biology

MDPI

Brief Report

Uncovering a Causal Connection between Gut Microbiota and Six Thyroid Diseases: A Two-Sample Mendelian Randomization Study

Jiahao Chen [1,†], Yu Wang [2,†], Hang Yao [3], Yuxin Li [2] and Hong Song [1,*]

[1] School of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, China; jiahao0017@163.com
[2] Graduate School of Jiangxi, University of Chinese Medicine, Nanchang 330004, China; wangyudpk@163.com (Y.W.); rongyu1107@163.com (Y.L.)
[3] School of Traditional Chinese Medicine, Binzhou Medical University, Yantai 264003, China; hanghang9703@163.com
* Correspondence: 20021017@zcmu.edu.cn
† These authors contributed equally to this work.

Simple Summary: Thyroid diseases, such as goiter and thyroid nodules, are prevalent and can significantly impact people's health and quality of life. Recent studies have indicated that the microorganisms in our gut, known as the gut microbiota, might influence the development of these conditions. However, whether these microorganisms cause thyroid diseases or are merely associated with them remains unclear. In this study, we employed genetic analysis to determine whether specific gut bacteria affect the risk of developing six common thyroid disorders. Our findings demonstrate that certain gut bacteria are indeed associated with these diseases—some bacteria seem to protect against thyroid conditions, while others may elevate the risk. This discovery highlights the important role that gut health plays in thyroid disease and suggests new possibilities for prevention and treatment. By targeting gut bacteria through dietary changes, probiotics, or other interventions, it may be feasible to reduce the risk of developing thyroid diseases. Our study underscores the vital connection between gut health and thyroid function, and its potential impact on overall well-being.

Abstract: Background: Recent studies have established associations between the gut microbiota (GM) and thyroid diseases (TDs). However, their causal relationships remain elusive. Methods: To investigate this causality, we conducted a two-sample Mendelian randomization (MR) analysis using genome-wide association study (GWAS) data from MiBioGen and FinnGen, with GM as the exposure and six TDs as outcomes. Results: We identified 32 microbial taxa linked to the risk of six TDs. The *Clostridium innocuum group*, *Ruminiclostridium5*, and *Lachnoclostridium* exhibited protective effects against nontoxic diffuse goiter (NDG). Conversely, an increased risk of NDG was associated with *Ruminococcaceae UCG002*, *Alistipes*, *Methanobrevibacter*, *Marvinbryantia*, and *Ruminococcaceae UCG014*. *Bifidobacterium* and *Sutterella* were protective against nontoxic multinodular goiter (NMG), while the *Ruminococcus gauvreauii group* and *Rikenellaceae RC9 gut group* heightened NMG risk. Protective effects against nontoxic single thyroid nodule (NSTN) were observed with *Defluviitaleaceae UCG011*, *Ruminococcus1*, and *Ruminococcaceae UCG010*, whereas increased risk was linked to *Alistipes*, the *Ruminococcus gauvreauii group*, and *Lachnospiraceae UCG010*. *Ruminiclostridium9*, *Victivallis*, and *Butyricimonas* offered protection against thyrotoxicosis with Graves' Disease (GD), while the *Eubacterium rectale group*, *Desulfovibrio*, *Bifidobacterium*, *Collinsella*, *Oscillospira*, and *Catenibacterium* were risk factors. For thyrotoxicosis with Plummer Disease (PD), protective taxa included *Butyricimonas* and *Lachnospira*, whereas *Dorea*, *Eggerthella*, *Odoribacter*, *Lactobacillus*, *Intestinimonas*, and *Phascolarctobacterium* increased risk. Lastly, *Parasutterella* was protective against thyrotoxicosis with toxic single thyroid nodule (TSTN), while increased risk was associated with *Sutterella*, *Oscillibacter*, and *Clostridium sensu stricto1*. Conclusions: Our findings support a causal relationship between specific GM and TDs at the genetic level, laying the foundation for future research into potential mechanisms and the identification of novel therapeutic targets.

check for updates

Citation: Chen, J.; Wang, Y.; Yao, H.; Li, Y.; Song, H. Uncovering a Causal Connection between Gut Microbiota and Six Thyroid Diseases: A Two-Sample Mendelian Randomization Study. *Biology* **2024**, *13*, 714. https://doi.org/10.3390/biology13090714

Academic Editor: Susana Sangiao-Alvarellos

Received: 5 August 2024
Revised: 3 September 2024
Accepted: 5 September 2024
Published: 11 September 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Keywords: gut microbiota; causal relationship; thyroid diseases; mendelian randomization analysis; gut-thyroid axis

1. Introduction

Thyroid diseases (TDs) encompass various disorders of the thyroid gland, including nontoxic diffuse goiter (NDG), nontoxic multinodular goiter (NMG), nontoxic single thyroid nodule (NSTN), Graves' disease (GD), Plummer disease (PD), and thyrotoxicosis with toxic single thyroid nodule (TSTN). These conditions are prevalent organ-specific disorders. Their etiology may be linked to congenital factors, insufficient iodine intake, or autoimmune responses [1–3]. Recent data indicate that TDs affect approximately 5% of the general population, with a higher incidence in females [4]. These conditions significantly influence morbidity and quality of life [5]. Although the pathogenesis of these diseases is not fully elucidated, it is thought to be influenced by a combination of genetic and environmental factors.

The gut microbiota (GM) comprises the microbial community within the human gut, forming a complex microecosystem, particularly within the gastrointestinal tract [6]. It is estimated that the human gut hosts about 1000 species of microbes, constituting 78% of the total microbial cell count in the body [7]. The GM is responsible for various regulatory functions, such as maintaining the integrity of the gut mucosal barrier and modulating the immune system [6]. Disruptions in this microbial balance can compromise the host's normal physiological functions, leading to various diseases. The link between the GM and the thyroid is referred to as the "gut-thyroid axis" [8]. Previous studies have demonstrated that the GM and its metabolites may influence thyroid function through the modulation of trace element absorption in the gut and through inflammatory and immune responses [9].

Mendelian randomization (MR) is a genetics-based method crucial for exploring the causal connections between the GM and TDs [10]. In the absence of feasible randomized controlled trials, MR has emerged as a significant alternative for assessing the causal relationship between theGM and disease risk. Its primary advantage lies in using naturally occurring genetic variations that are assigned randomly during fertilization, thereby minimizing confounding in causal analysis, and remaining largely unaffected by the disease process. Thus, it facilitates a robust assessment of the causal relationships between the GM and various TDs. MR has previously established a causal relationship between genetically predicted GM and conditions such as thyroid cancer [11], hypothyroidism [12], and Hashimoto's thyroiditis [13]. However, the causal role of the GM in many other TDs has not yet been reported.

To identify potential GM links to the etiology of TDs, we utilized the most recent and comprehensive GWAS summary data for a two-sample MR analysis. This investigation advances our understanding of the pathogenesis of TDs by exploring the causal connections between the human GM and TDs.

2. Materials and Methods

2.1. Study Overview

This study employed a two-sample MR analysis to assess the causal relationship between the GM and six TDs. GWAS summary-level data for the GM and TDs were acquired separately, treating each genus-level GM as an independent exposure, and grouping four thyroid nodules and two solitary nodules as outcomes. For accurate results, this MR analysis must meet three criteria: (1) the selected instrumental variables (IVs) must demonstrate a strong association with the GM; (2) the IVs must not be associated with any potential confounding factors; (3) the IVs should influence the risk of TDs solely through the GM, without any other intervening mechanisms [14,15]. Figure 1 illustrates the analytical process.

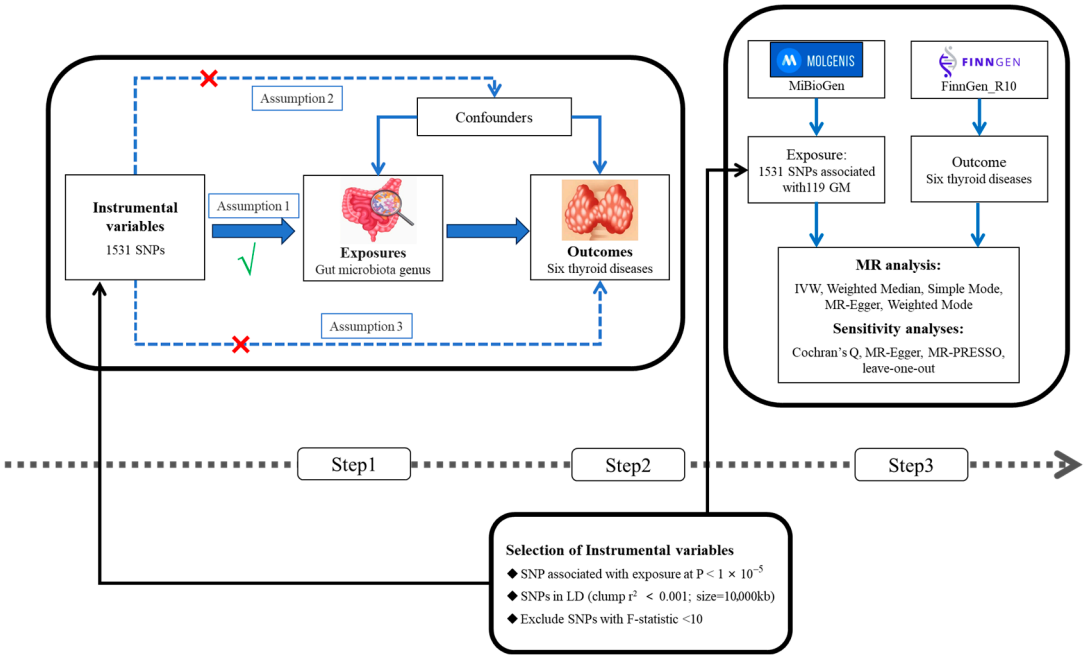


Figure 1. (Step 1) Assumptions of the study. (Step 2) Selection of instrumental variables. (Step 3) Analytical process.

2.2. Data Sources

Initially, GWAS data for the GM were sourced from the MiBioGen consortium, which involved 18,340 participants [16]. Twelve unknown genera were excluded, resulting in 119 genera included for analysis [17]. GWAS data for various TDs, including NDG, NMG, NSTN, GD, PD, and TSTN, were sourced from the FinnGen R10 database [18], with all data deriving from European populations. The summary data utilized in this study were obtained from public databases, and each GWAS had received ethical approval from its respective institution, as detailed in Table 1.

Table 1. Sources of data for the study.

Trait	Data Type	N_Cases	N_Controls	Consortium	Phenocode
GM	Exposure	18,340		MiBioGen	
NDG	Outcome	906	349,717	FinnGen_R10	E4_GOITREDIFF
NMG	Outcome	6699	349,717	FinnGen_R10	E4_GOITREMULTINOD
NSTN	Outcome	2203	349,717	FinnGen_R10	E4_GOITRENOD
GD	Outcome	4846	349,717	FinnGen_R10	E4_THYTOXGOITDIF
PD	Outcome	1717	403,309	FinnGen_R10	E4_THYTOXGOITMULT
TSTN	Outcome	246	403,309	FinnGen_R10	E4_THYTOXNOD

2.3. Selection of Instrumental Variables

Initially, single nucleotide polymorphisms (SNPs) strongly associated with specific genera were selected as IVs using a significance threshold ($p < 1 \times 10^{-5}$). Next, linkage disequilibrium (LD) analysis was performed using European genome sample data, with parameters set to kb = 10,000 and $r^2 < 0.001$ [19], while excluding palindromic SNPs to prevent allelic effects on the outcomes. Finally, the strength of the IVs was evaluated by calculating the F-statistic; an F-value greater than 10 indicates the absence of weak instrument bias, and IVs with an $F \leq 10$ were excluded. The F-value is calculated as follows: $F = \beta^2$ exposure/SE^2 exposure [17].

2.4. Mendelian Randomization Analysis and Sensitivity Analysis

This study conducted a comprehensive assessment of the potential causal links between the GM and six TDs using five different MR methods: inverse variance weighted (IVW), weighted median, simple mode, MR-Egger, and weighted mode, with IVW as the primary method [20].

Cochran's Q test was used to evaluate the heterogeneity of the results, with a *p*-value greater than 0.05 indicating no heterogeneity. Horizontal pleiotropy was assessed using MR-Egger and MR-PRESSO tests, where an intercept *p*-value above 0.05 suggested no horizontal pleiotropy. A leave-one-out analysis was conducted to determine the impact of any single SNP on the study results, and funnel plots and forest plots were utilized for visualization to ensure the robustness of the findings [21]. The analysis was performed using R-4.3.2 software.

3. Results

3.1. Instrumental Variables and Mendelian Randomization Results

Following the selection criteria, a total of 1531 SNPs from 119 GM genera were identified as IVs [22], all exhibiting an F-statistic greater than 10. This confirms that our study is not susceptible to weak instrument bias, with details provided in Supplementary Table S1. The MR analysis results for each of the 119 genera in relation to the six TDs are displayed in Figure 2 and Supplementary Tables S2–S7.

3.2. Causal Relationships between Gut Microbiota and Thyroid Diseases

The results from the IVW analysis revealed that 32 genera in the GM are associated with a risk of six TDs. Specifically, the *Clostridium innocuum group* (OR = 0.716, 95% CI: 0.530–0.967), *Ruminiclostridium5* (OR = 0.618, 95% CI: 0.402–0.951), and *Lachnoclostridium* (OR = 0.585, 95% CI: 0.362–0.947) were negatively associated with the risk of NDG. Conversely, *Ruminococcaceae UCG002* (OR = 1.470, 95% CI: 1.039–2.078), *Alistipes* (OR = 1.909, 95% CI: 1.123–3.243), *Methanobrevibacter* (OR = 1.503, 95% CI: 1.088–2.075), *Marvinbryantia* (OR = 1.875, 95% CI: 1.077–3.266), and *Ruminococcaceae UCG014* (OR = 1.808, 95% CI: 1.250–2.614) were associated with increased NDG risk. *Bifidobacterium* (OR = 0.771, 95% CI: 0.662–0.898) and *Sutterella* (OR = 0.830, 95% CI: 0.698–0.988) provided protection against NMG, while the *Ruminococcus gauvreauii group* (OR = 1.307, 95% CI: 1.033–1.655) and *Rikenellaceae RC9 gut group* (OR = 1.131, 95% CI: 1.034–1.237) increased NMG risk. *Defluviitaleaceae UCG011* (OR = 0.746, 95% CI: 0.570–0.976), *Ruminococcus1* (OR = 0.710, 95% CI: 0.532–0.948), and *Ruminococcaceae UCG010* (OR = 0.649, 95% CI: 0.462–0.913) had protective effects against NSTN, while *Alistipes* (OR = 1.509, 95% CI: 1.074–2.122), the *Ruminococcus gauvreauii group* (OR = 1.454, 95% CI: 1.100–1.923), and *Lachnospiraceae UCG010* (OR = 1.357, 95% CI: 1.000–1.840) were associated with increased NSTN risk. *Ruminiclostridium9* (OR = 0.749, 95% CI: 0.586–0.957), *Victivallis* (OR = 0.847, 95% CI: 0.745–0.964), and *Butyricimonas* (OR = 0.824, 95% CI: 0.698–0.972) were negatively associated with the risk of GD, while the *Eubacterium rectale group* (OR = 1.305, 95% CI: 1.049–1.624), *Desulfovibrio* (OR = 1.216, 95% CI: 1.006–1.468), *Bifidobacterium* (OR = 1.246, 95% CI: 1.051–1.476), *Collinsella* (OR = 1.301, 95% CI: 1.023–1.655), *Oscillospira* (OR = 1.231, 95% CI: 1.003–1.510), and *Catenibacterium* (OR = 1.331, 95% CI: 1.035–1.710) increased GD risk. *Butyricimonas* (OR = 0.743, 95% CI: 0.564–0.978) and *Lachnospira* (OR = 0.560, 95% CI: 0.329–0.952) provided protection against PD, while *Dorea* (OR = 2.262, 95% CI: 1.529–3.346), *Eggerthella* (OR = 1.292, 95% CI: 1.017–1.641), *Odoribacter* (OR = 1.832, 95% CI: 1.151–2.918), *Lactobacillus* (OR = 1.344, 95% CI: 1.016–1.777), *Intestinimonas* (OR = 1.355, 95% CI: 1.046–1.756), and *Phascolarctobacterium* (OR = 2.007, 95% CI: 1.452–2.774) increased PD risk. *Parasutterella* (OR = 0.490, 95% CI: 0.254–0.943) was negatively associated with the risk of TSTN, while *Sutterella* (OR = 3.178, 95% CI: 1.317–7.671), *Oscillibacter* (OR = 2.056, 95% CI: 1.130–3.742), and *Clostridium sensu stricto1* (OR = 3.367, 95% CI: 1.292–8.773) increased TSTN risk. These findings are visually represented in Figure 3 and the scatter plots in Supplementary Figure S1.

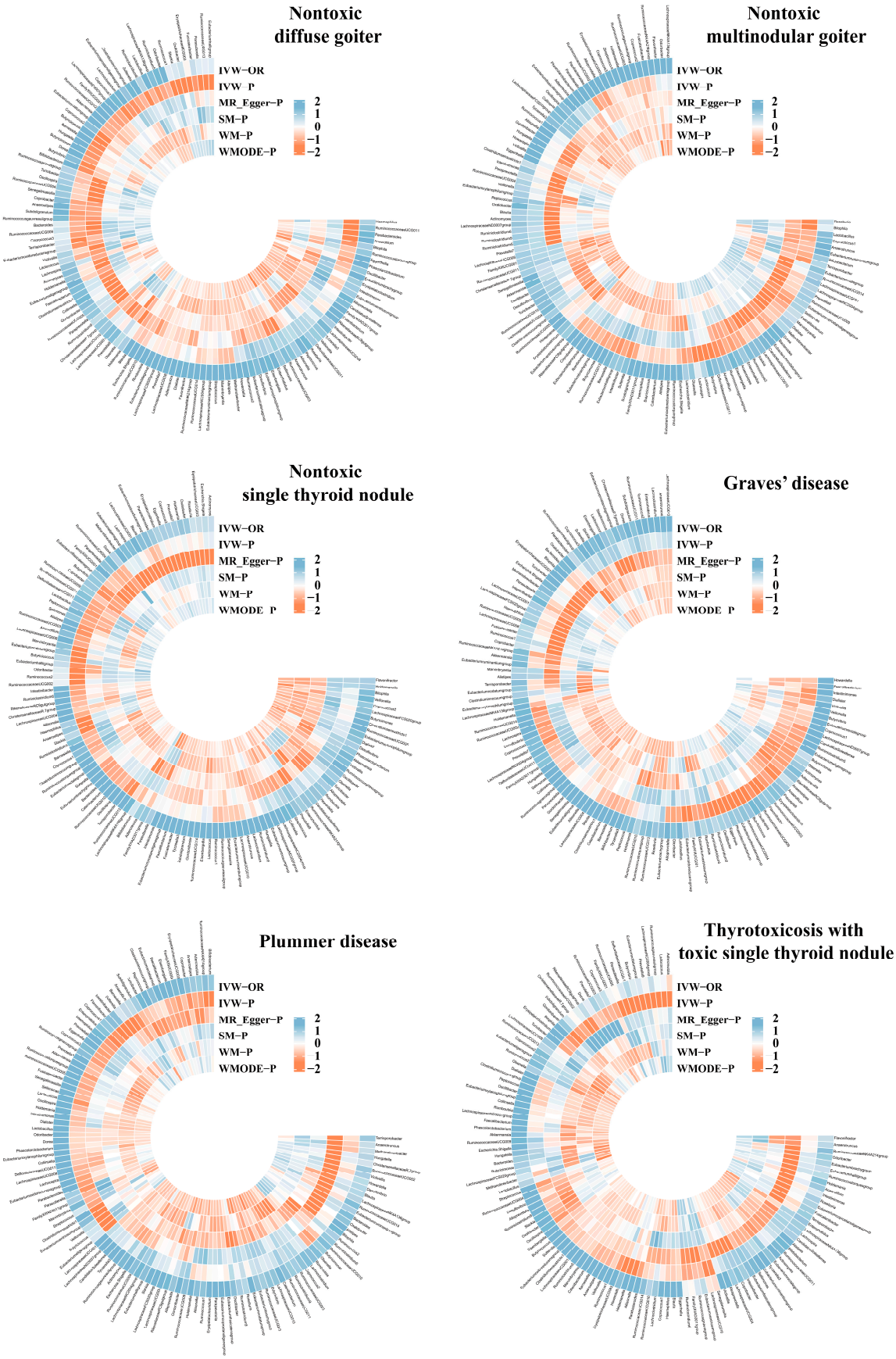


Figure 2. Circus plot showing the MR results for all GM.

Outcome	Exposure	Method	nSNP	P.value	OR(95%CI)
Nontoxic diffuse goitre	*Clostridium innocuum group*	IVW	10	0.030	0.716(0.530 to 0.967)
	Ruminococcaceae UCG002	IVW	23	0.029	1.470(1.039 to 2.078)
	Alistipes	IVW	14	0.017	1.909(1.123 to 3.243)
	Ruminiclostridium5	IVW	14	0.029	0.618(0.402 to 0.951)
	Methanobrevibacter	IVW	8	0.013	1.503(1.088 to 2.075)
	Marvinbryantia	IVW	10	0.026	1.875(1.077 to 3.266)
	Ruminococcaceae UCG014	IVW	17	0.002	1.808(1.250 to 2.614)
	Lachnoclostridium	IVW	14	0.029	0.585(0.362 to 0.947)
Nontoxic multinodular goitre	*Bifidobacterium*	IVW	14	0.001	0.771(0.662 to 0.898)
	Ruminococcus gauvreauii group	IVW	13	0.026	1.307(1.033 to 1.655)
	Sutterella	IVW	12	0.036	0.830(0.698 to 0.988)
	Rikenellaceae RC9gut group	IVW	12	0.007	1.131(1.034 to 1.237)
Nontoxic single thyroid nodule	*Defluviitaleaceae UCG011*	IVW	10	0.032	0.746(0.570 to 0.976)
	Alistipes	IVW	14	0.018	1.509(1.074 to 2.122)
	Ruminococcus gauvreauii group	IVW	13	0.009	1.454(1.100 to 1.923)
	Lachnospiraceae UCG010	IVW	12	0.050	1.357(1.000 to 1.840)
	Ruminococcus1	IVW	13	0.020	0.710(0.532 to 0.948)
	Ruminococcaceae UCG010	IVW	8	0.013	0.649(0.462 to 0.913)
Graves' disease	*Ruminiclostridium9*	IVW	14	0.021	0.749(0.586 to 0.957)
	Eubacterium rectale group	IVW	11	0.017	1.305(1.049 to 1.624)
	Desulfovibrio	IVW	10	0.043	1.216(1.006 to 1.468)
	Bifidobacterium	IVW	14	0.011	1.246(1.051 to 1.476)
	Victivallis	IVW	12	0.012	0.847(0.745 to 0.964)
	Butyricimonas	IVW	16	0.022	0.824(0.698 to 0.972)
	Collinsella	IVW	11	0.032	1.301(1.023 to 1.655)
	Oscillospira	IVW	9	0.046	1.231(1.003 to 1.510)
	Catenibacterium	IVW	4	0.026	1.331(1.035 to 1.710)
Plummer disease	*Dorea*	IVW	12	0.000	2.262(1.529 to 3.346)
	Eggerthella	IVW	10	0.036	1.292(1.017 to 1.641)
	Odoribacter	IVW	8	0.011	1.832(1.151 to 2.918)
	Lactobacillus	IVW	8	0.038	1.344(1.016 to 1.777)
	Butyricimonas	IVW	16	0.034	0.743(0.564 to 0.978)
	Intestinimonas	IVW	18	0.021	1.355(1.046 to 1.756)
	Phascolarctobacterium	IVW	11	0.000	2.007(1.452 to 2.774)
	Lachnospira	IVW	7	0.032	0.560(0.329 to 0.952)
Thyrotoxicosis with toxic single thyroid nodule	*Sutterella*	IVW	12	0.010	3.178(1.317 to 7.671)
	Oscillibacter	IVW	16	0.018	2.056(1.130 to 3.742)
	Clostridium sensu stricto1	IVW	9	0.013	3.367(1.292 to 8.773)
	Parasutterella	IVW	16	0.033	0.490(0.254 to 0.943)

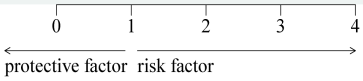


Figure 3. Forest plot of the associations between genetically predicted GM and 6 types of TDs risk using IVW methods.

3.3. Sensitivity Analysis

In the NMG, the *Ruminococcus gauvreauii group* displayed heterogeneity and horizontal pleiotropy (Cochran's Q *p*-value = 0.014, MR-PRESSO *p*-value = 0.024), potentially impacting the final study results. After the outliers rs2047242 and rs289410 were removed, a subsequent sensitivity analysis was conducted. The revised results indicate that the *Ruminococcus gauvreauii group* no longer exhibited heterogeneity or horizontal pleiotropy (Cochran's Q *p*-value = 0.503, MR-PRESSO *p*-value = 0.567). Horizontal pleiotropy was absent in other genera, as documented in Table 2. The leave-one-out analysis, along with funnel plots and forest plots, did not identify any significant outliers, confirming the reliability of the MR study findings, as illustrated in Supplementary Figures S2–S4.

Table 2. Sensitivity analysis results for this study.

Outcome	Exposure	Heterogeneity		Directional Pleiotropy		MR-PRESSO
		Cochran's Q	*p*-Value	Egger Intercept	*p*-Value	*p*-Value
NDG	*Clostridium innocuum group*	9.361	0.313	−0.111	0.323	0.358
	Ruminococcaceae UCG002	20.528	0.488	−0.015	0.672	0.561
	Alistipes	6.909	0.864	0.082	0.323	0.853
	Ruminiclostridium5	8.642	0.733	−0.007	0.870	0.842
	Methanobrevibacter	2.917	0.819	−0.014	0.881	0.882
	Marvinbryantia	10.957	0.204	0.019	0.858	0.282
	Ruminococcaceae UCG014	12.087	0.672	0.012	0.775	0.786
	Lachnoclostridium	10.957	0.533	0.027	0.648	0.598
NMG	*Bifidobacterium*	12.431	0.412	0.019	0.271	0.421
	Ruminococcus gauvreauii group	23.652	0.014	−0.030	0.446	0.024
		8.311	0.503	−0.022	0.418	0.567
	Sutterella	4.991	0.892	−0.004	0.892	0.933
	Rikenellaceae RC9 gut group	3.956	0.949	−0.007	0.863	0.974
NSTN	*Defluviitaleaceae UCG011*	3.316	0.913	0.051	0.360	0.914
	Alistipes	10.944	0.534	−0.014	0.787	0.639
	Ruminococcus gauvreauii group	7.889	0.723	−0.027	0.547	0.778
	Lachnospiraceae UCG010	4.287	0.933	−0.006	0.875	0.965
	Ruminococcus1	6.486	0.839	0.021	0.524	0.865
	Ruminococcaceae UCG010	1.724	0.943	0.010	0.795	0.973
GD	*Ruminiclostridium9*	16.142	0.185	−0.027	0.540	0.239
	Eubacterium rectale group	8.752	0.461	−0.026	0.262	0.487
	Desulfovibrio	7.665	0.467	0.013	0.667	0.602
	Bifidobacterium	10.787	0.547	0.004	0.835	0.668
	Victivallis	14.821	0.139	0.065	0.281	0.138
	Butyricimonas	11.642	0.635	0.033	0.238	0.605
	Collinsella	6.405	0.699	0.003	0.924	0.781
	Oscillospira	7.463	0.382	0.002	0.958	0.520
	Catenibacterium	4.388	0.111	−0.177	0.501	0.225
PD	*Dorea*	6.328	0.787	−0.062	0.149	0.680
	Eggerthella	8.349	0.400	0.019	0.768	0.516
	Odoribacter	5.514	0.480	−0.085	0.155	0.351
	Lactobacillus	6.446	0.375	−0.050	0.267	0.382
	Butyricimonas	10.576	0.719	−0.058	0.213	0.695
	Intestinimonas	11.493	0.778	0.013	0.671	0.833
	Phascolarctobacterium	8.348	0.500	−0.015	0.793	0.659
	Lachnospira	4.236	0.516	0.133	0.192	0.389
TSTN	*Sutterella*	9.669	0.470	−0.006	0.965	0.591
	Oscillibacter	13.415	0.494	0.032	0.763	0.596
	Clostridium sensu stricto1	6.494	0.483	0.090	0.473	0.554
	Parasutterella	14.629	0.404	−0.003	0.968	0.504

4. Discussion

Numerous studies have indicated that dysbiosis within the GM may precipitate various TDs [23–25]. However, definitively ascertaining a causal link between GM and TDs is problematic due to inherent limitations in both observational and experimental methodologies. Recent advances in sequencing technologies have shed light on the integral role of GM in disease regulation, encompassing immune function, metabolic processes, and pharmacokinetics. Utilizing the concept of the gut–thyroid axis, researchers have begun to investigate how GM affect thyroid activity through their impact on micronutrient absorption and immune modulation.

The present research is pioneering in its use of the MR approach to explore causal relationships between GM and the incidence of six distinct TDs. The GM, characterized as a dynamic and multifaceted ecosystem, represents a novel area of inquiry [26]. Our results suggest that specific microbial taxa are causally linked to the prevalence of these TDs.

This study also delineates the intricate genetic interactions contributing to the emergence of TDs, which frequently manifest as chronic thyroid enlargement and are commonly referred to as goiter. Goiters are classified into NDG and NMG, along with hyperthyroid conditions such as GD and multiple PD. At the genus level, we identified 32 GM taxa correlated with these conditions. Notably, the *Clostridium innocuum group*, *Ruminiclostridium5*, and *Lachnoclostridium* appear to exert protective effects against NDG. Conversely, taxa such as *Ruminococcaceae UCG002*, *Alistipes*, *Methanobrevibacter*, *Marvinbryantia*, and *Ruminococcaceae UCG014* elevate NDG risk. For NMG, *Bifidobacterium* and *Sutterella* have been found to protect against it, whereas the *Ruminococcus gauvreauii group* and *Rikenellaceae RC9 gut group* increase NMG susceptibility. Protective agents for GD include *Ruminiclostridium9*, *Victivallis*, and *Butyricimonas*, while taxa such as the *Eubacterium rectale group*, *Desulfovibrio*, *Bifidobacterium*, *Collinsella*, *Oscillospira*, and *Catenibacterium* are linked to a heightened risk of GD. Furthermore, *Butyricimonas* and *Lachnospira* offer protection against PD, while *Dorea*, *Eggerthella*, *Odoribacter*, *Lactobacillus*, *Intestinimonas*, and *Phascolarctobacterium* are associated with increased PD risk.

The GM influences thyroid health through mechanisms such as immune regulation, anti-inflammatory responses, and increased intestinal permeability. It is essential to highlight that *Bifidobacterium*, prevalent in both human and animal guts and part of the Firmicutes phylum, is crucial for maintaining intestinal health and immune balance. Nonetheless, its association with TDs, especially NMG and GD, involves intricate mechanisms tied to inflammatory responses and immune regulation. Our findings indicate that *Bifidobacterium* provides protection against NMG—likely via its metabolic byproducts, such as short-chain fatty acids—which could reduce intestinal inflammation and autoimmunity, thus potentially decreasing NMG risk [27–29]. Conversely, evidence from this study suggests there is an increased risk of GD associated with *Bifidobacterium*, challenging prevailing views. GM might play a critical role in GD's pathogenesis [30]. For example, research shows that combining *Bifidobacterium* with methimazole may affect neurotransmitters and blood micronutrients through the gut–brain and gut–thyroid axes, thereby enhancing thyroid function in GD patients [31].

Furthermore, while the roles of some microbial taxa in specific TDs remain unreported, *Lachnoclostridium*—an anaerobic, Gram-positive bacterium from the Firmicutes phylum—is known to offer protective effects against NDG. This protection may be attributed to its production of anti-inflammatory butyrate salts and the upregulation of tight junction proteins, thus enhancing the gut barrier [32]. Similarly, *Sutterella*, a genus of Gram-negative bacteria from the Bacteroidota phylum, appears to provide protection against NMG, possibly by adhering to intestinal cells and modulating immune responses [33,34]. Both GD and PD lead to excessive hormone production by the thyroid, resulting in hyperthyroidism and thyroid enlargement. GD typically manifests as diffuse thyroid enlargement, while PD features multiple nodular enlargements [35,36]. Studies have indicated significantly lower levels of *Butyricimonas* in thyroid nodules [37]. Further research into the relationship between the gut microbiome, its metabolites, and thyroid nodules reveals a marked reduction in

Butyricimonas in patients with thyroid nodules and thyroid cancer [9]. Research in hyperthyroid gerbils has shown diminished levels of beneficial *Butyricimonas*, which is involved in regulating the host's resting metabolic rate and food intake, suggesting that thyroid hormones might alter host thermogenesis by modifying the gut microbiome [38]. Our findings corroborate these observations, showing that *Butyricimonas* also offers protection against GD and PD. NSTN and thyrotoxicosis with TSTN both involve a single thyroid nodule, with the primary distinction being whether the nodule induces hyperthyroidism. An NSTN is generally asymptomatic, whereas a TSTN leads to symptoms of hyperthyroidism. Additionally, our study expanded the analysis to include NSTN and TSTN. Protective taxa against NSTN include *Defluviitaleaceae UCG011*, *Ruminococcus1*, and *Ruminococcaceae UCG010*, while taxa such as *Alistipes*, the *Ruminococcus gauvreauii group*, and *Lachnospiraceae UCG010* are associated with an increased risk of NSTN. *Parasutterella* offers protection against TSTN, whereas *Sutterella*, *Oscillibacter*, and *Clostridium sensu stricto1* are linked to a higher TSTN risk. Notably, *Alistipes*, a Gram-negative anaerobic bacterium from the Rikenellaceae family within the *Bacteroidetes* phylum, is identified as a pathogenic microbial modulator and is associated with an increased risk of NDG and NSTN [39]. Continued research is necessary to define the specific mechanisms through which other microbial taxa impact TDs.

5. Conclusions

In summary, our research has established a causal link between the GM and six TDs. However, several inherent limitations in our study must be acknowledged. First, the dataset was limited to a European population, which may not be representative of other ethnic groups. Second, the analysis of the GM was conducted at the genus level, rather than at a more detailed or specialized level. Third, our analysis did not address gender differences in the relationship between GM and the six types of TDs. In future studies, gender will be considered as a potential factor. Finally, the reliance on publicly available GWAS data may introduce various biases and limitations that warrant careful consideration. Further in-depth analysis will be required to address these issues and enhance our understanding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology13090714/s1, Figure S1: Scatter plots of causal estimates of gut microbiota on thyroid diseases; Figure S2: Leave-one-out stability tests causal estimates of specific GM on thyroid diseases; Figure S3: Funnel plots for causal effects of specific GM on thyroid diseases risk with individual SNPs; Figure S4: Forest plots for causal effects of specific GM on thyroid diseases risk with individual SNPs; Table S1: Detailed information of instrumental variables used in MR analyses; Table S2: The MR results of the associations between GM with NDG; Table S3: The MR results of the associations between GM with NMG; Table S4: The MR results of the associations between GM with NSTN; Table S5: The MR results of the associations between GM with GD; Table S6: The MR results of the associations between GM with PD; Table S7: The MR results of the associations between GM with TSTN.

Author Contributions: Conceptualization, J.C. and Y.L.; Methodology, J.C. and H.Y.; Software, J.C. and H.Y.; Validation, J.C.; Formal analysis, J.C. and H.Y.; Investigation, Y.W.; Resources, Y.W.; Data curation, Y.W.; Writing—original draft, J.C., Y.W., H.Y. and Y.L.; Writing—review & editing, H.S.; Visualization, H.S.; Supervision, H.S.; Project administration, H.S.; Funding acquisition, H.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional Review Board Statement: The summary data used in the article were sourced from public data. Each GWAS mentioned in the article received ethical approval from their respective institutions.

Informed Consent Statement: This study is based on publicly available summarized data. The informed consent of each genome-wide association study was obtained from participants or from a caregiver, legal guardian, or other proxy.

Data Availability Statement: The data from this study have been deposited in online repositories. The repository/repositories and corresponding accession number(s) can be located in the article's Supplementary Materials.

Acknowledgments: This study utilized data from the MiBioGen and FinnGen databases. The authors express their gratitude to everyone involved in the data collection and participation.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lisco, G.; De Tullio, A.; Triggiani, D.; Zupo, R.; Giagulli, V.A.; De Pergola, G.; Piazzolla, G.; Guastamacchia, E.; Sabbà, C.; Triggiani, V. Iodine Deficiency and Iodine Prophylaxis: An Overview and Update. *Nutrients* **2023**, *15*, 1004. [CrossRef] [PubMed]
2. Zimmermann, M.B.; Boelaert, K. Iodine Deficiency and Thyroid Disorders. *Lancet Diabetes Endocrinol.* **2015**, *3*, 286–295. [CrossRef] [PubMed]
3. Kawicka, A.; Regulska-Ilow, B.; Regulska-Ilow, B. Metabolic Disorders and Nutritional Status in Autoimmune Thyroid Diseases. *Adv. Hyg. Exp. Med.* **2015**, *69*, 80–90. [CrossRef] [PubMed]
4. Ludgate, M.E.; Masetti, G.; Soares, P. The Relationship between the Gut Microbiota and Thyroid Disorders. *Nat. Rev. Endocrinol.* **2024**, *20*, 511–525. [CrossRef] [PubMed]
5. Mendoza-León, M.J.; Mangalam, A.K.; Regaldiz, A.; González-Madrid, E.; Rangel-Ramírez, M.A.; Álvarez-Mardonez, O.; Vallejos, O.P.; Méndez, C.; Bueno, S.M.; Melo-González, F.; et al. Gut Microbiota Short-Chain Fatty Acids and Their Impact on the Host Thyroid Function and Diseases. *Front. Endocrinol.* **2023**, *14*, 1192216. [CrossRef]
6. Chen, Y.; Zhou, J.; Wang, L. Role and Mechanism of Gut Microbiota in Human Disease. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 625913. [CrossRef]
7. Zhu, Y.; Yang, Q.; Yang, Q.; He, Y.; Zhou, W. Intestinal Microbes and Hematological Malignancies. *Cancers* **2023**, *15*, 2284. [CrossRef]
8. Lerner, A.; Jeremias, P.; Matthias, T. Gut-Thyroid Axis and Celiac Disease. *Endocr. Connect.* **2017**, *6*, R52–R58. [CrossRef]
9. Jiang, W.; Lu, G.; Gao, D.; Lv, Z.; Li, D. The Relationships between the Gut Microbiota and Its Metabolites with Thyroid Diseases. *Front. Endocrinol.* **2022**, *13*, 943408. [CrossRef]
10. Venkatesh, S.S.; Ferreira, T.; Benonisdottir, S.; Rahmioglu, N.; Becker, C.M.; Granne, I.; Zondervan, K.T.; Holmes, M.V.; Lindgren, C.M.; Wittemans, L.B.L. Obesity and Risk of Female Reproductive Conditions: A Mendelian Randomisation Study. *PLoS Med.* **2022**, *19*, e1003679. [CrossRef]
11. Hou, T.; Wang, Q.; Dai, H.; Hou, Y.; Zheng, J.; Wang, T.; Lin, H.; Wang, S.; Li, M.; Zhao, Z.; et al. Interactive Association between Gut Microbiota and Thyroid Cancer. *Endocrinology* **2023**, *165*, bqad184. [CrossRef] [PubMed]
12. Wang, Z.; Wu, M.; Pan, Y.; Wang, Q.; Zhang, L.; Tang, F.; Lu, B.; Zhong, S. Causal Relationships between Gut Microbiota and Hypothyroidism: A Mendelian Randomization Study. *Endocrine* **2024**, *83*, 708–718. [CrossRef] [PubMed]
13. Xiong, Y.; Zhu, X.; Luo, Q. Causal Relationship between Gut Microbiota and Autoimmune Thyroiditis: A Mendelian Study. *Heliyon* **2024**, *10*, e25652. [CrossRef]
14. VanderWeele, T.J.; Tchetgen Tchetgen, E.J.; Cornelis, M.; Kraft, P. Methodological Challenges in Mendelian Randomization. *Epidemiology* **2014**, *25*, 427–435. [CrossRef] [PubMed]
15. Liu, Y.; Xu, H.; Zhao, Z.; Dong, Y.; Wang, X.; Niu, J. No Evidence for a Causal Link between Helicobacter Pylori Infection and Nonalcoholic Fatty Liver Disease: A Bidirectional Mendelian Randomization Study. *Front. Microbiol.* **2022**, *13*, 1018322. [CrossRef]
16. Kurilshikov, A.; Medina-Gomez, C.; Bacigalupe, R.; Radjabzadeh, D.; Wang, J.; Demirkan, A.; Le Roy, C.I.; Raygoza Garay, J.A.; Finnicum, C.T.; Liu, X.; et al. Large-Scale Association Analyses Identify Host Factors Influencing Human Gut Microbiome Composition. *Nat. Genet.* **2021**, *53*, 156–165. [CrossRef]
17. Xia, D.; Wang, J.; Zhao, X.; Shen, T.; Ling, L.; Liang, Y. Association between Gut Microbiota and Benign Prostatic Hyperplasia: A Two-Sample Mendelian Randomization Study. *Front. Cell. Infect. Microbiol.* **2023**, *13*, 1248381. [CrossRef]
18. Kurki, M.I.; Karjalainen, J.; Palta, P.; Sipilä, T.P.; Kristiansson, K.; Donner, K.M.; Reeve, M.P.; Laivuori, H.; Aavikko, M.; Kaunisto, M.A.; et al. FinnGen Provides Genetic Insights from a Well-Phenotyped Isolated Population. *Nature* **2023**, *613*, 508–518. [CrossRef]
19. Chen, S.; Zhou, G.; Han, H.; Jin, J.; Li, Z. Causal Effects of Specific Gut Microbiota on Bone Mineral Density: A Two-Sample Mendelian Randomization Study. *Front. Endocrinol.* **2023**, *14*, 1178831. [CrossRef]
20. Lee, C.H.; Cook, S.; Lee, J.S.; Han, B. Comparison of Two Meta-Analysis Methods: Inverse-Variance-Weighted Average and Weighted Sum of Z-Scores. *Genom. Inform.* **2016**, *14*, 173–180. [CrossRef]
21. Yao, H.; Chen, J.; Wang, Y.; Li, Y.; Jiang, Q. Assessing Causal Relationships between Gut Microbiota and Abortion: Evidence from Two Sample Mendelian Randomization Analysis. *Front. Endocrinol.* **2024**, *15*, 1415730. [CrossRef] [PubMed]
22. Yao, Y.; Hu, H.; Chen, L.; Zheng, H. Association between Gut Microbiota and Menstrual Disorders: A Two-Sample Mendelian Randomization Study. *Front. Microbiol.* **2024**, *15*, 1321268. [CrossRef] [PubMed]
23. Jiang, W.; Yu, X.; Kosik, R.O.; Song, Y.; Qiao, T.; Tong, J.; Liu, S.; Fan, S.; Luo, Q.; Chai, L.; et al. Gut Microbiota May Play a Significant Role in the Pathogenesis of Graves' Disease. *Thyroid* **2021**, *31*, 810–820. [CrossRef] [PubMed]
24. Liu, Q.; Sun, W.; Zhang, H. Interaction of Gut Microbiota with Endocrine Homeostasis and Thyroid Cancer. *Cancers* **2022**, *14*, 2656. [CrossRef]

25. Ejtahed, H.-S.; Angoorani, P.; Soroush, A.-R.; Siadat, S.-D.; Shirzad, N.; Hasani-Ranjbar, S.; Larijani, B. Our Little Friends with Big Roles: Alterations of the Gut Microbiota in Thyroid Disorders. *Endocr. Metab. Immune Disord. Drug Targets* **2020**, *20*, 344–350. [CrossRef]
26. Zhang, L.; Zi, L.; Kuang, T.; Wang, K.; Qiu, Z.; Wu, Z.; Liu, L.; Liu, R.; Wang, P.; Wang, W. Investigating Causal Associations among Gut Microbiota, Metabolites, and Liver Diseases: A Mendelian Randomization Study. *Front. Endocrinol.* **2023**, *14*, 1159148. [CrossRef]
27. Gong, B.; Wang, C.; Meng, F.; Wang, H.; Song, B.; Yang, Y.; Shan, Z. Association Between Gut Microbiota and Autoimmune Thyroid Disease: A Systematic Review and Meta-Analysis. *Front. Endocrinol.* **2021**, *12*, 774362. [CrossRef]
28. Arpaia, N.; Campbell, C.; Fan, X.; Dikiy, S.; van der Veeken, J.; deRoos, P.; Liu, H.; Cross, J.R.; Pfeffer, K.; Coffer, P.J.; et al. Metabolites Produced by Commensal Bacteria Promote Peripheral Regulatory T-Cell Generation. *Nature* **2013**, *504*, 451–455. [CrossRef]
29. Furusawa, Y.; Obata, Y.; Fukuda, S.; Endo, T.A.; Nakato, G.; Takahashi, D.; Nakanishi, Y.; Uetake, C.; Kato, K.; Kato, T.; et al. Commensal Microbe-Derived Butyrate Induces the Differentiation of Colonic Regulatory T Cells. *Nature* **2013**, *504*, 446–450. [CrossRef]
30. Chang, S.-C.; Lin, S.-F.; Chen, S.-T.; Chang, P.-Y.; Yeh, Y.-M.; Lo, F.-S.; Lu, J.-J. Alterations of Gut Microbiota in Patients with Graves' Disease. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 663131. [CrossRef]
31. Huo, D.; Cen, C.; Chang, H.; Ou, Q.; Jiang, S.; Pan, Y.; Chen, K.; Zhang, J. Probiotic Bifidobacterium Longum Supplied with Methimazole Improved the Thyroid Function of Graves' Disease Patients through the Gut-Thyroid Axis. *Commun. Biol.* **2021**, *4*, 1046. [CrossRef] [PubMed]
32. Dai, W.; Cai, D.; Zhou, S.; Li, A.; Xie, J.; Zhang, J. Uncovering a Causal Connection between the Lachnoclostridium Genus in Fecal Microbiota and Non-Alcoholic Fatty Liver Disease: A Two-Sample Mendelian Randomization Analysis. *Front. Microbiol.* **2023**, *14*, 1276790. [CrossRef] [PubMed]
33. Hiippala, K.; Kainulainen, V.; Kalliomäki, M.; Arkkila, P.; Satokari, R. Mucosal Prevalence and Interactions with the Epithelium Indicate Commensalism of *Sutterella* spp. *Front. Microbiol.* **2016**, *7*, 1706. [CrossRef] [PubMed]
34. Legakis, I.; Chrousos, G.P.; Chatzipanagiotou, S. Thyroid Diseases and Intestinal Microbiome. *Horm. Metab. Res.* **2023**, *55*, 813–818. [CrossRef] [PubMed]
35. Barczyński, M. Current Approach to Surgical Management of Hyperthyroidism. *Q. J. Nucl. Med. Mol. Imaging* **2021**, *65*, 124–131. [CrossRef]
36. Smith, T.J.; Hegedüs, L. Graves' Disease. *N. Engl. J. Med.* **2016**, *375*, 1552–1565. [CrossRef]
37. Zhang, J.; Zhang, F.; Zhao, C.; Xu, Q.; Liang, C.; Yang, Y.; Wang, H.; Shang, Y.; Wang, Y.; Mu, X.; et al. Dysbiosis of the Gut Microbiome Is Associated with Thyroid Cancer and Thyroid Nodules and Correlated with Clinical Index of Thyroid Function. *Endocrine* **2019**, *64*, 564–574. [CrossRef]
38. Khakisahneh, S.; Zhang, X.-Y.; Nouri, Z.; Wang, D.-H. Cecal Microbial Transplantation Attenuates Hyperthyroid-Induced Thermogenesis in Mongolian Gerbils. *Microb. Biotechnol.* **2022**, *15*, 817–831. [CrossRef]
39. Yu, X.; Jiang, W.; Kosik, R.O.; Song, Y.; Luo, Q.; Qiao, T.; Tong, J.; Liu, S.; Deng, C.; Qin, S.; et al. Gut Microbiota Changes and Its Potential Relations with Thyroid Carcinoma. *J. Adv. Res.* **2022**, *35*, 61–70. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.