-Ab: 983.65 ± 1,717.64 IU/mL - TPO-Ab: 110.55 ± 118.50 IU/mL - TRAb: 6.62 ± 5.39 IU/L	- TSH: 1.90 ± 0.63 mIU/L - FT3: 5.05 ± 0.61 pmol/L - FT4: 11.51 ± 1.16 pmol/L - TG-Ab: 10.78 ± 1.70 IU/mL - TPO-Ab: 8.63 ± 6.63 IU/mL - TRAb: 0.30 ± 0.00 IU/L	Fecal	High-throughput sequencing, RT-PCR
Cornejo-Pareja *et al.* [14] 2020	- TSH: 0.0033 ± 0.0085 mIU/L - FT3: 5.5 ± 2.3 pmol/L - FT4: 15.2 ± 3.1 pmol/L - TPO-Ab: 792 ± 621.7 IU/mL	- TSH: 0.0022 ± 0.001 mIU/L - FT3: 4.8 ± 0.4 pmol/L - FT4: 15.2 ± 1.3 pmol/L - TPO-Ab: 160.3 ± 381.3 IU/mL	Fecal	High-throughput sequencing
Deng *et al.* [15] 2023	- TSH: 0.0034 ± 0.0019 mIU/L - FT3: 20.18 ± 12.34 pmol/L - FT4: 7.51 ± 5.66 pmol/L - TG-Ab: 408.37 ± 556.69 IU/mL - TPO-Ab: 161.14 ± 224.9 IU/mL - TRAb: 9.3 ± 12.05 IU/L	- TSH: 2.51 ± 1.15 mIU/L - FT3: 3.05 ± 0.44 pmol/L - FT4: 1.14 ± 0.21 pmol/L - TG-Ab: 6 ± 2.18 IU/mL - TPO-Ab: 23.54 ± 5.24 IU/mL - TRAb: 1.14 ± 1.09 IU/L	Fecal	High-throughput sequencing, RT-PCR
Ishaq *et al.* [8] 2018	NR	NR	Fecal	High-throughput sequencing, RT-PCR, PCR-DGGE
Jiang *et al.* [16] 2021	- TSH: 0.04 ± 0.11 mIU/L - FT3: 20.04 ± 8.69 pmol/L - FT4: 49.23 ± 24.46 pmol/L - TG-Ab: 296.51 ± 408.3 IU/mL - TPO-Ab: 217.37 ± 148.21 IU/mL - TRAb: 18.98 ± 14.71 IU/L	- TSH: 2.25 ± 0.99 mIU/L - FT3: 3.64 ± 0.47 pmol/L - FT4: 12.9 ± 1.37 pmol/L - TG-Ab: 5.19 ± 2.56 IU/mL - TPO-Ab: 0.9 ± 0.47 IU/mL - TRAb: 2.38 ± 1.29 IU/L	Fecal	High-throughput sequencing, PCR
Shi *et al.* [17] 2021	- TSH: 1 ± 1.58 mIU/L - FT3: 5.06 ± 1.12 pmol/L - FT4: 14.94 ± 2.77 pmol/L - TG-Ab: 49.07 ± 82.82 IU/mL - TPO-Ab: 64.07 ± 95.02 IU/mL - TRAb: 4.19 ± 5.23 IU/L	- TSH: 1.7 ± 0.89 mIU/L - FT3: 4.9 ± 0.72 pmol/L - FT4: 14.58 ± 4.38 pmol/L - TG-Ab: 8.96 ± 11.84 IU/mL - TPO-Ab: 9.68 ± 12.53 IU/mL - TRAb: <1.75 mIU/L	Fecal	High-throughput sequencing
Su *et al.* [18] 2020	- TSH: 0.005 ± 0.004 mIU/L - FT3: 16.9 ± 16.1 pmol/L - FT4: 48.9 ± 40.1 pmol/L - TG-Ab: 67.7 ± 280.9 IU/mL - TPO-Ab: 206.7 ± 928.2 IU/mL - TRAb: 11.9 ± 16.9 IU/L	- TSH: 1.9 ± 0.9 mIU/L - FT3: 4.85 ± 0.43 pmol/L - FT4: 15.83 ± 1.61 pmol/L - TG-Ab: 23.2 ± 7 IU/mL - TPO-Ab: 33.1 ± 6.37 IU/mL - TRAb: 0.54 ± 0.34 IU/L	Fecal	High-throughput sequencing, RT-PCR
Yang *et al.* [19] 2019	NR	NR	Fecal	High-throughput sequencing, RT-PCR
Yan *et al.* [20] 2020	- TSH: 0.00 ± 0.001 mIU/L - TT3: 6.07 ± 3.77 nmol/L - TT4: 219.48 ± 74.99 nmol/L - TG-Ab: 268.58 ± 336.61 IU/mL - TPO-Ab: 422.09 ± 420.33 IU/mL - TRAb: 15.76 ± 12.77 IU/L	- TSH: 1.31 ± 0.71 mIU/L - TT3: 1.55 ± 0.13 nmol/L - TT4: 76.9 ± 8.75 nmol/L - TG-Ab: 9.68 ± 29.81 IU/mL - TPO-Ab: 0.75 ± 1.6 IU/mL - TRAb: <0.3 IU/L	Fecal	High-throughput sequencing, RT-PCR

FT3: free triiodothyronine; FT4: free thyroxine; NR: not reported; PCR: polymerase chain reaction; rRNA: ribosomal ribonucleic acid; TG-Ab: thyroglobulin antibody; TPO-Ab: thyroperoxidase antibody; TRAb: thyrotrophin receptor antibody; TSH: thyroid-stimulating hormone; TT3: total triiodothyronine; TT4: total thyroxine; NR: not reported.

Shannon index

All of the included studies reported the alpha diversity of the gut microbiota based on Shannon index. The meta-analysis revealed that the studies showed that the Shannon index was significantly reduced in individuals with GD when compared with healthy individuals as the comparison group (SMD −0.71; 95% CI −1.25, −0.17, p=0.01, I^2=90%, random-effect model; Fig. 2C).

Relative abundance of the gut microbiota at the phylum level (secondary outcomes)

Firmicutes relative abundance

Our meta-analysis revealed that 9 studies showed a lower relative abundance of the Firmicutes phylum in patients with GD than in the healthy controls. The percentage of Firmicutes was 53% (95% CI 0.44–0.61) in the GD group, slightly lower than

Table 3. Newcastle-Ottawa quality assessment of observational studies

First author, year	Study design	Selection[a]	Comparability[b]	Outcome[c]	Total score	Result
Chang *et al.* [9] 2021	Case-control	****	**	***	9	Good
Chen *et al.* [13] 2021	Case-control	**	**	***	7	Good
Cornejo-Pareja *et al.* [14] 2020	Case-control	***	**	***	8	Good
Deng *et al.* [15] 2023	Case-control	***	**	***	8	Good
Ishaq *et al.* [8] 2018	Case-control	**	**	***	7	Good
Jiang *et al.* [16] 2021	Case-control	****	**	***	9	Good
Shi *et al.* [17] 2021	Case-control	***	**	***	8	Good
Su *et al.* [18] 2020	Case-control	**	**	***	7	Good
Yang *et al.* [19] 2019	Case-control	**	**	***	7	Good
Yan *et al.* [20] 2020	Case-control	**	**	***	7	Good

[a](1) is the case definition adequate; (2) representativeness of the cases; (3) selection of controls; (4) definition of controls.
[b](1) comparability of cases and controls on the basis of design or analysis, (maximum two asterisks).
[c](1) ascertainment of exposure; (2) same method of ascertainment for cases and controls; (3) non-response rate.

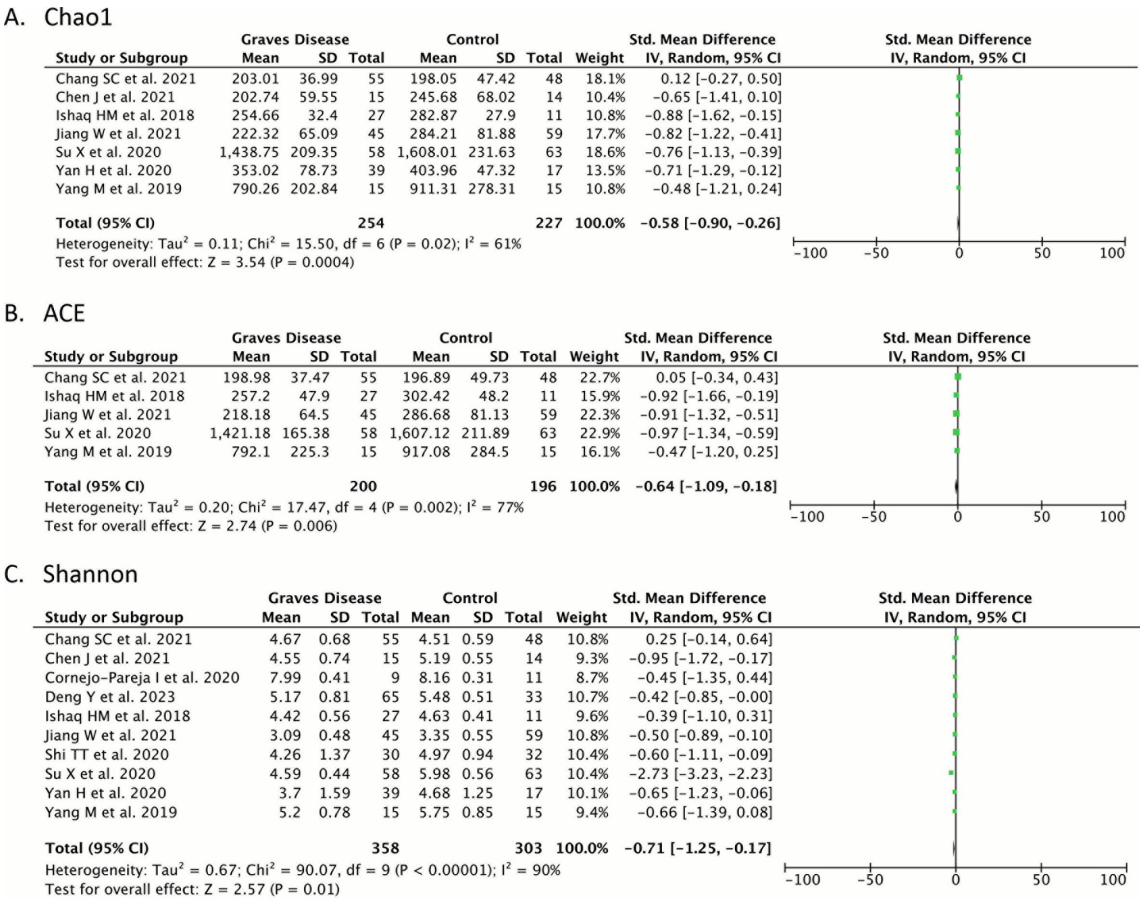

Fig. 2. Forest plot demonstrating the alpha diversity as indicated by the Chao1 (A), ACE (B), and Shannon (C) indices in patients with Graves' Disease (GD) when compared with healthy controls.

that in the healthy controls, who had 60% (95% CI 0.52–0.69; Fig. 3A).

Bacteroidetes relative abundance

Our meta-analysis revealed that 9 studies showed a higher relative abundance of the Bacteroidetes phylum in patients with GD than in the healthy controls. The percentage of Bacteroidetes was 36% (95% CI 0.25–0.48) in the GD group, slightly higher than in the healthy controls, who had 31% (95% CI 0.20–0.42; Fig. 3B).

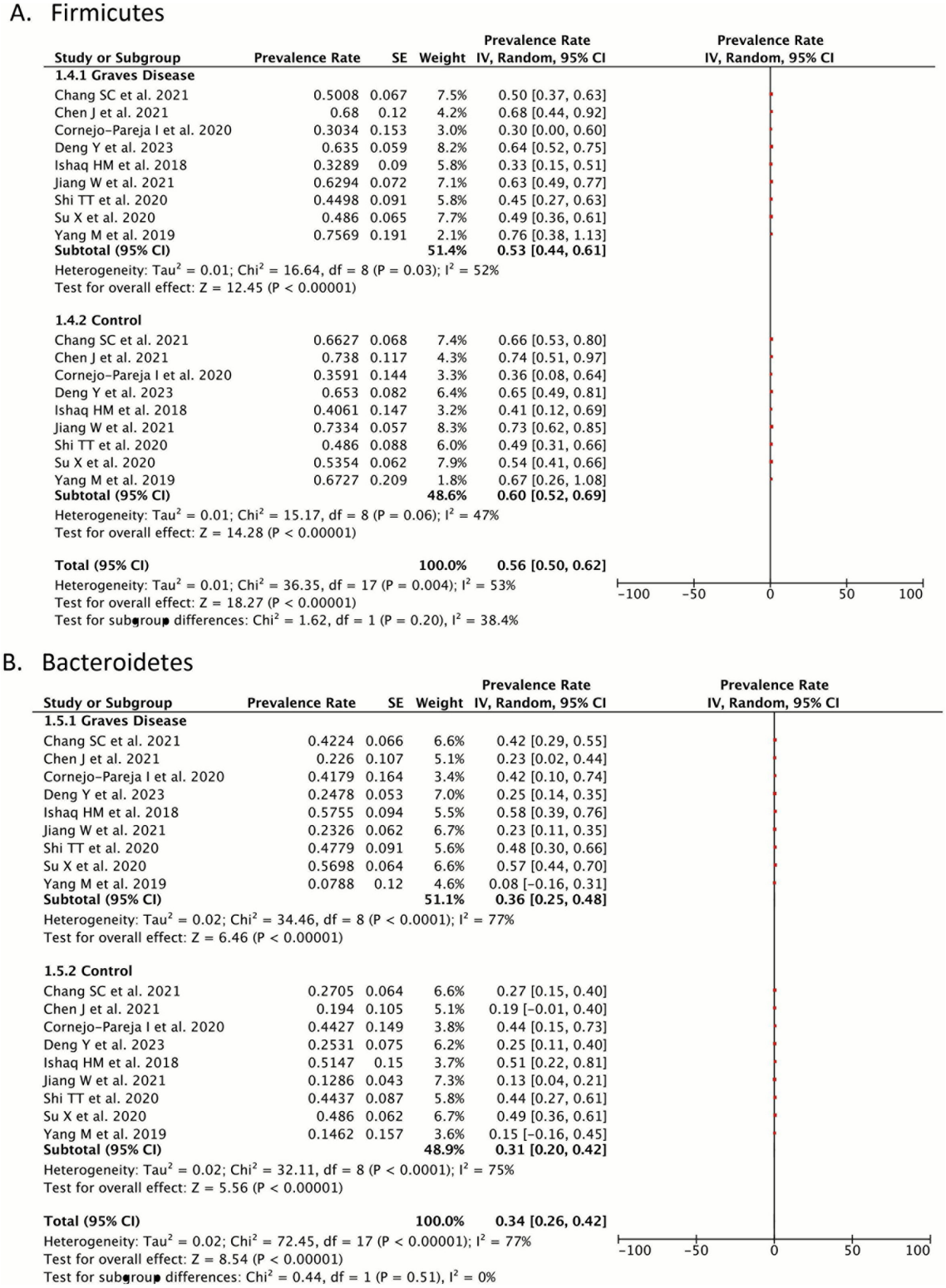

Fig. 3. Forest plot demonstrating the relative abundances of the Firmicutes (A) and Bacteroidetes (B) phyla in patients with Graves' Disease (GD) and healthy controls.

Relative abundance of the gut microbiota at the family level (secondary outcomes)

Prevotellaceae relative abundance

Our meta-analysis revealed that 4 studies showed a higher relative abundance of the *Prevotellaceae* family in patients with GD than in the healthy controls. The percentage of *Prevotellaceae* was 17% (95% CI 0.08–0.27) in the GD group, higher than in the healthy controls, who had 6% (95% CI 0.00–0.13; Supplementary Fig. 1A).

Lachnospiraceae relative abundance

Our meta-analysis revealed that 5 studies showed a lower relative abundance of the *Lachnospiraceae* family in patients with GD than in the healthy controls. The percentage of *Lachnospiraceae* was 16% (95% CI 0.03–0.29) in the GD group, slightly lower than in the healthy controls, who had 18% (95% CI 0.05–0.31; Supplementary Fig. 1B).

Relative abundance of the gut microbiota at the genus level (secondary outcomes)

Bacteroides *relative abundance*

Our meta-analysis revealed that 7 studies showed a lower relative abundance of the *Bacteroides* genus in patients with GD than in the healthy controls. The percentage of *Bacteroides* was 15% (95% CI 0.10–0.21) in the GD group, slightly lower than in the healthy controls, who had 16% (95% CI 0.08–0.23; Supplementary Fig. 2A).

Faecalibacterium relative abundance

Our meta-analysis revealed that 6 studies showed a lower relative abundance of the *Faecalibacterium* genus in patients with GD than in the healthy controls. The percentage of *Faecalibacterium* was 8% (95% CI 0.04–0.12) in the GD group, slightly lower than in the healthy controls, who had 9% (95% CI 0.05–0.13; Supplementary Fig. 2B).

Bifidobacterium *relative abundance*

Our meta-analysis revealed that 4 studies showed a higher relative abundance of the *Bifidobacterium* genus in patients with GD than in the healthy controls. The percentage of *Bifidobacterium* was 5% (95% CI 0.01–0.08) in the GD group, slightly higher than in the healthy controls, who had 3% (95% CI 0.00–0.06; Supplementary Fig. 2C).

Publication bias

Funnel plot analysis was employed to assess publication bias. The present investigation revealed a symmetrically inverted plot for the outcome of the Shannon index (Supplementary Fig. 3), suggesting the absence of publication bias. In the context of the ACE index, Chao1 index, and other secondary outcomes, an assessment of publication bias was not conducted due to the limited number of studies included (less than 10 studies). Consequently, the evaluation of publication bias lacks the same level of robustness as when there are more than 10 studies available for analysis [21, 22].

DISCUSSION

The findings of our meta-analysis indicate a reduction in gut microbiota diversity in patients with GD, as seen by a significant reduction in the Shannon index. Additionally, a decrease in gut microbiota richness, as indicated by a significant reduction in the Chao1 and ACE indices, was observed in individuals diagnosed with GD. At the phylum level, there was an observed decline in the Firmicutes/Bacteroidetes (F/B) ratio in individuals with GD. This decline was characterized by a reduction in the number of Firmicutes and a simultaneous increase in the abundance of Bacteroidetes. At the family level, the abundance of *Prevotellaceae* was increased while that of *Lachnospiraceae* was decreased. At the genus level, there was a slight decrease in the abundance of *Bacteroides* and *Faecalibacterium* as well as a slight increase in *Bifidobacterium* abundance in GD patients compared with healthy controls. All of these findings indicate changes in the gut microbiota in GD patients.

The development of GD may be influenced by many pathophysiologic mechanisms associated with the gut microbiome. Initially, alterations in the gut microbiota composition, commonly referred to as dysbiosis, can lead to detrimental effects on the integrity of the intestinal barrier, hence causing an elevation in intestinal permeability [7, 23]. Short-chain fatty acids (SCFAs), particularly butyrate, are significant metabolites synthesized by butyrate-producing bacteria, including Firmicutes [24, 25]. These SCFAs play a crucial role in enhancing the integrity of the intestinal barrier [24, 25]. Consequently, a reduction in the Firmicutes phylum will result in an elevation of intestinal permeability, facilitating the entry of antigens into the bloodstream and triggering immune system activation [23–25]. This phenomenon is particularly relevant in the context of GD, an autoimmune disorder [23–25]. Furthermore, the aforementioned circulating antibodies have the capability to interact with bacterial antigens, augmenting the activation of the inflammasome within the thyroid gland [25, 26]. Guo *et al.* [27] conducted a study that revealed a noteworthy upregulation in the expression of various components of the inflammasome, such as the NOD-like receptor (NLR) family pyrin domain containing 3 (NLRP3), AIM2, caspase-1, and IL-1β mRNA and protein, in patients diagnosed with autoimmune thyroid disease. This upregulation was found to be significantly influenced by the gut microbiota and its metabolic activities [27]. Finally, it is postulated that the immune system's development, functioning, and modulation may be significantly influenced by SCFAs, which are produced through the fermentation of dietary fiber by commensal bacteria [28–30]. One example of this involves butyrate, which is classified as a SCFA [28–30]. It has been observed that butyrate is linked to diminished concentrations of TNF-α and IL-6 as well as inhibited activation of the NLRP3 inflammasome through its interaction with GPR109A [31]. Therefore, a decrease in butyrate mediated by a decrease in the Firmicutes phylum could lead to immune system compromise and increased inflammatory processes, as seen in patients with GD.

The findings of our meta-analysis align with the prior meta-analysis conducted by Gong *et al.* [32] in 2021. In their meta-analysis, Gong *et al.* [32] demonstrated that GD is associated

with alterations in the gut microbiota. These alterations are characterized by a reduction in the Chao1 index and shifts in the abundance of specific gut microbiota species [32]. Nonetheless, there are some fundamental differences between our meta-analysis and the previous study by Gong *et al.* [32].

First, the earlier investigation conducted by Gong *et al.* [32] examined not only GD but also autoimmune thyroid disease as a whole term, encompassing both GD and Hashimoto's thyroiditis. In the present study, our primary focus was on GD alone, with the aim of conducting a thorough and complete meta-analysis, thereby facilitating a detailed analysis and debate of the topic.

Second, the earlier investigation conducted by Gong *et al.* [32] encompassed a limited number of papers, specifically five, that contained data pertaining to GD. Among the five studies under consideration, one study conducted by Zhou *et al.* [33] is deemed unsuitable due to its failure to precisely identify patients with GD. Instead, the study encompasses hyperthyroid individuals as a whole [33]. However, despite this limitation, Gong *et al.* [32] still incorporated this study into their analysis. The present meta-analysis comprised a greater number of studies, totaling 10 investigations, all of which explicitly indicated the inclusion of patients diagnosed with GD.

Third, the previous study by Gong *et al.* [32] only analyzed one indicator to show alpha diversity, namely the Chao1 index, which better describes microbiota richness. On the other hand, the present meta-analysis analyzed 3 indicators of alpha diversity, namely the Shannon index (diversity index), Chao1, and ACE index (richness index), so it could clearly obtain an overview of changes in microbiota diversity and richness in GD patients compared with healthy controls.

Fourth, one of the critical errors observed in the prior study conducted by Gong *et al.* [32] is the omission of the presentation of the outcomes of a risk of bias evaluation for the included studies within the Results section. Consequently, this deviation from the PRISMA guidelines raises concerns regarding the adherence to recommended reporting practices. Furthermore, the absence of information regarding the quality of all the included studies leaves uncertainty regarding their overall validity. The present meta-analysis used suitable tools to perform a comprehensive evaluation of the risk of bias in all the studies included. The outcomes of this assessment are presented in Table 3, which indicates that all the studies exhibited a high level of quality. Consequently, they were deemed suitable for inclusion in the meta-analysis.

The present investigation is not devoid of limitations. The research covered in this study was limited in its ability to show causation due to the use of an observational design. Second, the studies included in the analysis did not take into account the potential impact of seasonal fluctuations and food patterns on the composition of the gut flora. Third, a considerable number of the observational studies included in the analysis failed to assess the impact of thyroid hormone effects and thyroid autoantibodies on the makeup of the gut flora. Fourth, data regarding the relative abundance of intestinal microbiota stratified by race and severity of Graves' Disease were lacking in the included studies and therefore could not be analyzed further. Finally, the majority of the research included in this analysis was conducted in China, with just two studies originating from outside of China. Consequently, the generalizability of the findings may be constrained, particularly in relation to populations that are not of Asian descent. However, it is our contention that the findings derived from our comprehensive review and meta-analysis can offer valuable perspectives concerning enhancement of the identification and treatment of GD.

CONCLUSION

The findings of our systematic review and meta-analysis indicate a potential association between gut microbiota and the development of GD. Specifically, we observed a notable decrease in microbiota richness and diversity in individuals with GD compared with the healthy control group. The composition of certain microbiota was also altered at the phylum, family, and genus levels. Hence, restoration of the gut microbiota composition may emerge as a potential area of attention for future therapeutic interventions targeting GD. Nonetheless, additional meticulously planned investigations, especially from outside China and with data pertaining to the intestinal microbiota stratified by race and severity of GD, are still needed to validate the findings of our study.

ETHICS APPROVAL

This is a systematic review and meta-analysis study. The Research Ethics Committee, Faculty of Medicine, Syiah Kuala University, has confirmed that no ethical approval is required.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

AUTHOR CONTRIBUTIONS

Conceptualization, Hendra Zufry, Putri Oktaviani Zulfa, Timotius Ivan Hariyanto; methodology, Timotius Ivan Hariyanto; formal analysis and investigation, Hendra Zufry, Putri Oktaviani Zulfa, Timotius Ivan Hariyanto; writing - original draft preparation, Timotius Ivan Hariyanto; writing - review and editing, Hendra Zufry, Putri Oktaviani Zulfa; funding acquisition, Hendra Zufry, Putri Oktaviani Zulfa; resources, Timotius Ivan Hariyanto; supervision, Hendra Zufry, Putri Oktaviani Zulfa.

CONFLICT OF INTEREST

The authors report there are no competing interests to declare.

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