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The gut-skin axis: Interaction of gut microbiome and skin diseases

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Published in
Frontiers in Microbiology

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ISSN 1664-8714
ISBN 978-2-8325-5031-1
DOI 10.3389/978-2-8325-5031-1

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The gut-skin axis: Interaction of gut microbiome and skin diseases

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Citation

Chai, J., Wei, X., Zhao, J., eds. (2024). *The gut-skin axis: Interaction of gut microbiome and skin diseases*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-5031-1

Table of contents

frontiers | Frontiers in Microbiology

TYPE Editorial
PUBLISHED 04 June 2024
DOI 10.3389/fmicb.2024.1427770

Check for updates

OPEN ACCESS

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RECEIVED 04 May 2024
ACCEPTED 28 May 2024
PUBLISHED 04 June 2024

CITATION
Chai J, Deng F, Li Y, Wei X and Zhao J (2024) Editorial: The gut-skin axis: interaction of gut microbiome and skin diseases.
Front. Microbiol. 15:1427770.
doi: 10.3389/fmicb.2024.1427770

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Editorial: The gut-skin axis: interaction of gut microbiome and skin diseases

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KEYWORDS

gut-skin axis, microbiome, skin disease, skin epidermis, gut microbiome, microbiome-dynamics

Editorial on the Research Topic
The gut-skin axis: interaction of gut microbiome and skin diseases

More and more evidence has demonstrated that gut microbiome plays critical roles in modulating the development of diseases beyond the gastrointestinal tract, including skin disorders such as psoriasis. The gastrointestinal (GI) tract is one of the largest interfaces between the host and its environment, and is colonized by a large number of microbes, which have a big impact on host health (Deng et al., 2023; Chai et al., 2024). The skin epidermis, with appendage structures, such as sweat and sebaceous glands, have a total skin surface of about 25 m^2 and is one of the largest epithelial surfaces for interaction with microbes. Moreover, both gut and skin act as barriers for immune function. Recent multi-omics technological advances have revealed the importance of the microbiome in health and diseases. Thus, deep investigation of the roles of the microbiome in skin disease will contribute to unveil the mechanisms of the gut-skin axis. In addition, it is necessary to better characterize the skin microbiota and its modes of interaction with the host's immune system.

The new conception "gut-skin axis" refers to the bidirectional relationship between the gut microbiome and skin health. Several mechanisms, such as intestinal barrier, inflammatory mediators, and metabolites, have been proposed for gut-skin axis. Currently, there have been various studies regarding the presence of the gut-skin axis and its resulting inflammatory effect due to gut microbiome imbalance. In addition, dysbiosis of the skin and gut microbiota is also observed in skin disorders. Therefore, understanding the gut-skin axis, especially in terms of microbiome modulation, is important for the gut and skin health, which may lead to development of novel therapies for skin disease.

A total of 11 articles published in this Research Topic broaden our knowledge of microbiome and common skin diseases. Psoriasis, a common erythematous scaling skin disease with multiple skin manifestations and systemic involvement, can involve any skin site and occur at any age and in any geographic area, affecting more than 60 million adults and children worldwide. Zhu et al. summarized the latest information on the unique patterns of gut microbiota and co-metabolites involved in the pathogenesis of psoriasis and attempt to explore microbial-based therapeutic targets derived from mono-and polymicrobial probiotics,

fecal microbiota transplantation, pharmacomicrobiomics, and dietary interventions as diagnostic or therapeutic approaches promising to provide new options and long-term management for psoriasis. Another skin disease, alopecia areata, is a type of autoimmune inflammatory dermatological disease characterized by non-scarring hair loss of the scalp or body skin. Liu and Liu highlighted the relationship between alopecia areata and the gut microbiome or metabolome to provide novel directions for the prevention, clinical diagnosis and treatment of alopecia areata. Sánchez-Pellicer et al. reviewed the pathogenesis of rosacea in terms of gut-skin axis. Rosacea is a chronic skin disease affecting ~5.5% of the general population, mainly patients between 45 and 60 years old, which can severely impact the patient's quality of life as well as their mental health. Oral probiotic supplementation may be an alternative approach to improve the clinical evolution of rosacea. Systemic lupus erythematosus is a chronic autoimmune disease. Zhao et al. conducted a comprehensive analysis of 218 research articles and 118 review articles, and found that China and the United States have emerged as the most active contributors in this domain. They also concluded that recent research endeavors have predominantly focused on exploring the phenomenon of microbiota dysbiosis, specifically pertaining to gut microbiota dysbiosis, alongside elucidating the intricate mechanisms and applications associated with SLE, thereby establishing it as a prominent area of investigation.

In addition, four researches also revealed the link between gut microbiotas and skin diseases. Wu et al. found independent causal relationships between four gut microbes and acne vulgaris, and revealed a genetic association between acne vulgaris patients and gut microbiota. Modulation of these four gut microbes might be an effective way to prevent and treat acne vulgaris. The chronic inflammatory skin disease Hidradenitis suppurativa (HS) is strongly associated with Crohn's Disease (CD). However, gut microbiota composition and diet interaction have not been compared in HS and CD. Cronin et al. compared the fecal microbiota and habitual diet of previously reported subjects with HS, patients with CD and healthy controls, which reported that the fecal microbiota may help identify patients with HS who are at greater risk for development of CD. Xue et al. conducted a bidirectional Mendelian randomization (MR) which found a potential causative link between gut microbiota and hypertrophic scarring, opening up new ways for future mechanistic research and the exploration of nanobiotechnology therapies for skin disorders. Song et al. confirmed the gut microbiome and its metabolites could maintain the intestine barrier homeostasis to resist the intestinal damage caused by the skin ulceration syndrome. Whether alterations in the gut microbiota are a consequence or cause of skin diseases remains to be determined. However, it is clear that gut microbiota plays a critical role in progress or prevention of skin diseases.

Beyond gut microbiota, the microbiota inhabiting the host skin is also important for the skin barrier. Seo et al. identified that *Cutibacterium*, *Corynebacterium*, *Staphyloccocus*, *unclassified Neisseriaceae*, and *Streptococcus* were major skin microbial taxa at the genus level, and fluctuated with the seasons. Moreover, functional profiling using KEGG provided clues on the impact of *Cutibacterium* on the host skin barrier. This study enhances our understanding of the skin microbiome and its interplay with skin characteristics. Li et al. classified the microbiome and lipid profiles on forearm and cheek of humans, which provides insights on the potential correlation between skin microbiome and lipids. Another study conducted by Zhang et al. isolated potential skin probiotics, *Pantoea eucrina KBFS172*, and verified the positive effects of this strain on repairing UV damage. All these findings provide the insights that microbiota is associated with skin physiological characteristics and disease.

The present Research Topic highlights the tight links between the gut or skin microbiota and various skin diseases. It also reveals the contributions of microbial metabolites to skin disease. These recent advancements in the field of microbiome in skin disease provide insights into how to manipulate either gut or skin microbiota to improve host skin health in future studies. Isolations of probiotics from the host might be a new and effective research direction.

Author contributions

JC: Conceptualization, Data curation, Resources, Visualization, Writing – original draft. FD: Data curation, Resources, Writing – original draft. YL: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. XW: Conceptualization, Resources, Supervision, Writing – review & editing. JZ: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This project was supported by Agriculture and Food Research Initiative Competitive Grant No. 20196701629869 from the USDA National Institute of Food and Agriculture, National Natural Science Foundation of China (No. 32170430), Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding (2019B030301010), and Key Laboratory of Animal Molecular Design and Precise Breeding of Guangdong Higher Education Institutes (2019KSYS011).

Acknowledgments

The editor thanks the Frontiers admin team for their wonderful support with the Research Topic as well as the numerous peer reviewers.

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 17 October 2023
DOI 10.3389/fmicb.2023.1263731

Check for updates

OPEN ACCESS

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RECEIVED 25 July 2023
ACCEPTED 15 September 2023
PUBLISHED 17 October 2023

CITATION
Song M, Zhang Z, Li Y, Xiang Y and Li C (2023) Midgut microbiota affects the intestinal barrier by producing short-chain fatty acids in *Apostichopus japonicus*. *Front. Microbiol.* 14:1263731. doi: 10.3389/fmicb.2023.1263731

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Midgut microbiota affects the intestinal barrier by producing short-chain fatty acids in *Apostichopus japonicus*

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Introduction: The intestinal microbiota participates in host physiology and pathology through metabolites, in which short-chain fatty acids (SCFAs) are considered principal products and have extensive influence on intestine homeostasis. It has been reported that skin ulceration syndrome (SUS), the disease of *Apostichopus japonicus* caused by *Vibrio splendidus*, is associated with the alteration of the intestinal microbiota composition.

Method: To investigate whether the intestinal microbiota affects *A. japonicus* health via SCFAs, in this study, we focus on the SCFA profiling and intestinal barrier function in *A. japonicus* treated with *V. splendidus*.

Results and discussion: We found that *V. splendidus* could destroy the mid-intestine integrity and downregulate the expression of tight junction proteins ZO-1 and occludin in *A. japonicus*, which further dramatically decreased microorganism abundance and altered SCFAs contents. Specifically, acetic acid is associated with the largest number of microorganisms and has a significant correlation with occludin and ZO-1 among the seven SCFAs. Furthermore, our findings showed that acetic acid could maintain the intestinal barrier function by increasing the expression of tight junction proteins and rearranging the tight junction structure by regulating F-actin in mid-intestine epithelial cells. Thus, our results provide insights into the effects of the gut microbiome and SCFAs on intestine barrier homeostasis and provide essential knowledge for intervening in SUS by targeting metabolites or the gut microbiota.

KEYWORDS

short-chain fatty acids, intestinal permeability, acetic acid, *Vibrio splendidus*, *Apostichopus japonicus*

1. Introduction

The intestinal microbiota is a wild and diverse microbial ecosystem that has co-evolved with host species (Spiljar et al., 2017). In recent years, several studies have concentrated on the interaction between the host and the intestinal microbiota (Bonder et al., 2016; Wang et al., 2016; Rothschild et al., 2018). The microbial community was found to be affected by host genetic variation; for example, pathological shifts of the microbial community were found to be induced by host genetic susceptibilities in inflammatory bowel disease (IBD) (Plichta et al., 2019). Furthermore, the microbiota

and metabolites derived from the microbiota have adverse effects on the immune system and physiology of the host in IBD (Qin et al., 2018; Plichta et al., 2019). It has been demonstrated that the gut microbiome directly participates in the pathogenesis of various pathological activities, such as circulatory disease, obesity, IBD, and autism (Marchesi et al., 2007; Turnbaugh et al., 2007; Finegold, 2008; Holmes et al., 2008). Therefore, the intestinal microbiota performs various essential biochemical functions for the host, and disorders of the microbiome are associated with several human diseases. Given the interaction between the intestinal microbiota and host health in mammals by regulating physiological activities (Turnbaugh et al., 2006), immune response (Mazmanian et al., 2005), energy metabolism, and the response of intestinal homeostasis to epithelial cell injury (Rakoff-Nahoum et al., 2004), the intestinal microbiota is increasingly being valued for its crucial role in the maintenance of aquatic invertebrate health. Obvious correlations between the intestinal microbiota and physiological activities, such as neurotransmission, detoxification, oxidative stress, and intestinal epithelium integrity, were observed in aquatic invertebrates as well (Falcinelli et al., 2015). Recent studies have demonstrated that the gut microbiome of grass carp plays a role in carbohydrate turnover and fermentation, and the intestinal microbiota of *Nereis succinea* plays a role in biodegrading various organic pollutants (Wu et al., 2015; Wang et al., 2020). Moreover, gut microbiome disorders are associated with diverse aquatic animal disease processes. Enteritis in carps has been found to change the specific metabolic pathway, accompanied by a significant increase in members of the genera *Pseudomonas*, *Flavobacterium*, *Caulobacter*, *Rhodobacter*, *Planctomyces*, *Methylocaldum*, and *Dechloromona* (Tran et al., 2018). Hong et al. (2020) found that intestinal beneficial bacteria decreased and pathogens increased in Chinese mitten crabs exposed to imidacloprid. Zhang et al. (2018) demonstrated that *A. japonicus* afflicted with skin ulceration syndrome (SUS) had higher Firmicutes abundance and lower Verrucomicrobia abundance, while *Lactococcus garvieae* exhibited considerable abundance in sea cucumbers afflicted with SUS.

The sea cucumber *Apostichopus japonicus* (Skelenka) is an economically and ecologically important cultivation in coastal ecosystems of the western North Pacific Ocean (Zhang et al., 2018), which is known as the "ecosystem engineer" because it facilitates nutrient exchange and the carbonate cycle in the water–sediment interface (Schneider et al., 2011). However, SUS, a disease mainly caused by *Vibrio splendidus*, has led to huge economic losses and has been considered the main disease in *A. japonicus* aquaculture (Deng et al., 2008). Because SUS can induce intestinal microbiota dysbiosis, it is important to investigate the interaction between SUS and intestinal microbiota disorder. Then, we can develop a control strategy for SUS by taking advantage of the susceptibility of intestinal microorganisms and targeting them.

The digestive tract tissue of *A. japonicus* is composed of epithelial tissue, inner connective tissue, muscle layer, outer connective tissue, and peritoneum (Liao, 1997). Intestinal epithelium is the first protector of the host body against pathogenic microorganisms, and it is the structural basis for maintaining intestinal selective permeability and barrier function (Turner, 2006; Groschwitz and Hogan, 2009; Liang and Weber, 2014; Zhang et al., 2015). Therefore, the intestinal epithelium plays a significant role in preserving the stability of the host and external environment. Cell junctions in the intestinal epithelial tissue, such as the tight junction (TJ), gap junction (GJ), adhesion junction (AJ), and desmosome, jointly form the intestinal mechanical barrier. The tight junction is mainly composed of zonula occludens (ZO-1, ZO-2, and ZO-3), occludin, claudin, among other, and they play a vital role in maintaining the stability of the intestinal barrier structure and function (Haskins et al., 1998). Vaziri et al. (2013) found that the intestinal microbiota of chronic kidney disease patients changes rapidly and leads to damage, characterized by increased intestinal permeability in the intestinal barrier. Many studies have demonstrated that intestinal permeability significantly increases due to the downregulation of occludin and ZO-1 expression in IBD patients (Piche et al., 2008; Bertiaux-Vandaële et al., 2011). Furthermore, *Escherichia coli* and *Salmonella typhimurium* can disrupt the integrity of the intestinal barrier by altering TJ proteins' expression and arrangement (Berkes et al., 2003; Chin et al., 2006). The increase in probiotics *Lactobacillus sakei* OK67 abundance can reduce inflammation response and increase the ZO-1 expression (Lim et al., 2016).

Based on intestinal microecology, previous studies have continuously explored the effect of intestinal microbiota dysbiosis on the intestinal barrier. Therefore, it is important to search for substances that play an essential role in modulating the effect of intestinal microbiota on the intestinal barrier. The metabolites produced by the gut microbiota exert their effects on the host as signaling molecules and substrates for metabolic reactions. Short-chain fatty acids (SCFAs) could participate in host health as the most abundant microbiome-produced molecules that possess fewer than six carbons, including acetate, propionate, butyrate, pentanoate, and caproate. It has been reported that SCFAs are related to the maintenance of the microbial ecosystem (Duncan et al., 2007), and the profile variation of SCFAs can reflect the metabolic interaction between microorganisms. Some studies have discussed the effect of SCFAs on the intestinal barrier *in vivo* and *in vitro* (Miyoshi et al., 2008). SCFAs promote the recovery of the intestinal epithelium and induce a reversible reduction in paracellular permeability in a concentration-dependent manner *in vitro* (Wilson and Gibson, 1997; Mariadason et al., 1999). Butyrate promotes the intestinal barrier by modulating the combination of TJ in Caco-2 cell monolayers (Peng et al., 2009). Mátis et al. (2022) demonstrated that butyrate has positive modulatory effects on tight junction proteins, namely, claudin-1 and claudin-3, by oral butyrate supplementation in the chicken intestine.

The *A. japonicus* alimentary canal includes the foregut, midgut, and hindgut (Zamora and Jeffs, 2011). The foregut is used to transport food, the midgut is used to digest food and absorb nutrition, and the hindgut is used for excretion. The microbial diversity of the midgut and hindgut is greater than that of the foregut in matters of species and quantity (Gao et al., 2014; Wang et al., 2018). Compared with the hindgut, the midgut can better absorb and utilize SCFAs due to its striated border and villous epithelium. Furthermore, Dong et al. (2009) stated that pathogens could directly interact with and adversely affect the host by destroying the physical barrier of the midgut. Therefore, we regarded the midgut as the object to explore the interaction

between the intestinal microbiota and *A. japonicus* mediated by SCFAs. The invasion of *V. splendidus* significantly altered the intestinal microbiota abundance and species, accompanied by a change in metabolomic profiles (Zhang et al., 2018). Therefore, we hypothesized that SCFA profiles are altered during the occurrence of SUS, that the intestinal microbiota can interact with *A. japonicus* via SCFAs, and that SCFAs can alleviate the effect of *V. splendidus* on the intestinal barrier.

To address the hypothesis above, in the present study, a comprehensive exploration of the variation in the content of SCFAs in *A. japonicus* was achieved by combining the intestinal microbiota aberrations under *V. splendidus* infection. The protective effect of SCFAs on the intestinal barrier was first validated by investigating the tight junction expression and rearrangement. These valuable findings could provide a new prevention and control strategy for SUS.

2. Materials and methods

2.1. Infection experiment

A. japonicus with an average weight of 200 ± 15 g was collected from a farm in Dalian (Liaoning Province, China). The collected *A. japonicus* was acclimatized for 1 week before the experiment in a clear pool, and it was fed with a mixture of feed. One-third of the water was exchanged every day. *Vibrio splendidus* was grown in 2216E media at 28°C to $OD_{600} = 1.0$ for the bacterial suspension. The supernatant of the bacterial suspension was removed by centrifuging at 6,000 × g for 10 min. The bacterial precipitation was suspended in seawater and used to infect the sample *A. japonicus* with appropriate concentrations (1×10^7 CFU ml^{-1}) (Zhang et al., 2018). Fifty sea cucumbers were infected with 1×10^7 CFU ml^{-1} of *V. splendidus* in a tank containing 1,000 L seawater. We collected eight samples at 0, 12, 48, and 96 h after *V. splendidus* infection and SUS. The midgut tissues were collected, and the contents were sampled for the following experiments.

2.2. Histopathological and immunofluorescence analyses

Abdominal incisions were performed on three *A. japonicus* in each group of 0, 12, 48, 96 h, and SUS to collect midgut tissues, and then, the midgut was cutoff using small surgical scissors. The midgut tissues were fixed with paraformaldehyde and embedded with paraffin. The slices (5 μm-thick) were stained with hematoxylin and eosin (H & E) and analyzed under a light microscope (Carl Zeiss, German). For the midgut tissue immunofluorescence analysis, the midgut tissues of three *A. japonicus* in each group of 0, 12, 48, 96 h, and SUS were embedded in an OCT compound (optimal cutting temperature compound), frozen with liquid nitrogen, and stored in −80C. Slices of 10 μm were cut, fixed with paraformaldehyde for 20 min, penetrated with PBS containing 0.5% Triton X-100 for 20 min, blocked for 30 min in an antibody diluent, and stained with the primary antibodies ZO-1 and occludin (1:200, Beyotime Biotechnology, China) for 30 min in the antibody diluent. After three washes, the sections were stained with secondary antibodies Alexa Fluor 555-labeled Goat-anti-Rabbit IgG (1:1,000, Biotechnology, China) and Alexa Fluor 488-labeled Goat-anti-Rabbit IgG (1:1,000, Biotechnology, China) for 30 min. After three washes, the nucleus was stained using DAPI (1:10,000, Beyotime Biotechnology, China) for 5 min. The slices were mounted, and the images were analyzed under a Zeiss LSM 880 confocal laser scanning microscope (Carl Zeiss, German).

2.3. Midgut microbiota analysis

Gut content samples were collected from eight *A. japonicus* infected with *V. splendidus* at 0, 12, 48, 96 h, and SUS, and genomic DNA extraction was performed. The primer pairs 338F (5′-GTACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3-V4 region of the 16S rRNA gene (Zhang et al., 2018). The 16S rRNA sequence was assembled and filtered to obtain valid data (clean data). QIIME2 was used to control the quality of the clean data. The sequences were clustered and classified into operational taxonomic units (OTUs) at a 97% threshold in DADA2 in the 0, 12, 48, 96 h, and SUS groups, wherein the feature sequence was annotated by employing the blast method according to the SILVA database (Quast et al., 2013; Abellan-Schneyder et al., 2021). Bacterial classification and analysis were performed by referring to our previous study (Zhang et al., 2018). Spearman's correlation analysis was conducted to analyze the correlations between the key taxa and the SCFAs using R (version 4.1.0) (Livak and Schmittgen, 2001; Fagan et al., 2007).

2.4. Midgut content collection and targeted SCFAs profiling

Eight *A. japonicus* were taken from each group of 0, 12, 48, and 96 h after *V. splendidus* infection and SUS. The abdominal anatomy of the *A. japonicus* was immediately examined after sterilizing the body surface with 75% alcohol. The entire intestine was taken from the body cavity of the sea cucumbers, and the esophagus, stomach, and hindgut were cut off in a sterile environment. Subsequently, the contents of the midgut were squeezed into a 2-mL sterile tube and immediately immersed in liquid nitrogen. The SCFA concentration in the midgut contents was measured by gas chromatography/mass spectrometry (GC/MS). For the SCFA assay, 50 mg gut content was homogenized for 1 min with 50 μl 15% phosphoric acid, 100 μl 125 μg/ml isohexanoic acid solution, and 400 μl ether; then, it was centrifuged at 12,000 rpm at 4°C for 10 min. The supernatants were used for GC/MC (TRACE 1310-ISQ LT, Thermo, USA) analysis. Ether was used to prepare pure standards of acetic acid (CAS 64-19-7), propionic acid (CAS 79-09-4), butyric acid (CAS 107-92-6), isobutyric acid (CAS 79-31-2), valeric acid (CAS 109-52-4), isovaleric acid (CAS 503-74-2), and caproic acid (CAS 142-62-1) in ten mixed standard concentration gradients: 0.1, 100, 10, 0.02, 250, 25, 2, 0.5, 500, and 50 μg/ml. A chromatographic column Agilent HP-INNOWAX capillary column (30 m × 0.25 mm ID × 0.25 μm) was used to separate the metabolites. Helium was passed through the column as a carrier gas at a constant flow rate of 1 ml/min.

The initial temperature was 90°C, which was ramped to 120°C at a rate of 10°C/min, 150°C at a rate of 5°C/min, 250°C at a rate of 25°C/min, and finally kept at 250°C for 2 min. The linearity was investigated by taking the concentration of the standard as the abscissa and the peak area ratio of the standard to the internal standard as the ordinate. The obtained linear regression equation of each substance is shown in Supplementary Table 1. The correlation coefficient was $r > 0.99$.

$$\text{SCFAsconcentration}(\mu g/ml) = (\text{peakarea ratio} - b)/a$$

SCFAs content (μg/g)= concentration (μg/ml)×volume (ml)/sampling amount (mg) × 1,000

*(The values of a and b are shown in Supplementary Table 1).

The SCFA data were analyzed using SIMCA-P software.

2.5. RNA analysis

The midgut tissues of four individuals were sampled as described above, and the RNA was extracted using TRIzol reagent (Takara, Otsu, Japan). The RNA was reversely transcribed into cDNA using the Reverse Transcription Kit (Takara, Otsu, Japan) by following the manufacturer's protocol. qRT-PCR was used to analyze the occludin and ZO-1 expression, and it was executed on the Applied Biosystems 7500 real-time PCR system (Supplementary Table 2). The total volume of the reaction was 20 μl, containing 10 μl of TB GREEN^{TM} Premix Ex Taq II, 8 μl of cDNA, 0.8 μl each of forward and reverse primers, and 0.4 μl of ROX (Takara, Otsu, Japan). The reaction process consisted of the following steps: 5 min of initial denaturation at 95C, 15 s of denaturation at 95C, and 30 s of annealing and extension at 60C, comprising 45 cycles in total. The result was represented by $2^{-\Delta\Delta Ct}$, and the data were normalized by using β-actin as a reference gene (Evans and Surprenant, 1992).

2.6. Western blotting and ELISA

Hundred milligrams of midgut tissues from three individuals were washed with cold PBS, and protein was extracted from them using RIPA lysis (Sangon, China); the protein concentration was quantified using the BCA assay kit. Equal amounts of protein (50 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose filter (NC) membranes. After blocking the NC membranes with 5% non-fat milk in TBS at room temperature for 1 h, the membranes were incubated with the following primary antibodies at 4°C overnight: ZO-1 (1:2,000, Beyotime Biotechnology, China), occludin (1:2,000, Beyotime Biotechnology, China), and β-actin (1:5,000, Proteintech Group, USA). The membranes were incubated with HRP-conjugated Goat-anti-Rabbit IgG at room temperature for 1 h after washing three times with TBST (Beyotime Biotechnology, China). The immunoreactive proteins were analyzed using the Omega Lum C imaging system (Aplegen, California, USA). The band intensity was quantified using the Image J software.

The midgut tissues were collected, and 1 mL PBS homogenate was added. The supernate was kept after centrifugation for 10 min at 3,000 rpm. The ZO-1 and occludin were measured in the supernate following standard protocols, with three replicates in each group. The rat tight junction protein 1 (ZO-1) ELISA Kit (KT, China) and the Rat occludin ELISA Kit (KT, China) were used for the detection of ZO-1 and occludin.

2.7. Acetate treatment

A. japonicus with an average weight of 200 ± 15 g was acquired from a farm in Dalian (Liaoning Province, China). The collected *A. japonicus* was acclimatized for 1 week before the experiment in a clear pool, and it was fed with a mixture of feed. One-third of the water was exchanged every day. The sample *A. japonicus* was randomly assigned to four groups (four sea cucumbers per group): the control group (Control), the acetate feeding group (Acetate), the *V. splendidus* infection group (*V. splendidus*), and the acetate feeding + *V. splendidus* infection group (Acetate + *V. splendidus*). *A. japonicus* in the acetate and acetate + *V. splendidus* groups was orally administered with 500 mg/kg acetate in their normal feed, while *A. japonicus* in the control and *V. splendidus* groups was orally administered normal feed made with sea mud powder, *Sargassum thunbergii* powder, and *Spirulina* powder. After 8 weeks, midgut tissues were collected from each *A. japonicus* for histopathological analysis, immunofluorescence staining, qRT-PCR, and Western blotting for examining the occludin and ZO-1 expression, as described in Section 2.2.

2.8. Cell culture

To culture the primary midgut epithelial cells of *A. japonicus*, the body surface of three *A. japonicus* individuals was sterilized with 75% ethanol. Then, the midgut tissues of the three individuals were collected; the mesentery was removed, and the lumen of the midgut segments was flushed thrice with D-Hanks, which contained 200 U/ml penicillin and 200 μg/ml streptomycin sulfate. The midgut segments were turned over so that the intestinal mucosa would face outward. After washing the intestinal mucosa with the D-Hanks cleaning solution, it was placed in a culture dish, and scraped with a sterile scalpel; the cleaning solution was used to repeatedly clean the scraped epithelial cells (Peerapen and Thongboonkerd, 2021). The cells were seeded on 12-well microplates and grown in Leibovitz's L-15 cell medium (Invitrogen, USA) containing 100 U/ml penicillin and 100 μg/ml streptomycin sulfate at 16°C. Three wells were first treated with one treatment and later treated with acetate (0.07 g/ml) and LPS (10 μg/ml) for 24 h.

For the immunofluorescence analysis of intestinal epithelial cells, the cells were fixed with paraformaldehyde and incubated with ZO-1 antibody (1:200, Beyotime Biotechnology, China) and Coralite®488-conjugated Goat Anti-Rabbit IgG (1:200, Proteintech Group, USA). The nucleus was stained using DAPI (1:10,000, Beyotime Biotechnology, China) for 5 min. Analysis was performed under a Zeiss confocal microscope (Carl Zeiss,

LSM880, Germany) and using the Image J software by calculating cell fluoroscence.

2.9. Statistical analysis

SPSS V23.0 statistical software (SPSS Co., Ltd., Chicago, Illinois, USA) was used for statistical analysis. All data were expressed as mean ± standard deviation. If there were more than two groups, a two-sided unpaired *t*-test was performed for analysis. The differences among the three groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's range tests. Pearson correlation analysis was also performed. The difference was considered to be within a *p*-value of <0.05.

3. Results

3.1. *Vibrio splendidus* infection modulates permeability of midgut in *A. japonicus*

To confirm whether *V. splendidus* can influence the intestine barrier, we analyzed the variation in the midgut morphology and the disruption of the intestinal tight junction before and after *V. splendidus* infection. The midgut morphology results showed that the intestinal striated border gradually fell off, and the morphological structure of the epithelial tissue was disordered. The absorbing cells became necrotic, they shed, and the cell arrangement gradually loosened as the infection progressed over time (Figure 1A). There was obvious shedding of the intestine villi and striated borders in SUS *A. japonicus*. To determine if *V. splendidus* mediated intestinal barriers by targeting ZO-1 and occludin, their protein expressions were assayed. The result indicated that the fluorescence signal of ZO-1 and occludin gradually disappeared during *V. splendidus* infection. Consistently, the occludin and ZO-1 mRNA levels were significantly decreased; they reached the lowest level in SUS *A. japonicus* and only 0.02- and 0.07-fold, respectively, in the groups treated for 0 h (Figures 1C, D). Furthermore, the Western blotting results confirmed that occludin and ZO-1 downregulation was induced by *V. splendidus* (Figures 1E–G), suggesting that *V. splendidus* caused damage to the intestinal barrier by downregulating tight junction proteins.

3.2. *Vibrio splendidus* infection altered midgut microbiota profiles

Our previous study revealed differences in the microbiota community in the mid-intestine between healthy and diseased *A. japonicus* (Zhang et al., 2018). To characterize the alteration of the midgut microbiota during the *V. splendidus* infection, the 16S rRNA gene sequence data of *A. japonicas*, which were treated with *V. splendidus* for 0, 12, 48, and 96 h, were further analyzed. Seven predominant phyla (*Proteobacteria*, *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, *Cyanobacteria*, *Desulfobacterota*, and *Campilobacterota*) were detected, and all of them displayed significant alterations in abundance after *V. splendidus* treatment (Figure 2A; Supplementary Table 3). Taxonomic analysis of the phylum distribution showed significant reductions in Actinobacteriota, Cyanobacteria, and Desulfobacterota after *V. splendidus* treatment (Figure 2B). At the family level, Rhodobacteraceae increased in abundance, while Nocardiaceae, Halieaceae, Ilumatobacteraceae, Halomonadaceae, and Alteromonadaceae were obviously decreased in abundance in the infection group compared to the control group (Figures 2C, D; Supplementary Table 4). In addition, *Ilumatobacter*, *Rhodococcus*, *Halioglobus*, and *Pediococcus* increased significantly in the infected *A. japonicus* (Figures 2E, F). These variations in microbial abundance were synergistic with the tight junction protein expression. Therefore, the alteration of mid-intestine permeability was closely related to the midgut microbiome.

3.3. SCFA contents in the midgut were altered in response to the *V. splendidus* infection

The result suggested that there were varying degrees of changes in the SCFA contents in the midgut. Acetic acid gradually decreased and reached the lowest level in the SUS sea cucumber, at 14.84 μg/g (Figure 3A). The propionic acid content in the 12 and 48 h groups was significantly lower than that in the 0 h group ($p < 0.05$), and it decreased to the lowest level in the 96 h and SUS groups, at 2.10 and 2.09 μg/g, respectively (Figure 3B). The butyric acid content of the infection groups was remarkably lower than that of the 0 h group ($p < 0.05$), but there was no remarkable difference among the four infection groups (Figure 3D). The isobutyric acid content in the 12, 48, and 96 h groups was significantly higher than that in the 0 h group ($p < 0.05$), and it increased to the highest level in the SUS group, at 0.71 μg/g (Figure 3C). The isovaleric acid content decreased afterward to the lowest level at 12 and 48 h, both at 0.31 μg/g, and it increased to a slightly higher level at 96 h and in the SUS group but remained remarkably lower than in healthy *A. japonicus* (Figure 3E). There were no significant differences in valeric acid and caproic acid levels associated with pathogen infection, but they showed a substantial increase in SUS-diseased *A. japonicus* (Figure 3F). Therefore, acetic acid had the highest content and the most regular variation among the seven SCFAs.

PCA analysis revealed that the 0 h samples were distinct from those of the SUS group, using the quantification of seven SCFAs as variables and two principal component scores of PC1 and PC2 (31.4 and 24.6% of the explained variance, respectively; Figure 3H). The PCA plot showed a clear separation in the midgut SCFAs between the healthy and SUS sea cucumbers; it also showed that acetic acid was significantly correlated with healthy populations, and valeric and caproic acids were correlated with SUS populations. Then, we employed the OPLS-DA model on SCFAs and observed that the VIP values of acetic acid, valeric acid, caproic acid, and isobutyric acid were >1; among them, the acetic acid VIP value of 1.14 was the highest, as shown in Supplementary Table 5.

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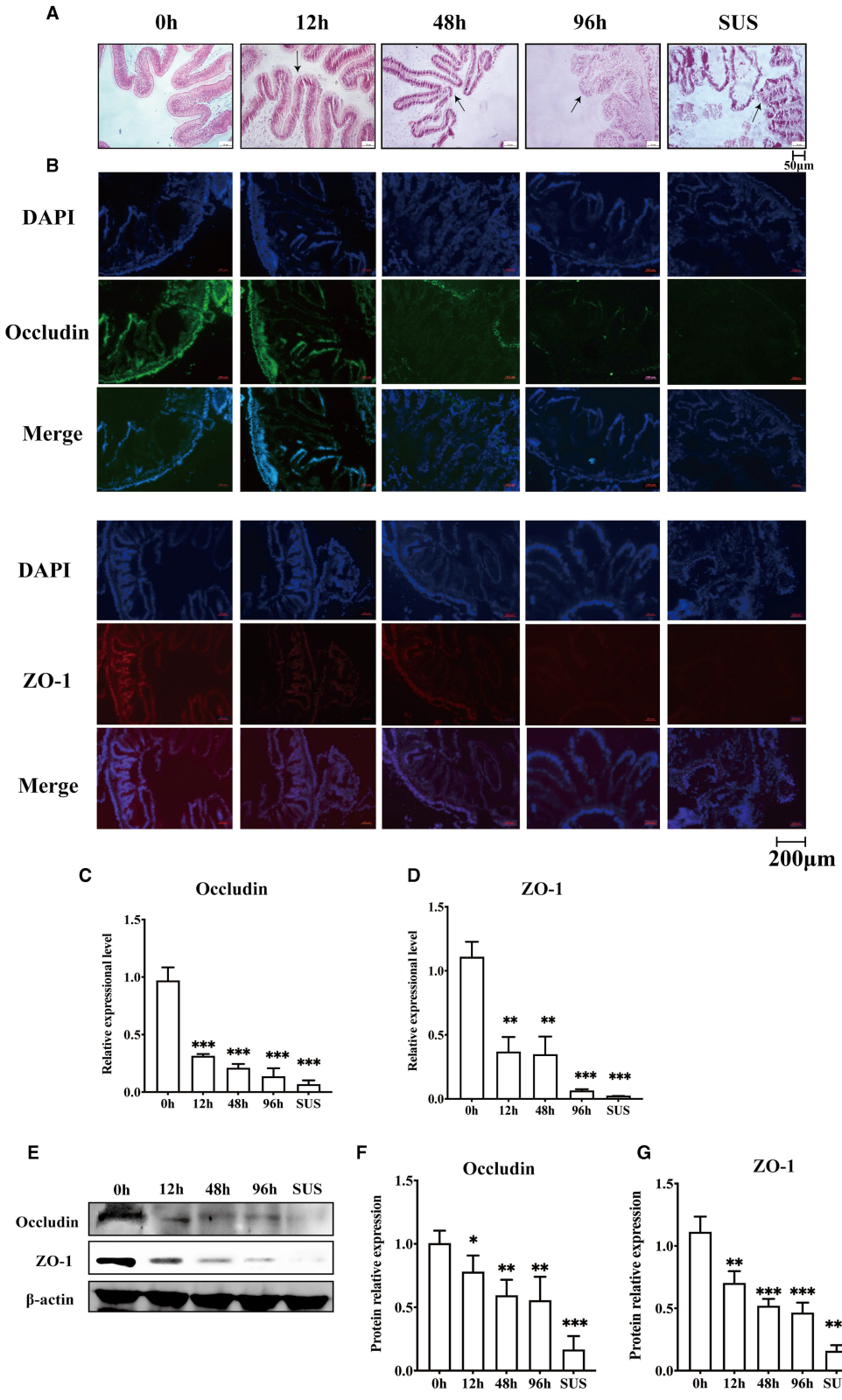

FIGURE 1
Changes in the intestinal mechanical barrier of *A. japonicus* with *V. splendidus* infection. **(A)** H&E staining of the section of the *A. japonicus* intestine. **(B)** Immunofluorescence of ZO-1 and occludin in the *A. japonicus* intestine. **(C, D)** RT-PCR analysis of occludin and ZO-1 transcript levels from the intestine tissue. **(E–G)** The western bloting was used to determine the expression level occludin and ZO-1 with β-actin as the reference in the intestine tissue. Asterisks indicate significant differences: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

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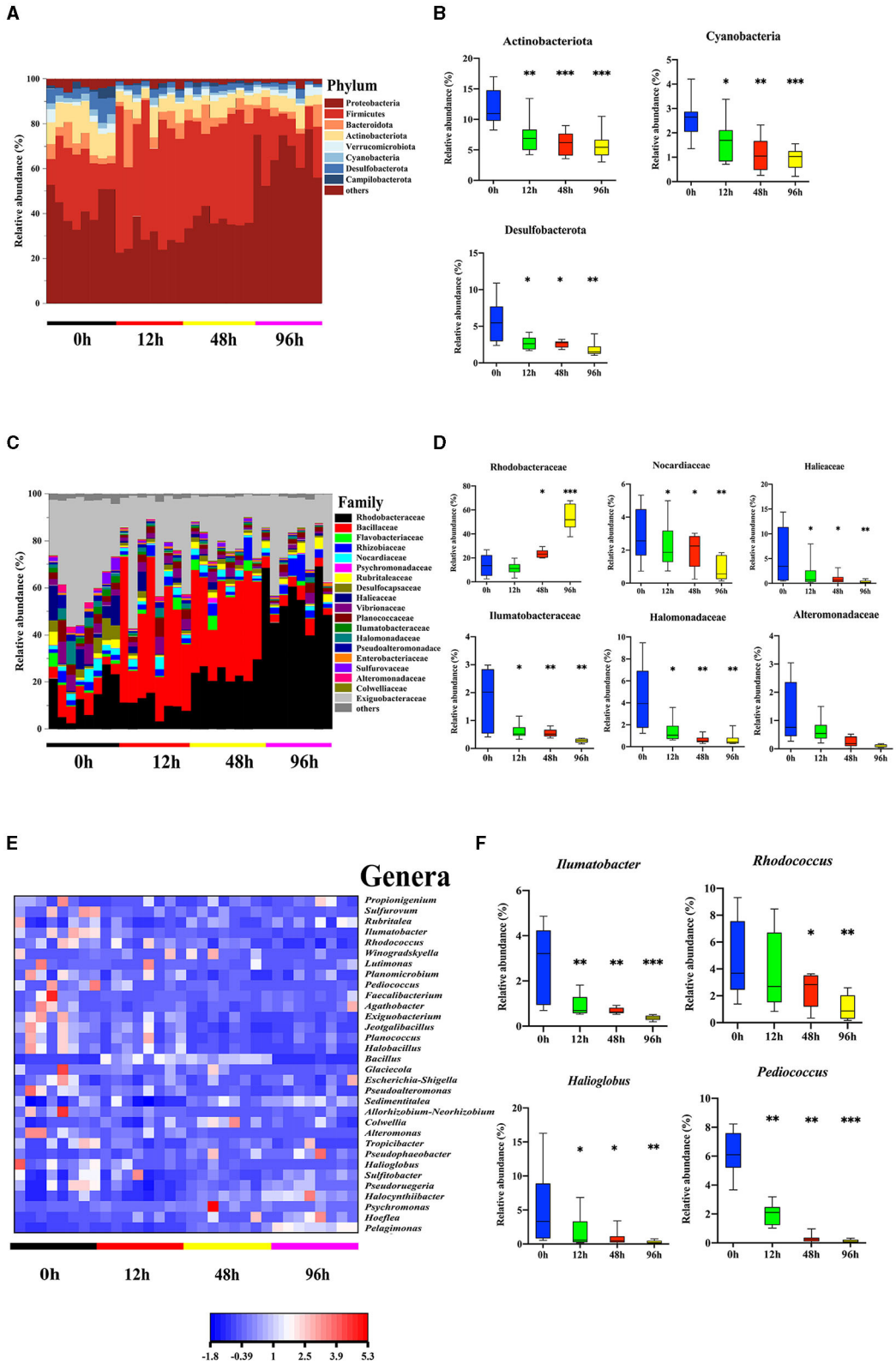

FIGURE 2
Vibrio splendidus infection modulated the midgut microbiota in the sea cucumber. **(A, B)** Microbial abundance and significant differences in the bacterial taxonomy at the phylum level. **(C, D)** Microbial abundance and significant differences in the bacterial taxonomy at the family level. **(E, F)** Relative abundance of the genera heatmap and significant differences in the bacterial taxonomy at the genus level. Asterisks indicate significant differences: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

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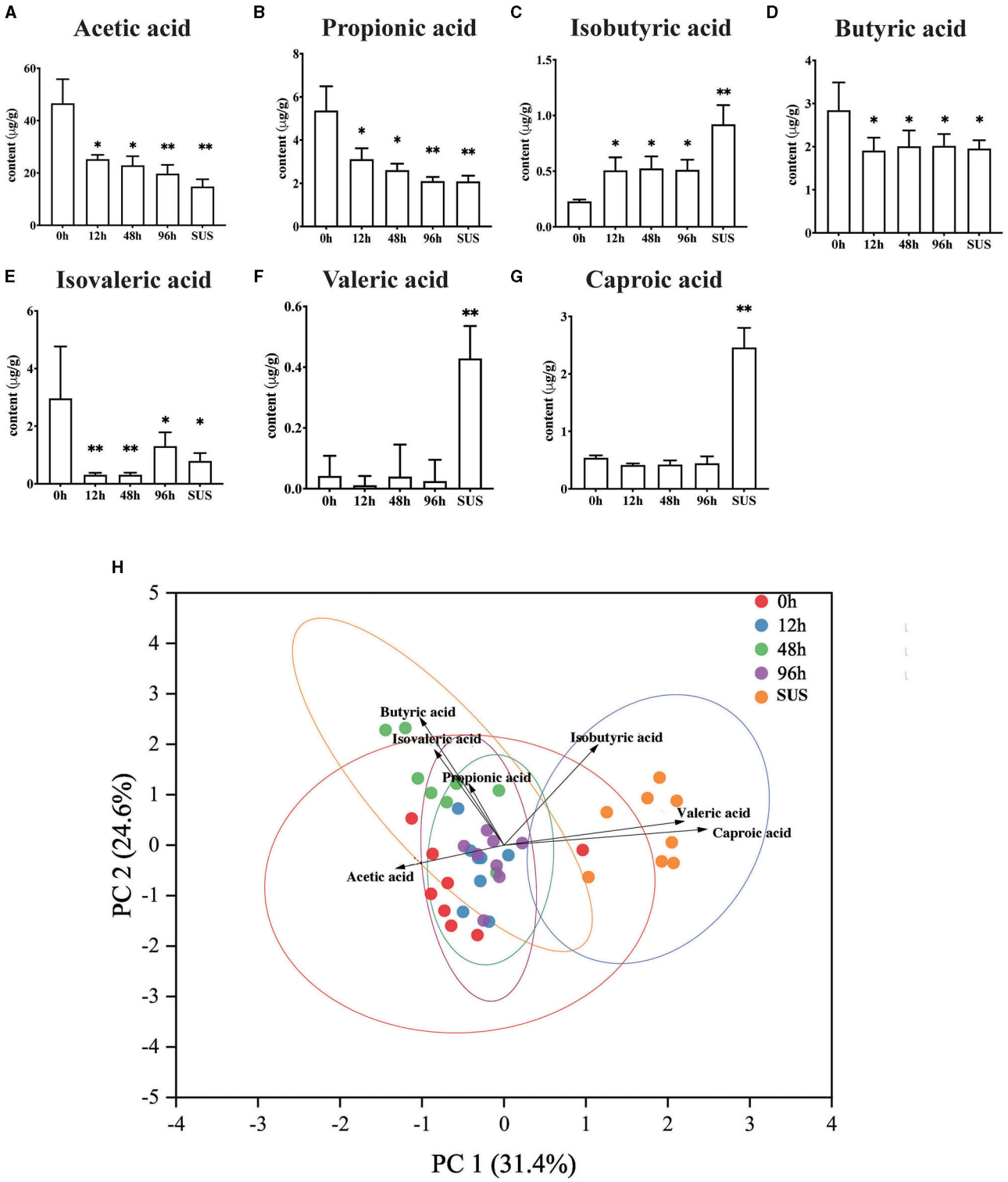

FIGURE 3
Effect of *V. splendidus* infection on SCFAs in the *A. japonicus* mid-intestine. **(A)** Acetic acid, **(B)** Propionic acid, **(C)** Isobutyric acid, **(D)** Butyric acid, **(E)** Isovaleric acid, **(F)** Valeric acid, and **(G)** Caproic acid. **(H)** Principal component analysis of targeted SCFAs. Asterisks indicate significant differences: $*p < 0.05$ and $**p < 0.01$.

3.4. Corelationship analysis between the midgut microbiota and SCFAs

To explore which resident taxa interact with the SCFAs, we performed the conjoint analysis in R software to investigate the correlation between the midgut microbiota and seven SCFAs. We observed 34 major genera of bacteria associated with the seven SCFAs, of which 17 genera were significantly positively correlated with acetic acid, including *Winogradskyella*, *Alteromonas*, *Halioglobus*, *Bacillus*, *Pediococcus*, *Ilumatobacter*, and *Desulfobacterota* ($p < 0.05$), and only *Hoeflea* and *Pelagimons* were significantly negatively correlated ($p < 0.01$) (Figure 4). *Rhodococcus* had a significant correlation with propionic acid, and butyric acid had an obvious negative correlation with only *Allorhizobium* ($p < 0.05$). Similar to the bacteria that showed a significant negative correlation with isobutyric acid, isovaleric acid ($p < 0.05$), and valeric acid, most other bacteria were positively correlated with acetic acid, including *Winogradskyell*, *Desulfobacterota*, *Rhodococcus*, and *Sulfurovum*. Both valeric acid and caproic acid were positively correlated with *Agathobacter* and *Lutimonas*. Additionally, valeric acid had a significant positive correlation with *Pelagimonas*, and caproic acid had a significant positive correlation with *Faecalibacterium* ($p < 0.05$) (Supplementary Table 6).

3.5. Crosstalk analysis between SCFAs and the tight junction

To further investigate the relationship between SCFAs and intestinal permeability, we analyzed the crosstalk data and compared them between the midgut SCFAs and tight junction proteins based on Spearman's correlation coefficients. As shown in Figure 4 and Supplementary Table 7, there was a strong correlation between the protein levels of SCFAs and the tight junction. Acetic acid was positively correlated to the level of ZO-1 ($r = 0.93548$, $p < 0.05$) and occludin ($r = 0.89606$, $p < 0.01$). Propionic acid ($r = 0.84230$, $p < 0.01$) and isovaleric acid ($r = -0.54783$, $p < 0.01$) were only positively correlated to the level of ZO-1, but there was no significant correlation with occludin. Isovaleric acid was only remarkably negatively correlated with ZO-1 ($r = -0.41219$, $p < 0.05$). Isobutyric acid, butyric acid, and caproic acid had no notable correlation with the tight junction (Figure 4).

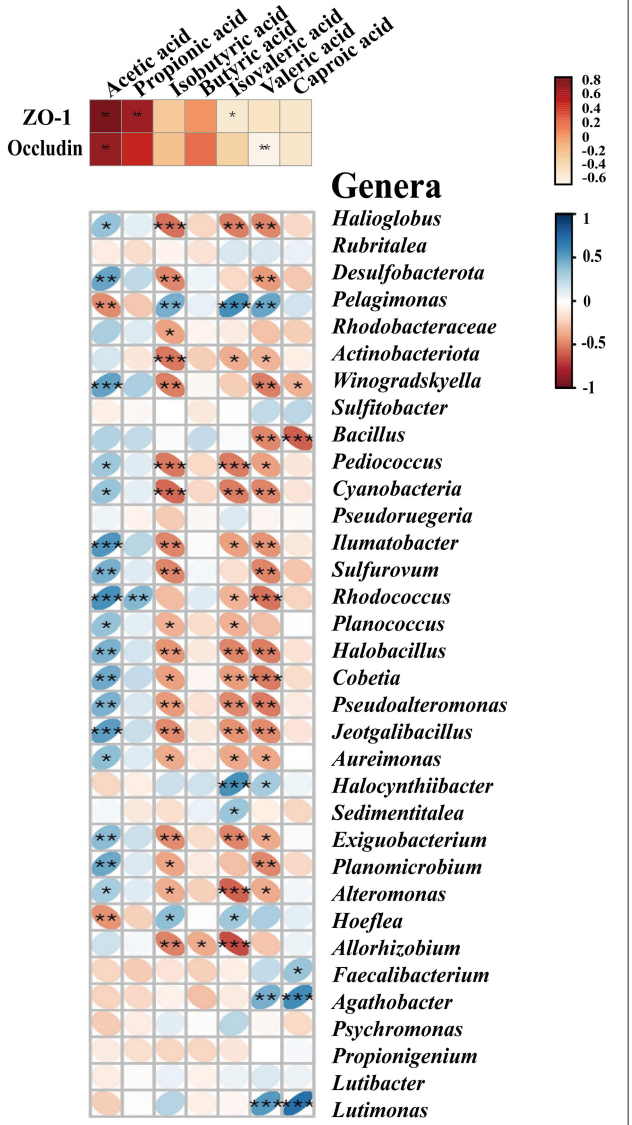

FIGURE 4
SCFAs exhibit correlations with tight junction proteins and the major midgut microbiota by Spearman's correlation analysis. The color and thickness of the ellipse represent the correlation coefficient value. Asterisks indicate obvious correlations. Asterisks indicate significant differences: *$p < 0.05$, **$p < 0.01$, and **$p < 0.001$.

3.6. Acetate-regulated midgut barrier function

We further studied the regulatory effect of acetate on the intestinal barrier integrity in the midgut by adding acetate to the *A. japonicus* normal feed. The results showed that acetate alleviated the damage caused by *V. splendidus* to the midgut barrier function (Figure 5A). The immunofluorescence result showed that the occludin and ZO-1 fluorescence signals aggravatingly increased compared with the control group (Figure 5A) and almost disappeared in *V. splendidus*. We further measured the mRNA level of occludin and ZO-1 in the midgut tissue by qRT-PCR after acetate treatment; the mRNA significantly increased to 9.22- and 3.57-fold, respectively, in the acetate group compared with the control group ($p < 0.01$; $p < 0.01$). *Vibrio splendidus* significantly reduced the mRNA level, and acetate restored the tight junction mRNA level to the control level (Figures 5B, C). The Western blotting results suggested that acetate restored the occludin and ZO-1 expression levels of the control group in the presence of *V. splendidus* (Figures 5D–F).

3.7. Acetate-altered F-actin and ZO-1 localization and organization

It was well-documented that actin participated in modulating the tight junction structural integrity via actin reorganization

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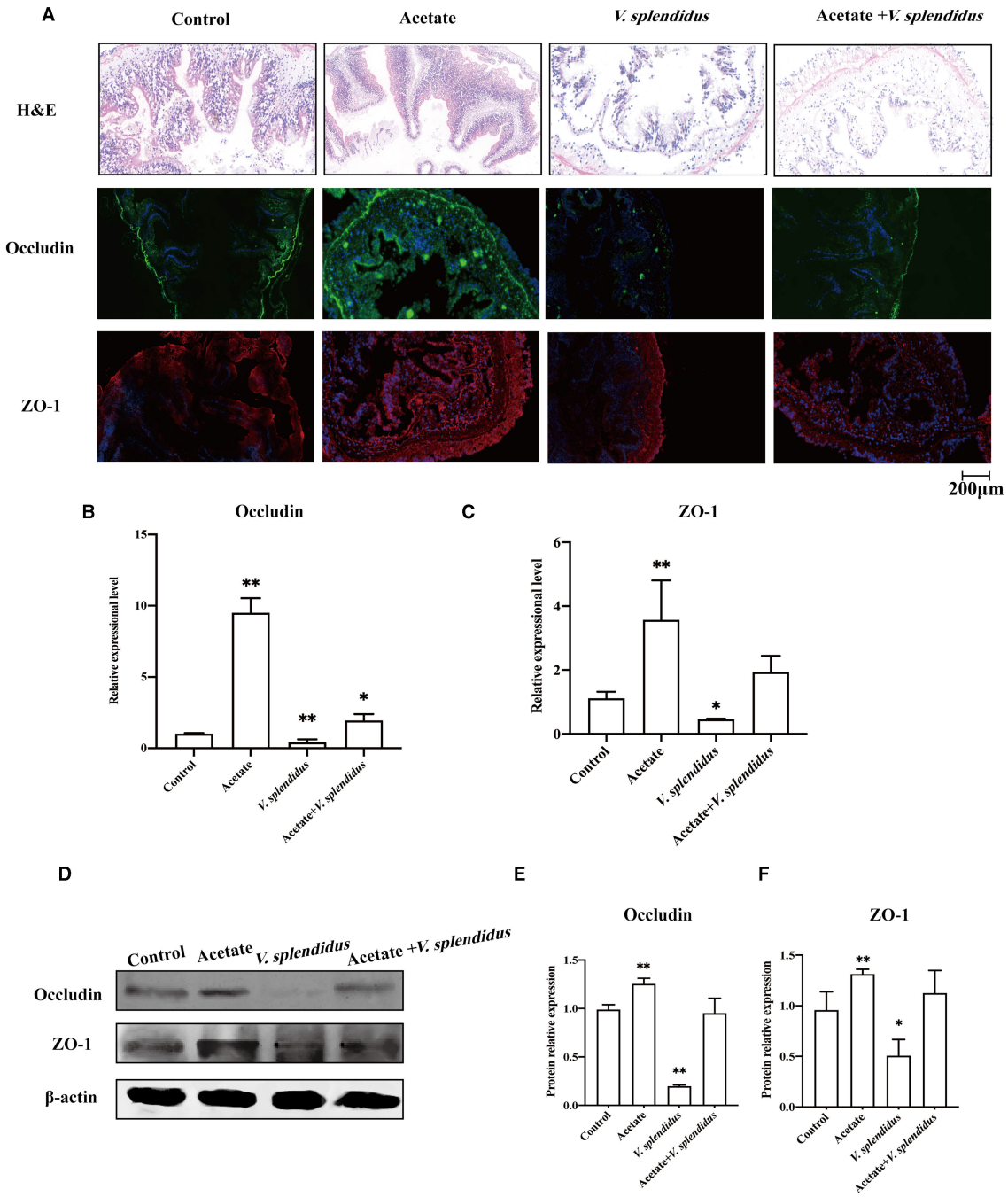

FIGURE 5
Acetate administration reinforces the integrity of the epithelial barrier during *V. splendidus* infection. **(A)** H&E staining of the section of the *A. japonicus* midgut. **(B)** Immunofluorescence of ZO-1 and occludin in the *A. japonicus* mid-intestine. **(C, D)** RT-PCR analysis of the occludin and ZO-1 transcript levels from the mid-intestine tissue after acetate treatment. **(E, F)** The Western blotting was used to determine the expression level of occludin and ZO-1 with β-actin as the reference in the mid-intestine tissue. Asterisks indicate significant differences: $*p < 0.05$ and $**p < 0.01$.

(Zeng and Chi, 2015). To preliminarily understand the detailed mechanism of acetate-mediated midgut permeability, we evaluated the effects of acetate on ZO-1 distribution and F-actin expression in the midgut epithelial cell by separating the epithelial cells from the midgut tissue and culturing *in vitro*. We only evaluated the tight junction ZO-1 expression in our *in vitro* experimental study. The *A. japonicus* intestinal epithelial cells did not form cell lines, and occludin was located at the cell junction. Immunofluorescence analysis revealed that ZO-1 and F-actin in the intestinal epithelial cells showed that acetate reinforces the intestine barrier integrity (Figure 6A). Fluorescence images of the control group showed that ZO-1 was located on the cell border and colocalized with F-actin in the mid-intestinal epithelial cells. In contrast, the cells treated with LPS suggested that ZO-1 and F-actin were weakly expressed

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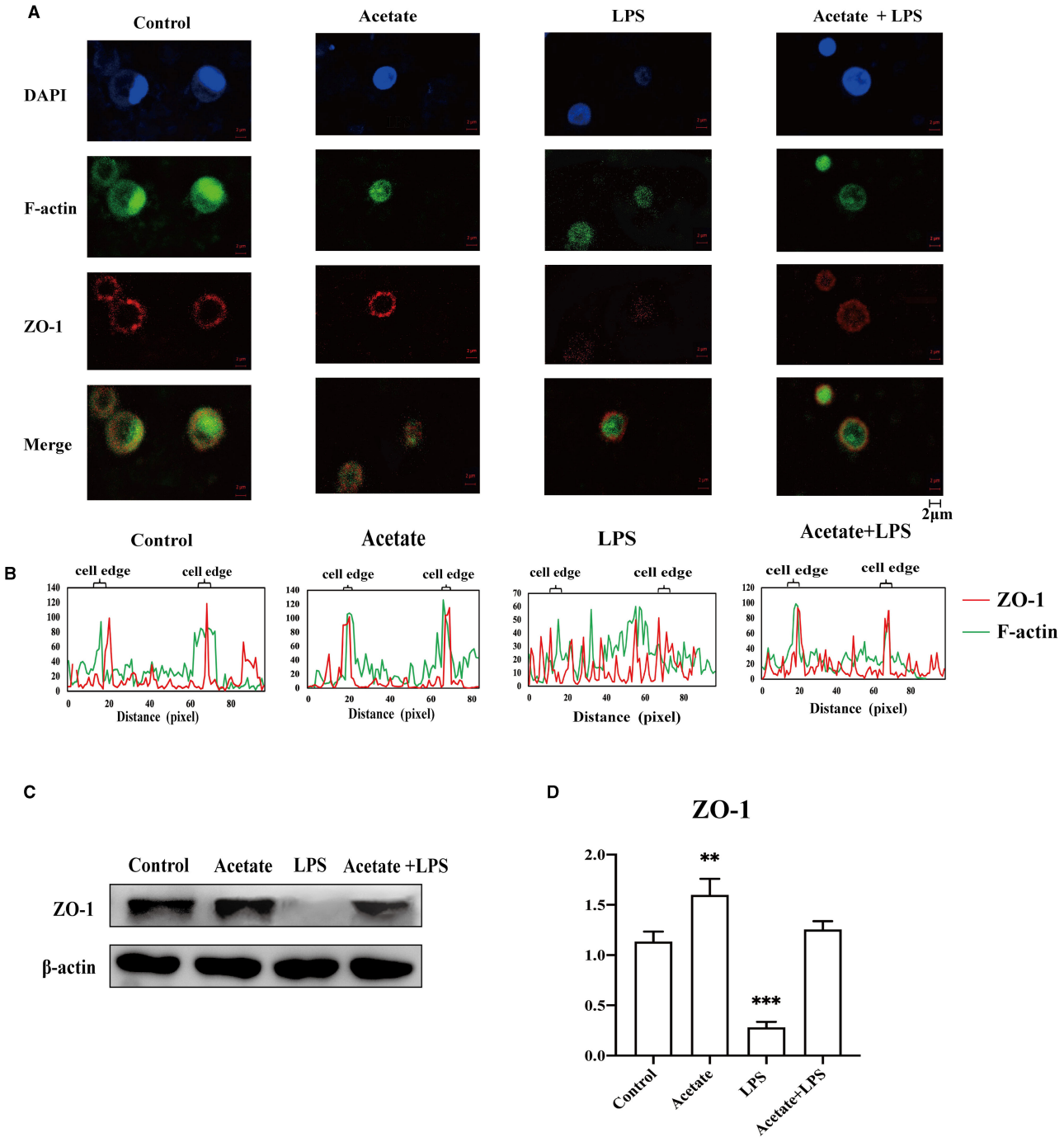

FIGURE 6
Acetate prevented LPS-induced F-actin and ZO-1 dissociation and reorganization in mid-intestinal epithelial cells. **(A)** Immunofluorescence of ZO-1 and F-actin in the intestinal epithelial cells. **(B)** Fluorescence colocalization analysis for F-actin and ZO-1 of the intestinal epithelial cells analysis. **(C, D)** The Western blotting was used to determine the ZO-1 in the intestinal epithelial cells. Asterisks indicate significant differences: $^{**}p < 0.01$ and $^{***}p < 0.001$.

in the cell edge and diffused to the cytoplasm (Figures 6A, B). The mid-intestinal epithelial cells incubated with acetic acid showed F-actin and ZO-1 colocalization, and the fluorescence signal at the cell edge was strong (Figure 6B). Compared with the acetate treatment group, the mid-intestinal epithelial cell fluorescence signal of the acetate and LPS treatment group was weak. The Western blotting results suggested that the effect of acetic acid increased the ZO-1 expression in the mid-intestinal epithelial cells (Figures 6C, D). Compared with the LPS-treated mid-intestinal epithelial cells, the combination of acetic acid and LPS could increase the expression of the ZO-1 protein, but it was still lower than that of the control group. Therefore, acetic acid affects the mid-intestinal permeability of *A. japonicus* by affecting F-actin rearrangement and decreasing TJ protein expression.

4. Discussion

The most vital function of the intestinal epithelium is being a barrier to prevent intestinal bacteria from entering the blood circulation from the intestinal cavity. The tight junction is regarded as the side gate between the two adjacent cells because it limits the access of molecules according to charges and sizes; it can also regulate paracellular permeability (Bhat et al., 2019). Moreover, the tight junction also plays a palisade function and maintains the polarization of epithelial cells by protecting them from the disorder displacement of apical and basolateral membrane proteins in mammals (Bhat et al., 2019; Heinemann and Schuetz, 2019; Otani and Furuse, 2020). More and more pieces of evidence suggest that configuration and expression changes of TJ proteins are caused by all kinds of stimuli, including toxins (Patterson et al., 2020), ROS (Kim et al., 2019), and nitric oxide (Logsdon et al., 2018). Alterations of the TJ protein level and location are related to the increase in the permeability of the epithelium monolayer bypass (Schilpp et al., 2021). ZO-1 is a cytosolic scaffolding protein, and it is the key molecule in TJ complexes (Lynn et al., 2020). It interacts with other member tight junction compounds (Schwayer et al., 2019) and forms a tight junction complex with the cytoskeleton. In our present study, the mid-intestine barrier integrity could be observed by H&E; the result indicated that *V. splendidus* seriously damaged the mid-intestinal epithelial tissue. The occludin and ZO-1 expression levels were decreased by *V. splendidus* in the midgut tissue, as examined by Western blotting and immunofluorescence (Figure 1). It was demonstrated that the decrease in the occludin and ZO-1 expression levels were involved in the defective barrier morphology and function induced by various pathological factors (Zeng and Chi, 2015). Among pathological factors, cytoskeletal remodeling was caused by the contraction of actin, which destroyed the TJ structure to increase intestinal permeability. The junctional ring of F-actin and myosin II supporting the tight junction is essential for the regulation of physiological and pathophysiological barriers (Madara and Pappenheimer, 1987). For instance, the activation of peripheral myosin light chain kinase (MLCK) is sufficient for enhancing cell bypass permeability (Shen et al., 2006). The activation of MLCK can cause phosphorylation of the myosin light chain (MLC); it can mediate actin contraction and destroy the tight junction, thereby increasing the permeability of epithelial cells. The mechanism by which *V. splendidus* destroys the intestinal barrier and reduces the occludin and ZO-1 expression remains to be further studied. *V. splendidus* might regulate MLCK by activating NF-κB to redistribute tight junction proteins and the surrounding cytoskeletal proteins, resulting in the displacement of the tight junction structure as well as an increase in intestinal permeability. At present, whether tight junction protein downregulation is caused by pathogenic microorganisms or a pathological state is not clear. Some studies indicate that the MAPK signaling pathway can regulate the transport of intestinal epithelial cells by modulating the expression of tight junction proteins and their phosphorylation degree (Basuroy et al., 2006).

In addition to the destruction of the intestinal barrier, we also found that the infection of *V. splendidus* altered the intestinal microbial community structure and microbiota-derived SCFA profiling (Figures 2, 3), The result indicated that microbiota-derived SCFAs may exert a function in SUS *A. japonicus*. *Vibrio splendens* invasion could influence the intestinal microbiota homeostasis by decreasing the alpha diversity of the intestinal microbiota in *A. japonicus* and decreasing the abundance of gram-positive Firmicutes and gram-negative Desulfobacterota as the most abundant phyla in the *A. japonicus* gut (Zhang et al., 2018). The imbalance of the intestinal microbiota may lead to the proliferation of previously conditioned pathogens in the gut (Mallon et al., 2015). The decrease in the bacterial community diversity may lead to a decrease in the functional stability of bacterial communities, resulting in an increased disease risk (Xiong et al., 2019). Ecological evidence suggests that a decrease in gut bacterial diversity can provide a vacancy for microbial invaders (Ruben et al., 2005). Recent studies have revealed that many illnesses are always accompanied by the alteration of the intestinal microbiota aberrations and SCFA profiling (Madara and Pappenheimer, 1987). In this study, the characterization of the intestinal bacteria of *A. japonicus* revealed a dominance of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteriota, which are typical dominant members of the vertebrate gut, particularly in mammals (Turnbaugh et al., 2007; Mahowald et al., 2009). The finding regarding these dominant phyla was similar to that of other studies involved in the gut microbiota of *A. japonicus* (Yang et al., 2015; Kwong et al., 2018; Pagán-Jiménez et al., 2019). At the genus level, *Rhodococcus* was obviously decreased during the *V. splendidus* infection, which was representative of the Actinobacterio phylum and can degrade harmful organic substances. Meanwhile, the decrease also occurred in Rhodobacteraceae, which is an aquatic photosynthetic probiotic for the intestinal epithelial cells of tilapias (Zhou et al., 2020). The decrease in Rhodobacteraceae and *Rhodococcus* during the *V. splendidus* infection suggested that *V. splendidus* destroyed the midgut microbiota homeostasis by reducing the abundance of these probiotics. Additionally, the correlation analysis revealed that the bacteria with a significant negative correlation with valeric acid were positively correlated with acetic acid. Many reports have demonstrated that Proteobacteria and Firmicutes are the dominant bacteria producing acetic acid and butyric acid (Kwong et al., 2018), but the variation trend in Proteobacteria and Firmicutes abundances was completely different from that of the valeric acid content. Hence, we speculated that valeric acid and acetic acid might exert a different function in the intestine via the midgut microbiota alteration.

Emerging pieces of evidence suggest that acetic acid and butyric acid derived from the microbiota can influence the host's immune response (Denning et al., 2000; Moore et al., 2001). Specifically, it has been demonstrated that butyric acid maintains mucosal integrity and has an anti-inflammatory effect on the intestine due to its significant antineoplastic properties (Bagheri et al., 2018). Many reports have demonstrated that butyric acid plays a beneficial role in the epithelial barrier formation in humans with IBD or IBS (Sokol et al., 2009; Eeckhaut et al., 2013; Machiels et al., 2014). Zeng and Chi (2015) demonstrated the ability of microbial-derived butyrate to promote the epithelial barrier function through claudin-2 repression in human epithelial cells. However, our results found that propionic acid and butyric acid do not play any role in

maintaining the intestinal barrier homeostasis, and they were not identified as marker metabolites in the SCFA profile analysis results (Figure 4). In particular, butyric acid had only one phyla bacterium with a significant correlation. We found that acetic acid had significant differences with the *V. splendidus* infection. There were differences in the living environment and food composition between higher animals and our research subjects. The differences led to changes in the midgut microbiota community and functions and then altered the metabolites profile. Hence, we considered that the synergy between acetic acid and the intestinal permeability variation in the *A. japonicus* midgut is not accidental, but the inevitable result was induced by *V. splendidus* infection in the sea cucumbers. Hu et al. (2023) reported that *Lactobacillus reuteri* abundance significantly decreased in the HCC mice intestine, accompanied by the reduction of SCFA levels, especially acetate.

The mid-intestine tissue morphology alteration and microbiota-derived SCFA profiling indicate that we should seek to understand the correlation between SCFAs and the intestinal epithelial barrier. The result of the association analysis between SCFAs and the tight junction confirmed that there was a strong correlation between SCFAs and the intestinal barrier (Figure 4), but only acetic acid was significantly correlated with both occludin and ZO-1. Meanwhile, microbial aberration and SCFA contents also indicate that acetic acid plays an essential role in SUS occurrence. To test the function of acetic acid on the intestinal barrier, we added acetate to the normal forage to feed the sea cucumbers. Our study found that acetate could maintain the normal mid-intestine barrier integrity by increasing the occludin and ZO-1 expression under *V. splendidus* invasion (Figure 5). The result was consistent with those of other studies, which demonstrated that SCFAs secure the intestinal barrier function by regulating the tight junction expression (Yang et al., 2010; Arpaia et al., 2013). These data indicate that *V. splendidus* reduced the expression levels of occludin and ZO-1, whereas acetate successfully prevented the *V. splendidus*-induced effects. However, the microbiota-derived SCFAs' mechanism that led to the improvement of the intestinal barrier and protected the intestinal barrier from exogenous bacteria is unknown. To further confirm the association between acetic acid and the mid-intestine barrier function regulated by the tight junction expression and distribution, acetate was employed to treat mid-intestinal epithelial cells. This study found that the ZO-1 and F-actin disaggregation at the cell border and F-actin recombination occurred in the LPS-treated cells, and acetate could maintain the normal distribution of F-actin and ZO-1 to cope with the challenge posed by LPS (Figure 6). Peerapen and Thongboonkerd (2021) also indicate that various stimuli induce F-actin reorganization. Cytoskeleton, composed of F-actin and other partner proteins, plays a key role in the modulation and formation of the cell shape (Schakenraad et al., 2020) and locomotion (Svitkina, 2018). The reorganization of F-actin has been reported to influence the tight junction structure integrity due to the direct connection of ZO-1 with F-actin (Odenwald et al., 2017; Otani and Furuse, 2020). F-actin contractile is driven by MLCK/MLC pathway activation, which causes tight junction contraction and structural displacement, leading to increased permeability (Hecht et al., 1996; Yang et al., 2010). Therefore, acetate might protect the F-actin organization and the tight junction structure from LPS-induced damage by inhibiting the nuclear import of NF-κB p65 and activating the MLCK/MLC pathway.

5. Conclusion

Overall, our results provide evidence that the alteration of the midgut microbiota and SCFA profiling is accompanied by intestinal barrier damage and tight junction downregulation during *V. splendidus* infection in sea cucumbers. Moreover, the SCFA profiling analysis suggested that acetic acid was a characteristic metabolite in sea cucumbers. The feeding experiment also confirmed that acetic acid has a profound effect on the intestinal barrier integrity and tight junction structure integrity.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The animal study was approved by Experimental Animal Ethics Committee of Ningbo University, China. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CL: Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review and editing. MS: Investigation, Methodology, Validation, Writing—original draft. ZZ: Funding acquisition, Supervision, Validation, Writing—review and editing. YL: Formal analysis, Validation, Writing—review and editing. YX: Supervision, Writing—review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by the National Natural Science Foundation of China (42106122 and 32073003) and KC Wong Magna Fund in Ningbo University.

Acknowledgments

We thank reviewers for their suggestions in writing and analysis. We thank Dalian Ocean University for providing experimental sites and materials.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1263731/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 19 October 2023
DOI 10.3389/fmicb.2023.1273902

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OPEN ACCESS

EDITED BY
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RECEIVED 07 August 2023
ACCEPTED 29 September 2023
PUBLISHED 19 October 2023

CITATION
Zhang Z, Ran H, Hua Y, Deng F, Zeng B, Chai J and Li Y (2023) Screening and evaluation of skin potential probiotic from high-altitude Tibetans to repair ultraviolet radiation damage. *Front. Microbiol.* 14:1273902. doi: 10.3389/fmicb.2023.1273902

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Screening and evaluation of skin potential probiotic from high-altitude Tibetans to repair ultraviolet radiation damage

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Human skin microbes play critical roles in skin health and diseases. Microbes colonizing on the skin of Tibetans living in the high-altitude area for generations may have a stronger ability to resist the harsh environment, such as high ultraviolet radiation (UV). Isolation of a potential probiotic from Tibetans skin is beneficial for resistance of skin disease for humans in the world. In this study, the signature microbiota for Tibetan skin were characterized compared to low-altitude humans. Next, using culture-omics, 118 species were isolated. The culturability of high-altitude of Tibetan skin microbiome reached approximate 66.8%. Next, we found that one strain, *Pantoea eucrina*, had the greatest ability to repair UV damage to the skin as the lowest pathological score was observed in this group. Interestingly, another animal trial found this bacterium resisted UV rather than its metabolites. Using whole genome sequencing, this strain *P. eucrina KBFS172* was confirmed, and its functions were annotated. It might involve in the metabolic pathway of carotenoid biosynthesis with anti-oxidative stress properties, which plays critical roles in UV-damage repair. In conclusion, we characterized the signature microbes of skin in high-altitude Tibetans, isolated a skin bacterium of *Pantoea eucrina KBFS172* which could repair UV damage via involving the metabolic pathway of carotenoid biosynthesis. Our results provide a new potential skin probiotic for skin disease prevention or sunburn.

KEYWORDS

skin microbiota, skin disease, UV damage, high altitude, culturomics, probiotic

Introduction

Skin, the largest organ of the human body, has over 30 m^2 surface area, and serves as the first line to defense against the harsh external environment (Yoshio et al., 2004; Ross et al., 2017). Variable microbes colonize on the surface of skin (Gallo, 2017). Advances in sequencing and bioinformatics have unraveled the mysteries of the skin microbiome (Gao et al., 2008; Grice et al., 2008), including the characterization of commensal and pathogenic microorganisms on human skin (Roszkowski et al., 1990; Tang and Stratton, 2010). Numerous studies have demonstrated the crucial roles of skin commensal bacteria in maintaining the overall health of the skin and the host (Shu et al., 2013; Wang et al., 2014; Knackstedt et al., 2020). However, the

specific roles of skin microbiota associated with common diseases (e.g., skin cancer) are still unclear.

Qinghai-Tibet Plateau (QTP), known as "the roof of the world," is the highest plateau on earth, with a resident population exceeding 12 million as early as 2006 (Li et al., 2019). The living environment in QTP is extreme, with high ultraviolet radiation (UV), hypobaric pressure, and hypoxia (Jablonski and Chaplin, 2010). In spite prolonged exposure to high-intensity UV may cause skin cancer (Armstrong et al., 2001), the adaptation of Tibetans may have ability to resist extreme environment (e.g., UV) or even skin cancer. In term of this, some studies started to investigate the skin microbiota in subjects living in high and low altitudes. Previous studies have shown that high altitudes impacted the composition and structure skin microbes (Muletz Wolz et al., 2018; Li et al., 2019). Our previous study suggested that altitudes have a significant effect on the skin microbiome of Tibetans (Zeng et al., 2017). However, whether commensal bacteria on the skin of Tibetans could resist UV and what is the mechanism are still unknown.

Thus, we hypothesized that skin microbiota isolated from humans living in QTP may have the ability to resist and repair the UV damage. In this study, we first compared the human skin microbiomes form high and low altitude by re-analyzing our previous published data. Then we isolated and purified high-altitude Tibetan skin microbes using the culturomics method, and validated the potential functions of bacterial isolations on the repair of the UV damage by a mice model. Furthermore, we utilized whole-genome sequencing to unveil the mechanism of this isolate to resist UV. This current isolated a potential skin probiotic successfully, which benefits the protection of disease, such as skin cancer and sunburn.

Materials and methods

The experiment protocol was approved by the Animal Ethics and Humane Animal Care of the Foshan University (protocol#: FOSU2021010).

Sample collection

Ten healthy Tibetans (Supplementary Table S1), living in the Daocheng area (high altitude, 3,750 masl, latitude 27°58' N, longitude 99°56′ E) in China and having long-term outdoor activities (e.g., agricultural production), were recruited for this study. All human subjects had no skin disease and free access of antibiotics within 3 months when sampled. All the participants were given the written informed consent. Relevant characteristics, including gender, age, and sampling site, were recorded to exclude individuals with interfering factors in this experiment. Skin samples were collected by swabbing the forehead of the Tibetans. Regarding sampling, all sterile swabs were pre-wet with SCF-1 buffer [50 mM Tris buffer [pH 7.6], 1 mM EDTA [pH 8.0], 0.5% Tween-20], and were rubbed vigorously on the forehead over 30 times to collect skin microbes. Then, the forehead swabs were cut off and placed into 2 mL centrifuge tubes, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis.

Pure culture

The samples were thawed from −80°C and transferred to 4°C for 30 min. After the microorganisms were revived, the swab heads were immersed in sterile PBS buffer in the EP tube to shake out the bacteria. The resulting bacterial suspension was used for further pure culture experiments.

Five culture media, namely Nutrient Agar (Van der Weele et al., 2000), R-2A Agar (Reasoner et al., 1985; Massa et al., 1998), TSBYS (Peeters et al., 2011; Nicholson et al., 2013), BHIA (Osawa, 1990) and Agar medium J (Pagnanelli et al., 2000), were selected for pure culture. Each medium had six replicates. The bacterial suspension was mixed well with sterile PBS buffer (pH = 7.0) at a ratio of 0.1:9.9. Then, 200 µL of the bacterial suspension was spread evenly onto each prepared culture medium plate using a coating rod. The plates were then incubated in an inverted position at 28°C for microbial growth. The plates were observed every 12 h until no new colonies appeared. Three plates of each culture medium for each sample were randomly selected for subculture, and the plates were eluted with sterile PBS buffer. The eluted liquid was then sequenced directly to obtain the V3-V4 region of the 16S rRNA gene.

DNA extraction, sequencing, and bioinformatics for pure culture sample

For pure culture samples, the DNA of all the single strain were extracted using the boiling method (Dashti et al., 2009). The full-length sequence of 16S rRNA gene was amplified using the 27f/1492r primers (27f: 5'-AGAGTTTGATCCTGGCTCAG-3′, 1492r: 5'-TACGGYACCTTGTTACGACTT-3′). The quality of the PCR products was assessed by 1% gel electrophoresis, and qualified samples were sequenced on an Illumina MiSeq platform (Sangon Biotech Co., Ltd., Shanghai, China). Raw sequencing reads were quality controlled using Seqman software (Swindell and Plasterer, 1997). The high-quality sequences were aligned using Blastn in the NCBI database, and similar sequences of genes of single bacteria and their similarities were obtained. The isolated strains were then preliminarily divided into groups.

For the non-culture group that was sequenced directly from the swab heads, the total bacterial DNA was extracted using a Mo Bio PowerFecal DNA isolation kit according to the manufacturer's recommendations. DNA concentration (µg/µl) was quantified using a NanoDrop 2000C ultra-micro spectrophotometer. The general primers 341F (5'-CCTAYGGGRBGCASCAG-3′) and 806R (5'-GGACTACNNGGGTATCTAAT-3′) were used to amplify the V3-V4 region of the 16S RNA gene. The quality of the PCR products was checked by 1% gel electrophoresis, and qualified samples were sequenced on an Illumina MiSeq platform (NovogeneCo, Ltd., Beijing, China). The raw sequences were denoised, dereplicated, and filtered for chimeras using QIIME2 (Bolyen et al., 2019) (dada2 Package). The previous data (Zeng et al., 2017) (16S rRNA, v4 region, 128 samples) were also quality controlled using QIIME2 (deblur Package) (Bolyen et al., 2019). The taxonomy was annotated using the Greengenes database (gg_13_8) (Cannone et al., 2002).

UV irradiation trial #1

Five-week-old SPF-class female ICR mice ($n = 38$, body weight = 23 g) were used for this trial. The mice were divided into eight groups: a blank control group ($n = 3$) that received no treatment, negative control group ($n = 5$), and experimental groups A–E ($n = 5$ for each group) that received bacterial therapy with different candidates (A: *Arthrobacter gandavensis*; B: *Bacillus psychrosaccharolyticus*; C: *Pantoea eucrina*; D: *Paenibacillus amylolyticus*; E: *Paenibacillus terrae*) (Supplementary Table S3). After a preparation period, the area on the back of each mouse, measuring 2 cm × 3 cm, was depilated and smeared with the corresponding bacterial suspension for 3 days.

Before the trial, the mice were pre-feed with SPF pellet feed for one week. All feeding utensils were sterilized twice a week, and the ambient temperature was controlled at 20–25°C. Each mouse was housed in an individual cage with *ad libitum* access to food and water. After the mice were adaptively fed for a week, and bacterial colonization of each mouse was checked by bacterial culture of the back. Further details regarding the grouping and treatment conditions can be found in Supplementary Table S3. All mice were exposed to the same combination-rays light, and the changes in the skin morphology of each group were observed and recorded daily throughout the trial. Two types of UV light (UVA, UVB) boxes were used to irradiate the depilated mice.

A UV facility comprises a UVA lamp (40 W, with a wavelength range of 320 to 400 nm and a peak wavelength of 365 nm, from Philips, Germany) and a UVB lamp (20 W, with a wavelength range of 290 to 320 nm and a peak wavelength of 297 nm, also from Philips, Germany) positioned side by side. The distance between the lamps and the backs of the mice was set at 30 to 40 cm, and the irradiation intensity was measured using a UV-radiometer (UVAB/ST-513, SENTRY, Taiwan, China). We followed the subacute light injury model used in animal experiments (Wei et al., 2002) and optimized it based on our specific experimental conditions. After preheating the tubes for 20 min, the mouse cages were placed under the lamp box. During the trial, the position of the mouse cages was rotated to ensure equal irradiation dose for all mice. In the first week of irradiation, we performed daily UV irradiation, with a daily UVA dose of 3.96 J/cm^2 and a UVB dose of 252 mJ/cm^2. During the second and third weeks of irradiation, we performed UV irradiation every other day for a duration of 6 h, with UVA and UVB doses of 10.8 J/cm^2 and 1.08 J/cm^2, respectively. After each irradiation, we applied the corresponding bacterial suspension on the depilated area of the mice's backs. Overall, the total amount of UVA irradiation in this trial was 114.12 J/cm^2, and for UVB, it was 8.964 J/cm^2.

At the end of the trial, the mice were euthanized by cervical dislocation. The skin on their backs was immediately peeled off and immersed in a 4% formaldehyde solution, and stored at 4°C. The skin was then cut into 5 µm thickness sections, stained with H&E (hematoxylin–eosin staining), and observed under a microscope to record the histopathological changes (Figures 1A–D).

Mice UV irradiation trial #2

Pantoea eucrina has been shown to significantly repaired the UV damage by the first trial, we thus designed the second validation trial. Five-week-old SPF-class female ICR mice ($n = 40$) were used for this trial. The mice were divided into five groups, with eight mice per group: BS group (bacterial suspension: Suspension of *Pantoea eucrina*), BL group (bacterial lysate: lysate of *Pantoea eucrina*), NC group (negative control), and VE group (vitamin E). To prepare the bacterial suspension, the bacteria were activated and then incubated overnight in nutrient broth with shaking at 28°C. The concentration of the suspension was adjusted to 1×10^8 CFU/mL by counting on the plate. To prepare the bacterial lysate, the bacterial suspension was taken into a centrifuge tube and subjected to three cycles of freeze-thawing (−20°C, 30 min; 20°C, 20 min). The bacteria were then lysed using an ultrasonic cell disruptor (JY96-IIN, ShangHai JinXin). The lysate was then cooled in an ice-water bath for 30 min, filtered through a 0.22 µm filter, and stored at 4°C. For further details on the experimental grouping and treatment conditions, please refer to Supplementary Table S4. The trial procedures were the same as those of the previous trial (Mice UV Irradiation Trial #1).

After the trial, the skin on the back of the mice was stained using the same method as in the previous trial. The observations and recordings were conducted in a similar manner.

The whole genome sequencing

The bacterial specie (*Pantoea eucrina*) in group C were isolated to perform whole genome sequencing in order to identify the bacterial strain and its potential functions. The sequencing was performed on a single strain using the Nanopore sequencing technology platform. The raw data was subjected to format conversion and filtering. Canu v1.5 (Koren et al., 2017) was utilized for assembling the filtered subreads and Pilon (Walker et al., 2014) was used to correct any errors in the assembled genomes. Prodigal (Hyatt et al., 2010) was employed to predict the encoded genes in the assembled genome. The default parameters for these three softwares were chosen. Finally, the assembled contig sequences were aligned with the NT database to determine the chromosome type.

The predicted gene sequences were aligned using the BLAST algorithm (Altschul et al., 1997) to the Non-Redundant Protein Database (Nr database). Subsequently, the functional annotation of the Gene Ontology (GO) database (Ashburner et al., 2000) was performed using the Blast2GO software (Conesa et al., 2005) based on the alignment results with the Nr database. In addition, KEGG (Kanehisa et al., 2004) metabolic pathway enrichment analysis and GO functional enrichment analysis were carried.

Macroscopic and microcosmic evaluation

The changes in the mice's skin were observed and recorded daily, and the degree of skin damage on their backs was evaluated based on Supplementary Table S5. After the UV Irradiation Trial, the mice were sacrificed, and pathological sections of their backs were made. The degree of pathological changes was evaluated using Supplementary Table S6 as a reference.

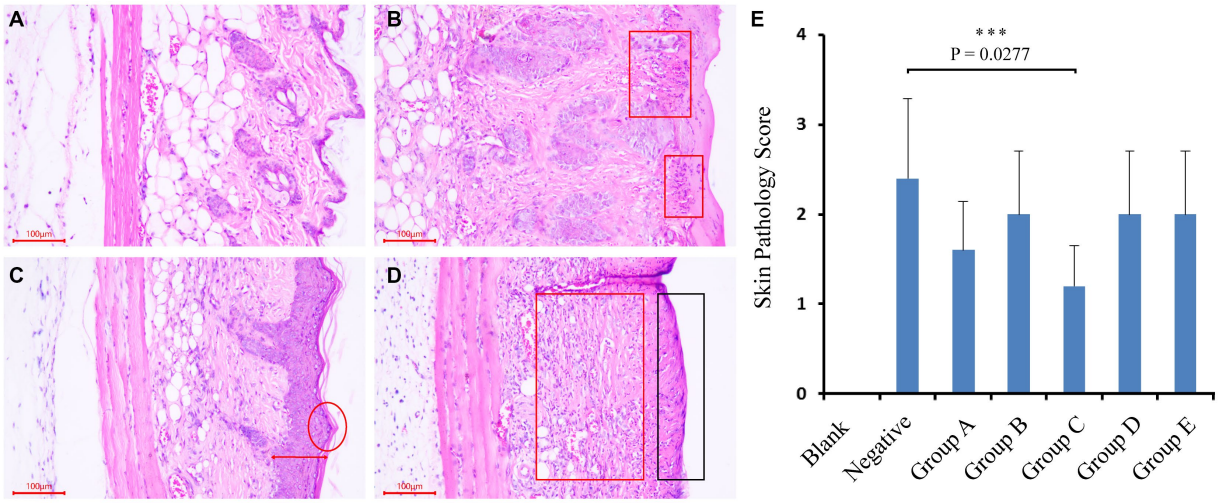

FIGURE 1
Mice trial #1: pathological changes of skin and sections, and scoring of skin pathological changes. **(A–D)** As followed, no obvious pathological changes in mouse skin; mild lesions, a small amount of inflammatory cell infiltration; moderate lesions, epidermal thickening, inflammatory cell infiltration and severe lesions, exposed subcutaneous tissue, and a large number of inflammatory cell infiltration. **(E)** Scores of pathological changes in skin sections of mice in each group. Blank (blank control group): without UV and smear bacteria treatment; Negative (negative control group): only UV treatment without smear bacteria suspension treatment; Group A–E: separately smear the suspension of *Arthrobacter gandavensis, Bacillus psychrosaccharolyticus, Pantoea eucrina, Paenibacillus amylolyticus*, and *Paenibacillus terrae*.

Statistical analyses

The algorithm of Linear Discriminant Analysis (LDA) coupled with effect size (LEfSe) (Paulson et al., 2013) was employed to determine features with significantly different abundances between high and low altitude human groups by re-analyzing our previous data (SRP065099, Sequence Read Archive (SRA) in NCBI).

The alpha and beta diversity of the high-altitude Tibetan skin microbiome of the 16S sequencing data were measured using the QIIME2 platform. The differences in alpha and beta diversity between groups were calculated using Student's t-test. Statistical significance was determined at $p < 0.05$ for all analyses. All figures were generated using the R (Wickham, 2011).

Results

Skin microbial differences in high- and low-altitude humans

Our previously published 16S rRNA data (Zeng et al., 2017) (128 samples) of skin samples from high and low- altitude humans were re-analyzed at the ASV level to identify signature bacteria between groups using linear discriminant analysis (LDA) effect size (LEfSe) (Figure 2). The main skin-featured bacteria at high altitude were *Aerococcus* (ASV6), *Staphylococcus* (ASV2, ASV10, ASV35), *Stenotrophomonas* (ASV14), *Acinetobacter* (ASV4), *Arthrobacter* (ASV22, ASV46), *Sanguibacter* (ASV20), *Pantoea* (ASV31), *Bacillus* (ASV25), *Paenibacillus* (ASV18, ASV29), *Pseudomonas* (ASV47) and *Enterobacteriaceae unclassified* (ASV1). In the low altitude group, the main signature included *Burkholderia* (ASV9), *Staphylococcaceae* (ASV16), *Enhydrobacter* (ASV11, ASV13), *Sphingomonas* (ASV23), *Acinetobacter* (ASV28), *Lactococcus* (ASV26, ASV30), *Brevundimonas* (ASV37, ASV48), *Corynebacterium* (ASV40, ASV51, ASV53), *Micrococcus* (ASV56) and *Neisseriaceae unclassified* (ASV69).

Culturability of high-altitude human skin microbes

Next, to achieve the goal of bacterial isolation from high-altitude human skin that may resist and repair UV damage, the cultivation method using five different medias were performed. Compared to 1,438 ASVs from next-generation sequencing method (non-culture group), 1,054 ASVs were observed in the cultivation methods (pure culture group). In the meantime, 961 shared ASVs between the cultivation and next-generation sequencing methods (Supplementary Figure S1A), and then the culturability of high-altitude human skin microbes is 66.83% (961/1,438) at the ASV level. After annotation, we obtained 628 and 502 genera in the non-culture and pure culture groups, respectively, with 475 genera co-existing in both groups (Supplementary Figure S1B). Furthermore, non-culture group had higher alpha diversity than pure culture group (Figure 3). Correspondingly, beta diversity based on Bray-Curtis distance showed significant differences between pure culture (cult) and non-culture (skin).

At the phylum level, the dominant bacteria in the non-culture group were *Proteobacteria* (44.48%), *Firmicutes* (29.19%), *Actinobacteria* (13.04%), *Bacteroidetes* (9.57%) and *Acidobacteria* (0.92%) (Figure 4). The dominant genera were *Pseudomonas* (10.20%), *Enhydrobacter* (8.76%), *Chryseobacterium* (6.90%), *Staphylococcus* (5.85%), *Acinetobacter* (3.68%), *Clostridium sensu stricto 1* (3.32%), *Bacillus* (2.89%), *Sphingomonas* (2.37%), *Peptoclostridium* (2.35%), *Psychrobacter* (2.26%), *Glutamicibacter* (1.42%), *Kocuria* (1.31%) and *Arthrobacter* (1.12%) from the non-culture group (Figure 5). In the pure culture group, the dominant phyla were *Firmicutes* (55.82%),

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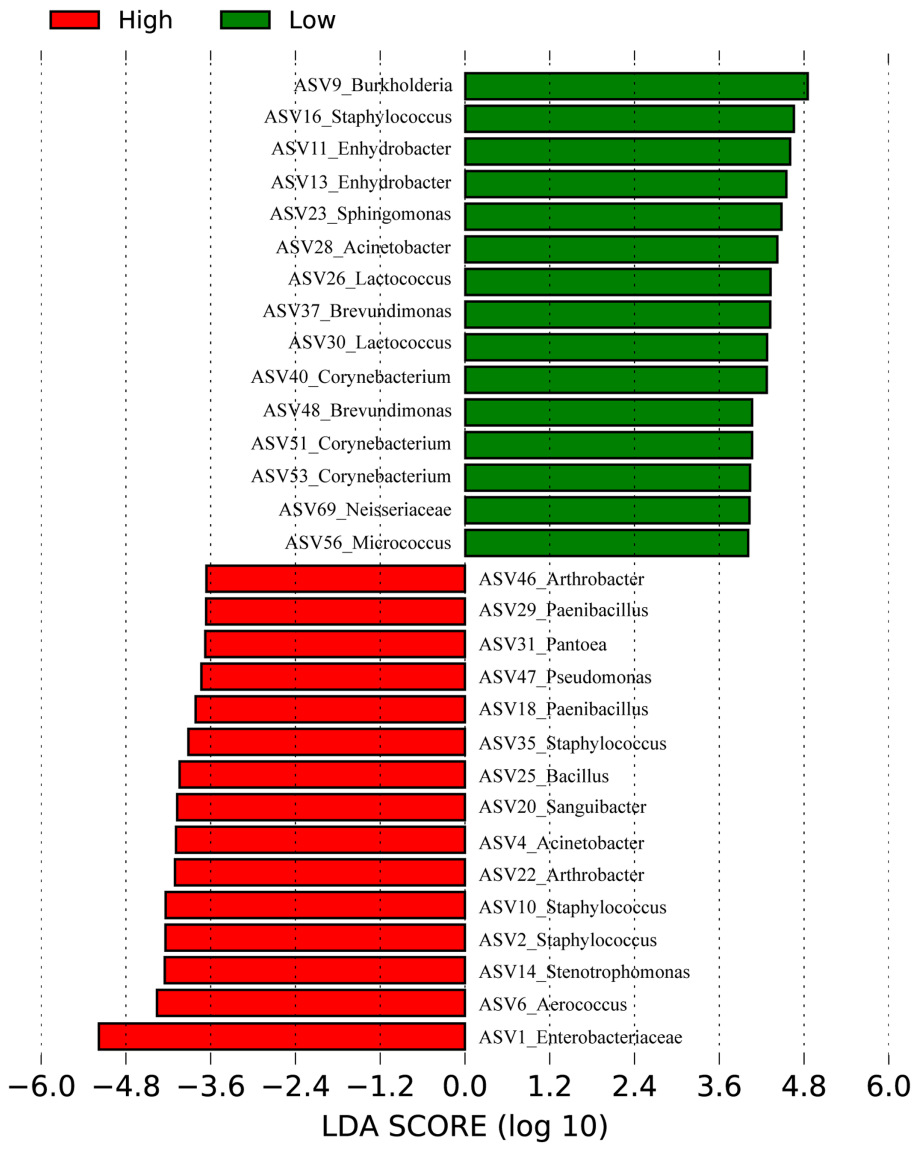

FIGURE 2
Bacteria differentiating high and low altitudes Humans by Linear discriminant analysis (LDA) coupled with effect size (LEfSe). Red represents the high-altitude group and green represents the low-altitude group.

Proteobacteria (22.14%) and *Actinobacteria* (21.02%). Then, we identified 10 genera with relative abundance greater than 1%, which were considered to be the main genera that could be cultivated from all culture samples. These genera were *Staphylococcus* (30.81%), *Bacillus* (15.19%), *Arthrobacter* (5.65%), *Psychrobacter* (5.51%), *Glutamicibacter* (5.27%), *Kocuria* (4.09%), *Acinetobacter* (3.88%), *Psychrobacillus* (3.19%), *Enhydrobacter* (2.08%) and *Micrococcus* (1.27%).

There was a total of 72 genera with a relative abundance greater than 0.1% in the non-cultivation group, and except for *Endobacter*, we were able to acquire 71 genera through cultivation. Among these, 29 genera (Supplementary Table S3) of strains were obtained through isolated and purified culture. Additionally, we also obtained strains of 9 genera through isolated and purified culture, whose relative abundance was less than 0.1% in the non-culture group.

Isolation of skin bacterial candidates to repair UV damage

We subsequently cultivated and isolated 340 single strains from these medias. Through sequencing the full-length of the 16S genes, a taxonomic map was generated. All isolations of strains were preliminarily divided into 4 phyla, 25 families, 42 genera, and 118 species (Table 1). We were able to cultivate 3 phyla (Firmicutes, Proteobacteria, Actinobacteria; $n = 339$), with Firmicutes ($n = 175$) and Actinobacteria ($n = 148$) being the most dominant (Figure 5). At the genus level, the majority of the isolated strains were *Kocuria* ($n = 64$), *Staphylococcus* ($n = 54$), *Bacillus* ($n = 39$), *Micrococcus* ($n = 28$), *Macrococcus* ($n = 19$), *Pantoea* ($n = 16$), *Rhodococcus* ($n = 12$), and *Paenibacillus* ($n = 12$). Furthermore, combining with results of Figure 2 and references, several bacterial candidates that may repair UV damage were selected for the below animal trial (Supplementary Table S3).

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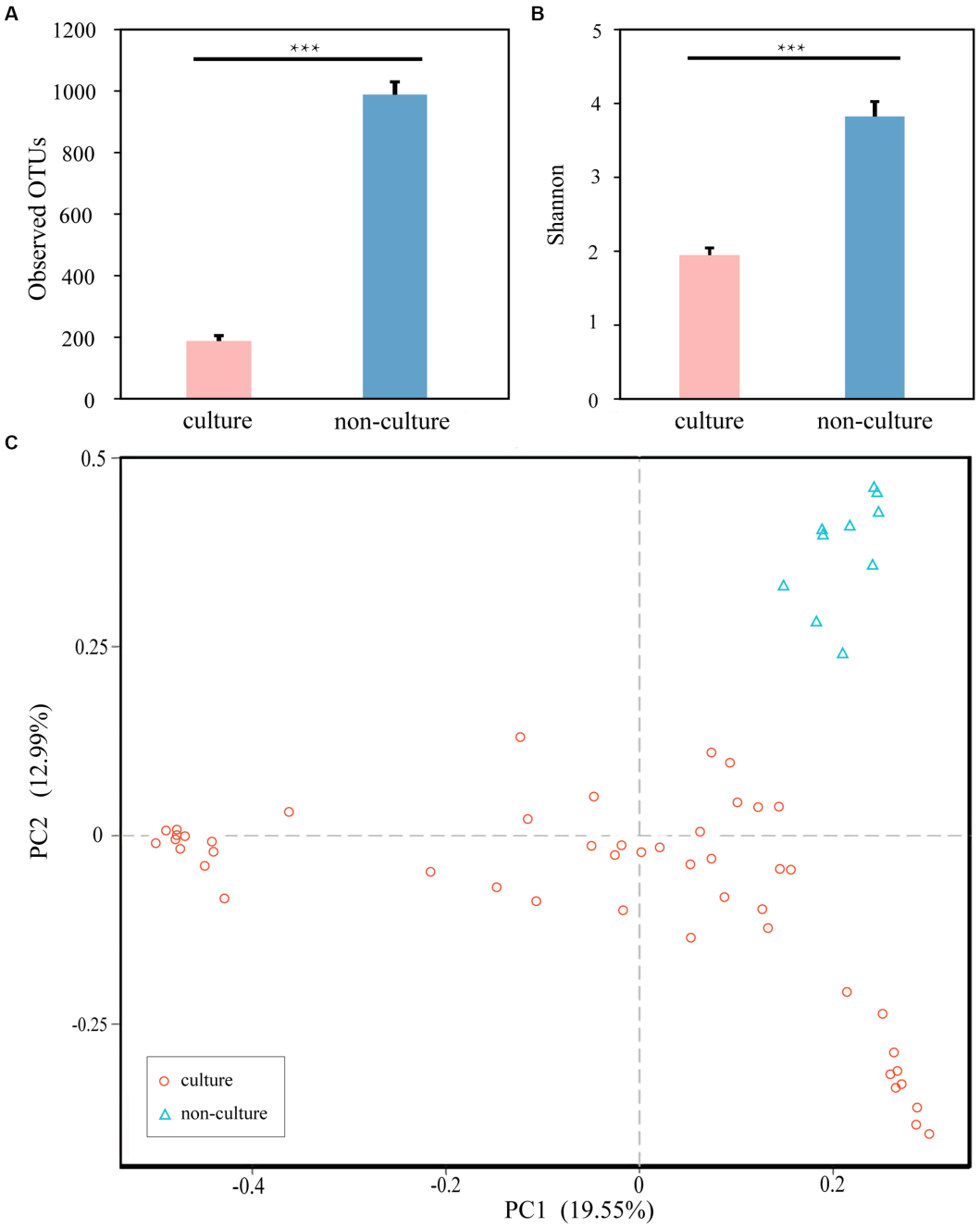

FIGURE 3
Microbial differences between non-culture and pure culture groups. **(A)** Observed OTUs. **(B)** Shannon Index. **(C)** PCoA plot based Bray-Curtis distance. Colors of red and blue represent samples on pure culture group and non-culture group. Pure culture group: using cultivation methods; non-culture group: using next-generation sequencing methods (***$p < 0.05$).

Animal trial to test UV-damage repair isolations

To test UV damage repair functions of isolations, bacterial suspension of *Arthrobacter gandavensis, Bacillus psychrosaccharolyticus, Pantoea eucrina, Paenibacillus amylolyticus* and *Paenibacillus terrae* were applied to the mice skin hurt by UV lamp. The skin of all the negative control mice visually thickened, and numerous inflammatory cells infiltrated in the dermis and subcutaneous tissue layers. The skin pathological changes in experimental groups (A, B, D, E) were similar to those in the negative control group (Supplementary Figure S2). We scored the skin pathological changes of each mouse in the groups (Figures 1A–D) and found that experimental group C (*Pantoea eucrina*) had the lowest pathological score except blank control group, indicating the lowest degree of skin damage (for detailed score statistics, Supplementary Table S7) (Figure 1E). Notably, there was a significant difference ($p = 0.027$) in the skin pathological section scores between the experimental group C and the negative control group, with only a small amount of inflammatory cell

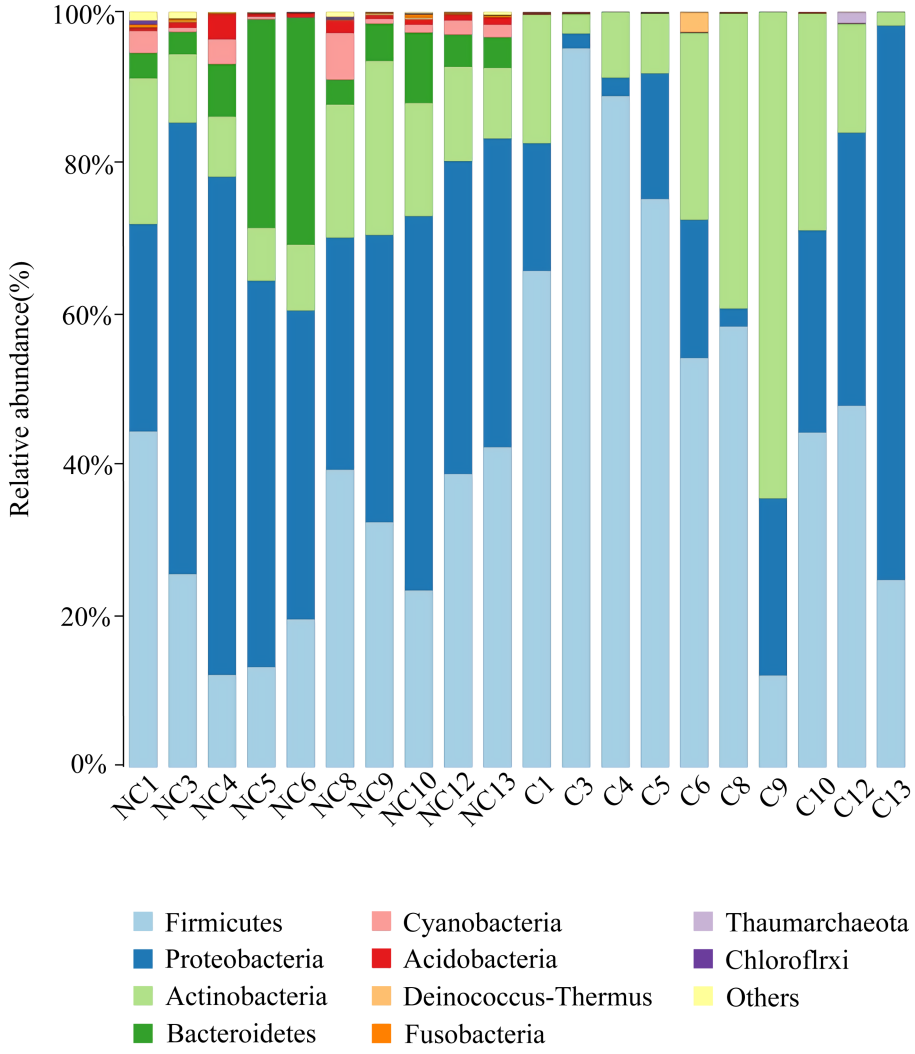

FIGURE 4
Microbial composition at the phylum level of each sample (top 10 taxon). NC (non-culture group): using next-generation sequencing methods; C (pure culture group): using cultivation methods. The same number represents from the same sample.

infiltration in the dermis and subcutaneous tissue layers (Figure 1B). Therefore, the bacteria in group C (*Pantoea eucrina*) have the potential to repair skin UV damage.

Validation of UV-resistant functions of *Pantoea eucrina KBFS172*

To further investigate whether the *Pantoea eucrina* strain or its metabolites could repair skin UV damage, a mice trial #2 was conducted. Firstly, in visual observation, the skin of the mice in the BS group (bacterial suspension: Suspension of *Pantoea eucrina*) appeared chapped erythema, while other control groups (NC group: negative control, BL group: bacterial lysate: lysate of *Pantoea eucrina*, and VE group: vitamin E, Supplementary Table S4) showed varying degrees of ulceration, dandruff production, and scarring. From the appearance of the mice skin, it appeared that the skin damage situation of the mice in the BC group was indeed better than that in the NC group (Figures 6B,C). The appearance and tissue pathological damage scores in BS groups were the best compared to NC Group, BL Group, and VE group (Figure 6), and the trial details were documented in Supplementary Tables S4–S6, S8, S9, and Supplementary Figure S2. In addition, we observed that BS groups had a better UV-resistant effect than NC and BL groups, indicating that *Pantoea eucrina* strain plays critical roles in UV-resistant rather than its metabolites.

Functional annotation of *Pantoea eucrina KBFS172* using whole genome sequencing

Through whole genome sequencing technology, a total of 4,053,112 bases of *Pantoea eucrina KBFS172* was generated, which included 1 chromosome sequence and 4 plasmid sequences, and predicted 3,871 genes (Supplementary Table S10). A total of 107 potential functional pathways were recognized and quantified, which included the metabolic pathways of carotenoid synthesis, ascorbate metabolism, geraniol degradation and streptomycin biosynthesis, etc.

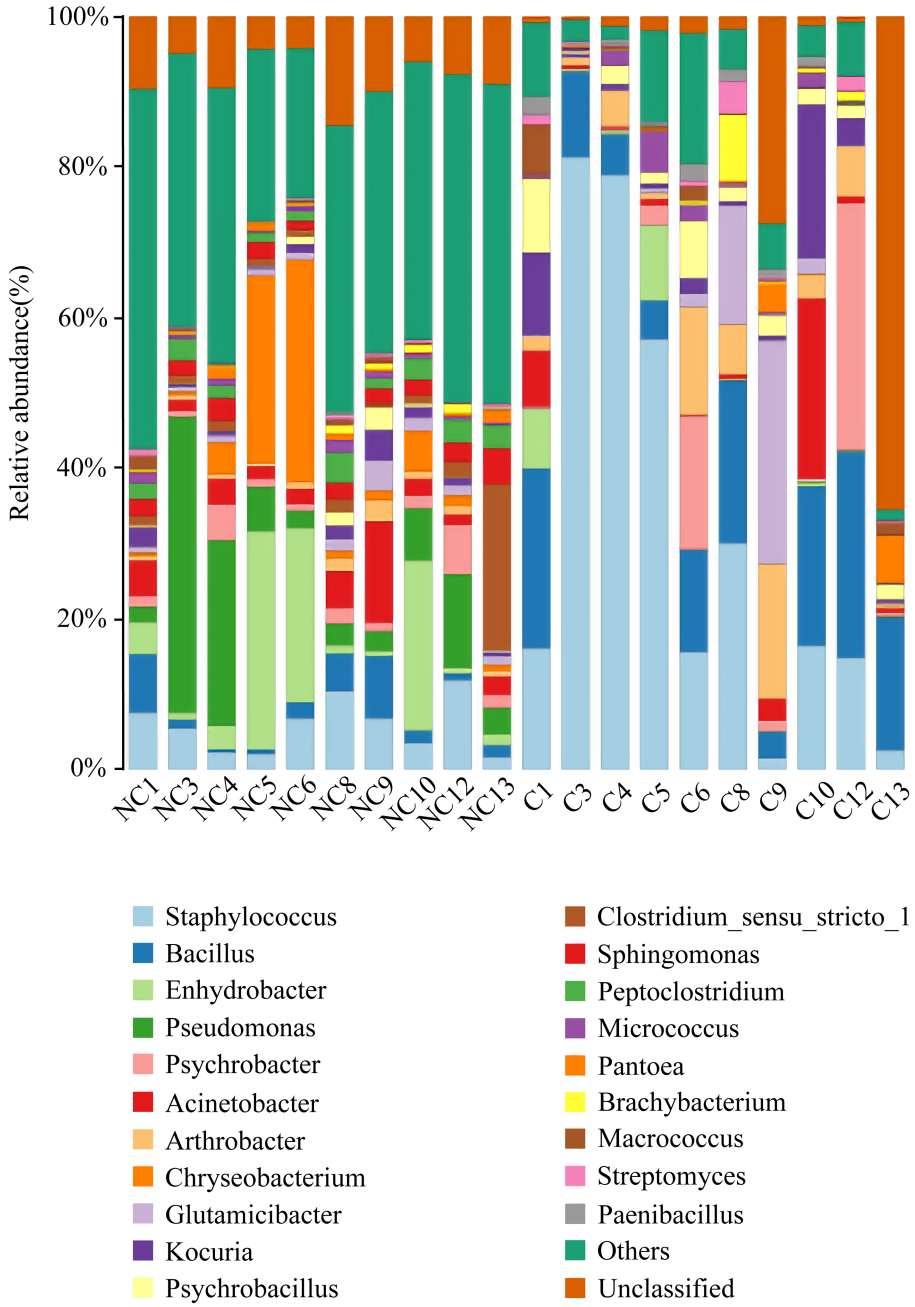

FIGURE 5
Microbial composition at the genus level of each sample (top 20 taxon). NC (non-culture group): using next-generation sequencing methods; C (pure culture group): using cultivation methods. The same number represents from the same sample.

Carotenoids have been shown to act as an antioxidant against oxidative stress and increase bacterial tolerance to UV radiation (Ordenes-Aenishanslins et al., 2016; Portero et al., 2019; Reis-Mansur et al., 2019; Sedlacek et al., 2019). In this study, *Pantoea eucrina KBFS172* had a large number of genes enriched in the metabolic pathway of carotenoid biosynthesis (Supplementary Figure S4, S5), among which beta-Carotene biosynthesis module was particularly prominent (Figure 7A). In addition, 6 enzymes were annotated to be closely related to carotenoid biosynthesis, including *geranylgeranyl pyrophosphate synthase (crt_E; GE03214), zeaxanthin glucosyltransferase (crt_X; GE03215), lycopene cyclase (crt_Y; GE03216), phytoene dehydrogenase (crt_I; GE03217), phytoene synthase (crt_B; GE03218), and beta-carotene hydroxylase (crt_Z; GE03219)*. Through consultation of the key gene set for carotenoid synthesis in *Pantoea* (Wang et al., 2007; Mohammadi et al., 2012; Choi et al., 2021), we speculated that these 6 enzymes were involved in the pathway of carotenoid biosynthesis in the order shown in Figure 7B.

Discussion

Although many studies focus on isolations of beneficial bacteria of gut microbiota (Deng et al., 2023; Xu et al., 2023; Zhao et al., 2023), the crucial role of skin microbes in humans have been highlighted.

10.3389/fmicb.2023.1273902

TABLE 1 Single bacteria obtained by isolation and purification.

Phylum	Family	Genus
Actinobacteria	*Brevibacteriaceae*	*Brevibacterium*
	Micrococcaceae	*Rothia*
		Micrococcus
		Kocuria
		Arthrobacter
	Corynebacteriaceae	*Carnobacterium*
		Corynebacterium
	Microbacteriaceae	*Curtobacterium*
		Microbacterium
	Intrasporangiaceae	*Janibacter*
		Kytococcus
	Cellulomonadaceae	*Oerskovia*
	Dietziaceae	*Dietzia*
	Nocardioidaceae	*Aeromicrobium*
	Nocardiaceae	*Rhodococcus*
	Streptomycetaceae	*Streptomyces*
Proteobacteria	*Moraxellaceae*	*Moraxella*
		Acinetobacter
		Psychrobacter
	Enterobacteriaceae	*Erwinia*
	Methylobacteriaceae	*Microvirga*
	Rhodobacteraceae	*Paracoccus*
		Plantibacter
	Xanthomonadaceae	*Stenotrophomonas*
Firmicutes	*Bacillaceae*	*Virgibacillus*
		Oceanobacillus
		Solibacillus
		Psychrobacillus
		Lysinibacillus
		Exiguobacterium
		Bacillus
	Carnobacteriaceae	*Desemzia*
	Enterococcaceae	*Enterobacter*
	Enterobacteriaceae	*Pantoea*
	Staphylococcaceae	*Staphylococcus*
		Macrococcus
	Paenibacillaceae	*Brevibacillus*
		Paenibacillus
	Planococcaceae	*Planomicrobium*
	Planococcaceae	*Sporosarcina*
	Aerococcaceae	*Aerococcus*
Deinococcus Thermus	*Deinococcaceae*	*Deinococcus*

Interactions between certain skin microbiome and host cells can result in functional changes in the latter. Past research has shown that skin microbes are involved in anti-inflammatory processes, maintenance of skin homeostasis, and regulation of the immune system in both humans and animals (Ridaura et al., 2018).

Four dominant bacterial phyla in the human skin microbiota had been identified, including *Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes* (Grice et al., 2008; Li et al., 2019). Changes in the living environment could affect the skin microbial composition. A previous study found that the relative abundance of *Bacteroidetes* and *Firmicutes* was higher at high altitudes (Li et al., 2019) than at low altitudes (Grice et al., 2008). This difference suggests that altitude may be an important driving force for skin microbiota composition (Gao et al., 2007; Fierer et al., 2008; Grice et al., 2009). Our own results showed that the relative abundance of continued to increase compared to the previous study. Therefore, *Firmicutes* might be beneficial for humans living at high altitudes. At the genus level, previous studies have found the resident microbiota in human skin, such as *Propionibacterium, Corynebacterium, Staphylococcus, Streptococcus, Acinetobacter, Colwiebacteria,* and *Enhydrobacter* (Gao et al., 2007; Fierer et al., 2008; Grice et al., 2009; Leung et al., 2015). *Propionibacterium* and *Staphylococcus* have been found to be the predominant species in sebaceous sites (Grice et al., 2008). However, in our study, *Propionibacterium* was not found to be predominant and its relative abundance was less than 1%, suggesting that it may not be able to tolerate the high-altitude environment. Interestingly, we found two genera, *Pseudomonas* (10.20%, *Proteobacteria*) and *Chryseobacterium* (6.90%, *Bacteroidetes*), with high relative abundance that had rarely been found at such levels in previous studies on human skin. This indicates that these two genera may be tolerant to high-altitude environments. Previous studies (Seelam et al., 2021) have demonstrated that *Pseudomonas* has the potential ability to provide protective effects mediated by melanin as a sunscreen agent against UV-B radiation. However, *Pseudomonas* has also been reported as an opportunistic pathogen of human skin (Spernovasilis et al., 2021), *Chryseobacterium* has been less studied on human skin, but a few studies (Gurav and Jadhav, 2013; Venil et al., 2015; Hui et al., 2019) have reported that its metabolite flexirubins can treat chronic skin diseases, while other studies (Nulens et al., 2001; Cascio et al., 2005) have shown that it is closely associated with some human diseases. Therefore, further research is needed to elucidate the specific functions of these two genera at the species level.

In our study, we employed the cultivation method using five different media and were able to obtain approximately 66.83% (961/1,438) of operational taxonomic units (OTUs). However, in the non-cultured group, we observed that five genera, including *Pseudomonas, Chryseobacterium, Clostridium sensu stricto 1, Sphingomonas*, and *Peptoclostridium*, had a relative abundance greater than 1% but were uncultured. These genera need to be further cultivated using other media, particularly *Pseudomonas* (isolated referencing (Ravi et al., 2018)) and *Chryseobacterium* (isolated referencing (Nishioka et al., 2016)), which are speculated from our results to be closely associated with high altitude. The skin bacteria that we were able to cultivate were dominated by *Firmicutes*, with most isolates belonging to *Staphylococcus* (30.81%) followed by *Bacillus* (15.19%). These bacteria were relatively common (Timm et al., 2020; Fleming et al., 2021). Certainly, culturability is closely related to the culture conditions. Thus, more culture media and different culture conditions can be employed to further cultivate uncultivated bacteria.

Until now, there have been relatively few studies conducted on the skin microbiota of Tibetans living on the plateau. Our study has provided insight into the structure of the skin microbiota of Tibetans

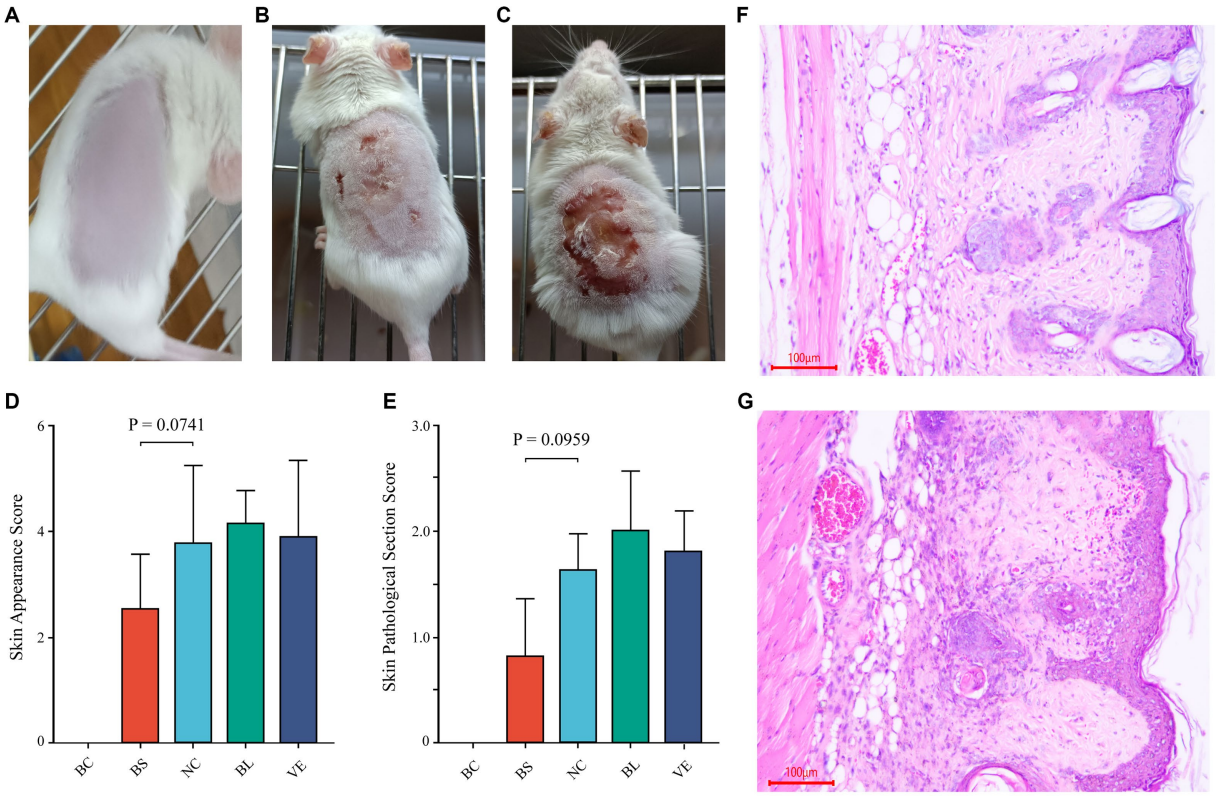

FIGURE 6
Mice trial #2: pathological changes of skin and sections, and scoring of skin pathological changes. **(A)** The healthy mouse skin appearance (BC). **(B)** Appearance of the skin of the mice smeared with BS. **(C)** Appearance of the skin of the mice in NC. **(D, E)** Statistical bar graph of skin appearance and skin pathological damage score in each group. **(F)** Representative diagram of skin section in the BS. **(G)** Representative diagram of skin section in the NC. BC, blank control; BS, bacterial suspension: Suspension of *Pantoea eucrina*; NC, negative control; BL, bacterial lysate: lysate of *Pantoea eucrina*; VE, vitamin E.

living on the plateau, with a focus on the culturomics of extremely rare high-altitude human skin microbes. This study serves as a reference for future investigations into human skin microbes. Additionally, we observed that approximately 66% of plateau Tibetan skin microbes could be cultivated using five different media (Supplementary Table S2). Although some of the isolated strains may be more commonly associated with soil or environmental sources, they were nonetheless found on the skin of healthy individuals. The contribution of these strains to the function of the skin microbiome is unknown, and further research in this area is warranted.

To further explore the function of skin microbes in plateau Tibetans and identify strains that can repair UV damage, we conducted mice trials using five selected strains from pure culture based on previous studies. Ultimately, we identified a strain (*Pantoea eucrina KBFS172*) that showed ability in repairing photodamage. Previous studies have demonstrated the photodamage repair function of skin bacteria, and they have multiple repair mechanisms, which have different repair mechanisms. Some studies have found that the metabolites of certain microorganisms can achieve the function of photodamage repair or alleviation, such as bioactive peptides (Wang et al., 2022), Vitamin C, ferulic acid, and phloretin, etc. (Oresajo et al., 2008). In our study, *Pantoea eucrina* was identified to associate with the metabolism of carotenoids. Carotenoids have been shown to have anti-oxidative stress properties, increase bacterial UV radiation tolerance (Ordenes-Aenishanslins et al., 2016; Reis-Mansur et al., 2019), and found that co-cultivation of carotenoids with bacteria can enhance the UV radiation tolerance of sensitive bacteria (Portero et al., 2019; Sedlacek et al., 2019). Additionally, we also found other species of the genus *Pantoea*, which could also produce carotenoids, such as *Pantoea ananatis, Pantoea stewartii subsp. Stewartii, and P. agglomerans Eho10* (Mohammadi et al., 2012; Sedlacek et al., 2019; Choi et al., 2021). This indicated that the *Pantoea* genus may have excellent potential for repairing photodamage. Moreover, *Micrococcus* (Hofer et al., 2011), *cyanobacteria* (Stege, 2001), and *Lactobacillus acidophilus* (Im et al., 2018) can also repair skin UV damage by synthesizing DNA damage repair enzymes or photolytic enzymes. Therefore, skin microbes can reduce the damage of ultraviolet rays to human skin in a variety of ways.

Conclusion

In conclusion, we characterized the skin microbiota of humans living in high altitude regions of China using 16S rRNA gene sequencing and culturomics methods. We identified and isolated strains that were effective in repairing UV damage through mouse trials, and obtained a potentially functional strain. The function of the strain was then verified by analyzing the whole-genome sequencing results. These findings gave the insights for further studies to explore

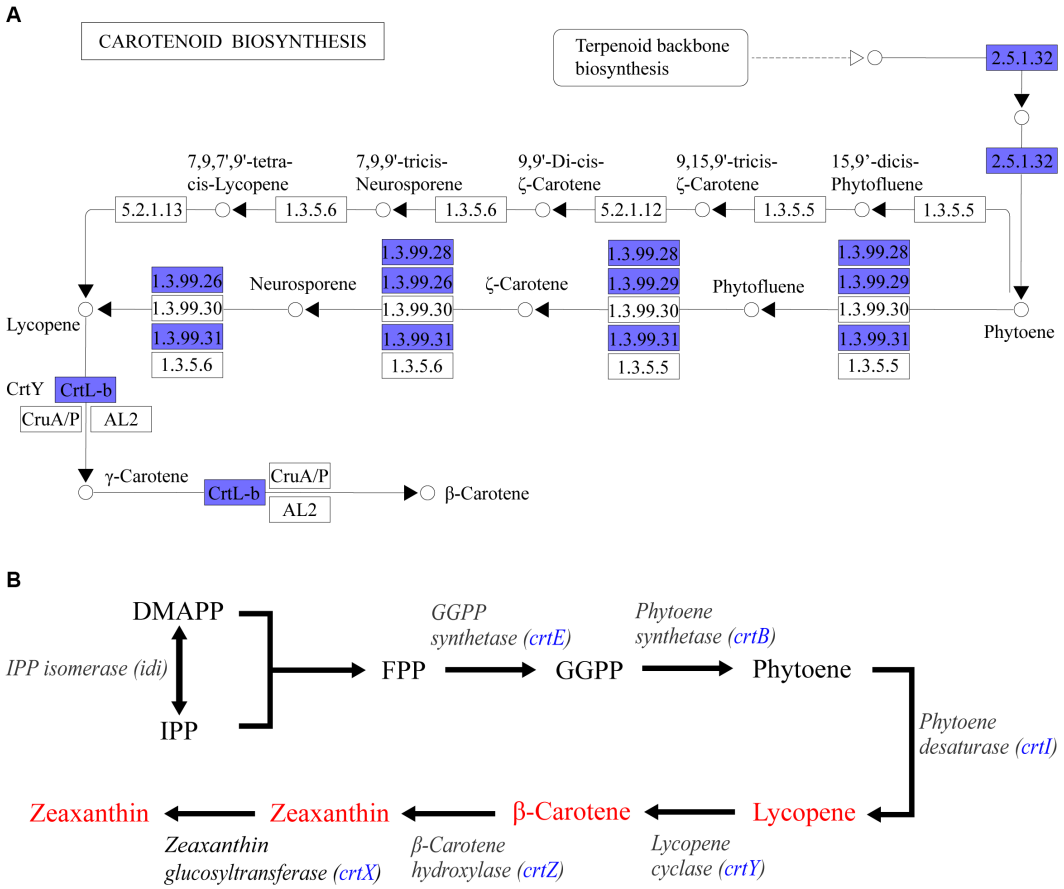

FIGURE 7
KEGG pathway enrichment analysis annotation results. **(A)** Beta-Carotene biosynthesis module (M00097) in carotenoid biosynthesis metabolic pathway. Blue filled were the annotated genes in *Pantoea eucrina KBFS172*. **(B)** Sketch of carotenoid synthesis pathway inferred from *P. agglomerans KFS-9* and *Pantoea ananatis*. The blue filled was the enzyme annotated by *Pantoea eucrina KBFS172*.

the potential of skin-associated microbial communities in high altitude adaptation of humans, and offer new insights into the development of human skin probiotic products to resist skin diseases, such as skin cancer or sunburn.

Data availability statement

The rumen metagenome sequences were deposited into NCBI Sequence Read Archive (SRA) under the accession number PRJNA1002829. The other sequences data in the study are included in the article/Supplementary material, and further inquiries can be directed to the corresponding authors.

Ethics statement

The experiment protocol was approved by the Animal Ethics and Humane Animal Care of the Foshan University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

ZZ: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. HR: Investigation, Writing – original draft. YH: Investigation, Writing – review & editing. FD: Resources, Visualization, Writing – review & editing. BZ: Investigation, Resources, Validation, Visualization, Writing – original draft. JC: Supervision, Validation, Visualization, Writing – review & editing. YL: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This project was supported by National Natural Science Foundation of China (32170430), Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding (2019B030301010), and Key

Laboratory of Animal Molecular Design and Precise Breeding of Guangdong Higher Education Institutes (2019KSYS011).

Acknowledgments

We greatly appreciate Tao Zhang, Xingyin Zhang, Donghao Liu, and Peini Wang for participating in animal trials and animal sample collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1273902/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 10 November 2023
DOI 10.3389/fmicb.2023.1289374

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OPEN ACCESS

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RECEIVED 06 September 2023
ACCEPTED 27 October 2023
PUBLISHED 10 November 2023

CITATION
Cronin P, McCarthy S, Hurley C, Ghosh TS, Cooney JC, Tobin A-M, Murphy M, O'Connor EM, Shanahan F and O'Toole PW (2023) Comparative diet-gut microbiome analysis in Crohn's disease and Hidradenitis suppurativa.
Front. Microbiol. 14:1289374.
doi: 10.3389/fmicb.2023.1289374

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Comparative diet-gut microbiome analysis in Crohn's disease and Hidradenitis suppurativa

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Introduction: The chronic inflammatory skin disease Hidradenitis suppurativa (HS) is strongly associated with Crohn's Disease (CD). HS and CD share clinical similarities and similar inflammatory pathways are upregulated in both conditions. Increased prevalence of inflammatory disease in industrialised nations has been linked to the Western diet. However, gut microbiota composition and diet interaction have not been compared in HS and CD.

Methods: Here we compared the fecal microbiota (16S rRNA gene amplicon sequencing) and habitual diet of previously reported subjects with HS ($n = 55$), patients with CD ($n = 102$) and controls ($n = 95$).

Results and discussion: Patients with HS consumed a Western diet similar to patients with CD. Meanwhile, habitual diet in HS and CD was significantly different to controls. Previously, we detected differences in microbiota composition among patients with HS from that of controls. We now show that 40% of patients with HS had a microbiota configuration similar to that of CD, characterised by the enrichment of pathogenic genera (Enterococcus, Veillonella and Escherichia_Shigella) and the depletion of putatively beneficial genera (Faecalibacterium). The remaining 60% of patients with HS harboured a normal microbiota similar to that of controls. Antibiotics, which are commonly used to treat HS, were identified as a co-varying with differences in microbiota composition. We examined the levels of several inflammatory markers highlighting that growth-arrest specific 6 (Gas6), which has anti-inflammatory potential, were significantly lower in the 40% of patients with HS who had a CD microbiota configuration. Levels of the pro-inflammatory cytokine IL-12, which is a modulator of intestinal inflammation in CD, were negatively correlated with the abundance of health-associated genera in patients with HS. In conclusion, the fecal microbiota may help identify patients with HS who are at greater risk for development of CD.

KEYWORDS

Hidradenitis suppurativa, Crohn's disease, gut microbiota, diet, inflammation

1. Introduction

Hidradenitis Suppurativa (HS) is an incompletely understood, painful chronic autoinflammatory skin disorder of the terminal hair follicle. The prevalence of HS is increasing globally (Vazquez et al., 2013; Ingvarsson, 2017; Alotaibi, 2023) and affects up to 1% of the population (Jemec et al., 1996; Cosmatos et al., 2013; Sung and Kimball, 2013; Jemec and Kimball, 2015; Miller et al., 2016; Alotaibi, 2023). However, estimates have varied widely due to the diversity in methodologies and sources utilised and in the USA it has been suggested that HS is prevalent in 0.1% of the population (Ingram, 2020). This debilitating disease is characterised by the development of abscesses, nodules and fistulae which typically occur at intertriginous sites of the body including the inframammary, axillary, gluteal and inguinal regions (Zouboulis et al., 2015; Saunte and Jemec, 2017; Sabat et al., 2020). Hereditary HS which is characterised by loss of function mutations to genes encoding the subunits of γ-secretase (NCSTN, PSENEN, and PSEN1) accounts for a minority of cases (Moltrasio, 2022) whilst environmental and lifestyle factors are thought to be the main driver. Typically, the first events occurring at the terminal hair follicle include infundibular acanthosis, hyperkeratosis and perifollicular immune cell infiltration (Wolk et al., 2020). It is thought that molecules associated with cell damage and bacteria can initiate inflammation leading to immune cell infiltration that clinically manifests as inflamed nodules and abscesses (Wolk et al., 2020). Epigenetic mechanisms including DNA methylation of miRNA genes has also been linked to development of HS (Radhakrishna, 2022). Importantly, the indigenous microbiota has been implicated in HS pathogenesis. We (McCarthy et al., 2022) and others (Ring et al., 2017) have shown significant alterations in the composition of the skin and faecal microbiota of patients with HS compared to controls. Significantly enriched taxa in the skin microbiota included: *Peptoniphilus lacrimalis*, *Peptoniphilus coxii*, *Anaerococcus murdochii*, *Anaerococcus obesiensis* and *Finegoldia magna* (Ring et al., 2017; McCarthy et al., 2022). *Finegoldia magna* is a pathogenic species that is associated with a pro-inflammatory response—thus likely to contribute to the pathogenesis of HS (Neumann et al., 2020). HS is also associated with co-morbidities including obesity, metabolic syndrome and Crohn's disease (CD; Sabat et al., 2012; Gold et al., 2014; Chen and Chi, 2019). The concurrent existence of inflammatory/metabolic gut and skin diseases has highlighted the potential involvement of the gut-skin axis as a contributor to HS (Eppinga et al., 2016; Balato et al., 2018; Kam et al., 2020; Lam et al., 2021). CD development is mediated by an altered inflammatory response which is typically characterised by alterations to innate immunity of the intestinal mucosa barrier and remodelling of the extracellular matrix through expression of metalloproteins and adhesion molecules (Petagna et al., 2020). This reshaping of the intestinal microenvironment increases leucocyte migration to areas of intestinal inflammation promoting a TH1 response through production of IL-12 and TNFα cytokines (Petagna et al., 2020). Interestingly, both HS and CD are characterised by shared disease manifestations. The HS faecal microbiome is enriched with *Ruminococcus gnavus* and *Clostridium ramosum* which have been previously detected in the CD microbiota and are associated with the upregulation of the inflammatory cytokine TNFα (Joossens et al., 2011; Hall et al., 2017; Nishino et al., 2018; Lloyd-Price et al., 2019; McCarthy et al., 2022). Other pro-inflammatory pathways are also upregulated in both disease states, mediated by the cytokines IL-6, IL-1, IL-17, IL-12 and IL-23 (Duerr et al., 2006; Schlapbach et al., 2011). A dysfunctional Th1 inflammatory response is a key characteristic of both HS and CD (Vossen et al., 2018; Grand et al., 2020). Furthermore, both HS and CD are characterised by shared manifestations such as sinus tract and abscess formation (Van Der Zee et al., 2014). Given the links and overlapping features of these inflammatory diseases it is unsurprising that patients with HS have a significantly higher risk for the development of CD (Deckers et al., 2017; Egeberg et al., 2017). Like HS (McCarthy et al., 2022), alterations to the gut microbiota in patients with CD has been well documented (Halfvarson et al., 2017; Pascal et al., 2017; Franzosa et al., 2018; Yilmaz et al., 2019). Although some of these changes in microbiota composition are likely secondary to intestinal inflammation, specific pro-inflammatory taxa have been implicated in CD through a number of clinical and experimental studies (Bernstein and Forbes, 2017; Nishida et al., 2017; Sheehan and Shanahan, 2017; Zuo and Ng, 2018; Ryan et al., 2020). It is currently unknown whether inflammation driving the development of HS occurs first at the epidermis or is extracutaneous. If extracutaneous inflammation occurs first, the intestinal microbiota could play an important role in its pathogenesis. Furthermore, given that a significant number of HS patients eventually present with CD, the microbiota may also have a role in the development of CD in patients with HS. To date, we are unaware of any study that has compared the gut microbiota of patients with HS with that of patients with CD.

The global increase in HS prevalence is most pronounced in industrialised countries, consistent with trends for other metabolic and inflammatory diseases including CD (Roda et al., 2020). This increased prevalence is also linked to consumption of the Western diet, which is low in dietary fibre and high in sugars, saturated fat and dairy (Cordain et al., 2002; Simopoulos and DiNicolantonio, 2016; Khan and Chang, 2022). Diet is now being recognised as an effective approach to help mitigate the burden of inflammatory disease. It has been suggested that dairy products (William Danby, 2015; Silfvast-Kaiser et al., 2019; Khan and Chang, 2022) and brewer's yeast (Silfvast-Kaiser et al., 2019; Khan and Chang, 2022) can aggravate HS symptoms. It is important to note that the reported associations between diet and HS are controversial. The few studies examining dairy and/or brewers' yeast in HS used small sample sizes and did not use formally validated assessment methods (placebo control; Cannistrà et al., 2013; Colboc et al., 2016). Currently it is not clear what role diet plays in HS, and little is known about day-to-day dietary intake patterns in patients undergoing treatment.

To address these questions, we undertook a study to compare faecal microbiota composition and habitual diet in patients with HS to patients with CD and healthy controls.

2. Materials and methods

2.1. Overview of study population

The study population for this analysis included 55 individuals with HS who were recruited as previously reported (Table 1; McCarthy et al., 2022). For comparative analysis we included data for 102 patients with CD obtained from a previously published dataset (Table 1; Clooney et al., 2021). The control group included 95 individuals with no known medical condition, the samples for which

TABLE 1 Study population and sample sizes.

	Patients (*n*)	Samples	Dataset
Controls	95	164	Clooney et al. (2021)* and McCarthy et al. (2022)*
Crohn's disease (CD)	102	212	Clooney et al. (2021)
Hidradenitis suppurativa (HS)	55	55	McCarthy et al. (2022)

*Controls had $n = 30$ patients (30 samples) from McCarthy et al. (2022) whilst rest of the controls was $n = 72$ (182 samples) obtained from Clooney et al. (2021).

were obtained from both studies (Table 1; Clooney et al., 2021; McCarthy et al., 2022). Patient data obtained from Clooney et al. (2021) included multiple longitudinal samples for some individuals. Whilst different datasets were used for analysis in this study, sample collection procedures, DNA extraction and 16S PCR protocols as well as sequencing methodology (Illumina MiSeq 2 × 300 bp chemistry) were uniform across both studies. Dietary data was previously collected using a food frequency questionnaire (FFQ) which we obtained on request from the authors.

2.2. Pre-processing of 16S amplicon sequence reads

16S rRNA amplicon raw reads sequenced from faecal samples were obtained from two previously published datasets which are publicly available on the European Nucleotide Archive (ENA) under accession number PRJEB43835 (McCarthy et al., 2022) and PRJNA414072 (Clooney et al., 2021; Table 1). Firstly, raw reads were processed using the filterAndTrim function in the DADA2 package with the parameters trimLeft = 19, maxEE = 2, truncLen = 240 (version 1.18; Callahan et al., 2016). We used the forward reads only for analysis owing to the lower quality present in the reverse reads, which is known to negatively impact sample inference downstream in the DADA2 pipeline. Read dereplication, learning of the error rates, and sample sequence variant inference with pooled samples followed by the construction of amplicon sequence variant (ASV) table and removal of chimaeras were performed using DADA2. Taxonomic assignment of reads was carried out using the SILVA database (v138.1) also in DADA2 (Quast et al., 2013). In order to remove any dataset specific noise (study effect) we conducted all analysis at the genus level. In addition, we also applied a quality filtering step removing rare genera from the analysis, keeping those present in 10% or more of samples.

2.3. Biostatistical analysis

All biostatistical analysis was carried out in Rstudio (version 4.1.1; RStudio Team, 2020). Both alpha (α) diversity and beta (ß) diversity (Bray–Curtis dissimilarity) were calculated using the phyloseq (version 1.36; McMurdie and Holmes, 2013) and the vegan (version 2.7; Oksanen et al., 2022) packages in R. To test for differences in ß-diversity between the groups we used Permutational Analysis of Variance (PERMANOVA) whilst controlling for the study effect and patient identifier. Principal component analysis (PCoA) was used to visualise differences in microbiota composition and conducted using the dudi.pco and s.class functions of the ade4 (version 1.7) package (Thioulouse et al., 2018). To perform quantitative comparative evaluation of differences observed in microbiota composition we use a median centroid testing methodology as previously employed by us (Cronin et al., 2022). This metric is obtained whereby the median PCoA coordinates of a specific group are calculated, and the distance of all other samples from this point is subsequently determined. In order to establish significantly differentially abundant genera between groups we used Analysis of Compositions of Microbiomes with Bias Correction (ANCOMBC; version 2.2.0; Breiman, 2001). The machine learning random forest approach was used to identify the most discriminatory features when comparing different groups using the randomForest package in R (version 4.7; Breiman, 2001). The approach used to identify the Co-Abundance Groups (CAGs) was carried out as previously reported by us (Flemer et al., 2017; Cronin et al., 2022). Briefly, we used ward-D2 clustering on the genera-level spearman correlation matrix and CAGs were subsequently identified by cutting the generated dendrogram to obtain four distinct clusters of genera. Differences in overall habitual diet were visualised on a PCoA using a Kendall tau distances. Spearman correlations were carried out using the corr.test function of the psych package (version 2.3.3; Revelle, 2017). Logistic regression was used to identify dietary ingredients, drugs or clinically relevant metadata factors which co-varied between groups. Associations between microbial genera and inflammatory markers were tested using the CCREPE package (version 1.36). Heatmaps were generated using the heatmap.2 function in the gplots package (version 3.3.1). Network plots were generated using the gephi (version 0.10.1; Bastian et al., 2009). Chord diagrams were generated using the chordDiagram function in the circlize package (Gu et al., 2014). All other graphics were produced using the ggplot2 package (version 3.4.2). Furthermore, where rank normalised genera abundances were used they were calculated using the formula [rank(x)-(min(rank(x)))/max(rank(x))-min(rank(x))]. Statistical significances (unless otherwise stated) were calculated by employing the non-parametric Kruskal–Wallis' test with Dunn's *post hoc* test where three or more groups were being compared. Where only two groups were compared, a Wilcoxon's test was used. All *p*-values presented in this study were FDR corrected using the Benjamini Hochberg method.

2.4. Inflammatory marker measurement

Serum samples corresponding to patients with HS from McCarthy et al. (2022) dataset were analysed for several markers of inflammation. For measurement of adipokines (leptin and adiponectin), growth-arrest specific 6 (Gas6), TNFα, IL-6, and C-reactive protein (CRP), venous blood was drawn into 2.5 mL vacuette tube (red top) which contained a serum separator clot activator (Grenier Bio-One International). Serum was allowed to clot at room temperature for approximately 30 min. Subsequently the serum was separated by centrifugation (5,000 rpm for 15 min). Serum concentrations for each inflammatory marker was determined using an enzyme-linked immunosorbent assay (ELISA; Protein Simple-Simple Plex Cartridge Kit, BioTechne). Samples were prepared and loaded into the cartridge

according to a standard procedure provided by the manufacturers with all steps in the immunoassay procedure automated by the Ella instrument (Biotechne). All ranges for detection and quantification are provided in detail for each of the proteins evaluated from the company website documentation. Human complement C5a levels in serum were also measured using an ELISA method (Abcam—ab193695). ELISA based calprotectin assay (R&D Inc. S100A8/S100A9) was performed with faecal material from each patient with HS as per the manufacturers protocol.

3. Results

3.1. Microbiota composition in most patients with HS differs significantly from that of patients with CD

Principal coordinate analysis (PCoA) was carried out using the Bray–Curtis dissimilarity β-diversity measure to investigate differences in microbiota composition at the genus level (Figure 1A). Given that the study population contained data from different sequencing runs as well as multiple longitudinal samples from some individuals, we adjusted for the study effect and patient identifier as confounders. Using this method, we identified statistically significant microbiome separation between all groups (PERMANOVA FDR-corrected $p < 0.0001$: $R^2 = 0.06$). Interestingly, large differences in microbiota composition were observed between patients with HS and patients with CD (Pairwise PERMANOVA FDR-corrected $p < 0.001$: $R^2 = 0.08$). In line with what was previously reported (Clooney et al., 2021; McCarthy et al., 2022), patients with either HS (Pairwise PERMANOVA FDR-corrected $p < 0.001$: $R^2 = 0.07$) or CD (Pairwise PERMANOVA FDR-corrected $p < 0.001$: $R^2 = 0.07$) both had a significantly different genus-level microbiota composition to controls. Statistically significant microbiome separation (Bray–Curtis dissimilarity) was also observed at the ASV level (PERMANOVA FDR-corrected $p < 0.0001$: $R^2 = 0.04$) after controlling for the study effect and patient identifier as confounders (Supplementary Figure 1). Next, we conducted a quantitative comparative evaluation of the reported differences in microbiota composition using a median centroid testing methodology calculated from the PCoA of Bray–Curtis dissimilarity in Figure 1A. This calculates the similarity between the microbiota of all faecal samples and the median PCo1 (X-axis) and PCo2 (Y-axis) coordinates (presented in Figure 1A) of the control group. Ultimately this calculation provides the distance between each sample and the control group median centroid. Statistically significant differences were observed between the HS and CD patient groups for this measure (Figure 1B). The majority of patients with HS harboured a microbiota that was a much smaller distance from the control median centroid than those with CD. In keeping with this observation, no significant differences could be found when comparing the HS and control study groups ($p = 0.37$). Interestingly, there was no significant difference in two α-diversity measures (Shannon and Simpson) between HS and CD (Supplementary Figures 1A,B). However, patients with HS or CD had a significantly lower microbiota α-diversity when compared to controls (Supplementary Figures 1A,B).

Given the differences in microbiota composition observed between HS and CD, we wanted to determine what specific genera were significantly differentially abundant between the groups. Thus, we conducted ANCOMBC differential abundance analysis controlling for the study effect (Figure 1C; Supplementary File 1). In total, 24 genera were significantly enriched or depleted between patients with HS and patients with CD. Of these genera, 4 were significantly enriched in the HS microbiota (Figure 1C). *Faecalibacterium* was the most significantly elevated of these taxa followed by *Subdoligranulum*, *Romboustia* and *Clostridium_senu_stricto_1, respectively* (Supplementary Figure 2). 20 genera were significantly more abundant in patients with CD compared to those with HS (Figure 1C; Supplementary Figure 2). Of these 20 taxa, *Defluviitaleaceae_UCG_011* and *Marvinbryantia* were the most significantly enriched in the CD microbiota. Other genera in this list included *Bacteroides* and *Escherichia_Shigella*, both of which have been associated with CD in previous studies. Whilst clear differences exist in the faecal microbiota of both HS and CD, we find here that the microbiome of both inflammatory disease states can be characterised by a shared set of 11 genera which are significantly enriched compared to the control group (Figure 1C; Supplementary Figures 3, 4). This core set of inflammatory disease associated genera included *Streptococcus*, *Veillonella*, *Eggerthella* and *Anaerotruncus*, amongst others. The microbiota of HS and CD was also characterised by a shared set of depleted taxa compared to controls (Figure 1C; Supplementary Figures 3, 4). This set of taxa included multiple genera in the *Ruminococcaceae* and *Lachnospiraceae* families as well as genera such as *Coprococcus_2* and *Christensenellaceae_R_7_group*.

A powerful approach for analysing and predicting complex interactions within microbiome data, particularly in the context of comparing disease states to healthy conditions, is the implementation of the machine learning algorithm Random Forest. Using this method to compare the faecal microbiota of HS and CD we were able to construct a predictive model with an error rate of 9% (91% accuracy). Interestingly, we found that the HS enriched taxon *Subdoligranulum* was the most important discriminatory taxon in classifying between the two inflammatory diseases (Figure 1D). Of the top 15 most discriminatory genera in this random forest classifier model, 12 genera were higher in abundance in patients with HS whilst only 3 were found in a higher abundance in those with CD (Figure 1D). For patients with HS this random forest classifier predicted 41% of patients as having a microbiota composition that resembles CD. Comparing the HS faecal microbiota to controls using this same methodology predicted that 43% of HS patients were classified as having a normal microbiota composition. Overall, this second model also exhibited high accuracy as the reported error rate was 14% (86% accurate). The HS enriched taxa *Eggerthella* and *Erysipelatoclostridium* were the most discriminatory genera in classifying between HS and healthy study populations (Supplementary Figure 5; Supplementary File 1). Other discriminatory genera (higher in controls) at the top of this list included members of the *Ruminococcaceae* family as well as *Coprococcus_1*, *Alistipes* and *Butyricimonas*. For context, repeating the random forest machine learning method but this time comparing the healthy and CD study population also resulted in a model with a low error rate of 12% (88% accuracy; see Supplementary Figure 6 for the top 15 most discriminatory genera).

Thus, whilst HS and CD microbiota share a set of common enriched or depleted genera compared with the control microbiota, there are significant differences in microbiota composition between

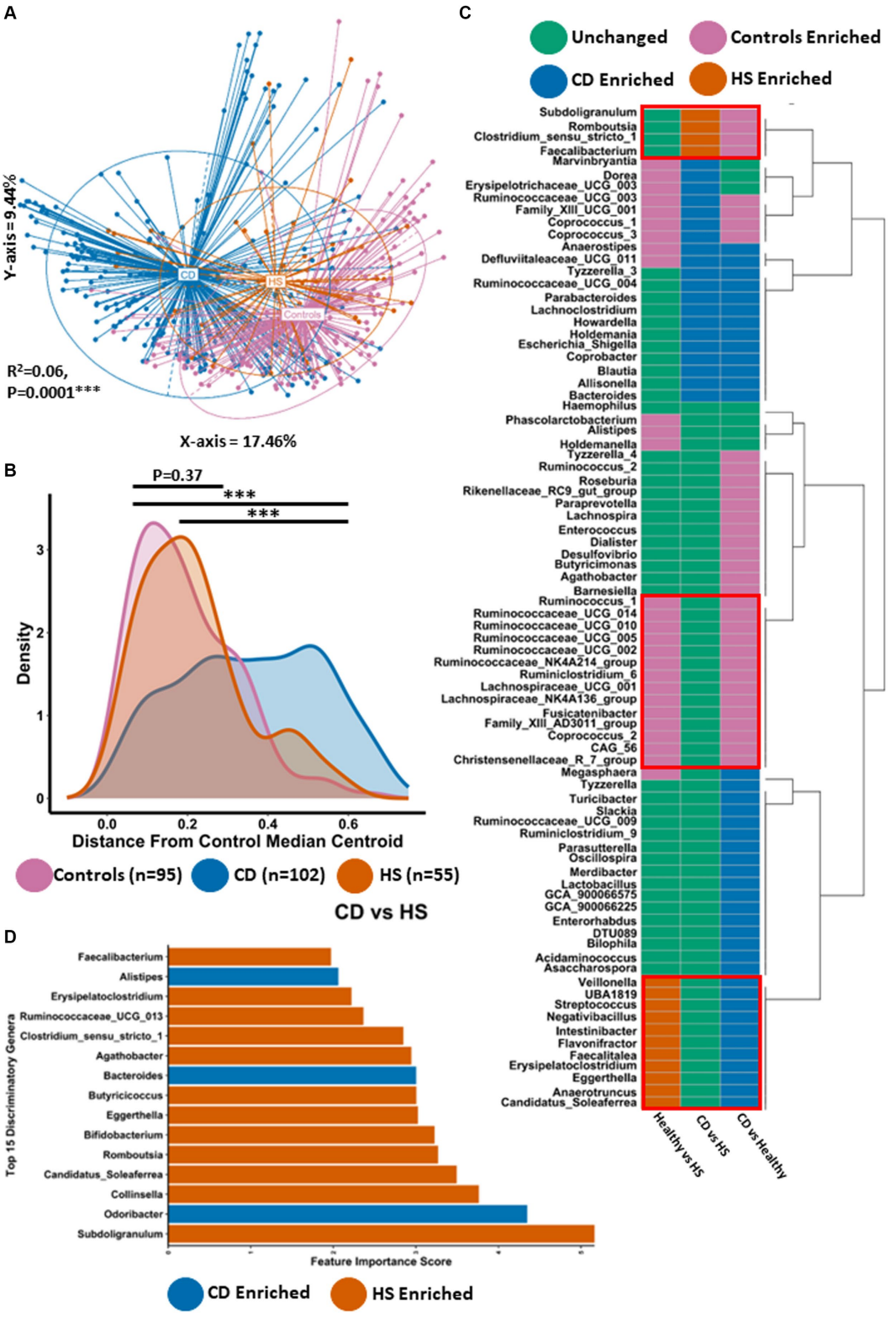

FIGURE 1
Microbiota composition in HS is significantly different to CD for most individuals. **(A)** Principal Component Analysis (PCoA) of β-diversity (Bray–Curtis dissimilarity) at the genus level (16S rRNA gene amplicon profiles). The *P*-value (0.0001) obtained using a PERMONOVA shows there is statistically significant microbiome separation between the groups even after controlling for the study effect and patient identifier as confounders. The eigen values are also reported which show the variation reported in the X-axis (17.46%) and Y-axis (9.44%) of the PCoA. **(B)** Using the PCoA coordinates from **(A)** the median control centroid was calculated and the distance of all samples from those coordinates was subsequently determined and displayed here as a density plot. Density is displayed on the Y-axis whilst the actual reported distance from the control median centroid is displayed on the X-axis. Kruskal-Wallis with Dunn's *post hoc* test was used to determine significant differences between the groups for this distance measure. The annotations

(Continued)

FIGURE 1 (Continued)
used for p-values are $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$***. All displayed p-values are FDR corrected. **(C)** ANCOMBC differential abundance analysis was sued to determine significantly differentially abundant genera between the groups controlling for the study effect and patient identifier. Each colour signifies whether that specific genera was unchanged (green), higher in controls (purple), higher in patients with CD (blue) or higher in patients with HS (orange). **(D)** Barplot depicting the top 15 most discriminatory genera from a machine learning random forest classifier comparing HS and CD. The colour indicates whether that genera is found in higher abundance in HS or CD.

the groups. However, the machine learning random forest approach indicates that some, but not all patients with HS have a similar microbiota composition to CD.

3.2. HS and CD have different distinctive co-abundance group microbiota profiles

To further understand compositional microbiota differences between the HS and CD inflammatory diseases, we employed a co-abundance group (CAG) analysis, which is an approach previously used successfully in our group (Flemer et al., 2017; Cronin et al., 2022). CAGs are clusters of microbial taxa that consistently occur together and exhibit similar abundance patterns across multiple samples. Briefly, the CAGs or clusters are determined using Spearman correlations of genera abundances followed by hierarchal clustering. CAGs provide further insight into the co-occurrence and interactions amongst different microbial genera within a community. Using this method, we identified four CAGs which we named based on their composition (Figure 2A), specifically, *Ruminococcus cluster*, *Lachnospiraceae cluster*, *Pathogen cluster 1* and *Pathogen cluster 2*. The detailed taxon memberships of each CAG are shown in Supplementary File 2.

To statistically test if a CAG was associated with a healthy microbiota composition, we measured Spearman correlations between CAG relative abundance and distance from the control median centroid position which was previously calculated for Figure 1B. Members of the *Ruminococcus cluster* were observed to have strong negative correlations with the distance from the control median centroid (Figure 2B). This indicates that the *Ruminococcus cluster* is associated with a microbiota composition characteristic of controls in this study, as the genera in this cluster have a higher abundance as a function of shorter distance from the control median centroid. The *Lachnospiraceae cluster* was significantly different to the *Ruminococcus cluster* for this measure (Figure 2B). However, the abundance of members in the *Lachnospiraceae cluster* were recorded as negatively correlating with the distance from the control median centroid indicating that this CAG is also health associated. It is also important to note that both CAGs associated with a normal (control) microbiome comprise genera which are thought to be putatively beneficial and capable of fibre-fermentation. Both CAGs (*Ruminococcus cluster* and the *Lachnospiraceae cluster*), were significantly different to *Pathogen cluster 1* and *Pathogen cluster 2, respectively* (Figure 2B). Members of the *Pathogen cluster 1* were observed to have strong positive correlations with the distance from the control median centroid. Similar findings were observed for *Pathogen cluster 2* albeit to a lesser degree. These findings indicate that *Pathogen cluster 1* and *Pathogen cluster 2* are not associated with a normal microbiota composition as the abundance of their members is found at a lower level the larger the distance from the control median centroid.

The findings presented in Figure 2B were strengthened when we compared the abundance of the CAGs across the three study groups (Figures 2C–F). The microbiota composition in controls was characterised by a high abundance of the *Ruminococcus cluster* and the *Lachnospiraceae cluster* as well as a low abundance of *Pathogen cluster 1* and *Pathogen cluster 2* (Figures 2C–F). Interestingly, for the *Ruminococcus cluster*, patients with HS had a significantly higher level than patients with CD (Figure 2C). Both inflammatory disease groups were observed to have a significantly lower abundance of this CAG when compared to individuals in the control group. Patients with HS also had a significantly higher level of the health associated *Lachnospiraceae cluster* when compared to patients with CD (Figure 2D). Interestingly, no significant difference was detected between the HS and control study groups for the abundance of the *Lachnospiraceae cluster* or *Pathogen cluster 1* (Figures 2D,F). CD had the highest abundance of *Pathogen cluster 1* and this was found to be significantly different to patients with HS as well as controls. Patients with HS had the higher abundance of *Pathogen cluster 2* which was significantly higher than patients with CD and controls (Figure 2E). Patients with CD also maintained a significantly higher level of this cluster compared with the control group.

Thus, CAG analysis reveals different microbiome profiles between the groups. Although patients with HS have a higher level of the health associated *Ruminococcus cluster* and the *Lachnospiraceae cluster* than patients with CD, their faecal microbiota is characterised by a high abundance of *Pathogen cluster 2*. Members of this CAG include the putatively pathogenic *Bilophila*, *Dialister*, *Parabacteroides* and *Anaerostipes*, amongst others (Supplementary File 2). The CD faecal microbiota is characterised by a high abundance of Pathogen cluster 1 whose members include a different set of pathogenic genera such as *Streptococcus*, *Escherichia_Shigella*, *Enterococcus* and *Veillonella* (Supplementary File 2).

3.3. Similar habitual Western diet in patients with HS and those with CD

We conducted PCoA using the Kendall tau distance measure to investigate differences in habitual diet (daily frequency of consumption) of the study datasets (Figure 3A). We observed statistically significant separation between the groups (PERMANOVA FDR-corrected $p < 0.0001$: $R^2 = 0.06$). Habitual diet in patients with HS was significantly different to controls. This finding was confirmed when we measured the similarity (distance) between individuals daily dietary pattern and the coordinates of the control median centroid as calculated from Figure 3A. As expected, patients with HS were a significantly larger distance from the median centroid than controls

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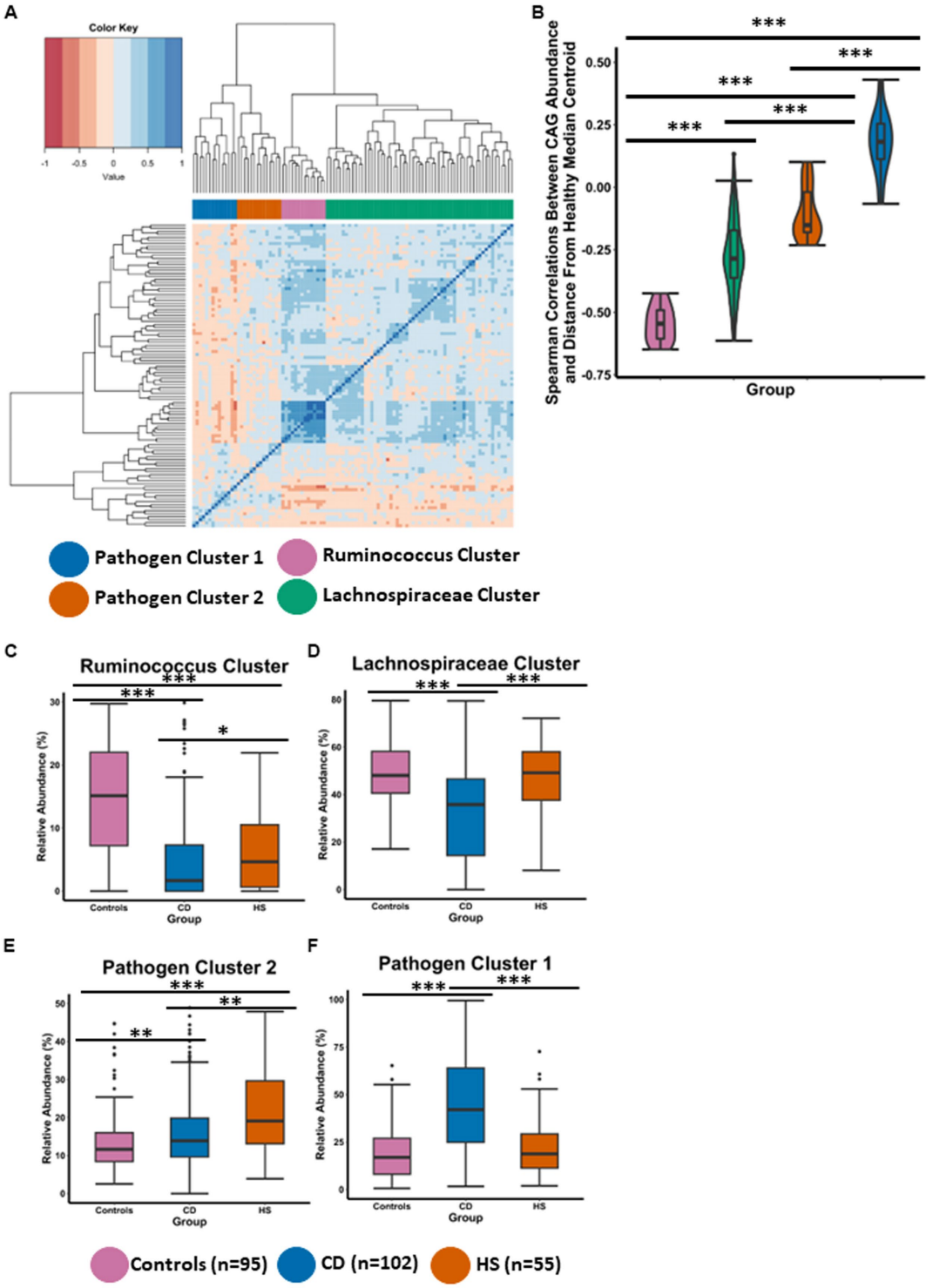

FIGURE 2
HS and CD are characterised by different distinctive co-abundance group (CAG) microbiota profiles. **(A)** Heatmap illustrates the ward.d2 clustering of Spearman correlation coefficients representing the relative abundance of genera in the faecal microbiota of individuals included in this study. Each distinct co-abundance group (CAG) is visually distinguished by its corresponding colour as indicated by the legend to the right. **(B)** Violin plot showing the spearman correlations between CAG abundance and the control median centroid which was previously determined for Figure 1B. **(C–F)** Boxplots showing the relative abundance (%) of each CAG across the three groups. Statistical significance was calculated using Kruskal-Wallis with Dunn's *post hoc* test. The annotations used for *p*-values are $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$***. All displayed *p*-values are FDR corrected.

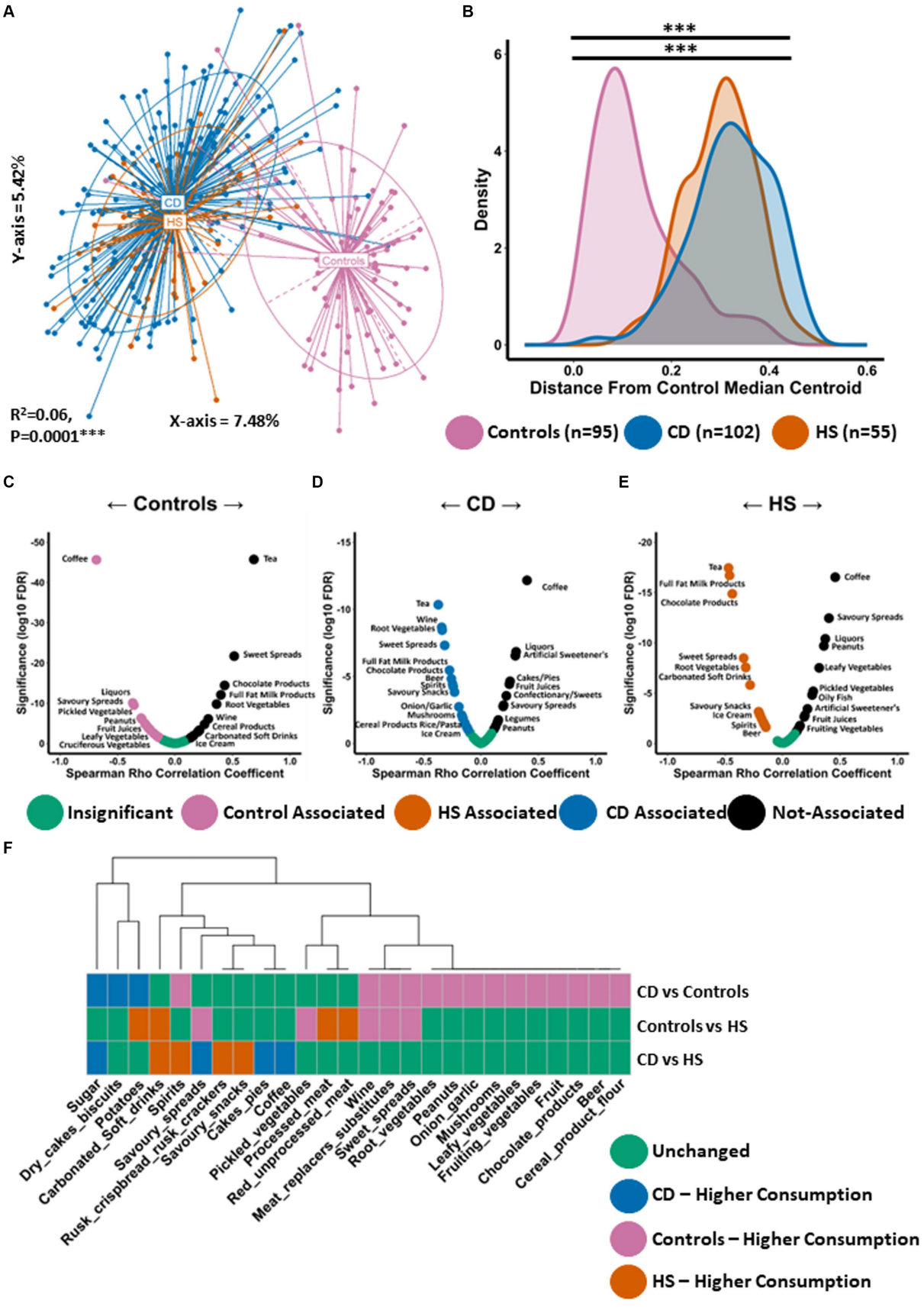

FIGURE 3
Habitual diet of patients with HS resembles the Western diet similar to patients with CD. **(A)** Principal Component Analysis (PCoA) of habitual dietary profiles (Kendall tau distance) based on daily frequency of consumption. The P Value (0.0001) obtained using a PERMONOVA shows there is statistically

(Continued)

FIGURE 3 (Continued)
significant separation between the groups. The eigen values are also reported which show the variation reported in the X-axis (7.48%) and Y-axis (5.24%) of the PCoA. **(B)** Using the PCoA coordinates from **(A)** the median control centroid was calculated and the distance of all samples from those coordinates was subsequently determined and displayed here as a density plot. Density is displayed on the Y-axis whilst the actual reported distance from the control median centroid is displayed on the X-axis. Kruskal-Wallis with Dunn's *post hoc* test was used to determine significant differences between the groups for this distance measure. The annotations used for *p*-values are $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$***. All displayed *p*-values are FDR corrected. **(C,D)** Volcano plot showing spearman correlations between the consumption of dietary ingredients and **(C)** the median control centroid, **(D)** the CD median centroid and **(E)** the HS median centroid. A significant negative correlation indicates that a specific dietary ingredient is associated with the median pattern of that patient group as its consumption increase the shorter the distance to the median centroid. Significant positive correlations indicate that a specific food item is not associated with the median dietary of that patient group as it consumption increases the larger the distance from the median centroid. **(F)** Wilcoxon test was conducted to determine what food items were differentially consumed between each pairwise comparison and shown in a colour coded heatmap. Each colour signifies whether that specific genera was unchanged (green), higher in controls (purple), higher in patients with CD (blue) or higher in patients with HS (orange).

(Figure 3B). Interestingly, the HS habitual diet was not significantly different to that of CD (Figures 3A,B).

Given the large difference in habitual diet between patients with HS and controls, we wanted to establish what specific dietary ingredients were most or least associated with the median dietary pattern of each group. We conducted Spearman correlation analysis between the consumption levels of each dietary ingredient with the distance from the median centroid of each study group respectively, as calculated from Figures 3A,C–E and Supplementary File 3. As expected, the control group was observed to have a strong negative correlation with the distance from the control median centroid for a number of food items considered to be health promoting and high in dietary fibre (Figure 3C). This list included a variety of vegetables (cruciferous, leafy and pickled) as well as fish (Figure 3C; Supplementary File 3). Dietary ingredients which were least associated (positive correlation) with controls using this approach included chocolate products, ice-cream and carbonated soft drinks, all of which are elements of the Western diet. In total, 29 different food types significantly correlated with the distance from the control median centroid (Supplementary File 3). Using this approach, an opposing trend was identified for CD (Figure 3D; Supplementary File 3) and HS (Figure 3E; Supplementary File 3). When exploring habitual dietary patterns in HS, we found 36 individual food items to significantly correlate with the distance from the HS median centroid (Supplementary File 3). A number of food items whose consumption is typical for the Western diet were found to negatively correlate with the distance from the HS median centroid (Figure 3E), including processed meat, sugar, carbonated soft drinks, ice-cream and chocolate. Several alcoholic beverages were also found to be associated with the HS diet including beer, wine and spirits. Dietary ingredients which were least associated with the HS diet (positive correlation) included fish, a variety of vegetables (pickled, leafy and fruiting) as well as fruit (Figure 3E; Supplementary File 3).

Next, we identified the specific food items that were differentially consumed between the groups. We performed a Wilcoxon test for all individual pairwise comparisons, the full results of which can be found in Supplementary File 3. Although overall habitual diet was similar between HS and CD, with both conditions being associated with a frequency of consumption pattern typical of the Western diet, there were a small number of food items differentially consumed. For example, patients with CD consumed significantly more sugar and cakes/pies than patients with HS (Figure 3F). The HS study group was found to consume larger quantities of carbonated soft drinks and alcohol (spirits), for example (Figure 3F). Compared to controls, patients with HS were found to consume significantly more carbonated soft drinks and processed meat (Figure 3F). Furthermore, patients with HS consumed significantly less pickled vegetables and meat replacer substitutes. The latter is likely an indication of vegetarianism amongst some individuals within the control study population.

Thus, the dietary pattern in patients with HS is a Western type diet, similar to that linked with CD. Dietary ingredients found to positively associate with HS were high in sugar and saturated fat. In addition, food items least associated with HS included vegetables and fruit which are high in dietary fibre and health promoting.

3.4. Some patients with HS have a microbiota configuration similar to that of patients with CD

In a previous study, we calculated the relatedness between samples (HS and controls) based on their microbiome composition (ASV level) using Spearman's rank correlation coefficient and subsequent hierarchical clustering with the Ward2 method (McCarthy et al., 2022). This hierarchical clustering revealed the presence of two HS microbiome clusters. The first cluster was composed exclusively of patients with HS. The second cluster was comprised of healthy controls as well as the remaining patients with HS. When we compared these two distinct microbiome groups of patients with HS, we found that one group was significantly older and had a lower microbiome α-diversity (McCarthy et al., 2022). At the time of publication, no other factor was detected as co-varying with the observed differences in microbiota composition. Building on this finding, we showed above using the machine learning (random forest) approach that 41% of patients with HS were predicted as having a microbiota composition resembling CD (Figure 1E). Furthermore, we also established using this same approach that 42% of patients with HS were predicted as having a microbiota composition similar to controls (Supplementary Figure 5). With respect to the findings from both McCarthy et al. (2022) and this current study, we wanted to further investigate differences in microbiota composition in patients with HS.

We used an improved approach for identifying differences in microbiota composition within a single study group which was in keeping with findings (in this current study) detected at the genus level. We calculated the relatedness between samples solely from patients with HS (not including healthy controls) using ranked genera abundances and subsequent Ward2 clustering (Figure 4A). Using this approach, we identified two groups which separated based on genus-level faecal microbiota composition. The first group represented the

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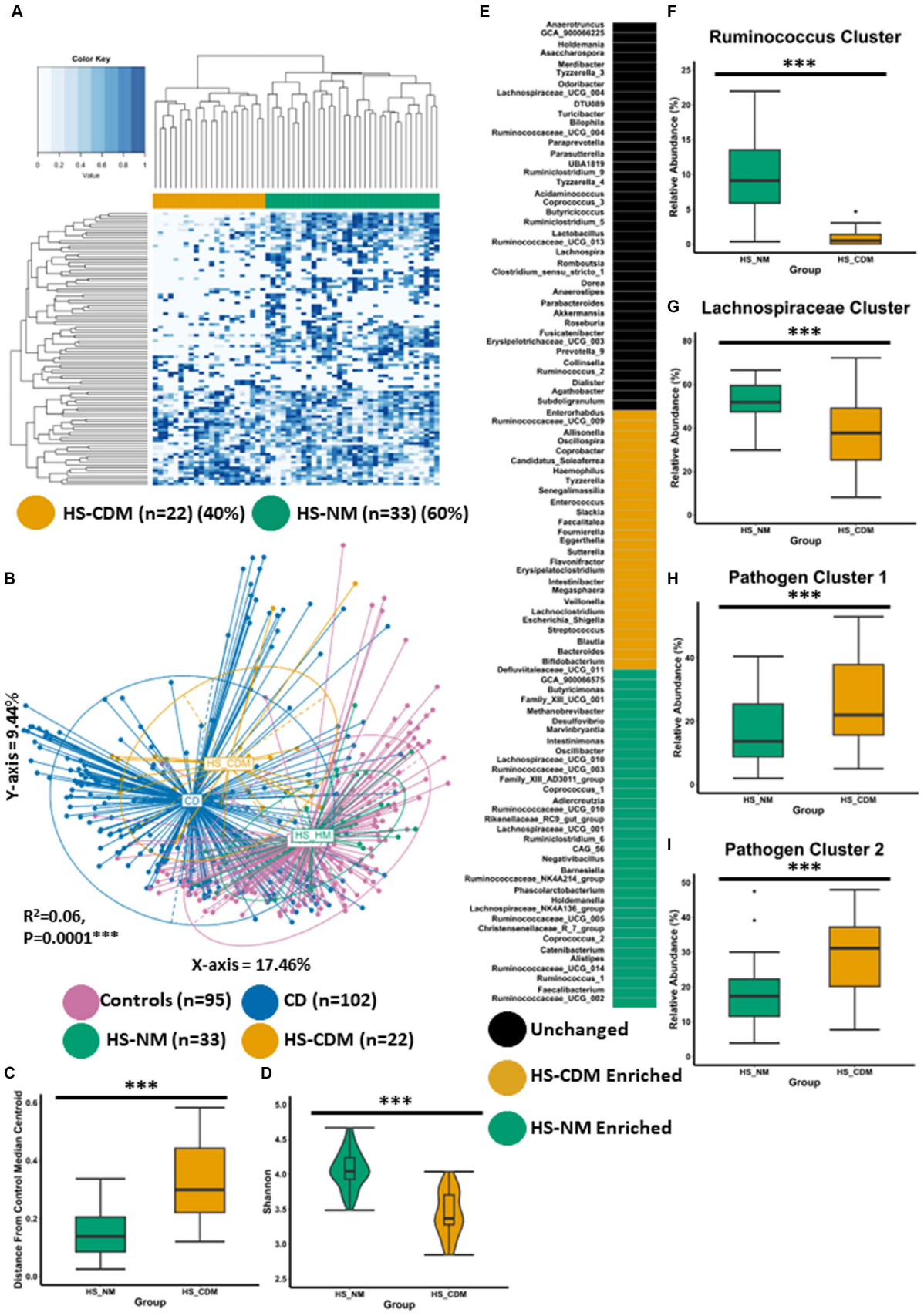

FIGURE 4
Some patients with HS have a microbiota configuration similar to patients with CD. **(A)** Heatmap showing the genus profiles of all patients with HS. Through Ward.d2 clustering two genus level microbiome groups were identified labelled HS-NM (HS-Healthy-like microbiota; green) and HS-CDM

(Continued)

FIGURE 4 (Continued)
(HS-Crohns disease-like microbiota; yellow). **(B)** Principal Component Analysis (PCoA) of β-diversity (Bray–Curtis dissimilarity) at the genus level (16S rRNA gene amplicon profiles). The *p*-value (0.0001) obtained using a PERMONOVA shows there is statistically significant microbiome separation between the groups even after controlling for the study effect and patient identifier as confounders. The eigen values are also reported which show the variation reported in the X-axis (17.46%) and Y-axis (9.44%) of the PCoA. **(C)** Using the PCoA coordinates from **(A)** the median control centroid was calculated and the distance of samples (HS-HM and HS-CDM) from those coordinates was subsequently determined and displayed here as a box plot. **(D)** Boxplot showing Shannon microbiome α-diversity between the groups. **(E)** ANCOMBC differential abundance analysis was sued to determine significantly differentially abundant genera between the groups. Each colour signifies whether that specific genera was unchanged (black), higher in HS-HM (green), higher HS-CDM (yellow). **(F–I)** Boxplots showing the relative abundance (%) of each CAG across the two groups of patients with HS. Wilcoxon test was used to determine significant differences between the groups. The annotations used for *p*-values are $p < 0.05$*; $p < 0.01$**; $p < 0.001$***. All displayed *p*-values are FDR corrected.

majority of patients with HS (60%) whilst the second group represented 40% of the study group (Figure 4A). Next, we wanted to better understand how these groups compared to the microbiota of controls and patients with CD. We conducted PCoA of β-diversity (Bray–Curtis dissimilarity) at the genus level showing statistically significant microbiome separation between the groups (PERMANOVA FDR-corrected $p < 0.0001$: $R^2 = 0.06$). We observed that one cluster of patients with HS had a microbiota composition most similar to the normal microbiota composition detected in controls and was subsequently named HS-NM (HS-Normal-like microbiota; $n = 33$; Figure 4B). The second group of patients with HS compromising 40% of the population was observed to have a microbiota composition most similar to patients with CD and was subsequently named HS-CDM (HS-Crohn's disease-like microbiota; $n = 22$; Figure 4B). This finding was further corroborated with the observation that the HS-CDM patient cluster was a significantly greater distance from the control median centroid than the HS-NM group (Figure 4C). Patients in the HS-NM group were also shown to have a significantly higher microbiome α-diversity (Figure 4D; Supplementary Figures 7A–C).

To identify the specific genera that are significantly differentially abundant between HS microbiome patient clusters, we conducted ANCOMBC differential abundance analysis whereby we identified 60 genera as being significantly different between the two groups (Figure 4E; Supplementary File 4). 34 genera were identified as being significantly higher in the HS-NM patient group as compared to HS-CDM. Many of these genera had also been identified as being significantly higher in controls when compared to patients with CD (Figure 1C). This included several members of the *Ruminococcaceae* and *Lachnospiraceae* families as well as, *Faecalibacterium*, *Buyricimonas* and *Coprococcus_1* (Figure 4E). Meanwhile, patients in the HS-CDM group were found to have significantly higher levels of several genera typically enriched in CD, including *Escherichia_Shigella*, *Bacteroides*, *Enterococcus*, *Streptococcus* and *Veillonella* (Figure 4E). When we compared CAG abundance between HS-NM and HS-CDM the results were strikingly similar to what we observed when comparing controls to patients with CD (Figures 2C–F). HS-NM had a significantly higher abundance of the *Ruminococcus cluster* and the *Lachnospiraceae cluster* than patients in the HS-CDM group (Figures 4F–G). Likewise, HS-CDM was observed to have a significantly higher level of the disease associated *Pathogen cluster 1* and *Pathogen cluster 2* (Figures 4H–I). These observations confirm that some patients with HS have a microbiota configuration that is strikingly similar to that of patients with CD and it builds upon our previous work identifying differential microbiome trajectories in HS.

3.5. Different microbiota configurations in HS can be replicated in a published dataset

To test if we could validate our findings, we obtained the only other publicly available 16S rRNA gene amplicon faecal microbiota dataset (Lam et al., 2021). We analysed faecal microbiota data from 17 individuals with HS obtained from Lam et al. (2021), in a pilot study that was conducted in the Netherlands. We used the same approach as this current study, calculating the relatedness between samples using ranked genera abundances and subsequent Ward2 clustering (Supplementary Figure 8). Using this method, we were able to identify two distinct microbiome clusters in line with our earlier findings (referred to as Lam-HS-NM and Lam-HS-CDM). First, we wanted to compare the abundance of genera which were associated with a normal microbiota composition between the two clusters. We found that the Lam-HS-NM patient group had significantly higher levels of "normal" genera, as identified in Figure 1C (when comparing controls to patients with CD), than the Lam-HS-CDM group (Supplementary Figure 9). Patients in the Lam-HS-CDM group had significantly higher levels of genera associated with CD (also identified in Figure 1C; Supplementary Figure 10). This analysis further validates the existence of two different microbiota configurations in patients with HS.

3.6. Medication and diet metadata associate with differences in microbiota composition in HS

Given the wide range of factors known to influence microbiota composition, we wanted to establish whether any clinically relevant metadata (including demographics, disease severity, treatment history, drug consumption and habitual diet) differed between the HS-NM and HS-CDM patients groups. The data available for this analysis is presented in Supplementary File 5. We found that patients in the HS-NM group were significantly older than individuals in the HS-CDM group (Supplementary Figure 11A). In addition, we also discovered that the HS-NM group were significantly older at the time of HS diagnosis than patients in the HS-CDM group (Supplementary Figure 11B). Naturally, both factors (age and age of diagnosis) strongly correlated (positive) with one another ($p = 0.003$). Hurley score which is a measure of disease severity in HS was not detected as being significantly different between the groups. In fact, when we compared the microbiota composition between the different levels of severity in HS (3 being the most severe) using PCoA of Bray–Curtis dissimilarity, we did not detect any significant differences

(Supplementary Figure 12). Thus, disease severity in HS is not associated with differences in faecal microbiota composition. Antibiotic usage (within the last year) was significantly higher in the HS-CDM patient group compared to HS-NM (Figure 5A). Given that specific species of skin microbiota may drive inflammation in HS, antibiotic treatment is one of the most common first line treatments for management of the disease. Interestingly, a number of patients from both groups were taking antibiotics at the time of sample collection (Supplementary File 5), although no significant difference in current use could be detected between the groups. Although other treatments used to manage HS in this study population are known to influence microbiota composition, including anti-TNFα therapy (infliximab), immunomodulator therapy and surgery, we could not detect any differences in the number of patients between the HS-NM and HS-CDM group using these treatments.

Next, we focused on identifying secondary drugs (i.e., drugs that are not directly used to treat HS such as antibiotics) whose consumption co-varied between the HS-NM and HS-CDM study groups. In order to do so effectively we only wanted to examine informative variables which had a large effect size. Thus, we conducted logistic regression on intake data for medications which were consumed in 20% or more of the study population (removing those with a small effect size), whilst adjusting for a number of demographic (gender, age, age at diagnosis) and clinically important characteristics (disease severity and treatment history; Supplementary File 6). Using this approach, we identified a group of cardiovascular drugs as significantly co-varying with differences in faecal microbiota composition in patients with HS (Table 2). This group of drugs includes aspirin, statins, beta-blockers, angiotensin II receptor antagonists and angiotensin-converting enzyme (ACE) inhibitors, all of which are used to treat cardiovascular disease. Furthermore, when we compared the consumption levels of cardiovascular drugs between the groups, we found it was significantly higher in patients from the HS-NM group (Figure 5B).

To examine the consumption of specific dietary ingredients with respect to differences in microbiota composition in HS (HS-NM and HS-CDM), we also used logistic regression adjusting for demographics (gender, age, age at diagnosis) and clinically relevant metadata (disease severity and treatment history). To do this effectively we removed dietary ingredients which had a small effect size (Cohen's $d < 0.5$) from our models. Using this method, we identified one dietary ingredient as co-varying with differences in microbiota composition in HS (Table 2). Specifically, we found that carbonated soft drink consumption was significantly higher in patients in the HS-CDM group than those in the HS-NM group (Figure 5C; Supplementary File 6). Interestingly, no significant difference was observed between HS-NM and HS-CDM for overall habitual diet (Supplementary Figure 13).

TABLE 2 Diet and drug consumption co-varies with differences in microbiota composition.

	Estimate	Std error	Z-value	Pr(>Chi)	Q-value
Cardiovascular medications					
Intercept	−1.51972	2.17419	−0.699		
Age	0.04841	0.06289	0.77	0.033877*	0.12
Age at diagnosis	−0.06025	0.0666	−0.905	0.041790*	0.12
Gender	0.70949	1.01584	0.698	0.774202	0.86
Hurley score	−0.58962	0.65072	−0.906	0.642929	0.82
Surgery for HS	0.97826	0.9454	1.035	0.890866	0.89
Antibiotics (last year)	2.4279	1.24088	1.957	0.049694*	0.12
Current anti-TNFα therapy	−1.03846	1.33051	−0.78	0.661523	0.82
Previous anti-TNFα therapy	−0.30019	1.35177	−0.222	0.323765	0.53
Immunomodulator therapy	20.91855	7375.77	0.003	0.09492	0.18
Cardiovascular medications	−20.4486	2805.03	−0.007	0.002749**	0.02*
Carbonated soft drinks					
Intercept	−1.32478	2.0772	−0.638		
Age	−0.00713	0.04909	−0.145	0.029712*	0.09
Age at diagnosis	−0.0633	0.05497	−1.151	0.088597	0.19
Gender	−0.23199	1.19189	−0.195	0.7154	0.89
Hurley score	−0.50905	0.65224	−0.78	0.974009	0.97
Surgery for HS	0.82643	0.99168	0.833	0.836732	0.92
Antibiotics (last year)	2.73648	1.49051	1.836	0.011686*	0.05
Current anti-TNFα therapy	−0.27681	1.17628	−0.235	0.604441	0.86
Previous anti-Tnfα therapy	−2.13611	1.46653	−1.457	0.211059	0.35
Immunomodulator therapy	19.28246	2732.849	0.007	0.097685	0.19
Carbonated soft drinks	3.55746	1.25323	2.839	0.001033**	0.01*

The annotations used for p values are $p < 0.05$*; $p < 0.01$**; $p < 0.001$***.

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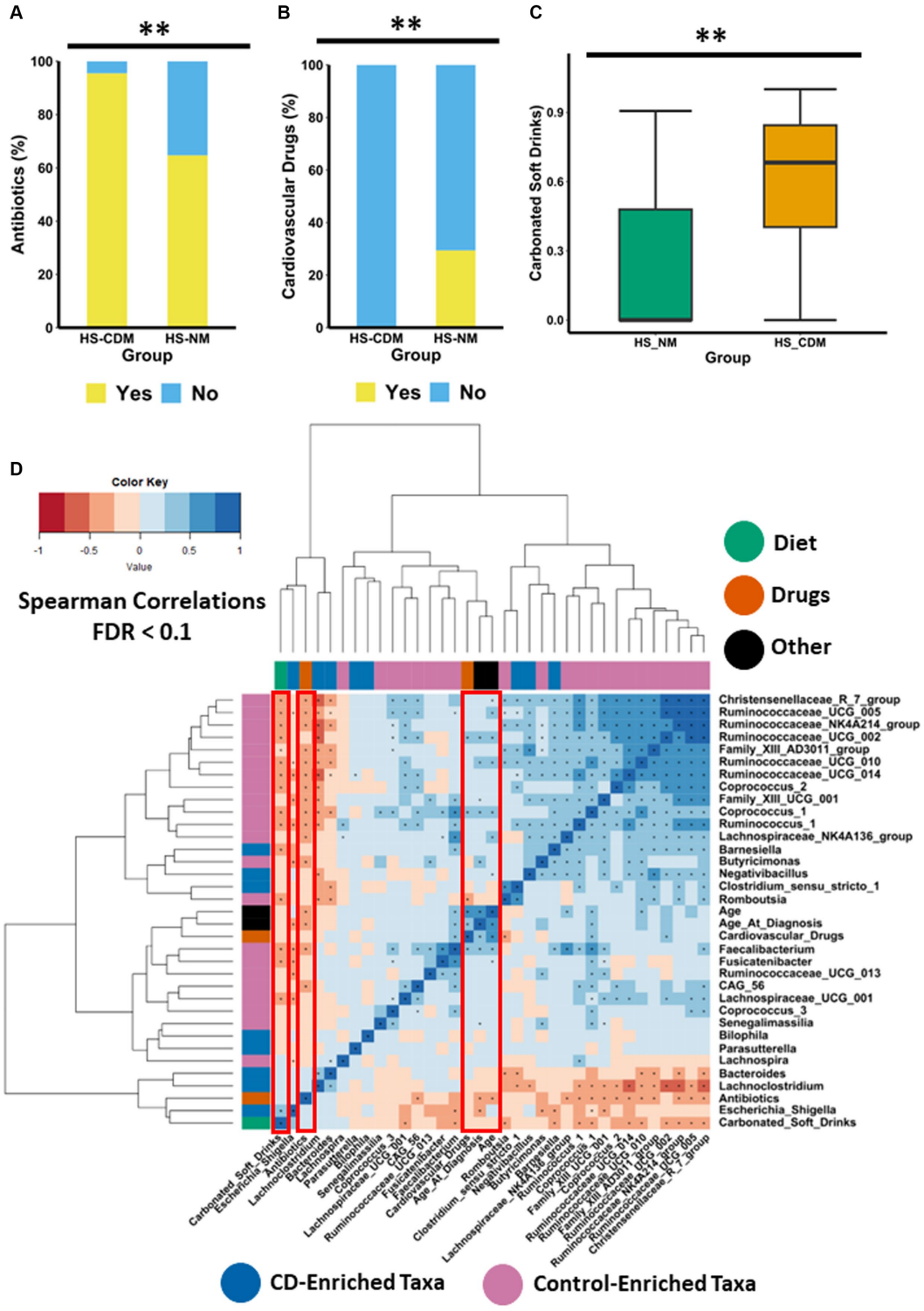

FIGURE 5
Drugs and diet associate with differences in microbiota composition in HS. Stacked barplot showing the consumption levels of **(A)** antibiotics and **(B)** cardiovascular drugs between the HS-NM and HS-CDM groups. Significance was tested using Fishers exact test. **(C)** Boxplot showing the daily frequency of consumption of carbonated soft drinks between the groups. Significance was tested using a Wilcoxon test. The annotations used for *p*-values are $p < 0.05$*; $p < 0.01$**; $p < 0.001$***. All displayed *p*-values are FDR corrected. **(D)** Heatmap showing correlations between specific taxa and age, age at diagnosis, antibiotics, carbonated soft drinks and antibiotics. The taxa used in this correlation analysis were identified as the top 30 discriminatory features from machine learning random forest classifier comparing controls to CD. Significance was assumed at <0.1* and all *p*-values are FDR corrected.

To identify host-associated factors that might associate with specific microbiota, we conducted spearman correlation analysis between diet, drugs, age and age at diagnosis with the top 30 genera from a random forest classifier comparing healthy and CD. Interestingly, we found that carbonated soft drink intake had a strong positive correlation with *Escherichia_Shigella* (Figure 5D). Carbonated soft drink consumption in HS was also observed to negatively correlate with 13 different genera identified as being enriched in controls, including *Christensenellaceae_R7_group*, *Coprococcus_2*, *Coprococcus_1*, *Faecalibacterium* and several genera in the *Ruminococcaceae* family (Figure 5D). Cardiovascular drugs were shown to positively correlate with the health associated *Coprococcus_2* and *Ruminococcaceae_UCG_002*. Antibiotics had strong negative associations with 16 genera enriched in the control faecal microbiota (Figure 5D). Interestingly, both age and age of diagnosis had strong positive correlations with numerous health associated genera, whilst the latter was also observed to have a strong negative association with *Escherichia_Shigella* (Figure 5D). To identify how these factors associated with CAGs, we determined spearman correlations (Supplementary Figure 14). As expected, carbonated soft drink consumption negatively correlated with the relative abundance of the *Ruminococcus cluster* and *Lachnospiraceae cluster* (Supplementary Figure 14). Antibiotics were also observed to have a negative association with the *Ruminococcus cluster*. Both cardiovascular drugs and age had a strong negative association with *Pathogen cluster 2*.

3.7. Faecal microbiota in HS associates with an inflammatory phenotype

Although changes to the microbiota in CD are likely secondary to disease development, there is clear evidence that a number of species enriched in the CD microbiota directly contribute a pro-inflammatory effect maintaining intestinal inflammation and disease progression (Sartor, 2008; Baker et al., 2014; Schirmer et al., 2019; Candelli et al., 2021; Pavel et al., 2021). Given that we detected a minority of patients with HS as having a microbiota composition similar to CD, we hypothesised that inflammatory phenotypes might also differ between the HS-NM and HS-CDM patients groups. To test this hypothesis, we quantified the concentration of 10 different inflammatory markers from serum in patients with HS. This included several cytokines (IL-12, IL-23, IL-6 and TNFα) as well as the adipokines leptin and adiponectin (Supplementary File 7). We also measured C-reactive protein (CRP), complement component 5a (C5a) and the anti-inflammatory, the vitamin-k dependent protein, Growth Arrest-Specific gene 6 (Gas6; Supplementary File 7). Furthermore, we also quantified the intestinal inflammatory marker faecal calprotectin which is commonly used as a detection method for CD (Vernia et al., 2020). When we compared the levels between patients in HS-NM and HS-CDM for all 10 markers of inflammation only Gas6 was found to be significantly different (Figure 6A and data not shown). Serum Gas6 (ng/mL) levels were significantly higher in patients in with a healthy-like microbiota composition (HS-NM; Figure 6A).

Next, we wanted to establish whether the levels of inflammation associated with the abundance of specific gut microbiota genera in patients with HS. To do so we conducted linear regression between the 10 inflammatory markers and the 60 genera which were identified as being significantly differentially abundant between the HS-NM and HS-CDM groups in Figure 4E. The complete results are shown in Supplementary File 7. Our models revealed a total of 55 different associations between genera abundance and inflammation in HS (Figure 6B). IL-12 which is an important cytokine in CD development had the most associations (12) of any inflammatory marker, followed by C5a (9) and IL-23 (7) (Figure 6B). In order to further investigate this relationship, we conducted CCREPE (Compositionality Corrected by REnormalization and PErmutation) analysis between the ranked inflammatory markers and genera abundances. We identified a number of associations between inflammation and taxa associated with CD (Figure 6C). IL-12 serum levels were found to positively correlate with the HS-CDM enriched *Eggerthella*, *Candidatus_Solefera* and *Erysipelatoclostridium* (Figure 6C; Supplementary File 8). IL-12 also negatively correlated with the abundance of 9 health associated genera enriched in the HS-NM patient group. This included *Faecalibacterium*, *Holdemanella*, *Lachnospiraceae_NK4A136_group*, *Lachnospiraceae_UCG_010* and *Ruminococcaceae_UCG_003*. C5a was also found to have a positive association with *Eggerthella* whilst calprotectin levels positively correlated with *Streptococcus* and *Candidatus_Solefera*. Adiponectin which is thought have be anti-inflammatory showed a positive association with the HS-NM enriched *Holdemanella* (Figure 6C; Supplementary File 8). The opposite was observed for both CRP and Leptin levels which had strong negative correlations with this health-associated genera. Interestingly, leptin also had a strong positive association with the HS-CDM enriched Bacteroides genera. Levels of Gas6 were found to co-vary with differences in microbiota composition in HS and they positively correlated with the abundance of the health associated *Ruminiclostridium_6*. Gas6 was also detected as negatively correlating with two CD associated genera, *Escherichia_Shigella* and *Erysipelatoclostridium*. In summary, the differences in faecal microbiota compositions detected were associated with different levels of inflammatory markers in patients with HS. These findings highlight that the HS-CDM microbiota associates with a greater inflammatory phenotype.

4. Discussion

In this current study we report that some patients with HS have a faecal microbiota configuration characteristic of CD. However, the majority of patients with HS were detected as having a "normal" microbiota composition most similar to controls. Antibiotics, which are a common first line treatment for HS, were a key covariate of distinct microbiota compositions in HS. We also detected several associations between the microbiota and inflammation levels in patients with HS, including IL-12, which is directly implicated in CD pathogenesis. These findings highlight the potential of the faecal microbiota as a biomarker in identifying patients with HS at risk for development of CD.

Specifically, we reported that the microbiota in 40% of patients with HS was enriched with genera such as *Escherichia_Shigella, Veillonella* and *Enterococcus* which is characteristic of CD. Several species belonging to these genera are known to promote intestinal inflammation. For example, adherent invasive *Escherichia coli* can produce outer membrane vesicles which increases the secretion of pro-inflammatory cytokines (Barnich and Darfeuille-Michaud, 2007; Eaves-Pyles et al., 2008; Rolhion et al., 2010; Kim et al., 2013; Agus

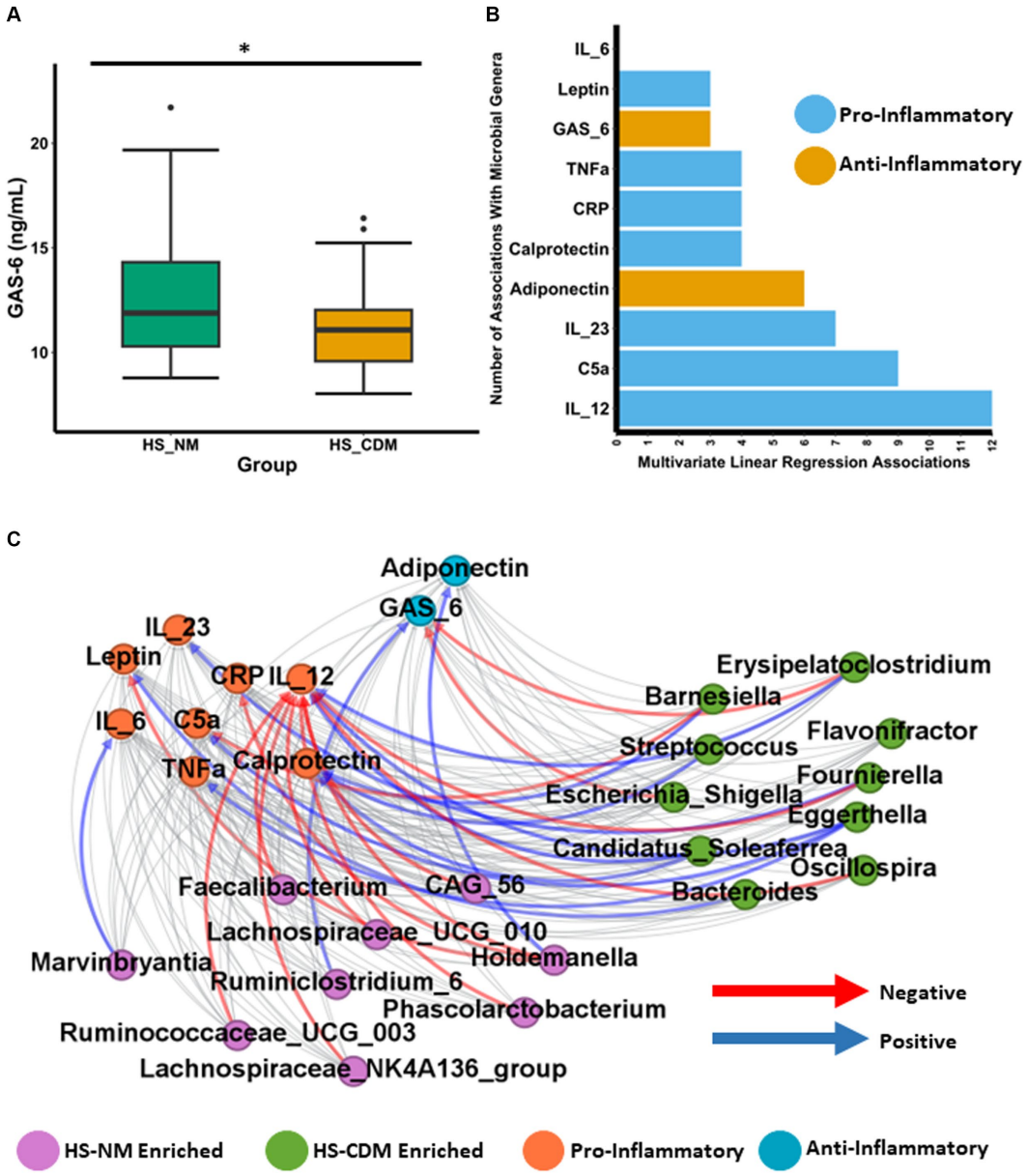

FIGURE 6
Faecal microbiota in HS associates with an inflammatory phenotype. **(A)** Boxplot showing the levels of Growth Arrest-Specific 6 (Gas6) in serum of patients with HS. Wilcoxon test was used to determine significance. The annotations used for p-values are $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$***. **(B)** Barplot showing the number of significant associations (as determined using linear regression) detected between each specific inflammatory marker and general identified as being significantly differentially abundant between the HS-NM and HS-CDM group through ANCOMBC. **(C)** Network plot showing significant correlations between inflammatory markers and specific genera as determined using CCREPE.

et al., 2014; Kaparakis-Liaskos and Ferrero, 2015; Lee et al., 2019; Mirsepasi-Lauridsen et al., 2019). *Enterococcus faecalis* can secrete metalloproteases (Steck et al., 2011; Zhou et al., 2016) which degrade the intestinal mucosa, an important event in CD pathogenesis (Marônek et al., 2021). Higher abundance of the oral bacteria *Veillonella parvula* which is common in CD, is directly linked with higher levels of the pro-inflammatory compound nitrate which promotes colonisation by this species (Rojas-Tapias et al., 2022). We also reported a depletion of beneficial microbiota (*Faecalibacterium* spp.) in patients with HS who had a CD microbiota configuration. Some of these taxa may exert a protective effect for the host. For example, *Faecalibacterium prausnitzii* secretes an anti-inflammatory protein which can inhibit the NF-κB pathway in intestinal epithelial cells (Quévrain et al., 2016). Our findings suggest that patients with HS who have a CD microbiota configuration may be at risk for higher levels of microbiota induced intestinal inflammation than patients with a microbiota composition resembling controls.

Drug consumption and diet were identified as important covariates of compositional differences in patients with HS. We identified that antibiotic use negatively associated with the abundance of several health associated microbiota in patients with HS. Antibiotics are directly associated with the risk of new-onset CD development (Ungaro et al., 2014; Theochari et al., 2018). Specifically, antibiotic use has been associated with lower numbers of protective commensals which can

open a niche for typically low abundant pathogenic species (Modi et al., 2014; Haak et al., 2019). Cardiovascular drug consumption was higher in patients with HS who have a "normal" microbiota composition. The group of drugs have been shown to significantly alter microbiota composition, including ACE inhibitors (Vich Vila et al., 2020), beta-blockers (Weersma et al., 2020), angiotensin II receptor antagonists (Zhernakova et al., 2016), aspirin (Prizment et al., 2020) and statins (Vieira-Silva et al., 2020). Interestingly, the *Bacteroides2* enterotype which is characterised by a high abundance of *Bacteroides* and low abundance of *Faecalibacterium* has been associated with systemic inflammation and is found in high levels in patients with IBD (Vandeputte et al., 2017; Vieira-Silva et al., 2019). An important study examining this inflammatory enterotype (*Bacteroides2*) in obesity showed that statin treatment negatively correlated with its abundance suggesting a beneficial role for this drug in the context of the microbiome (Vieira-Silva et al., 2020). Although no differences were reported in overall habitual diet between HS microbiome groups, we detected a significantly higher consumption of carbonated soft drinks in individuals with a CD-like microbiota configuration. High intake of carbonated soft drinks was reported to increase risk of CD development (Yang et al., 2019). Key ingredients of carbonated soft drinks include simple sugars (glucose, sucrose and fructose) and emulsifiers (Vilela et al., 2018). Both of these ingredients have independently been shown to alter microbiota composition and exacerbate inflammation in murine colitis models (Chassaing et al., 2015; Khan et al., 2020).

We also report significantly lower levels of the Gas6 protein in patients with HS who have a CD microbiota configuration. Gas6 interacts with TAM receptors maintaining immune homeostasis through TLR signalling (Lemke, 2013; Rizzi et al., 2023). Gas6 can exert an anti-inflammatory through activation of TAM receptors present on activated T regulatory cells (Lemke and Rothlin, 2008; Sainaghi et al., 2013; Bellan et al., 2016; Cohen et al., 2019; Rizzi et al., 2023). Interestingly, Gas6$^{-/-}$ mice exhibited more severe DSS-induced colitis (Akitake-Kawano et al., 2013). We also observe negative correlations between the levels of several putatively beneficial microbiota and the pro-inflammatory cytokine IL-12 in patients with HS. This is important as IL-12 promotes inflammation in CD by regulating the differentiation of naive CD4+ T cells into IFNγ-producing TH1 cells (Macdonald et al., 2016; Moschen et al., 2018; Petagna et al., 2020). Our findings indicate that patients with HS who have low levels of health associated taxa are more likely to have higher levels of IL-12 mediated inflammation.

Diet in HS was characterised by high level consumption of ingredients high in sugar and saturated fat, which is a staple of the Western diet. Consumption of food items high in dietary fibre (such as fruit and vegetables) were least associated with the typical dietary pattern in patients with HS. Interestingly, habitual diet in HS was strikingly similar to patients with CD. HS and CD subjects differed in their consumption of a small number of dietary ingredients, but these food items included sugar (differently consumed in CD) and carbonated soft drinks (differentially consumed in HS) the macronutrient composition of which is similar. Ultimately these findings highlight the need for greater nutritional support for patients with HS. Structured dietary advice and/or dietary intervention represents a promising avenue to alleviate disease burden. Diets high in fibre have anti-inflammatory potential which may help improve symptoms of HS.

One limitation of this study was that previously published data available to validate the findings was limited. Lam et al. (2021) analysed only a small number of samples which we could avail of to conduct replication analysis. A large-scale comparative study will be required to consolidate the findings of this study. Furthermore, comparing the skin microbiota between patients with HS with either a normal or CD-like microbiota configuration was not possible due to the small number of samples available at different dermatological sites. A future study will be conducted to determine whether the findings from this study also extend to the skin microbiome.

Whether alterations in the gut microbiota are a consequence or cause of intestinal inflammation remains to be determined. However, it is clear that intestinal inflammation and changes in oxygen tension can cause major disturbances in microbiota composition. Theoretically, patients with HS identified as having a CD-type microbiota configuration might be at greater risk for development of CD. Prospective longitudinal sampling of the faecal microbiota in HS might enhance our understanding of the role of the intestinal microbiota in the pathogenesis of HS and clarify direct or indirect links with risk of developing CD.

Data availability statement

Publicly available datasets were analysed in this study. This data can be found at: European Nucleotide Archive (ENA) under accession number PRJEB43835 and PRJNA414072.

Ethics statement

Ethical approval was not required for the studies involving humans because the data used was obtained from previously published studies. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

PC: Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. SM: Data curation, Writing – review & editing. CH: Formal analysis, Writing – review & editing. TG: Conceptualization, Writing – review & editing. JC: Conceptualization, Writing – review & editing. A-MT: Data curation, Writing – review & editing. MM: Data curation, Writing – review & editing. EO'C: Conceptualization, Writing – review & editing. FS: Writing – review & editing. PO'T: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Work in PWOTs laboratory was supported by Science Foundation Ireland through a Centre award (APC/SFI/12/RC/2273_P2) to APC Microbiome Ireland, and by the Health Research Board of Ireland

under Grant ILP-POR-2017-034. EO'C, PO'T, and PC were supported by the FIRM award scheme of the (Govt. Ireland; Department of Agriculture, Food and Marine) under grant code 17F251.

Acknowledgments

The authors would like to thank the authors of Clooney et al. (2021) for the data they provided which was integral to this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1289374/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 15 November 2023
DOI 10.3389/fmicb.2023.1281660

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OPEN ACCESS

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RECEIVED 22 August 2023
ACCEPTED 24 October 2023
PUBLISHED 15 November 2023

CITATION
Liu Z and Liu X (2023) Gut microbiome, metabolome and alopecia areata. *Front. Microbiol.* 14:1281660. doi: 10.3389/fmicb.2023.1281660

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Gut microbiome, metabolome and alopecia areata

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Alopecia areata (AA) is a type of dermatological disease characterized by rapid and non-scarring hair loss of the scalp or body skin that may be related to genetic, immunological and physiological factors. It is now believed that AA is associated with oxidative stress, autoimmune disease, neuropsychological factors, pathogens, immune checkpoint inhibitors and microecological imbalance under the premise of host genetic susceptibility. In recent years, studies have revealed the significant role of the gut microbiome or metabolome in many aspects of human health. Diverse studies have revealed that the gut microbiome and metabolome have an important influence on skin conditions. This review highlights the relationship between AA and the gut microbiome or metabolome to provide novel directions for the prevention, clinical diagnosis and treatment of AA.

KEYWORDS

alopecia areata, gut microbiome, metabolome, JAK–STAT signaling, TGF-β signaling, Wnt/β-catenin signaling, oxidative stress

1. Introduction

Alopecia areata (AA) is a type of autoimmune inflammatory dermatological disease characterized by non-scarring hair loss of the scalp or body skin (Zhou et al., 2021). Nearly 2% of the general population undergo AA at some point during their lifetime (Pratt et al., 2017). Although some patchy AA are self-limited, a large proportion of AA are typically relapsing and severe. In these patients, their hair loss is extensive and the course is persistent, which seriously affect their physical and psychological health (Pratt et al., 2017). In pathogenesis, increasing lines of evidence support the notion that under the premise of host genetic susceptibility, AA is triggered by disturbance of inflammatory pathway, oxidative stress, neuropsychological factors and pathogens, companying with comorbidities and microecological imbalance (Alessandrini et al., 2021).

In recent years, studies have revealed the significant role of the gut microbiome or metabolome in many aspects of human health (Gomaa, 2020). Diverse studies have revealed that the gut microbiome and metabolome have an important influence on skin conditions and inflammatory skin diseases (Mahmud et al., 2022), including AA (Sanchez-Pellicer et al., 2022a,b). Especially, fecal microbiota transplantation (FMT) and therapies that alter the gut microbiota or metabolites potentiate the effect of immunotherapies (Baruch et al., 2021; Huang J. et al., 2022), indicating promising application of therapies targeting gut microbiome or metabolome. Brzychcy et al. (2022) revealed a loss of overall richness and a decrease in taxonomic diversity across all the stool samples of the AA patients compared to healthy individuals. While other studies had found no significant changes in α or ß diversity of the gut microbiota structure of AA patients. However, they still hold the opinion that intestinal bacterial biomarkers associated with AA or part of the gut microbial communities should be further

studied as they may be involved in the pathophysiology of AA (Moreno-Arrones et al., 2020; Lu et al., 2021; Rangu et al., 2021).

In this review, we systematically evaluate current data regarding gut microbiome, metabolites and their effects on AA. We discuss potential mechanisms of the gut-skin axis in AA and the link between the gut microbiota or metabolites and oxidative stress (OS) or inflammatory pathways, such as Janus kinases and signal transducers and activators of transcription (JAK–STAT) signaling, transforming growth factor beta (TGF-β) signaling, and Wnt/β-catenin signaling. This review will increase our understanding of the impacts of gut microbiome on AA to aid in finding new medications for AA.

2. Effects of the gut-skin axis and gut metabolites on skin and hair

The existence of the microbiota-gut-brain axis (Cryan et al., 2019) and the gut-brain-skin axis has been investigated and studied in recent years (Mahmud et al., 2022; Sanchez-Pellicer et al., 2022a,b). These two axes play a crucial role in modulating intestinal health, emotional state and inflammation in the human body and skin (Sanchez-Pellicer et al., 2022a,b).

The gut microbiota and gut metabolites regulate both the innate and adaptive immune systems, which in turn influence the homeostasis of the skin (Sinha et al., 2021; Sanchez-Pellicer et al., 2022a,b). Numerous studies have shown that there is a bidirectionality between the gut microbiota and skin homeostasis. Dybiosis in the intestinal microbiome is linked to the development of skin diseases, such as psoriasis, acne vulgaris, atopic dermatitis, and even skin cancers (De Pessemier et al., 2021). Genes affecting gut microbial colonization may induce the Th1 response, leading to the production of interferon (IFN), which signals through the JAK–STAT pathway and then causes abnormal growth of hair follicle cells and ultimately the development of alopecia (Simakou et al., 2019).

3. The pathogenesis of alopecia areata

Although the pathogenesis of AA remains unclear, it is generally believed to be primarily related to loss of hair bulb privilege and autoimmune responses (Strazzulla et al., 2018; Olayinka and Richmond, 2021). AA has been identified as an organ-specific autoimmune disease of the hair follicle with a genetic background (Trueb and Dias, 2018; Olayinka and Richmond, 2021). In recent decades, new insights into the genetics, epigenetics, oxidative stress, autoimmune comorbidities, psychologic stress, and microbiome or metabolome of AA, have updated the etiopathogenetics of AA. These factors work together through several signaling pathways and collectively contribute to AA.

3.1. JAK–STAT signaling pathways

In AA, CD8 + NKG2D + T cells are activated by the stimulators mentioned above, and IFN-γ is produced via the JAK1 and JAK3 pathways, which further promote IL-15 production in follicular epithelial cells via JAK1 and JAK2. Then, IL-15 inversely combines with CD8 + NKG2D + T cells amplify a positive feedback loop that results in the IFN-γ storm (Olayinka and Richmond, 2021; Zhou et al., 2021). In addition, the JAK–STAT pathway can suppress the proliferation and activation of hair stem cells and reduce angiogenesis, therefore plays a part in the premature termination of the anagen phase in AA. Based on these discoveries, JAK inhibitors have been used as a new strategy for the clinical treatment of AA (Sterkens et al., 2021). After binding with JAK, the inhibitor makes it unable to bind and activate STAT and thereby inhibits the entry of the latter into the nucleus for transduction of cytokines, such as IFN-γ, IL-2, IL-7, IL-15 and IL-21, which play a crucial role in the pathogenesis of AA (Montilla et al., 2019).

3.2. TGF-β signaling pathway

TGF-β is a pleiotropic cytokine with regulatory and inflammatory activities (Waskiel-Burnat et al., 2021). TGF-β1 functions as a negative regulator of cell growth, inhibiting epithelial cell growth and influencing the functioning of the immune system by acting as a keratinocyte proliferation inhibitor and apoptosis inducer. In 2015, a genome-wide meta-analysis in AA uncovered new molecular pathways disrupted in AA, including JAK–STAT signaling, autophagy/apoptosis and TGF-β signaling, the latter functioning to induce the differentiation of Tregs that participate in hair biology (Betz et al., 2015). Many experiments and studies have evaluated the change in the serum level of TGF-β in patients with AA. Some results have shown that the serum level of TGF-β in patients with AA is higher than that in healthy controls (Manimaran et al., 2022) whereas others showed the opposite (Tembhre and Sharma, 2013; Alzolibani et al., 2016). Due to these conflicting results, further studies on the role of TGF-β in AA are needed.

Studies revealed that there is a crosstalk between insulin-like growth factor-1 (IGF-1) and TGF-β. Cogent evidence supports that the negative role of TGF-β in cell growth could be suppressed by the complicated influence of the IGF-1 signaling pathway. IGF-1 was proved to prohibit TGF-β-induced apoptosis by neutralizing or isolating IGF-BP3 (an IGF-1 binding protein) (Cohen et al., 2000). Numerous experiments suggested that the activation of IGF-1R could block early steps in TGF-β signaling pathway at the level of TGF-β receptors or the activation of Smad (Danielpour and Song, 2006). It was ever revealed that caffeine could promote hair shaft elongation, stimulate hair matrix keratinocyte proliferation, up-regulate IGF-1 gene expression and protein secretion, and down-regulate TGF-β protein secretion. This may also indicate that TGF-β has an inhibitory effect on hair follicle growth, while IGF-1 may stimulate hair follicle cell growth by interacting with it (Fischer et al., 2014).

3.3. Wnt/β-catenin signaling pathway

The Wnt/β-catenin pathway plays a central role in hair morphogenesis and cycling during the embryonic stage and adult stage. It is the most important pathway in the proliferation and differentiation of hair follicle stem cells (HFSCs) and promotes the renewal, proliferation and differentiation of HFSCs by binding to the lymphoid enhancer factor (Lef)/T-cell transcription factor in the nucleus (Li et al., 2015). AA, an autoimmune disease in which hair follicles prematurely enter the regressive and telogen stage during the anagen phase, may be related to an abnormal regulatory process of the Wnt/β-catenin pathway (Lee et al., 2021).

3.4. Oxidative stress

OS is correlated with the development of many dermal diseases, including AA. OS leads to the loss of immune privilege and facilitates autoimmunity in AA patients by inducing the upregulation of NKG2D ligands (Rajabi et al., 2018). The changes in OS biomarkers such as malondialdehyde, advanced glycation end-products, and ischemic-modified albumin confirm a general pro-oxidative status in AA patients (Peterle et al., 2023). Impaired melanocytes under OS may be the trigger site followed by an attack on hair follicles by the immune system (Xie et al., 2022).

4. Gut microbiome and metabolome and AA

4.1. Gut microbiome and AA

AA is an autoimmune disease characterized by high levels of proinflammatory cytokines that disrupt the anagen growth phase of hair, eventually leading to spot baldness (Mahmud et al., 2022). According to some relevant studies, the proportion of patients with ulcerative colitis is higher in AA patients than in healthy individuals (Simakou et al., 2019). Although several studies found no or very few significant changes in α or β diversity of the gut microbiota structure of AA patients (Moreno-Arrones et al., 2020; Lu et al., 2021; Rangu et al., 2021; Kang et al., 2022), they had found AA-related microbial biomarkers, including *Megasphaera*, *Achromobacter*, *Lachnospiraceae Incertae Sedis* (Lu et al., 2021), *Ruminococcus bicirculans* (Rangu et al., 2021), *Parabacteroides distasonis* and *Clostridiales vadin BB60* (Moreno-Arrones et al., 2020). Even more, the combination of *Parabacteroides distasonis* and *Clostridiales vadin BB60* could predict the disease status of alopecia universalis with an 80% accuracy (Moreno-Arrones et al., 2020). The above results suggest that there may be a critical link between gut microbiome and the genesis and development of AA, and these relative biomarkers therefore have the potential of being used as early diagnosis and therapeutic targets.

Other evidence regarding the relationship between the intestinal microbiome and AA is as follows: a meta-analysis showed that inflammatory bowel disease is a comorbidity of human AA (Maghfour et al., 2021); genes associated with AA may affect the gut microbiota, inducing the Th1 response that leads to IFN-γ production; C3H/HeJ mice are not only an animal model for AA but also a model for spontaneous colitis (Kopper et al., 2021); and epidemiological data have shown that the incidence of AA is related to diet. The prevalence of AA is 1.7% in the United States, which has a Western diet, and less than 1% in Japan, which has a predominantly soy-based diet, suggesting that dietary nutrients affect the onset and development of AA by altering the intestinal microbiota (Villasante Fricke and Miteva, 2015); comparable results could also be observed in animal models such as mice: the use of vancomycin resulted in the overgrowth of *Lactobacillaceae* in the gut of mice, which consumed and reduced other microflora in the gut. Feeding mice a biotin-deficient diet would cause hair loss, suggesting that *Lactobacillaceae* promote alopecia in a biotin-dependent way (Hayashi et al., 2017). In addition, case reports have shown hair regrowth in two AA patients treated with fecal microbiota transplantation, which supports the hypothesis that the gut microbiome plays a part in the pathophysiology of AA (Xie et al., 2019).

Concerning the relationship between the gut microbiome and AA, a generally accepted interpretation is that accumulating autoreactive T lymphocytes gain tolerance against cell apoptosis, which results in the production of inflammatory cytokines by autoreactive Th1 cells and further leads to prolonged chronic inflammation and hair loss. It is now believed that the functionality of T cells is also influenced by the skin microbiota (Edslev et al., 2020).

T-lymphocyte Tregs are an active part of the peripheral tolerance process and are crucial for the prevention of autoimmune diseases. Tregs are particularly abundant in hair follicles (Raugh et al., 2022). In 2017, a study demonstrated that hair follicle Tregs have the ability to accelerate the proliferation and differentiation of stem cells, which in turn stimulate hair regeneration (Ali et al., 2017). More recent studies have also provided evidence that Treg subpopulations from AA lesions are functionally different from those of healthy tissues (Hamed et al., 2019). Based on these facts, many studies have suggested that short-chain fatty acids (SCFAs) could influence both the number and function of gut Tregs, which have an impact on autoimmune diseases such as AA (Smith et al., 2013; Koh et al., 2016). SCFAs produced by gut bacteria such as *Bacteroides*, *Bifidobacterium* and *Lactobacillus* by fermentation from undigested polysaccharides could enhance the barrier function of the epithelium, lower the permeability of the intestinal barrier and interact with skin receptors to affect or modify dermal commensal bacteria (Martin-Gallausiaux et al., 2021). Low fiber intake and a decrease in SFCA-producing bacteria could cause a decrease in SFCA synthesis by gut bacteria. In this way, gut microbiota may have to do with the pathogenesis of AA.

In addition, dysfunction of the intestinal barrier, including destruction of tight junctions and damage to the mucosal layer, could result in increased intestinal permeability, which has also been termed "leaky gut" syndrome (LGS) (Kinashi and Hase, 2021). Under this circumstance of LGS, gut microbiota may translocate and produce a variety of neurotransmitters that have the ability to pass through the intestinal wall into the bloodstream, causing systemic responses and autoimmune diseases such as AA (De Vos et al., 2022). Nevertheless, the association between increased intestinal permeability and AA still lacks experimental or laboratory evidence, which remains to be further investigated.

4.2. Gut metabolome and AA

The gastrointestinal system contains a tremendous number of bacteria, which are capable of producing a huge amount of metabolites. Gut metabolites are molecules with two-sided effects on other organs and the body after entering the circulatory system. That is, the physiological and pathological condition of skin may be related to gut metabolites produced by gut microbiota. Recently, an increasing number of studies on molecular metabolites affecting the host metabolome have emerged, raising questions about the interaction between the gut metabolome and chronic skin diseases.

Human skin and its appendages are target organs of a wide range of neuroendocrine molecules, and we can therefore hypothesize that the abnormal serum levels of some associated neuropeptides and neurohormones have the potential to impact skin health and dermal diseases.

For instance, a study showed that feeding mice a strain of *Lactobacilli* significantly alleviated neurogenic skin inflammation and hair growth inhibition (Arck et al., 2010). This striking result may

be associated with several studies that focus on the production of neurotransmitters, including dopamine, GABA and serotonin, by gut microbiota. Resident gut microbiota carries genes encoding digestive enzymes that the host lacks and therefore provide vitamin K, vitamin B12, biotin, folic acid and other micronutrients that may be responsible for hair growth.

As an example, biotin, also known as vitamin B7, is highly dependent on bacterial production. Biotin is a necessary nutrient for human health, especially skin health and hair growth. Decades ago, scientists discovered that biotin deficiency is related to dermatologic diseases such as alopecia. In an experiment, biotin-deficient germ-free (GF) mice developed alopecia, while conventional mice did not. This indicates that in mice fed a biotin-deficient diet, some gut microbiota would deprive the host of biotin and therefore cause alopecia (Hayashi et al., 2017). Experiments on dopamine have also demonstrated the role it plays in the inhibition of hair growth by promoting catagen induction.

Although the association between gut metabolites and AA remains to be further studied, we can still infer that the gut metabolome is an important part of the pathogenesis of AA.

4.3. Relationship between the gut microbiome or metabolome and the aforementioned inflammatory pathways

4.3.1. Relationship between the gut microbiome or metabolome and the JAK–STAT signaling pathway

Current studies on the influence of the gut microbiome and metabolome on the JAK–STAT pathway are not sufficient. However, there are experiments showing that parenteral nutrition without going through the gut could change the level of phosphorylated JAK1 and STAT6 (Heneghan et al., 2013). Another study showed that the administration of an elemental enteral diet alone decreased the amounts of IL-4, IL-13, polymeric immunoglobulin receptor (pIgR), and sIgA that were positively associated with JAK1 and STAT6. According to an experiment on the effects of baicalin on atopic dermatitis, the activation of the NF-κB and JAK–STAT pathways in the skin of 2,4-dinitrochlorobenzene (DNCB)-treated mice could be inhibited by baicalin, accompanied by a restoration of the gut microbiome and epidermal barrier function. Furthermore, in these pseudo germ-free (GF) DNCB-treated mice, their dorsal skin thickness and EASI score were reduced, and their serum levels of IgE, histamine, TNF-α and IL-4 were inhibited when they received fecal microbiota from baicalin donors (Wang et al., 2022). LPS, a key outer membrane component of gram-negative bacteria in the gut microbiota, acts as a bridge between inflammation and high-fat diet-induced metabolic syndrome via JAK–STAT and AMPK-dependent cPLA2 expression (Chang et al., 2019).

During the development and use of novel medicines, scientists have revealed their biological effects on the JAK–STAT signaling pathway by affecting the gut microbiota or metabolome. IHS, a kind of spore powder, has been proven to influence the gut microbiota and serum metabolites, further affecting JAK–STAT signaling and the abundance of CD8+ T cells and subsequently showing anti-colorectal cancer (CRC) properties (Yang et al., 2022). MOP, purified from *Moringa oleifera* seeds, can remodel the intestinal mucosal barrier and ameliorate dextran sulfate sodium (DSS)-induced gut microbiota dysbiosis by inhibiting JAK–STAT pathway activation and regulating the gut microbiota and its metabolites (Hong et al., 2022). Moreover, JAK inhibitors such as tofacitinib, which have been put into use for the clinical treatment of hair or scalp diseases such as AA, were observed to influence mucosal immunity and gut microbiota abundance while alleviating the disease (Hablot et al., 2020).

Although evidence for the link between the gut microbiome or metabolome and the change in the JAK–STAT pathway is not strong enough thus far, we can still hypothesize that parenteral nutrition, LPS and some medicines that influence the JAK–STAT pathway could also change the gut microbiota or metabolites to a certain extent, then they further affect the expression level of JAK–STAT pathway in follicular epithelial cell via gut-skin axis (Figure 1).

4.3.2. Relationship between the gut microbiome or metabolome and the TGF-β signaling pathway

Previous experiments have shown that the gut microbiome or metabolome has an impact on the TGF-β pathway. For example, *Bifidobacterium breve* M-16 V may prevent the occurrence of allergies by affecting the gut flora, intestinal epithelial barrier and immune system and has potential beneficial effects on infant health. Even in preterm infants, it can stimulate Treg cells to produce regulatory TGF-β1. In addition, *Bifidobacterium breve* M-16 V could enter the gut and induce the maturation of the epithelial barrier, preventing pathogen infection and translocation and inducing anti-inflammatory processes by promoting the secretion of IFN-γ (Cukrowska et al., 2020). Another study demonstrated that the increased abundance of *Eggerthella* in the fecal samples of patients with premature ovarian insufficiency was reversed when receiving hormone replacement treatment. Furthermore, the abundance of *Eggerthella*, metabolic signatures and serum TGF-β1 levels were positively correlated in the same direction (Jiang et al., 2021). Inhalation of carbon nanotubes (CNTs) simultaneously causes lung inflammation, gut microbiota changes, and TGF-β induction, which indicates a modulatory role of gut dysbiosis and gut-to-lung communication (Bhattacharya et al., 2022). *Lactobacillus plantarum* HNU082 (Lp082) protects the gut mucosal barrier, regulates signaling pathways and increases the expression of MPO, TGF-β1 and TGF-β2 (Wu et al., 2022). Similarly, nisin, cecropin, and *P. chinense* have both the ability to change the abundance and composition of the fish gut microbiota and affect the expression of some anti-inflammatory cytokines, such as TGF-β (Ke et al., 2021). Probiotics such as *Lactobacillus plantarum* AR113 could influence liver generation by increasing the expression of TNF-α, HGF and TGF-β (Xie et al., 2021). All these experimental results indicate that there are correlations between the gut microbiota and the TGF-β signaling pathway; however, functional gain- and loss-of-function experiments are still lacking to confirm the direct relationship between the gut microbiome or metabolome and the TGF-β signaling pathway.

Additionally, several studies had shown that *Lactobacillus* appears to be a key microbes altering the levels of IGF-1 and members of it are well-known producers of SCFAs, which is observed to have direct inhibiting effects on GH production through the cAMP/PKA/CREB pathway (Wang et al., 2013; Jensen et al., 2020). Moreover, a few studies point to a role of microbiota on GH and IGF-1 levels which may be mediated through its production of SCFAs and microbial mimetics or impact on intestinal environment and immune system (Yan and Charles, 2018; Jensen et al., 2020).

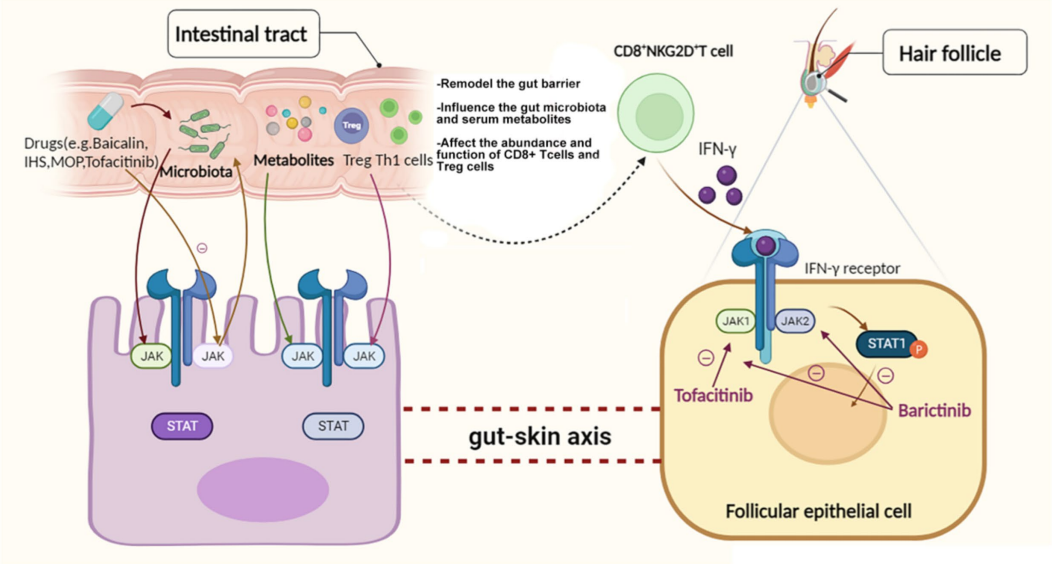

FIGURE 1
Hypothetical relationships among the gut microbiome or metabolome, the JAK–STAT pathway and AA.

4.3.3. Relationship between the gut microbiome or metabolome and the Wnt/β-catenin signaling pathway

Recently, some studies have proven that the gut microbiome plays a role in the onset of several diseases, such as colorectal cancer, by affecting the associated Wnt/β-catenin signaling pathway. Many studies have cast light on a gut metabolite called short-chain fatty acid (SCFA) butyrate, which serves as a histone deacetylase (HDAC) inhibitor. A number of studies have indicated that butyrate has the ability to induce Wnt/β-catenin activity and apoptosis. It is now confirmed that hyperactivation of the Wnt/β-catenin pathway is a major contributor to colorectal carcinogenesis (Tian et al., 2018). *Portulaca oleracea* extract (POE) treatment inhibits the development of colorectal carcinoma in mice and causes changes in the gut microbiota, among which 20 differential microbiota may participate in CRC development via the Wnt pathway (Yi et al., 2022). *Lactobacillus* species also inhibit the progression of CRC by modulating the Wnt/β-catenin pathway (Ghanavati et al., 2020). For intestinal stem cells (ISCs), gut microbiota and metabolites trigger a series of chain reactions involving 5-HT and PGE2, eventually promoting the regeneration of ISCs (Zhu et al., 2022). The Chinese herbal medicine Qingchang Wenzhong decoction has been proven to modulate gut microbiota and may further activate Wnt/β-catenin signals, thus promoting ISC renewal (Sun et al., 2021). Another experiment indicated that gut microbiota dysbiosis and hypoxia can inhibit low-density lipoprotein receptor-related protein 6 (LRP6) and the Wnt/β-catenin pathway, while drugs targeting LRP6 can protect the intestinal barrier and restore the gut microbiota by regulating the Wnt/β-catenin pathway (Liu et al., 2022). Since AA showed an abnormality in the regulation process of the Wnt/β-catenin pathway and a link with the intestinal system, we believe that the gut microbiota and metabolome are also associated with this abnormality.

4.4. Relationship between the gut microbiome or metabolome and OS

Recent experiments have revealed that in the presence of microbiota, gut epithelial cells generate physiological levels of OS and in turn interfere with the composition and function of the microbiota. Since the homeostasis of the gut barrier is affected by the redox ability of the gut microbiota, the permeability of the gut would increase, probably leading to LGS and causing autoimmune diseases such as AA. Emerging evidence has displayed the close interaction between OS and the gut microbiome or metabolome, which is involved in the pathogenesis of neurodegeneration and neuroprotection (Shandilya et al., 2022), inflammatory skin diseases (Ni et al., 2022), and inflammatory bowel disease (Bourgonje et al., 2020). According to recent opinions, gut dysbiosis could influence the redox state and cause inflammation, ultimately leading to inflammatory dermal diseases or previous systemic diseases via the gut-skin or gut-brain axis-related response (Ni et al., 2022). It was revealed by an MRL/lpr mouse model for autoimmune diseases that gut microbiome dysbiosis is associated with increased colonic OS and barrier dysfunction (Wang et al., 2021). Supplementation with a healthy microbiome and specifically *Lactobacillus reuteri* could reverse the redox balance and inhibit the proliferation of CRC cells via the induction of selective protein oxidation (Bell et al., 2022).

4.5. Relationship between the microbiota of hair follicles and the modulation of inflammatory process

There is a significant presence of microbial colonization within the hair follicle funnel, accompanied by an ongoing microbiota-immune system crosstalk at the scalp barrier. Furthermore, the microbiota

extends beneath the hair follicle funnel, particularly abundant in *Propionibacterium acnes* and *Staphylococcus epidermidis*, which may be responsible for conferring immune privilege (Skogberg et al., 2017). A study analyzing the microbiota of the hair follicle of patients with AA revealed that the diversity of hair follicle microbiota in AA patients is higher, with an increase in *Propionibacterium acnes*, and a decrease in *Staphylococcus epidermidis* (Lousada et al., 2021). They further uncovered that *Prevotella copri*, which is confirmed to be involved in the pathogenesis of rheumatoid arthritis (Alpizar-Rodriguez et al., 2019), expressed most abundantly in the subcutaneous tissue of AA, suggesting that *Prevotella copri* evokes the autoimmune disturbance of hair follicles in AA. Additionally, external stimuli can impact the activation state of the immune system dysregulation of the dynamic balance that maintains the immune privilege state for hair follicles. This dysregulation activates abnormal signaling pathways, modulating inflammatory process, resulting in damage to the microecology of stem cells and hindering hair regeneration (Constantinou et al., 2021). More interestingly and unexpectedly, skin-resident bacteria increases the glutamine metabolism in keratinocytes by induction of hypoxia through HIF-1α signaling, further to enhance the regeneration of skin and hair follicles (Wang et al., 2023).

5. Conclusion and perspectives

Alopecia areata is an autoimmune dermatological disease with complex and unclear etiology that causes great physical and psychological damage to patients. With more attention being paid to the gut-skin axis in recent years, we now believe that the gut microbiome and metabolome are likely to play a part in the development of AA. Evidence has increasingly demonstrated that the gut microbiome and metabolome are involved in the pathogenesis of AA. Although there are no direct and conclusive studies to confirm the specific mechanisms between the gut microbiome or metabolome and OS and inflammatory signaling pathways in AA, accumulating studies in other diseases have validated the effect of the gut microbiome or metabolome on the JAK–STAT, TGF-β, Wnt/β-catenin or other signaling pathways, which are also critical pathways involved in the etiopathogenesis of AA (Figure 2). However, many complementary studies need to be conducted to elucidate the complex interactions between the gut microbiota or metabolites and AA.

With the more and more in-depth researches on the impact of gut microbiota or metabolites, the application of interventional approaches, such as probiotics, prebiotics, fermenting microbes and FMT therapies as routine or adjuvant therapeutic strategies, hold promise for novel and effective treatments. Although there were only case reports of FMT from healthy donors by restoring the homeostasis of the gut flora in AA patients (Xie et al., 2019), accumulating clinical evidences support its potential efficacy in treatments of tumors (Davar et al., 2021; Routy et al., 2023), autoimmune diseases (Huang C. et al., 2022), inflammatory bowel diseases (Sokol et al., 2020), especially the latter two that their pathogenesis is also related to JAK–STAT signaling pathway. For applications of probiotics or prebiotics in the field of hair loss, it was reported that cheonggukjang probiotic product, fermented by *Bacillus*, *Lactobacillus*, *Leuconostoc* and *Enterococcus faecium*, could promote hair growth and reverse hair loss (Park et al., 2020). Furthermore, use of probiotics, such as heat-killed *Lacticaseibacillus paracasei* GMNL-653 (Tsai et al., 2023), fermenting microbes (Mayer et al., 2023) in hair care cosmetics, also exhibited the activities of

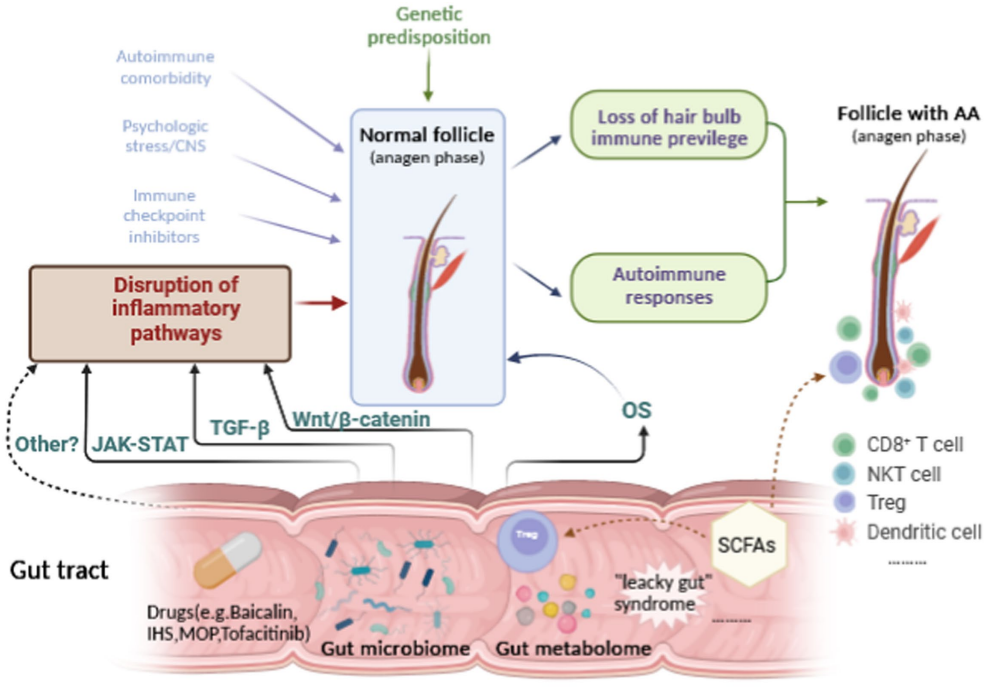

FIGURE 2
Hypothetical relationships among the gut microbiome or metabolome, the inflammatory pathways, OS and AA.

balancing hair microbiota and promoting hair growth. To our excitement, dietary fiber and probiotics improve host response to immune checkpoint blockade in tumor growth (Si et al., 2022; Bender et al., 2023), in addition to their robust efficacy in remodeling intestinal health (Sanders et al., 2019). Therefore, we are fairly confident that probiotics, prebiotics and FMT therapies will be a potential adjuvant therapeutic alternative for AA.

Still, microbial interventions also raise some questions and challenges. First all, although some AA-related microbial biomarkers were reported, there still lacks precise gut microbial biomarkers in AA, especially in alopecia universalis and alopecia totalis in a large number scale of specimens. Furthermore, the functions of these reported biomarkers were not validated by gain- or loss-functional experiments. In addition to *Lactobacillus* and *Bifidobacterium* species, and prebiotics of fructooligosaccharides and galactooligosaccharides, whether there are specific probiotics or prebiotics for AA, it also remains unknown. Secondly, expert consensus statements on FMT (Ng et al., 2020; National Institute of Hospital Administration, et al., 2022), probiotics (Hill et al., 2014) and (Gibson et al., 2017) have been published to guide the clinical use of microbial interventions. However, the administration time, the optimal dose and duration of these treatments for AA have not been completely determined. Therefore, it is essential to actively explore the mechanisms underlying the microbiota-gut-skin cross talk in AA, to precisely pinpoint the functions of the microbial products and their possible host interactions, and to strictly carry out the randomized controlled trials for these microbial interventions in AA.

Author contributions

ZL: Data curation, Visualization, Writing – original draft. XL: Conceptualization, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Natural Science Foundation of Zhejiang Province (LY22H110001).

Conflict of interest

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 16 November 2023
DOI 10.3389/fmicb.2023.1298632

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OPEN ACCESS

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RECEIVED 22 September 2023
ACCEPTED 30 October 2023
PUBLISHED 16 November 2023

CITATION
Seo JY, You SW, Gu K-N, Kim H, Shin J-G, Leem S, Hwang BK, Kim Y and Kang NG (2023) Longitudinal study of the interplay between the skin barrier and facial microbiome over 1 year. *Front. Microbiol.* 14:1298632. doi: 10.3389/fmicb.2023.1298632

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Longitudinal study of the interplay between the skin barrier and facial microbiome over 1 year

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Skin is a diverse ecosystem that provides a habitat for microorganisms. The skin condition and the skin microbiome interact each other under diverse environmental conditions. This study was conducted on 10 study participants for a one-year, from September 2020 to August 2021, to investigate the variability of skin microbiome and skin biophysical parameters [TEWL, hydration, and elasticity (R5)] according to season, and to understand the interplay between skin microbiome and skin characteristics. We identified that *Cutibacterium*, *Corynebacterium*, *Staphyloccocus*, unclassified genus within *Neisseriaceae*, and *Streptococcus* were major skin microbial taxa at the genus level, and fluctuated with the seasons. *Cutibacterium* was more abundant in winter, while *Corynebacterium*, *Staphylococcus*, and *Streptococcus* were more abundant in summer. Notably, *Cutibacterium* and skin barrier parameter, TEWL, exhibited a co-decreasing pattern from winter to summer and showed a significant association between *Cutibacterium* and TEWL. Furthermore, functional profiling using KEGG provided clues on the impact of *Cutibacterium* on the host skin barrier. This study enhances our understanding of the skin microbiome and its interplay with skin characteristics and highlights the importance of seasonal dynamics in shaping skin microbial composition.

KEYWORDS

skin microbiome, TEWL, skin biophysical characteristics, *Cutibacterium*, *Corynebacterium*, *Staphylococcus*, *Neisseriaceae*, *Streptococcus*

Introduction

The skin is a primary physical barrier against the invasion of pathogens, and also is a diverse ecosystem that can be a habitat for microorganisms including bacteria and fungi (Chiller et al., 2001; Fredricks, 2001). The ecology of the skin is varied topographically and influenced by various elements including environment and host factors (Fierer et al., 2008; Grice et al., 2009; Isler et al., 2023). The host's characteristics, such as age and sex, contribute to the diversity of the skin microbiome (Somerville, 1969; Grice and Segre, 2011). On the other hand, the environmental factors such as temperature and humidity, have been reported as stimuli of the growth of skin microbiome (Duncan et al., 1969). The ultraviolet radiation stimulates the overproduction of oil glands and thickens the outermost layer of skin (Pearse et al., 1987; Lesnik et al., 1992), which may lead to the growth of lipophilic microorganisms (Dréno et al., 2018; Kobayashi et al., 2019).

Recent studies have revealed that the skin microbiome plays an important role in maintaining skin health under specific environmental conditions (Gallo and Nakatsuji, 2011; Byrd et al., 2018). The commensal skin microbiome produces substances similar to antimicrobial peptides that contribute to the development and maintenance of the skin's immune system (Cogen et al., 2010). The disruption of the balance between commensal and pathogenic microorganisms can lead to the onset of skin diseases (Fitz-Gibbon et al., 2013; Liu et al., 2020). Additionally, the barrier function of the skin can be influenced by skin microbiome that produces metabolites activating aryl hydrocarbon receptor, which promotes epithelial differentiation and integrity (Uberoi et al., 2021). Typically, studies investigating the associations between microbial community and specific skin phenotypes divide subjects into case and control groups. However, even within these groups, individual physiological variations persist, potentially impacting research outcomes. Intra-individual microbiome assessments are therefore useful for examining substantial associations between microbial variation and the host's skin condition by controlling for individual intrinsic traits. A longitudinal examination of the skin microbiome may reveal the interplay between microbial dynamics and skin characteristics.

To investigate compositional variation of skin microbiome, several studies analyzed bacterial and fungal communities of human skin over various time ranges. The longitudinal studies conducted within 6 months demonstrated that composition and diversity of skin microbiome can vary temporally depending on the skin sites and subjects (Grice et al., 2009; Flores et al., 2014). On the other hand, studies conducted over a longer period of time (1–2 years), which are the most extended time scale for investigation of skin microbiome, showed that the compositional features of the skin microbiome tend to remain relatively stable (Oh et al., 2016; Hillebrand et al., 2021; Schmid et al., 2022). However, although several longitudinal studies on the skin microbiome have been performed, understanding of the complex relationship between skin microbiome and skin characteristics is still limited. This is largely due to the fact that most long-term studies on skin characteristics have been conducted independently of those on the skin microbiome (Youn et al., 2005; Nam et al., 2015; Dolečková et al., 2021), which has resulted in a lack of comprehensive understanding of their interplay.

To investigate the interactions between skin microbiome and skin characteristics, we conducted a longitudinal study tracking changes in the facial skin microbiome and various skin biophysical parameters, over a period of 1 year. We collected microbiome data at weekly intervals with a higher frequency compared to previous researches, enabling the capture of detailed changes in the microbial community and mitigating potential biases in microbiome sampling. Additionally, the skin characteristics of subjects were collected at monthly intervals simultaneously. Our study aims to offer a more comprehensive understanding of longitudinal alterations in microbiome composition and skin properties. It may help understand the interplay between these two traits and estimate the impact of microbial changes.

Abbreviations: TEWL, transepidermal water loss; CCA, Canonical Correspondence Analysis; PERMANOVA, permutational analysis of variance; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Materials and methods

Study participants

Participants with the following characteristics were excluded: (1) those who used systemic or topical antibiotics within 3 months prior to the study, (2) those who had cutaneous disease on the skin, and (3) those who had sensitive skin. A total of 10 healthy Korean participants (7 males and 3 females) voluntarily contributed in study. The mean age of the participants was 32.4 years. During the study period (12 months, from September 2020 to August 2021), all study participants wore a mask at least 8 h a day due to COVID-19. Facial skin microbiome samples were collected by having subjects swab their left cheek once a week using a Copan swab 480 CE (Copan, Brescia, Italy), following an infographic-guided procedure: (1) sampling right after waking up and before washing face, (2) rubbing the left cheek for 1 min. The use of cosmetics was prohibited at least 8 h before skin microbiome sample collection. All participants were required to wear a mask under routine office work conditions and to maintain cosmetic and hygiene routines as consistently as possible. All samples were stored at −80°C until further processing. Three facial skin biophysical parameters of study participants were evaluated once a month with several measurements as follows: Skin surface TEWL was measured using Tewameter® TM 300 (Courage and Khazaka GmbH, Cologne, Germany) and was expressed in grams per square meter per hour (g/m^2/h) (Gardien et al., 2016). Skin hydration was calculated using Corneomter CM 825 (Courage and Khazaka GmbH, Cologne, Germany). Changes in the capacitance of the stratum corneum were measured and were expressed in arbitrary units (CM) (Dąbrowska and Nowak, 2021). Skin surface elasticity was quantified using Cutometer MPA 580 (Courage and Khazaka GmbH, Cologne, Germany). R5 [immediate retraction (Ur)/immediate distension (Ue)], representing the net elasticity of the skin was used as elasticity index (Ohshima et al., 2013). This study was approved by the institutional review board at the LG H&H Research Center (Seoul, South Korea) and all study participants provided an institutional review board-approved written consent form (No. LGHH-20201210-AB-03).

Skin microbiome sequencing

Genomic DNA was extracted from swab sample using the QIAamp mini kit (QIAGEN, Hilden, Germany). Quantity of extracted DNA was measured with a Qubit 4 Fluorometer (Invitrogen, Massachusetts, USA). 16S rRNA gene amplicon libraries were constructed following the instructions of Illumina's 16S rRNA metagenomics sequencing library preparations with some adaptation (Illumina, 2014) as follows: (1) locus specific amplification with two specific primers for v3- v4 variable regions of 16S rRNA gene, 341F (5′ - CCTACGGGNGGCWGCAG - 3′) and 805R (5′ - GACTACHVGGGTATCTAATCC - 3′), (2) purification of the amplicon with AMPure XP beads (Beckman Coulter, Krefeld, Germany), (3) amplification for sample indexing with Nextera XT Index kit (Illumina, California, USA), (4) purification of the amplicon with AMPure XP beads (Beckman Coulter, Krefeld, Germany), (5) validation of constructed library quantity and quality (size and integrity) with Qubit 4 Fluorometer and 2100 Bioanalyzer (Agilent,

California, USA), respectively. Final library of each sample was then normalized and pooled. Pooled final library was loaded into MiSeq Reagent Kit v3 (600-cycle) (Illumina) and was sequenced on an Illumina MiSeq with 2 × 300 bp paired-end read chemistry (Illumina) (Fadrosh et al., 2014). Quality of raw sequence reads were checked using the FastQC (Babraham Institute, Cambridge, UK) and Sequencing Analysis Viewer (Illumina).

16S rRNA gene sequences taxonomy classification

Demultiplexing of sequence reads was conducted by Illumina Miseq Reporter Software automatically. Subsequent pre-processing and clustering of demultiplexed paired-end sequence reads to obtain the clean amplicon sequence variants were processed by Quantitative Insights into Microbial Ecology 2 pipeline (2021.2.0) (Bolyen et al., 2019). In detail, the primer sequences used to amplify the v3- v4 variable regions of 16S rRNA gene were trimmed using q2-cutadapt plugin based on Cutadapt (2021.2.0) (Kechin et al., 2017). After trimming, the denoising of paired-end sequence reads, removal of chimeric sequences, and read fusion were conducted through q2-dada2 plugin based on DADA2 (2021.2.0) (Callahan et al., 2016). With the scikit-learn naïve-bayes model based pre-trained taxonomy classifier on GreenGene 13.8 99% reference database, all processed reads were matched to proper microbiome taxonomy. Further statistical analysis for the facial microbiome in this study was conducted on genus level and skin microbiome samples with low sequencing quality (read count ≦ 12,000, passing filter ≦ 80%) were excluded.

Statistical analysis

All statistical analyses were performed using R version 3.6.0. Relative abundance of microbiome was obtained by dividing the number of reads of one genus by the number of reads of all genus per subject. In order to obtain robust data, the mean value of the relative abundance of the microbiome within each participant per month (each participant was sampled per week, accumulating 4–5 samples in 1 month) was used, and four seasons were classified according to the month (spring: March, April, May; summer: June, July, August; fall: September, October, November; winter: December, January, February). The objective of the study was to observe changes in the overall microbiome. Therefore, the major five genera were selected based on their average relative abundance, which was greater than 1% in at least one-third of the samples. To compare the relative abundance of major taxa between summer and winter, Wilcoxon rank sum test was used.

Shannon diversity was calculated to examine microbiome evenness and richness of each sample (α-diversity) (Olszewski, 2004). Jensen-Shannon distance was also calculated for measuring dissimilarity between microbiome compositions of each sample (β-diversity) (Lin, 1991). To assess the statistical significance of β-diversity, permutational analysis of variance was used on the Jensen-Shannon distance matrices with 999 permutations in vegan 2.5–7 package in R (Anderson, 2001).

Normalization through Z-score transformation was conducted on measured skin parameters and the relative abundance of *Cutibacterium, Corynebacterium*, *Staphylococcus*, unclassified genus within *Neisseriaceae* (F), and *Streptococcus*. Canonical Correspondence Analysis (CCA) (Braak, 1986) was conducted to explain the dispersion of the microbiome communities with reference to factors including weather information, skin biophysical parameters and relationships between these factors. Jensen-Shannon distance matrix was used in microbiome 1.8.0 (Lahti and Shetty, 2017), vegan 2.5-7 (Oksanen et al., 2022), phyloseq 1.30.0 packages in R (McMurdie and Holmes, 2013). All statistical analyses were performed with R software (version 3.6.3.), and the ggplot2 package was used to visualize results (Wickham, 2009). To elucidate the association of skin biophysical parameters and the relative abundance of each five genera, linear regression analysis was conducted while adjusting for individual differences. Linear regression analysis adjusted for weather information (temperature, humidity: downloaded from Korea Meteorological Administration) (Supplementary Figure S1) was also performed. We considered *p*-value significant if less than 0.05. *p*-values were adjusted using the false discovery rate correction method by Benjamini-Hochberg procedure for multiple testing. Kruskal-Wallis test was used to test for significant differences in α-diversity between four season groups. Pairwise Wilcoxon rank sum test was used to compare the relative abundance of each major genus or skin biophysical parameters between pairs of seasons.

Functional profiling of skin microbiome

Functional profiling of the skin microbiome was conducted using q2-picrust2 (2021.2), which is based on PICRUSt2 (Douglas et al., 2020). PICRUSt2, tool for predicting functional abundances based on marker gene sequences, predicted the enriched pathway by inferring the functional profile of the facial skin microbiome based on Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology gene family database (Kanehisa et al., 2004, 2012). For the purpose of identifying the pathway that can explain the difference between groups, the process was applied: comparing the KEGG orthology annotation results from PICRUSt analysis between the groups using the Kruskal-Wallis test for whole seasons, and using Wilcoxon rank-sum test for comparing summer and winter while adjusting individual factors.

Results

Seasonal variation in skin microbiome composition and diversity

We collected a total of 358 skin microbiome data from ten participants by weekly swab sampling and measured individual skin characteristics monthly. The workflow of this study is presented in Supplementary Figure S2 and a summary of the skin biophysical parameters of the study participants is presented in Supplementary Table S1. The skin microbiome compositions at the genus level of study participants on a monthly basis for 1 year are shown in Figure 1. The microbiome composition varied longitudinally, with each individual exhibiting a distinct microbial profile. Among

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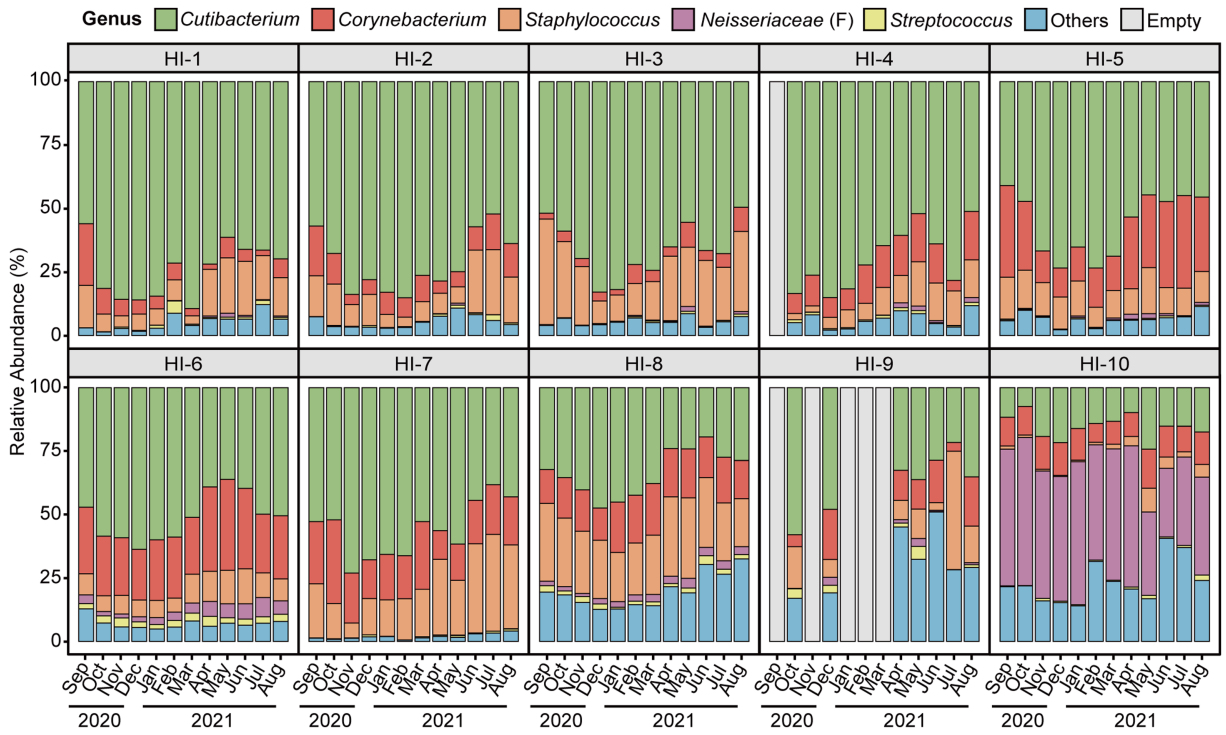

FIGURE 1
The skin microbiome composition of study participants by month of the year. Average per month the relative abundance of major skin microbiome genera (*Cutibacterium*, *Corynebacterium*, *Staphylococcus*, unclassified genus within *Neisseriaceae* (classified only at the family level), and *Streptococcus*) of study participants. Microbial taxa which are found in less than one-third of study participants and having low abundance taxa (<1%) are grouped as 'others'. HI, healthy individual.

various microbiome genera, *Cutibacterium* was the most dominant genus among 9 participants, with an average relative abundance of from 34 to 74% per individual. Additionally, one individual showed unclassified genus within *Neisseriaceae* (classified only at the family level) is the most dominant taxa.

At the genus level, *Cutibacterium*, *Corynebacterium*, *Staphylococcus*, unclassified genus within *Neisseriaceae* (F) and *Streptococcus* were major skin microbial taxa, which accounted for approximately 90% of the skin microbial community composition, and exhibited seasonal fluctuations (Figures 1, 2). In particular, the relative abundance of *Cutibacterium* was statistically significantly more abundant in winter than in summer (63.97 and 45.50%, respectively, p-value = 2.5E-10), while *Corynebacterium*, *Staphylococcus*, and *Streptococcus* were more abundant in summer than in winter (p-value <0.05).

The α-diversity of the microbial community exhibited seasonal variation (Figure 3A), with significant differences observed between the four seasons (p-value = 1.6E-10, Figure 3B). Notably, there were significant differences between most pairs of seasons (p-value <0.05) except for comparing spring 2021 and summer 2021 (Figure 3B). The β-diversity based on Jensen-Shannon distance plots according to season, month, and individuals are presented in Supplementary Figure S3. To examine skin microbiome composition varied longitudinally, a permutational analysis of variance (PERMANOVA) was performed by season and month, respectively (Supplementary Tables S2, S3). The results of the PERMANOVA analysis showed that there were significant differences in the microbiome compositions among the four seasons for all pairwise comparisons, except for the comparison between fall 2020 and winter 2021 (p-value <0.05; Supplementary Table S2). Furthermore, the distance within seasons is significantly closer than the distance between seasons, indicating the clustering of microbial communities by season (Wilcoxon rank sum test p-value <2.2E-16). Also, the microbiome compositions were clearly separated by each individual (p-value <0.05) (Supplementary Table S4).

Association analysis of skin barrier parameters and the skin microbiome

Seasonal variations were also observed in skin biophysical parameters, including TEWL, hydration, and elasticity (Figure 4; Supplementary Table S1). Especially, TEWL, which is a measure of skin barrier function, was significantly higher in the winter than in the summer (22.33 and 15.08, respectively, p-value = 5.0E-05). No significant differences in hydration were found between seasons, but elasticity showed significant differences in all pairs of seasons (Figures 4B,C). We noted longitudinal variations in both the five major microbial taxa and skin biophysical parameters, with remarkable differences between the two seasons (Figure 5). Notably, the abundance of *Cutibacterium* and TEWL exhibited a co-decreasing pattern from winter to summer and while the abundance of *Corynebacterium* and *Staphylococcus* showed the opposite pattern to TEWL (Figures 5A,D,G). In terms of hydration, there were coordinated patterns with *Corynebacterium* and *Staphylococcus* across the seasons, but an opposite pattern was observed in *Cutibacterium*

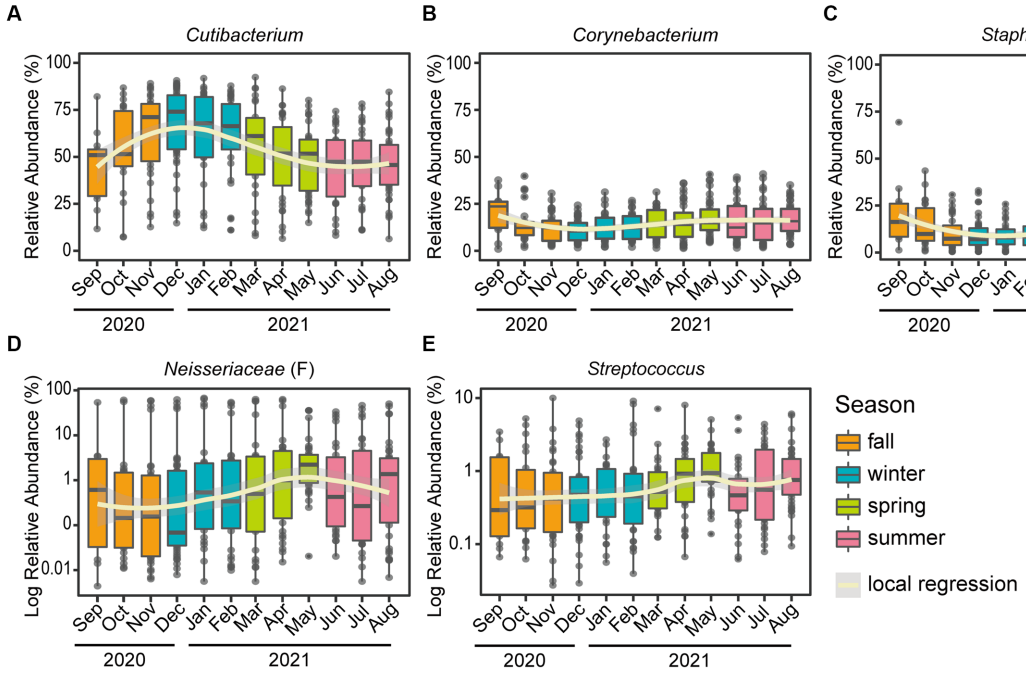

FIGURE 2
Seasonal variation of the top five skin microbiome at the genus level. Boxplots of the relative abundance for the five major genera: **(A)** *Cutibacterium*, **(B)** *Corynebacterium*, **(C)** *Staphylococcus*, **(D)** unclassified genus within *Neisseriaceae* (classified only at the family level) and **(E)** *Streptococcus*. A polynomial local regression line was added to boxplots to enhance visualization of the trend. Relative abundances of panel **(D)** unclassified genus within *Neisseriaceae* and **(E)** *Streptococcus* were converted to a logarithmic scale (log10), to increase legibility.

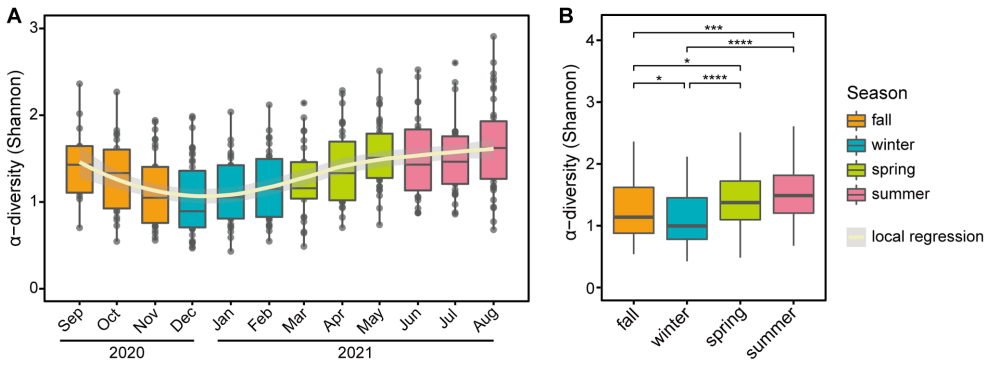

FIGURE 3
Seasonal variation of α-diversity at the genus level. Boxplots of α-diversity based on panel **(A)** month and **(B)** season. In panel **(A)**, a polynomial local regression line was added to boxplots to enhance trend visualization. In panel **(B)**, significant differences in pairwise comparison are marked with stars (*p-value <0.05, **p-value < 0.01, ***p-value < 0.001, and ****p-value < 0.0001).

with regards to hydration (Figures 5B,E,H). Elasticity showed co-increasing patterns with *Staphylococcus* and *Streptococcus* from winter to summer, but conversely, the opposite pattern was observed with *Cutibacterium* (Figures 5C,I,O).

The Canonical Correspondence Analysis (CCA) was used for investigating the correlation between skin microbiome composition, weather conditions, and skin biophysical parameters (Supplementary Figure S4). The total inertia was 0.94 and the constrained inertia was 0.18, of which 9.6% was explained by the CCA1 axis and 3.8% by CCA2 axis. The length of each arrow reflects the strength of the variable in explaining the observed dispersion of the microbiome. Specifically, TEWL and hydration had a substantial impact on the dispersion of the microbiome.

To investigate the association between skin parameters and the skin microbiome that changes with seasonal changes, linear regression analyses were conducted using the whole collected skin microbiome data and skin biophysical data in study period. As a result, TEWL was significantly associated with the relative abundance of *Cutibacterium*, *Corynebacterium*, and *Staphylococcus*, respectively (p-value <0.05) (Table 1). Elasticity (R5) was also significantly associated with the relative abundance of *Cutibacterium*. After adjusting with false discovery rate, only *Cutibacterium* was significantly associated with

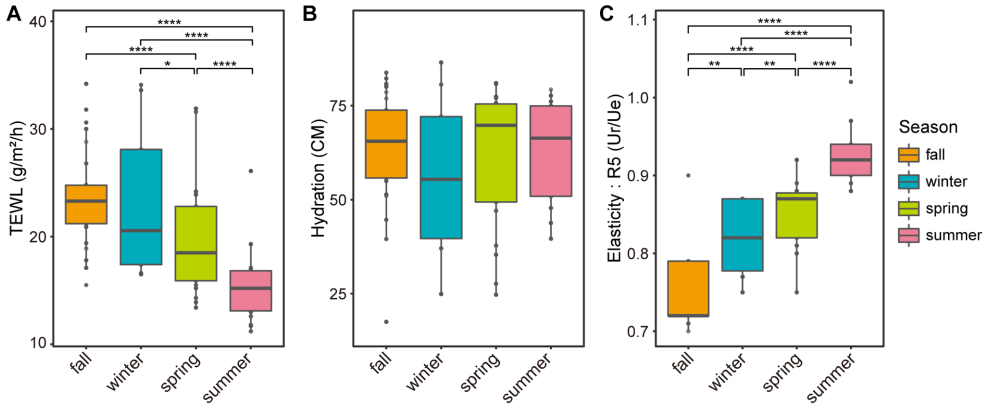

FIGURE 4
Boxplot of skin biophysical parameters based on the season. Boxplots of panel **(A)** skin surface transepidermal water loss, **(B)** skin hydration, and **(C)** skin surface elasticity (R5, Ur/Ue) based on the season. Ur: immediate retraction, Ue: immediate distension. Significant differences in pairwise comparison are marked with stars (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, and ****p-value < 0.0001).

TEWL (false discovery rate-adjusted p-value = 1.5E-05). *Cutibacterium* was also significantly associated with TEWL after adjusting for individual, temperature, and humidity (p-value = 0.02).

Functional analysis of skin microbiome according to season

To identify functional differences in the skin microbiome across different seasons, we conducted a predictive analysis of metagenome function by inferring enriched pathways based on sequences using PICRUSt2, which is based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. At the second level of analysis, among 40 pathways, 36 pathways showed significant differences between seasons (false discovery rate-adjusted p-value <0.05) (Supplementary Figure S5). In particular, 33 pathways showed noticeable differences between summer and winter (Supplementary Table S5). For instance, pathways related to cell motility, the circulatory system, aging, and signal transduction were enriched in summer (Supplementary Figure S5). At the third level of analysis, among 390 pathways, 264 pathways were significantly different between summer and winter. Notably, pathways related to glycosaminoglycan degradation, oxidative phosphorylation, and porphyrin and chlorophyll metabolism were enriched in winter (Supplementary Table S6).

Discussion

Understanding the skin microbiome is important as it plays an essential role in maintaining skin's homeostasis by protecting against pathogens or controlling the immune system (Schommer and Gallo, 2013; Kobayashi et al., 2019). The impact of external environmental factors on both the skin condition and skin microbiome has been extensively investigated in previous studies (Leeming et al., 1984; Grice and Segre, 2011; Kobayashi et al., 2019; Isler et al., 2023). Since temperature and humidity obviously fluctuate with the season (Supplementary Figure S1), it is important to investigate skin microbiome and skin biophysical parameters along with seasonal changes. However, most previous studies have not investigated skin conditions and skin microbiome together longitudinally. Therefore, we conducted a one-year longitudinal study on skin biophysical parameters and skin microbiome, simultaneously.

Previous studies suggested that the bacterial community of the skin remains stable for approximately 1–2 years. However, these studies performed sampling only once or twice a year, which was insufficient to observe the annual fluctuations of the microbial community (Flores et al., 2014; Hillebrand et al., 2021). In a recent longitudinal study conducted by Oh et al. (2016), the annual variation in the mycobiota of healthy individuals was examined by collecting samples once a month, revealing that the mycobiota remained relatively stable throughout the year. However, it should be noted that this study focused solely on fungi and did not investigate bacteria and skin characteristics. Although these findings provide valuable insight into mycobiota dynamics in healthy individuals, further research is needed to fully understand the complex interplay between the microbiome and skin health.

In our study, we collected skin microbiome samples once a week and skin biophysical parameters once a month, which allowed us to collect a large amount of data and ensure the accuracy of our study results to study the relationship between skin and skin microbiome. As a result, we discovered the composition of the skin microbiome fluctuated with the seasons, in particular, that observed in the major skin microbial taxa, including *Cutibacterium*, *Corynebacterium*, and *Staphylococcus*. Especially, the HI-10 participant exhibited a distinct most abundant taxon, an unclassified genus within *Neisseriaceae*, which has been reported as one of the dominant microbial taxa in human skin (Qiao et al., 2021). The β-diversity plot also showed a distinct cluster of HI-10 samples from the others. Functional studies should be conducted further to untangle the potential relationships between dominant taxa and human skin properties. The α-diversity showed seasonal variation and β-diversity analysis revealed that microbial communities were distinct by season. Moreover, the Jensen-Shannon distance within seasons is significantly closer than between seasons suggests that microbial communities undergo seasonal changes, highlighting the importance of seasonal dynamics in shaping skin microbial composition. Seasonal variations were also observed in skin biophysical parameters, including TEWL, hydration, and elasticity. The relative abundance of *Cutibacterium* and TEWL was

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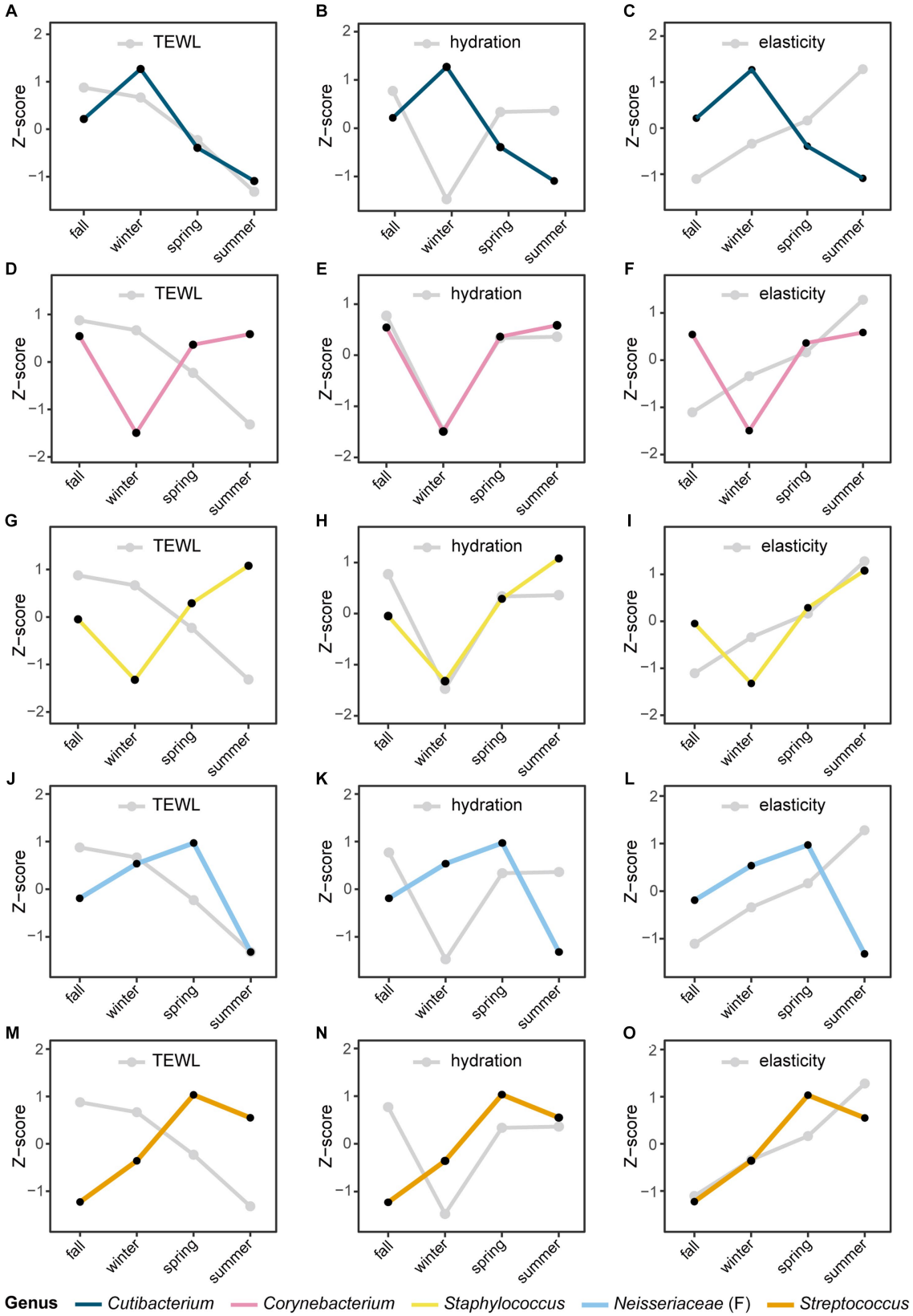

FIGURE 5
Seasonal variation of the microbial taxa and skin biophysical parameters. The longitudinal movement of the relative abundance of panels **(A–C)** *Cutibacterium*, **(D–F)** *Corynebacterium*, **(G–I)** *Staphylococcus*, **(J–L)** *Neisseriaceae* (classified only at the family level), and **(M–O)** *Streptococcus* at the genus level were compared with skin biophysical parameters [**(A,D,G,J,M)**: TEWL, **(B,E,H,K,N)**: skin hydration, **(C,F,I,L,O)**: skin elasticity]. The relative abundance of microbial taxa and skin biophysical parameters were normalized to the *Z*-score; each point represents the mean value for each season.

TABLE 1 Association analysis of the relative abundance of microbial taxa at the genus level with skin biophysical parameters.

Skin biophysical parameters	*Cutibacterium*			*Corynebacterium*			*Staphylococcus*		
	Beta	P^a	$P_{corr}{}^b$	beta	P^a	$P_{corr}{}^b$	beta	P^a	$P_{corr}{}^b$
TEWL	0.14	$\mathbf{9.7\times10^{-7}}$	$\mathbf{1.5\times10^{-5}}$	−0.14	$\mathbf{8.8\times10^{-3}}$	ns	−0.09	**0.02**	ns
Hydration	–	ns	ns	–	ns	ns	–	ns	ns
Elasticity (R5)	-9.6×10^{-4}	**0.04**	ns	–	ns	ns	–	ns	ns

Significant associations are shown in bold face (p-value < 0.05).
ns, not significant; TEWL, transepidermal water loss.
[a]p-value of linear regression analysis by adjusting for individuals as covariates.
[b]p-value after adjusted with false discovery rate.

significantly high in winter and low in summer. In particular, our study's CCA and linear regression result provide clues that can explain the association between skin microbiome and skin biophysical characteristics. The results of CCA analysis demonstrate that TEWL and hydration have more impacts on microbiome dispersion, and the relative abundance of *Cutibacterium* is correlated with TEWL. Especially, there was a significant association between *Cutibacterium* and TEWL, even after adjusting for individual differences and weather indicating a dependent relationship.

The epidermal barrier function is known to vary depending on the season, and the difference in the integrity of the skin barrier is mainly caused by epidermal ceramide, which is a major component of the skin barrier (Coderch et al., 2003; Wei et al., 2016; Pappas et al., 2018). The level of epidermal ceramide is widely recognized to be regulated by various factors such as skin microbiome and skin disease (Zheng et al., 2022; Schachner et al., 2023). Several studies have demonstrated that the skin microbiome can have an impact on the physical barrier of the skin (Liu et al., 2020; Harris-Tryon and Grice, 2022). Considering previous studies, we found that the major taxa that fluctuated with the seasons, may regulate the epidermal ceramide levels and subsequently impact the skin barrier function. *S. epidermidis*, one of the *Staphylococcus* species, helps maintain skin barrier integrity through the secretion of sphingomyelinase, which is crucial for the production of ceramide (Zheng et al., 2022). *C. acnes* is known to account for more than 90% of *Cutibacterium* in human skin (Li et al., 2021) and can partially hydrolyze triglycerides in sebum which acts as a pathway for water diffusion into the epidermis (Riegels-Nielsen et al., 1989; Grice and Segre, 2011; Rocha and Bagatin, 2018; Kobayashi et al., 2019). In addition, we performed functional profiling of the skin microbiome across seasons using KEGG to interpret how the skin microbiome may affect skin barrier function. As a result, it was possible to partially interpret and infer the effect of *Cutibacterium*, which accounts for more than half of the total individual's microbiome composition, on the host skin barrier. For instance, porphyrin metabolism was elevated in winter, which is known to be produced by *Cutibacterium acnes*, can induce oxidative stress and cause skin inflammation (Barnard et al., 2020; Spittaels et al., 2021; Stødkilde et al., 2022). Although the resulting pathways operate at the cellular level and the functional relationship is not clear, the increase in glycosaminoglycan degradation pathway during winter may play an important role in the elevated loss of water content in the epidermal skin. Hyaluronic acid, one of the extracellular matrix components in the epidermis and dermis, is a type of glycosaminoglycan and is known to be degraded by hyaluronate lyase produced by *C. acnes* (Nazipi et al., 2017; Li et al., 2021; Mayslich et al., 2021). Moreover, a previous anti-acne treatment study has reported improvement in skin barrier function (reduced TEWL) and simultaneously the relative abundance of *Cutibacterium* (formerly *Propionibacterium*) tended to decrease (Shao et al., 2023). These results can partially explain the impact of the skin microbiome on skin characteristic such as skin barrier function.

Our study has the advantage of longitudinally observing variations by concurrently collecting skin microbiome and skin biophysical characteristics. This approach enables us to explore the intricate association between skin microbiome and host skin characteristics. Nonetheless, it is imperative to acknowledge the limitations of our study. The composition of the microbiome can be influenced by various factors such as the surrounding environment and host's lifestyle (Prescott et al., 2017; Moitinho-Silva et al., 2021). While information about environments regarding humidity, and temperature, is available, details about the host's lifestyle are lacking in our study. Since we consistently collected skin microbial samples and conducted skin measurements in the same facial area, we consider that the aspects related to personal hygiene habits such as mask-wearing and cosmetics were controlled. Specifically, throughout the entire duration of the study, mask-wearing was required and their cosmetic and hygiene routines were maintained consistently. However, to investigate a more systemic relationship between host and skin ecology, we propose the collection of survey data about host lifestyle information. By considering such factors, we anticipate a more comprehensive understanding of the relationship between host skin and skin microbiome.

In conclusion, our long-term study found that there was seasonal variation in skin microbial composition while maintaining an individual's unique microbiome profile, and a significant association between the abundance of *Cutibacterium* and skin barrier parameter, TEWL. This study can contributed to a better comprehension of the skin microbiome and its intricate interplay with skin characteristics. Further functional studies on the changes in microbiome composition and skin barrier functions are needed for a deeper understanding of the skin microbiome's contributions to skin biophysical characteristics.

Data availability statement

The datasets presented in this study are deposited in the NCBI repository under accession number PRJNA1034158.

Ethics statement

The studies involving humans were approved by the institutional review board at the LG H&H Research Center. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JS: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. SY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. K-NG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. HK: Conceptualization, Investigation, Validation, Visualization, Writing – review & editing. J-GS: Conceptualization, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. SL: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. BH: Conceptualization, Methodology, Validation, Writing – review & editing. YK: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. NK: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by a grant funded by LG H&H.

Acknowledgments

Supplementary Figure S2 was created with BioRender.com (license number MC258I2B6Y).

Conflict of interest

JS, SY, K-NG, HK, J-GS, SL, BH, YK, and NK were employed by LG H&H Co., Ltd.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1298632/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 16 November 2023
DOI 10.3389/fmicb.2023.1192543

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OPEN ACCESS

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RECEIVED 27 March 2023
ACCEPTED 01 November 2023
PUBLISHED 16 November 2023

CITATION
Zhu Q, Wu K, Yang Q, Meng B, Niu Y and Zhao F (2023) Advances in psoriasis and gut microorganisms with co-metabolites. *Front. Microbiol.* 14:1192543. doi: 10.3389/fmicb.2023.1192543

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Advances in psoriasis and gut microorganisms with co-metabolites

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This review summarizes the potential role of gut microbes and their metabolites as novel mediators of psoriasis, including their composition and function in disease pathogenesis, progression, and management. Gut microbiota network analysis, colony construction, and *in vivo* large-scale interaction experiments showed that different degrees of damage and repair in psoriasis, both in animals and humans, involve cross-border homeostasis of the microbial community. Which gut microbiota interactions are present in psoriasis and how they collaborate with immune cells and influence psoriasis development via the gut-skin axis remain incompletely elucidated. In this article, we review the latest information on the unique patterns of gut microbiota and co-metabolites involved in the pathogenesis of psoriasis and attempt to explore microbial-based therapeutic targets derived from mono-and polymicrobial probiotics, fecal microbiota transplantation, pharmacomicrobiomics, and dietary interventions as diagnostic or therapeutic approaches promising to provide new options and long-term management for psoriasis.

KEYWORDS

psoriasis, gut microorganisms, metabolites, immunity, diet

1. Introduction

Psoriasis, a common erythematous scaling skin disease with multiple skin manifestations and systemic involvement, can involve any skin site and occur at any age and in any geographic area, affecting more than 60 million adults and children worldwide (Griffiths et al., 2021; Figure 1; Supplementary Tables S1, S2). Based on different clinical manifestations of psoriasis, it is usually classified into five types: plaque-dominated psoriasis vulgaris, punctate (droplet) or hemorrhagic psoriasis, pustular psoriasis (represented by sterile pustules), arthritic psoriasis (with arthritis as the main manifestation), and erythrodermic psoriasis with systemic involvement, of which psoriasis vulgaris is the most common type, accounting for approximately 90% of cases (Boehncke and Schön, 2015). Immunological and genetic studies confirmed IL-17 and IL-23 as key drivers in psoriasis pathogenesis (Ghoreschi et al., 2021). However, psoriasis is currently incurable due to its lingering and recurrent nature. A plethora of studies found that psoriasis is no longer considered a disease that affects only the skin but is seen as a systemic inflammatory disorder (Gulliver, 2008; Reich, 2012; Elnabawi et al., 2019; Gelfand and Wang, 2023), which is associated with multiple comorbidities, including colorectal cancer, metabolic syndrome, obesity, nonalcoholic fatty liver disease, and cardiovascular disease (Griffiths and

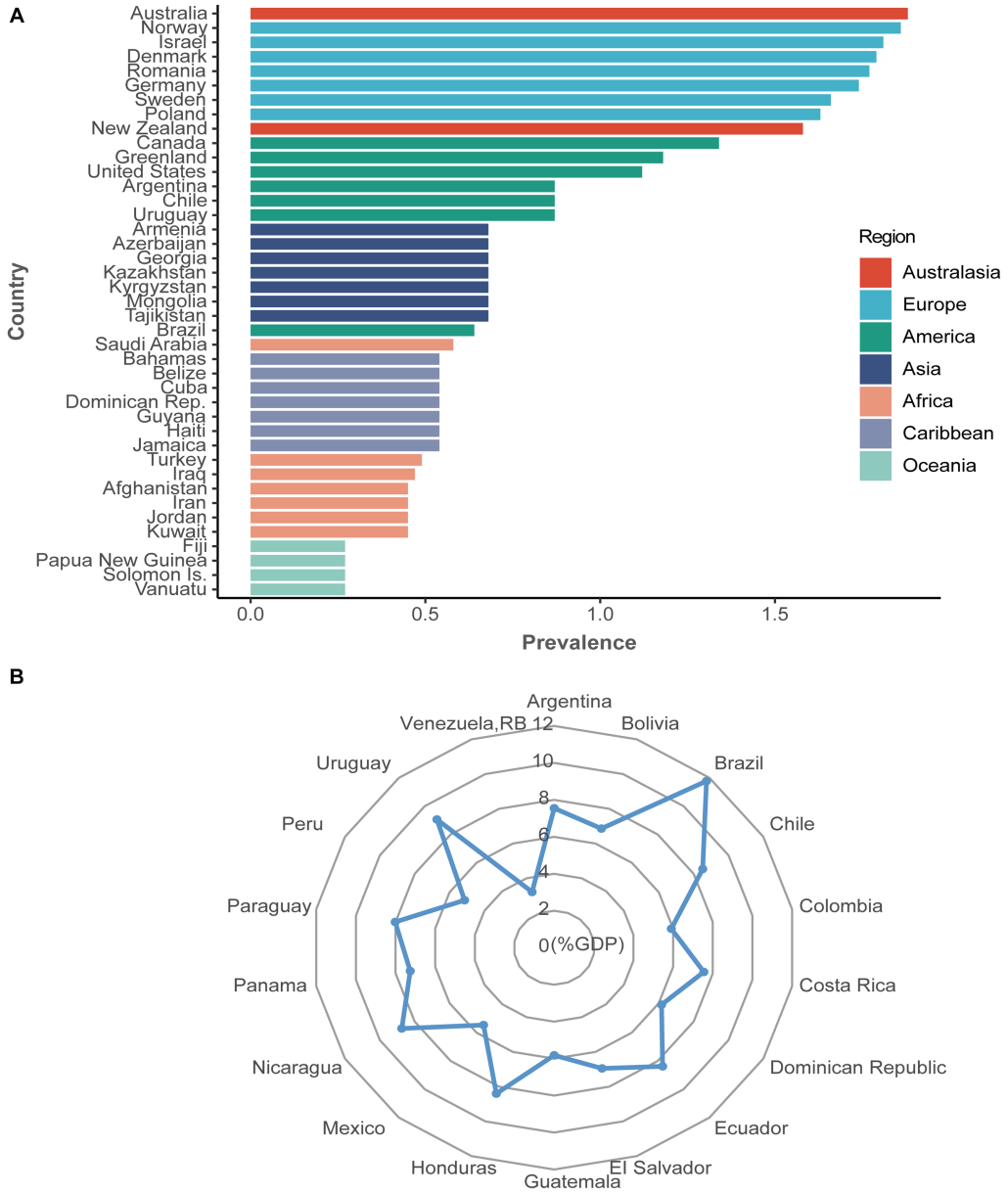

FIGURE 1
The prevalence (%) and health expenditure (%GDP) of psoriasis worldwide. **(A)** The prevalence of psoriasis from 169 countries. Seven colorful blocks represent seven continents, and each country from the belonging continent is ranked from highest to lowest prevalence, with only the top seven countries shown. The prevalence of psoriasis varied from 0.27% in Oceania to 1.88% in Australasia. **(B)** Health expenditure (%GDP) of psoriasis from 18 available countries. Health expenditure for psoriasis varied from 3.21% in Venezuela, RB, to 11.77% in Brazil. Data from the Global Psoriasis Atlas (https://www.globalpsoriasisatlas.org/). For further details about how the prevalence data is calculated, please visit https://www.bmj.com/content/369/bmj.m1590.

Barker, 2007; Figure 2). Fu et al. (2018) published a large meta-analysis of data from nearly 7.8 million people, which showed that psoriasis was significantly associated with both Crohn's disease and ulcerative colitis. Two years later, another meta-analysis on the results of eight cohort studies (10,544,609 subjects in total) found a significantly increased risk of colorectal cancer in women with psoriasis (but not men), suggesting that patients with psoriasis exhibiting gastrointestinal symptoms should undergo colonoscopy (Fu et al., 2021).

Microbial infections are considered an important etiology of psoriasis, especially upper respiratory tract infections with streptococci, which are strongly associated with the development of psoriasis vulgaris (De Jesús-Gil et al., 2020). However, as the understanding of psoriasis improved, both *in vivo* experiments and clinical trials demonstrated that certain common genetic and environmental factors and immune pathways might be present in psoriasis and inflammatory diseases due to intestinal dysbiosis (Schreiber et al., 2019; Okada et al., 2020; Paine et al., 2023). Indeed, the gut microbiota is a community of trillions of symbiotic organisms that work together in metabolically active endocrine-like organs now known to contribute to host physiology through the digestion of many nutrients, vitamin synthesis, and production of bioactive metabolites (Benson et al., 2023). Therefore, the association of human gut

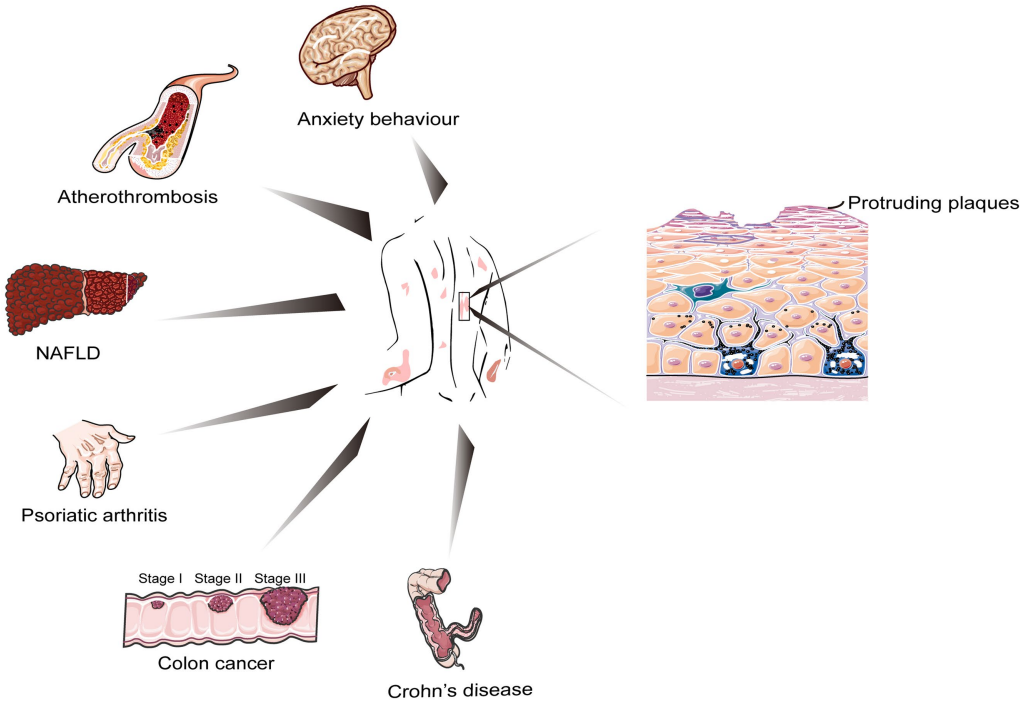

FIGURE 2
Cellular biology of psoriasis and other closely related chronic and serious health conditions.

microbiota and commensal metabolites with psoriasis is receiving attention. Additionally, studies showed that various microbiota metabolites, such as short-chain fatty acids (SCFAs), tryptophan metabolites, and amine derivatives, including trimethylamine N-oxide (TMAO), play an important regulatory role in autoimmune diseases (Stec et al., 2023). The composition of gut microbes influences nutrition, inflammation, natural immune function, and, to some extent, people's skin condition.

Given that intestinal dysbiosis is involved in the physiopathology of inflammatory and immune diseases, the correction of intestinal dysbiosis and the maintenance of intestinal microecological balance are new targets for the prevention and treatment of psoriasis. Therefore, in this paper, we explored microbial therapy for psoriasis, including mono-and polymicrobial probiotics, fecal microbiota transplant (FMT), pharmacomicrobiomics, and dietary interventions. More importantly, we not only summarized the effective cure rate, prognosis with single or combined probiotics, and molecular mechanisms by which the gut microbiota might be engaged in drug metabolism in the treatment of psoriasis but also evaluated the effects of probiotics interacting with exogenous dietary factors on psoriasis, providing evidence for microbial immunotherapy as well as dietary interventions.

2. Gut microbiome and psoriasis

In the human body, the skin and the intestine are the two organs with the most abundant microbiota, and the gut microbiota is involved in the occurrence and development of human systemic diseases in many ways. Many reports confirmed the close association between gut microbiota and diseases such as cancer (Cheng et al., 2020), type 2 diabetes (Qi et al., 2022), mental illness (Sampson et al., 2016), obesity (Liu et al., 2017), and autoimmune diseases, including lupus erythematosus and rheumatoid arthritis (RA; Miyauchi et al., 2023). The gut microbes have a crucial role in maintaining the integrity of the intestinal mucosal barrier, immune homeostasis, and the dynamic balance of energy and helping the host absorb vitamins; hence, the mesenteric system is also considered the largest immune organ in the body (Jacobs and Braun, 2014).

A strong bidirectional link between the gut and skin has been demonstrated, with many studies linking gastrointestinal health to dynamic homeostasis and heterogeneity of the skin. Patients with psoriasis have a reduced diversity of intestinal microbiota and possible dysbiosis compared to healthy controls, as evidenced by the changes in the microbiota at different levels of categorization, as detailed in Table 1 (Scher et al., 2015; Eppinga et al., 2016; Codoñer et al., 2018; Tan et al., 2018; Hidalgo-Cantabrana et al., 2019; Huang et al., 2019; Shapiro et al., 2019; Dei-Cas et al., 2020; Xiao et al., 2021; Zhang et al., 2021). Hidalgo et al. sequenced and analyzed 16S rRNA in the fecal samples of 19 patients with psoriasis and 20 healthy individuals from the same region and found a low diversity and dysbiosis of gut microbiota in patients with psoriasis (Hidalgo-Cantabrana et al., 2019). The structural changes in the microbiota of psoriasis patients in this study were specific and elucidated the mechanisms of gut microbiota-host interactions in psoriasis. Kiyohara et al. induced psoriasis-like dermatitis using TLR7 agonist in the skin of mice, affecting intestinal immune cell and microbiota composition, which, in turn, led to an exacerbation of DSS-induced colitis (Kiyohara et al., 2018). Additionally, a study found that type IIA secreted phospholipase A2 (sPLA2-IIA) modulates gut microbiota in mice by degrading bacterial membranes, altering the expression of intestinal immune and metabolism-related genes, and regulating the levels of multiple

TABLE 1 Intestinal dysbiosis in psoriasis.

Study	Subjects	Sample	Gut Microbiota Analysis Technique	Gut microbiota alterations
Hidalgo-Cantabrana et al. (2019)	19 psoriasis patients; 20 healthy individuals	Fecal samples	16S rRNA	**Phylum**: *Actinobacteria, Firmicutes, Bacteroidetes, Proteobacteria* **Genus**: *Blautia, Bifidobacterium, Collinsella, Ruminococcus, Subdoligranulum, Slackia; Bacteroides, Parabacteroides, Barnesiella, Alistipes, Paraprevotella, Faecalibacterium*
Zhang et al. (2021)	30 psoriasis patients; 30 healthy controls	Fecal samples	16S rRNA	**Family**: *Veillonellaceae, Ruminococcaceae; Lachnospiraceae* **Genus**: *Faecalibacterium, Megamonas*
Scher et al. (2015)	15 patients with psoriasis of the skin;17 healthy subjects	Fecal samples	16S rRNA	**Genus**: *Parabacteroides, Coprobacillus*
Huang et al. (2019)	35 psoriasis patients;27 healthy controls	Fecal samples	16S rRNA	**Phylum**: *Bacteroidetes; Firmicutes* **Genus**: *Bacillus, Bacteroides, Sutterella, Lactococcus, Lachnospiraceae_UCG004, Lachnospira, Mitochondria_norank, Cyanobacteria_norank, Parabacteroides; Thermus, Streptococcus, Rothia, Granulicatella, Gordonibacter, Allobaculum, and Carnobacterium*
Tan et al. (2018)	14 patients with vulgaris psoriasis; 14 healthy controls	Fecal samples	16S rRNA	**Phylum**: *Verrucomicrobia, Tenericutes* **Genus**: *Enterococcus, Bacteroides; Akkermansia*
Xiao et al. (2021)	30 psoriatic patients;15 healthy subjects	Fecal samples	Metagenomic sequencing	**Phylum**: *Actinobacteria, Firmicutes, Verrucomicrobia; Bacteroidetes, Proteobacteria* **Genus**: *Faecalibacterium, Bacteroides, Bifidobacterium, Megamonas,Roseburia; Prevotella, Alistipes, Eubacterium*
Eppinga et al. (2016)	29 psoriasis patients; 33 healthy individuals	Fecal samples	quantitative polymerase chain reaction	**Species**: *Escherichia coli; Faecalibacterium prausnitzii*
Codoñer et al. (2018)	52 plaque psoriasis;300 healthy individuals extracted from the human microbiome project (http://hmpdacc.org/)	Fecal samples	16S rRNA	**Genus**: *Faecalibacterium, Akkermansia* spp.*; Bacteroides*
Shapiro et al. (2019)	24 psoriasis patients; 22 healthy individuals	Fecal samples	16S rRNA	**Phylum**: *Firmicutes, Actinobacteria* **Species**: *Ruminoccocus gnavus, Dorea formicigenerans, Collinsella aerofaciens; Prevotella copri, Parabacteroides distasonis*
Dei-Cas et al. (2020)	55 psoriasis patients; 27 healthy individuals	Fecal samples	16S rRNA	**Phylum**: *Firmicutes; Bacteroidetes* **Genus**: *Faecalibacterium, Blautia; Bacteroides, Paraprevotella*

Red font – increased level; blue font – decreased level.

blood metabolites and fecal bacterial lipids to suppress bacterial infections and alleviate DMBA/TPA-induced skin cancer while worsening imiquimod (IMQ)-induced psoriasis (Miki et al., 2022). These findings suggest that in the gut-skin axis, skin inflammation can affect gut health, and gut microbiota can affect skin health, shedding new light on the relevance of psoriasis to inflammatory bowel disease. Similar results were observed by Schneeweiss et al., who analyzed more than 240,000 patients with chronic inflammatory skin disease and nearly 2.4 million controls and found that the risk for ulcerative colitis and Crohn's disease was significantly higher in patients with septic sweat glands and risk for Crohn's disease was significantly higher in patients with psoriasis (Schneeweiss et al., 2022).

Exploring the changes and functions of the gut microbiota in psoriasis can help provide new targets for the diagnosis and treatment of psoriasis. Studies showed that helper T cells (Th17) are important mediators of intestinal epithelial barrier integrity and can drive both intestinal inflammation and extraintestinal autoimmune disease progression through the microbiota, acting as a bridge between host microbiota and immune-mediated inflammatory diseases; thus, the heterogeneity and plasticity of Th17 are expected to be a breakthrough in the mechanisms of psoriasis development and refinement of "litmus test" therapeutic strategies (Bellone et al., 2020; Schnell et al., 2023). For example, different species of the same genus differ in their regulation of Th17 in different environments. *P. histicola* alleviates RA (Marietta et al., 2016), and *P. copri* exacerbates arthritis (Scher et al., 2013). Ito et al. found that *Staphylococcus cohnii*, a commensal bacterium present in mouse and human skin, could suppress skin inflammation in several mouse models of dermatitis by inducing the expression of anti-inflammatory genes and glucocorticoid-related pathway genes (Ito et al., 2021). Oral administration of *Lactobacillus pentosus* GMNL-77 significantly decreased the interleukin (IL)-23/IL-17A axis–associated cytokines and erythematous scaling lesions in the skin of IMQ-treated mice, suggesting that artificial alteration of the gut microbiota might be relevant for reducing the systemic inflammatory response in the skin of psoriasis patients (Chen et al., 2017).

Although biologics have achieved high skin lesion clearance in psoriasis, the current process of treating psoriasis with biologics has been a source of secondary failure problems and safety issues have been a major concern for clinicians. Clinical evidence showed that

IL-23 inhibitors reachive remission in patients who do not respond well to IL-17 monoclonal antibodies (Sofen et al., 2014; Reich et al., 2019). Significant differences in the relative abundance of bacteria taxa between responders and non-responders suggested that IL-23 and IL-17 inhibitors may functionally interact with gut microbiota to reduce cutaneous inflammation (Huang et al., 2023). However, the specific mechanisms and applications of the microbiome in psoriasis treatments require more attention.

3. Fungi and psoriasis

In addition to bacteria that can influence various physiopathological processes from immune development to phenotype, mounting studies demonstrated the involvement of intestinal fungi in the regulation of immune homeostasis (Doron et al., 2021; Li B. Z. et al., 2022). Fungi are non-negligible members of the gut microbiota. Composition changes of intestinal fungi were found in various diseases, such as systemic lupus erythematosus, RA, and colitis (Sokol et al., 2017; Li H. V. et al., 2022). However, the effects of fungi on host health and their mechanisms of action are still poorly understood. Adult intestinal fungi are mainly composed of 10 genera in the phylum Cysticercus (70%) and Streptomyces (30%) and are influenced by environmental and dietary factors (Richard and Sokol, 2019). There are two main types of fungi found in the human body: environmental fungi, such as yeasts and molds, which are usually harmless to healthy people, and commensal fungi. The latter live in the human skin or body and can improve intestinal health. Conversely, changes in the composition of intestinal fungal (called "fungal dysbiosis") can lead to colon cancer, alcoholic liver disease, and allergic respiratory disease (Wheeler et al., 2016; Lang et al., 2020; Papon et al., 2021). Therefore, there is a possible role of a balanced intestinal fungal community in maintaining the dynamic balance of host immunity and human health.

Although gut bacteria and development of psoriasis have bidirectional regulatory mechanisms, core questions regarding the involvement of fungal biota are only beginning to be investigated. A case–control study found that early skin infections, including cutaneous viral, bacterial, and fungal infections, and microbial ecological dysbiosis were significantly associated with psoriasis in children (Chen Y. J. et al., 2021). Th cells play different roles in fungal infection and colonization (Speakman et al., 2020). Hurabielle et al. found that colonization by skin commensal fungi, such as *Candida albicans*, worsened psoriasis-like skin inflammation by enhancing the response of Th17 cells, neutrophils, and Langerhans cells and inducing changes in the skin transcriptome of mice similar to those in lesional skin of psoriasis patients (Hurabielle et al., 2020). Besides, another study showed the presence of a specific fungal community in the intestinal mucosa of humans and mice and that mucosa-associated fungi protect the intestine from damage and infection through the induction of IL-22 by Th17 cells (Leonardi et al., 2022). In addition to this, *Pseudomonas tropicalis* can promote colorectal tumorigenesis through various mechanisms, such as modulation of host immunity, induction of inflammatory vesicle production, myeloid-derived suppressor cell differentiation, and IL-22 secretion by ILC3 cells (Wang et al., 2018). Due to their large size, fungi can also compete with bacteria in the intestine and inhibit the proliferation of probiotics, thus, promoting tumorigenesis and progression (Narunsky-Haziza et al., 2022).

The mechanisms of intestinal fungi involvement in psoriasis have rarely been reported, but these aforementioned findings revealed the strain specificity of host-fungal interactions and highlighted new diagnostic and therapeutic targets for diseases of inflammatory origin. It is thus clear that deciphering the numerous interactions between bacteria and fungi in the gut and other ecological settings is one of the most explored areas of research in the gut-skin axis, and a deeper understanding of the mechanisms of immune regulation mediated by fungal-bacterial and fungal-fungal interactions will help elucidate their role in the development of psoriasis.

4. Commensal metabolites and psoriasis

One of the main mechanisms by which gut microbes play a role in autoimmunity and stimulation of immune responses is the alteration of microbial metabolites with immunomodulatory functions (Arpaia et al., 2013). Thus far, only a few metabolites have been identified and extensively studied, such as SCFAs, tryptophan, and secondary bile acids, which play specific roles at the cellular and even systemic level by interacting with different receptors on immune and skin cells (Campbell et al., 2020; Choi et al., 2020; Schwarz et al., 2021). Understanding how microbial-host symbiotic metabolites affect the skin and immune cells may be a milestone in deciphering the mechanisms of the gut-skin axis.

Gut microbiota has an important role in the maintenance of systemic immune homeostasis and can affect skin health in many ways, including immune regulation and strain/metabolite transfer. A recent study has found that sodium butyrate treatment alleviated skin inflammation, decreased IL-17 expression, and increased IL-10 and Foxp3 expression in a mouse model of psoriasis. In Treg isolated from the blood of patients with psoriasis, sodium butyrate restored the acetylation level of its inhibitory agent H3 histone. In the lesional skin of patients with psoriasis, sodium butyrate restored Treg numbers and dysregulated levels of certain cytokines, such as IL-17 (Schwarz et al., 2021).

Notably, patients with psoriasis often have impaired intestinal barrier integrity and altered gut microbiota and are seven times more likely to develop inflammatory bowel disease (IBD) than the general population, although the responsible mechanisms are unclear. A recent study has found that psoriasis disrupts gut microbiota, causing the production of succinate and pro-inflammatory ligands by intestinal microorganisms, which induce the proliferation and activation of colonic CX3CR1hi macrophages, ultimately exacerbating colitis (Pinget et al., 2022). Additionally, there is an important link between the metabolism and immune responses of the body. A study showed that a high-fat diet causes changes in the metabolic state of the body, which in turn, alter the activation of Toll-like receptor (TLR)-dependent dendritic cells, increases IL-23, and ultimately exacerbates the inflammatory symptoms of psoriatic skin (Mogilenko et al., 2019). Therefore, intestinal microorganisms and their metabolites might break through the damaged intestinal barrier to enter the body circulation and, thus, directly or indirectly regulate distant organs, including the skin and joints, and play an important role in the process of establishing, dysregulating, and re-establishing homeostasis in the gut-skin axis.

Trimethylamine (TMA) is generated from dietary carnitine and choline in fish, eggs, and beef products by intestinal microbiota choline TMA lyase. Then, TMA enters the liver via the portal circulation and is oxidized by the hepatic enzyme flavin-containing monooxygenase 3 to form TMAO (Cho and Caudill, 2017). Hazen's team initially discovered that increased TMAO levels are associated with an increased risk of incident major adverse cardiovascular events (Wang et al., 2011; Tang et al., 2013). A recent prospective cohort study of 6,785 participants followed for approximately 17 years confirmed that higher plasma TMAO levels were associated with a higher risk of all-cause mortality, risk of cardiovascular disease mortality, and risk of renal failure mortality and were not significantly associated with risk of cancer and dementia mortality (Wang et al., 2023). Researchers at the University of Cincinnati, USA, who enrolled 2,129 individuals from 2 independent cohorts, confirmed that plasma TMAO levels are positively correlated with the risk of abdominal aortic aneurysm (AAA) onset and progression and elucidated the mechanism by which gut microbiota-derived TMAO enhances endoplasmic reticulum stress and apoptosis in smooth muscle cells of the aortic wall, leading to the development of AAA in mouse experiments (Benson et al., 2023). The American College of Cardiology/American Heart Association identified psoriasis as an independent risk factor for atherosclerosis, myocardial infarction, and stroke (Grundy et al., 2019). Whether circulating TMAO, also an independent risk factor for cardiovascular disease, is involved in the pathogenesis of psoriasis remains a mystery. Several clinical trials demonstrated that TMAO could be used as an indicator of psoriasis severity by measuring TMAO in the serum of psoriasis patients by high-performance liquid chromatography-mass spectrometry (Coras et al., 2019; Sikora et al., 2021; Sun et al., 2022). In a mouse model of systemic lupus erythematosus, the intestinal microbial metabolite, TMAO, contributes to TLR7-induced autoimmune and vascular dysfunction through the activation of pro-inflammatory Th17 lymphocytes and an increase in B cell differentiation (González-Correa et al., 2021). It remains to be determined whether TMAO plays a marker or mediator role in the etiology of psoriasis, whether its high concentration can activate the immune system, and whether it can coexist with factors of homeostasis within the circulatory system.

New biologic treatments have recently been added to psoriasis treatment options (e.g., bimekizumab, secukinumab, and ixekizumab; Burkett and Kuchroo, 2016). Although effective, clinical randomized controlled trials demonstrated that adverse events were reported in 86.1% of patients receiving bimekizumab and 81.4% of patients receiving secukinumab between 48 weeks, with bimekizumab leading to a higher incidence of ulcerative colitis, oral candidiasis, and suicide risk (Reich et al., 2021). Therefore, the highly heterogeneous disease profile of various biologics still requires more exploration in terms of efficacy and safety. As increasing evidence supports the importance of the microbiome for our health, the desire to promote a healthy microbiome becomes stronger. In addition to TMAO, there are many favorable gut microbiota-derived metabolites such as butyrate (Wen et al., 2021), propionic acid (Duscha et al., 2020), and tryptanthrin (Shankar et al., 2020). These metabolites, as postbiotics for the therapeutic purpose of autoimmune diseases, are mainly aimed at correcting dysbiosis and the imbalance between resident microorganisms and the immune system that contribute to health risks. Postbiotics are the latest trend in gut health, promising to improve our skin (Rawal and Ali, 2023), enhance our physique (Akatsu, 2021), and even reverse the signs of aging (Iglesia et al., 2022).

5. Drug-microorganism interference

As pharmacomicrobiomic studies progressed, it was discovered that gut microbiota could be used as a biomarker for predicting therapeutic response. Additionally, modulating microbiota could increase the bioavailability and efficacy of drugs, and inhibiting the enzymatic activity of specific bacteria could prevent them from metabolizing drugs into toxic products. The heterogeneity of the microbiome among individuals could determine the clinical efficacy of certain drugs or reduce the occurrence of adverse events, which is well used in RA, psoriatic arthritis, and ankylosing spondylitis (Scher et al., 2020).

Methotrexate, used to treat colon cancer and psoriasis, is also used in the treatment of RA (Marsh et al., 1991; van Huizen et al., 2022; Zhang et al., 2022), but about 50% of patients with RA do not respond adequately to methotrexate therapy. A clinical study (Artacho et al., 2021) comparing pretreatment differences in gut microbiota between RA patients who responded and those who did not respond to methotrexate therapy found that a microbiota-based model could more accurately predict patient response to methotrexate therapy. Additionally, *in vitro* co-culture of gut microbiota with methotrexate suggests that the metabolism or clearance of methotrexate by gut microbiota might inhibit the therapeutic effect of methotrexate. Another study (Ventin-Holmberg et al., 2021) comparing the differences in fecal microbiota (both bacterial and fungal) before and after infliximab treatment found that non-responders had lower abundance of short-chain fatty acid-producing bacteria (especially *Clostridium*) and higher abundance of pro-inflammatory bacteria and fungi (such as *Candida* spp.) compared to responders, and that response to infliximab treatment was more accurately predicted based on bacterial taxa.

Zimmermann et al. (2019) systematically analyzed the metabolism of 271 orally administered drugs by 76 species/strains of human intestinal bacteria, identified bacterial genes and their products involved in drug metabolism, and validated some of the findings in mouse models and human gut microbiota cultures, deepening the understanding of the molecular mechanisms involved in drug metabolism by gut microbiota and providing insights into individualized drug interventions targeting microbiota. Furthermore, since psoriasis and IBD are highly heterogeneous diseases, more precise and in-depth phenotyping is needed to identify specific subgroups and their molecular signatures as human microbiome research advances. Scientists are beginning to identify core and variant microbiomes (enteric, vagal, and metabolic; Rizkallah et al., 2010), how different functional groups can be precisely combined with drugs, and how interventions (e.g., microbiome editing) and clinical practice (e.g., microbiome testing) can be administered to treat autoimmune diseases. Although a huge challenge, there is no doubt that the translation of pharmacomicrobiomics into routine healthcare applications is just around the corner (Figure 3).

6. Microbiotherapy and psoriasis

A retrospective analysis showed that 83.7% of psoriasis patients had 100% improvement in PASI scores after 24 weeks of treatment with *Streptococcus salivarius* K-12 and that efficacy continued to improve with longer treatment duration (Zangrilli et al., 2022). Besides, a randomized controlled trial found that continuous oral administration of *Bifidobacteria infantis* (*B. infantis*) 35,624

significantly improved the progression of psoriasis and reduced the expression of C-reactive protein and tumor necrosis factor (TNF)-α, showing that the immunomodulatory effects of the microbiota in humans are not limited to the mucosal immune system but extend to the systemic immune system (Groeger et al., 2013). In addition to this, there are also animal studies that demonstrate that individual probiotics can improve the symptoms of psoriasis (Chen et al., 2017, 2023). GMNL-77 decreased skin erythema and scaling, inhibited hyperplastic suprabasal keratinocytes, suppressed mRNA expression of pro-inflammatory cytokines, including TNF-a, IL-6, and the IL-23/IL-17A axis–associated cytokines (IL-23, IL-17A/F, and IL-22), in skin lesions, decreased the spleen weight, and also reduced the numbers of IL-17/IL-22-producing $CD4^+$ T cells in the spleen (Chen et al., 2017). Furthermore, a probiotic mixture can also improve the symptoms of psoriasis, but the mechanism behind it is complex and variable (Navarro-López et al., 2019; Chen H. L. et al., 2021; Choy et al., 2023).

Recent study has found that the transfer of intestinal microbiota from mice with severe psoriasis-like skin phenotype exacerbated psoriasiform skin inflammation in mice with mild symptoms, including increasing the infiltration and differentiation of Th17 and the abundance of Prevotella while decreasing that of *Parabacteroides distasonis* in the colon. These alterations affected fatty acid metabolism, increasing the abundance of oleic and stearic acids. In turn, administration of oleic and stearic acids exacerbated psoriasis-like symptoms and increased Th17 and monocyte-derived dendritic cell infiltration in the skin lesion areas *in vivo*, as well as increased the secretion of IL-23 by stimulating dendritic cells (DCs) *in vitro*. Therefore, the influence of exogenous dietary factors has to be considered while using probiotics (Zhao et al., 2023). Additionally, do probiotics have side effects, can probiotics colonize the intestine, and what is the quality of survival? How long does it take for probiotics to work? Can it interact with other microorganisms and their metabolites in different intestinal segments? The mechanism of probiotics is still to be fully analyzed (Suez et al., 2019). After the mechanism of single probiotics is clearly explained, the application of probiotics in combination and the corresponding standards and methods still need to be explored. We have different diets and environments, which might cause differences in gut microbiota. Hence, when to use probiotics and their precise application according to different individuals are still to be further developed.

Single strains have made many advances in the treatment of diseases with localized lesions, such as plaque psoriasis. Meanwhile, multistrain therapy, such as FMT, is considered a method to correct gut microbiota dysbiosis and re-establish intestinal microecological balance. Such therapy has been used in recent years to treat psoriasis (Yin et al., 2019), systemic lupus erythematosus (Huang C. et al., 2022), irritable bowel syndrome (El-Salhy et al., 2020), and Parkinson's disease (Zhao et al., 2021), and even influences the response to cancer immunotherapy (O'Leary, 2021). Furthermore, Chen et al. found that the imiquimod-induced psoriasis in mice with healthy donors stool exhibited effective antipsoriatic skin inflammation and even two individual humanized mice almost completely abrogated skin lesion progression (Chen Y. J. et al., 2021). However, the effectiveness of FMT treatment varies depending on the different diseases, the form and number of grafts, the route of administration, and the donor used (Green et al., 2020; Ianiro et al., 2022). An exploratory randomized placebo-controlled trial of 31 patients with active peripheral psoriatic arthritis (PsA) underwent either fecal transplantation or sham surgery. Twenty-six weeks of clinical evaluation showed that fecal transplantation had a good safety profile but had a higher rate of treatment failure than sham surgery and was inferior or non-inferior to sham surgery on secondary measures, such as HAQ-DI scores and ACR20 (Kragsnaes et al., 2021). Moreover, the response rate at 6 months after FMT in patients with irritable bowel syndrome was only 27.5% (Huang H. L. et al., 2022). Although FMT is known to multiply recurrent Clostridioides difficile (mrCDI), greater interest has been drawn to whether the altered microbiota of the recipient affects their risk of other diseases (e.g., psoriasis). Based on a comparison of data from 1,165 CDI patients treated with FMT and 3,692 mrCDI patients who did not undergo FMT, it was found that FMT had no significant association with diabetes, hypertension, or psoriasis, but it increased the risk of myocardial infarction by 68% (Dawwas et al., 2022).

7. Dietary nutrition and psoriasis

Drugs and probiotics alone are not effective in all cases. It has been confirmed that diet has a significant impact on gut microbiota diversity in skin disease patients and normal people (Simpson et al.,

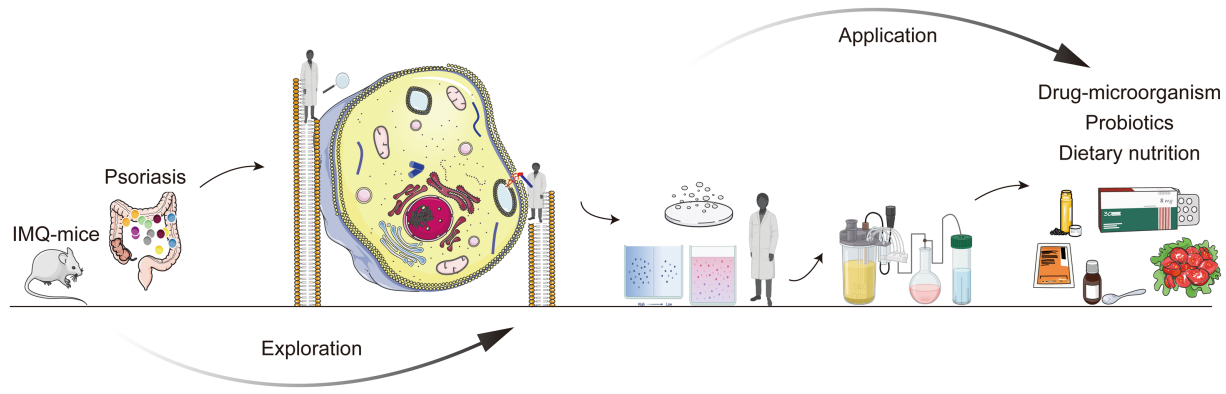

FIGURE 3
Schematic representation of "ignorance to knowledge" of gut microbiota and psoriasis.

2022; Barati et al., 2023). The most recent research found that high-fat diets rather than carbohydrates or proteins exacerbate psoriatic skin inflammation by altering the mucus barrier and gut microbiota, resulting in an enhanced systemic IL-17 response, which exacerbates psoriasis (Sonomoto et al., 2023). However, the fact that a high-fat diet exacerbates tissue inflammatory diseases such as psoriasis may also stem from the molecular mechanisms by which dietary components and tissue lymphocyte responses interact. It has been shown that IL-17-producing γδ T cells in the skin need to sense cholesterol metabolites (hydroxysterols) via GPR183 to maintain their thymic development and skin homeostasis, and that dietary cholesterol promotes the activation of these cells and worsens skin inflammation in mice (Frascoli et al., 2023). A recent study (Shi et al., 2020) has found that mice fed a Western-style diet for a short period of time exhibited IL-17A-mediated skin inflammation before significant weight gain occurred. Mechanistically, the Western diet induces psoriasis-like dermatitis by disrupting the homeostasis of IL-23 and bile acid signaling pathways, promoting γδT cell infiltration at the skin and enhancing their ability to produce IL-17A. Furthermore, a high-fat diet increases free fatty acids, which inhibit TLR-activated hexokinase activity and interfere with the tricarboxylic acid cycle, thereby enhancing the production of mitochondrial reactive oxygen species (mtROS), increasing the unfolded protein response, altering cellular transcription, and increasing IL-23, ultimately exacerbating the inflammatory symptoms of psoriatic skin (Mogilenko et al., 2019). In spite of this, it is still unclear what the long-term health effects of dietary fat will be. Shi et al. demonstrated that an isocaloric moderately high-fat diet extends lifespan in male rats and Drosophila (Shi et al., 2021). As compared to those on an high-carbohydrate, low-fat (HCLF) diet, T2DM patients on a 6-month, calorie-unrestricted, low-carbohydrate, high-fat (LCHF) diet had greater clinically meaningful improvements in their glycemic control and weight. In order to truly benefit your health, you need to adhere to dietary changes over time, as the changes were not sustained 3 months after intervention (Hansen et al., 2023). In addition, high-protein diets may improve glucose homeostasis *in vivo* by promoting glucose tolerance via upper small intestinal peptide transporter 1 and inhibiting gluconeogenesis (Dranse et al., 2018). However, intake of different sources of dietary protein has different associations with long-term cause-specific mortality and chronic disease prevalence. Higher intake of plant proteins is associated with significantly lower cardiovascular disease risk and mortality (Huang et al., 2020). Replacement of red meat with high-quality plant protein foods (legumes and nuts, etc.) improves lipid and lipoprotein indices and inflammatory burden (Guasch-Ferré et al., 2019; Hruby and Jacques, 2019).

Psoriasis, a chronic inflammatory skin disease, has significant associated morbidity and impact on quality of life. In addition to phototherapy, biologic agents, and microbial therapies, it is important to determine whether dietary interventions can help reduce disease severity in psoriasis. Although both short-and long-term very low–calorie ketogenic diets (VLCKDs) have certain side effects, such diet significantly reduces inflammation and is an effective means of relieving symptoms in obese psoriasis patients, possibly related to the microbiota-gut-skin axis (Barrea et al., 2022). A French questionnaire cohort study of 35,000 people, using the MEDI-LITE score to assess adherence to the Mediterranean diet, showed a significant negative association between the MEDI-LITE score and severe psoriasis, suggesting that the Mediterranean diet might slow psoriasis progression (Phan et al., 2018). Moreover, the Medical Board of the National Psoriasis Foundation offers scientifically sound and detailed recommendations for the diet of adults with psoriasis or PsA, including gluten-free diet in psoriasis, dietary weight reduction, and dietary supplements (e.g., fish oil, vitamin D, selenium, and micronutrient supplementation; Ford et al., 2018).

8. Conclusion and future perspectives

In this article, we provided current evidence on the role of the gut microbiome and metabolites in psoriasis and discussed their potential implications for diagnosis and treatment. Significant progress has been made in characterizing the composition of gut microbes and their relevance to inflammatory diseases of the skin, as well as in resolving whether microbes interact with host cells through various small molecules and signaling peptides. Furthermore, a number of emerging microbial interventions/therapeutic strategies and protocols for their clinical application emerged. However, there are still many challenges in facing the important scientific issue of "relapse after drug withdrawal" in psoriasis. Therefore, further exploring the pathogenesis of psoriasis and screening for new targets, new candidate commensal bacteria, and their metabolite molecules that are safe and effective in prolonging the time to relapse are greatly significant.

Author contributions

QZ and KW collected the data research from PubMed and proposed the structure of the manuscript. QZ wrote the first manuscript draft. QY and BM contributed to funding acquisition. FZ and YN had primary responsibility for the final content. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1192543/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 08 January 2024
DOI 10.3389/fmicb.2023.1323644

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OPEN ACCESS

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RECEIVED 18 October 2023
ACCEPTED 26 December 2023
PUBLISHED 08 January 2024

CITATION
Sánchez-Pellicer P, Eguren-Michelena C, García-Gavín J, Llamas-Velasco M, Navarro-Moratalla L, Núñez-Delegido E, Agüera-Santos J and Navarro-López V (2024) Rosacea, microbiome and probiotics: the gut-skin axis.
Front. Microbiol. 14:1323644.
doi: 10.3389/fmicb.2023.1323644

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Rosacea, microbiome and probiotics: the gut-skin axis

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Rosacea is an inflammatory skin disease involving diverse symptoms with a variable clinical progress which can severely impact the patient's quality of life as well as their mental health. The pathophysiological model of rosacea involves an unbalanced immune system predisposed to excessive inflammation, in addition to vascular and nervous alterations, being certain cutaneous microorganisms' triggers of the symptoms onset. The gut-skin axis explains a bidirectional interaction between skin and gut microbiota in some inflammatory skin diseases such as atopic dermatitis, psoriasis, or rosacea. The introduction and consolidation of the next-generation sequencing in recent years has provided unprecedented information about the microbiome. However, the characterization of the gut and skin microbiota and the impact of the gut-skin axis in patients with rosacea has been little explored, in contrast to other inflammatory skin diseases such as atopic dermatitis or psoriasis. Furthermore, the clinical evolution of patients with rosacea is not always adequate and it is common for them to present a sustained symptomatology with frequent flare-ups. In this context, probiotic supplementation could improve the clinical evolution of these patients as happens in other pathologies. Through this review we aim to establish and compile the basics and directions of current knowledge to understand the mechanisms by which the microbiome influences the pathogenesis of rosacea, and how modulation of the skin and gut microbiota could benefit these patients.

KEYWORDS

rosacea, microbiome, probiotic, gut microbiota, skin microbiota, gut-skin axis

1 Introduction

Rosacea is a chronic skin disease affecting approximately 5.5% of general population, mainly patients between 45 and 60 years old, regardless of sex (Gether et al., 2018). Rosacea mainly appears in the cheeks, nose, chin, and forehead, with alternating periods of remission and aggravation. Cutaneous symptoms comprise persistent erythema, papules, pustules, telangiectasia, flushing, sebaceous glands hypertrophy, and fibrosis (characteristically referred to as phyma) (van Zuuren, 2017). In addition, more than 50% of rosacea patients present ocular rosacea even with absent or mild forms of cutaneous symptomatology. Symptoms and signs of ocular rosacea include dryness, burning, itching, photophobia, blurred vision, foreign body sensation, lid margin, conjunctival telangiectasia, meibomian glands collapse, and in

severe forms can be developed corneal inflammation and perforation, scars, or vision loss (Redd and Seitzman, 2020).

Connecting with the chronic nature of rosacea, there is considerable evidence to support its association with various systemic comorbidities which could indicate a systemic inflammatory state. In this context, rosacea is linked with hypertension, dyslipidemia, atherosclerosis, other cardiovascular diseases, gastrointestinal diseases (this issue will be further developed), migraine, anxiety, depression, and even several malignancies (Morss-Walton and McGee, 2021).

The variable clinical spectrum mentioned above is illustrated by the multifactorial origin of rosacea. Therefore, several phenotypes of this diseases are recognized as part of an ongoing inflammatory process. On this basis, with current knowledge of its pathophysiology, the national rosacea society (NRS) published in 2017 a new standard classification system (Gallo et al., 2018). The previous 2002 classification yet identified the main signs and symptoms of rosacea and categorized them into four subtypes: erythematotelangiectatic (ETR), papulopustular (PPR), phymatous, and ocular rosacea, being ETR and PPR the top phenotypes diagnosed (Barakji et al., 2022). The problem with this system was that it did not consider the frequent coexistence of phenotypes and progression from one subtype to another. The updated 2017 classification requires at least one diagnostic criteria (fixed centrofacial erythema in a characteristic pattern that may periodically intensify, phymatous changes) or two major or primary phenotypes (papules and pustules, flushing, telangiectasia, ocular manifestations). In addition, secondary signs, and symptoms (burning, itching, edema, dryness) may also appear in conjunction with one or more diagnostic or primary phenotypes. The global rosacea consensus panel (ROSCO) recommendations supported in 2019 this NRS classification (Schaller et al., 2020). Importantly, rosacea can progress not only to additional phenotypes, but also in severity.

The treatment of rosacea should be based on these phenotypes (according to new NRS classification) (Gallo et al., 2018) and severity. Thus, there are several first-line treatments and in some cases a maintenance schedule could be justified depending on the clinical evolution and background of the patient. Usually in moderate to severe cases a combined approach of oral and topical therapy is required. Moreover, there are a variety of important general instructions for the management of several manifestations of rosacea, including non-aggressive hygiene measures, frequent use of moisturizers and photoprotectors, and elimination or mitigation of recognized aggravating factors (e.g., heat and some foods) (Schaller et al., 2017a; Salleras et al., 2019; Clanner-Engelshofen et al., 2022). However, rosacea is a difficult disease to keep under control. Some studies have shown that approximately 80% of rosacea patients consider their facial erythema to be unpredictable (Dirschka et al., 2015). In many cases the rosacea patient presents a history of therapeutic failure or insufficient results, but it should always be insisted for 6–8 weeks until an exacerbation treatment was considered ineffective (Schaller et al., 2017a; Salleras et al., 2019; Clanner-Engelshofen et al., 2022). At present, not all patients achieve complete resolution of symptoms. Therefore, there is still a need to find more effective treatments (van Zuuren et al., 2021).

A main reason for seeking new therapeutics approaches in rosacea is that its symptomatology can have an emotional impact and even on social relationships resulting in stigmatization. Numerous studies have highlighted a negative impact on the health-related quality of life in rosacea patients (van der Linden et al., 2015). Interestingly, in many patients the severity of rosacea does not correlate with psychosocial severity. Moderate cases already can have a severe psychosocial impact due to the facial location of the symptoms (Oussedik et al., 2018). Common psychosocial comorbidities of rosacea include depression and anxiety (Chang et al., 2022). In relation to associated mental health problems, in a descriptive study involving 827 European rosacea patients, one third reported feelings of stigmatization. These rosacea patients were more likely to avoid social situations (54.2% vs. 2.0%) and presented a higher rate of depression (36.7% vs. 21.1%) than patients without feelings of stigmatization (Halioua et al., 2017). In any case, stigmatization leads to even more difficulties, creating a vicious cycle. Moreover, and directly related to the psychosocial impact, almost half of patients with rosacea-associated facial erythema feel that it interferes with their work life (Bewley et al., 2016).

Finally, and focusing definitively on the objective of this review, it is mandatory to introduce the concept of the gut-skin axis and related promising therapeutic applications. The gut-skin axis explains how inflammatory skin diseases such as atopic dermatitis, psoriasis, acne vulgaris, hidradenitis suppurativa, or rosacea, are the consequences of a sophisticated interplay between genetic, lifestyle, and immune system in continuous synchronization with the nervous and endocrine systems (De Pessemier et al., 2021). Importantly, the cutaneous and gut microbiota play a key role in these relationships, as both skin and colon present constant interaction between microorganisms and the immune system (Salem et al., 2018). In addition, the introduction and consolidation of the next-generation sequencing (NGS) in recent years has enabled to obtain unprecedented information about the microbiome (Gilbert et al., 2018). Therefore, the aim of this narrative review is to analyze the role of gut and skin microbiota in the pathophysiology of rosacea (mainly in cutaneous rosacea), their composition and characteristics in these patients, and to overview the role of probiotics as a potential therapeutic target.

2 Pathophysiology of rosacea

The pathophysiology of rosacea remains incompletely understood. The current rosacea pathophysiological approach suggests an unbalanced immune system predisposed to an excessive inflammation (Wladis and Adam, 2021) (Figures 1, 2), in conjunction with a vascular and neuronal dysfunction, and extrinsic or intrinsic triggers or exacerbating factors such as dysbiosis or several microorganism-related factors (Holmes, 2013; Chang and Huang, 2017), heat-cold, psychological stress, ultraviolet (UV) radiation, alcohol (Searle et al., 2021), smoking (Yuan and Yin, 2021), spicy food (Searle et al., 2021) among others (Wollina, 2019). Next, we will develop the main points of view on the pathophysiology of rosacea and its association with the microbiome.

2.1 Pathophysiology of rosacea: an immunological point of view

The pathophysiology of rosacea begins by the activation of cutaneous immune and nervous systems in response to physical, chemical, or biological stimuli. This subsequently leads to

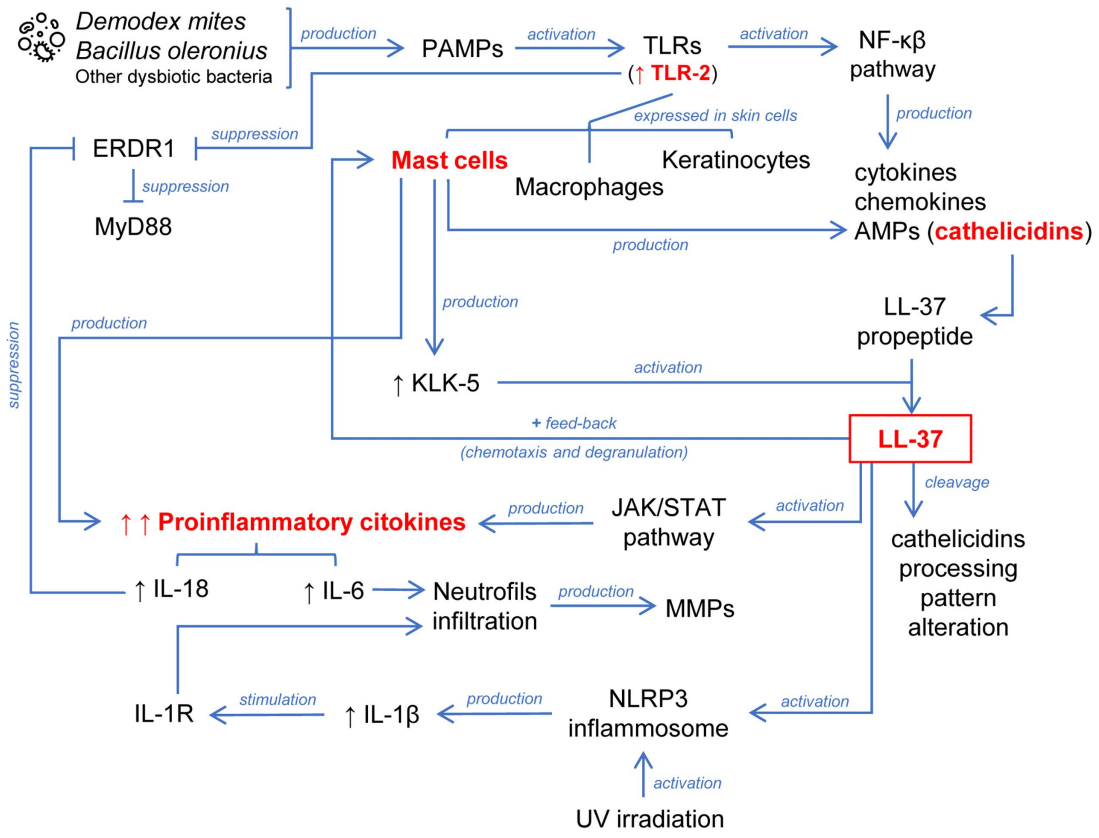

FIGURE 1
Schematic representation of the abnormally sustained innate immune response in the pathophysiology of rosacea. A key process is the activation of the cathelicidin LL-37 generated by different cells localized in the skin. This leads to chemotaxis and degranulation of mast cells through positive feedback. LL-37 is additionally involved in the inflammasome and JAK/STAT pathway activation. The release of proinflammatory cytokines converging these mechanisms leads to the infiltration of neutrophils.

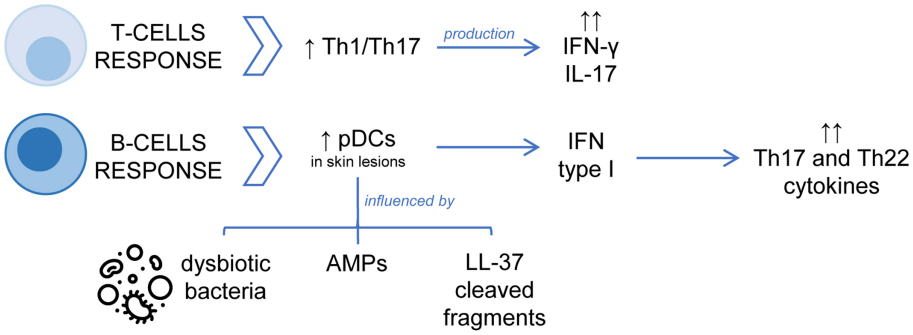

FIGURE 2
Schematic representation of the dysregulation of the adaptative immune response in the pathophysiology of rosacea. The T-cell response is dominated by Th1/Th17 cells, with significant increase of IFN-γ or IL-17. Concerning the B cell-mediated response, the accumulation and activation of pDCs with overexpression of type I IFN in skin lesions are important processes.

morphological changes observed in skin affected by rosacea such as facial erythema, telangiectasia, papules, or pustules. Activation of immune-mediated inflammatory pathways plays a central role in the pathogenesis of rosacea, involving several cell types, especially mast cells, and the release of certain proinflammatory mediators (Ahn and Huang, 2018; Marson and Baldwin, 2020; Wladis and Adam, 2021) (Figures 1, 2).

Pathogen-associated molecular patterns (PAMPs) derived from bacteria such as *Bacillus oleronius* (O'Reilly et al., 2012) or *Demodex* mites (Moran et al., 2017) are biological triggers which activate Toll-like receptors (TLRs) including TLR-2. TLRs play a key role in innate immunity and are expressed in keratinocytes, macrophages, and mast cells of the skin (Kumar, 2021). TLR stimulation leads to activation of the nuclear factor kappa B

(NF-κB) pathway and consequent production of cytokines, chemokines, and antimicrobial peptides (AMPs). The problem begins when uncontrolled activation of the innate immune system implies a detrimental effect. Notably, TLR-2 is overexpressed in keratinocytes of rosacea patients (Yamasaki et al., 2011) enhancing skin sensitivity to external triggers because its stimulation activates an inflammatory cascade.

Cathelicidins are a class of AMPs stored in innate immune system and skin epithelial cells, which mediates leukocyte infiltration (Scheenstra et al., 2020), playing a major role in mammalian innate immune protection against bacteria. Moreover, cathelicidins are also overexpressed in the facial skin of rosacea patients (Yamasaki et al., 2007). Cathelicidin LL-37 (the only human cathelicidin) is secreted by keratinocytes like a biologically inactive propeptide form (called human cationic antibacterial protein of 18 KDa), requiring the action of kallikrein-5 (KLK-5) to release the biologically active peptide (Yamasaki et al., 2006). KLK-5 enzyme activity is enhanced in the skin of rosacea patients, which also explains the increased levels of LL-37 (Yamasaki et al., 2006, 2011). LL-37 could be further cleaved, which would affect its subsequent activity. In this regard, it has been observed that rosacea skin presents a different cathelicidin-processing pattern compared to healthy skin (Yamasaki et al., 2007).

Therefore, an exacerbated innate immune response is established in the skin of rosacea patients due to TLR-2 stimulation involving the production of the active form of the cathelicidin LL-37. In a healthy skin, activation of an innate immune response via TLRs would induce a controlled secretion of cytokines, chemokines, and AMPs, with recruitment and activation of leukocytes to eradicate the threat but without tissue damage. Rosacea patients do not experience the same balanced inflammatory response, so that there is a sustained anomalous innate immune response. In this regard, the role of mast cells in the pathogenesis of rosacea is remarkable (Wang et al., 2019). Mast cells are one of the major sources of cathelicidins and KLK-5 in the skin and are highly active in rosacea patients. In turn, released LL-37 exerts a powerful stimulus on the activity of mast cells inducing their chemotaxis, degranulation, and release of proinflammatory cytokines, generating a positive feedback mechanism. LL-37 has been injected intradermally into mast cell-deficient mice and no inflammation has been observed unlike in wild-type mice. However, when these mast cell-deficient mice have been supplied with mast cells and then injected with LL-37, they have exhibited inflammation (Muto et al., 2014). Moreover, inflammatory mediators secreted by LL-37-activated mast cells such as interleukin 6 (IL-6) lead to an infiltration of neutrophils that continue to amplify the feedback process releasing matrix metalloproteinases (MMPs) (Marson and Baldwin, 2020). KLK-5 can also be stimulated by MMP-9 in the skin of rosacea patients (Jang et al., 2011).

Mast cells also participate in the fibrosis processes observed in certain patients with phymatous rosacea. Histamine and tryptase secretion from mast cells can promote fibrosis by recruiting fibroblasts. Fibroblasts carry on stimulating mast cells causing a release of MMP-1, which can also influence more fibroblasts, facilitating fibrosis (Wang et al., 2019).

The Janus kinase/Signal transducers and activators of transcription (JAK/STAT) pathway has been also involved in the LL-37-mediated inflammatory mechanism of rosacea. Li et al. observed through an *in vitro* study with a keratinocyte cell line treated with LL-37, an increase in JAK2 and STAT3 activity in conjunction with an increase in the production of proinflammatory cytokines (Li et al., 2018).

The erythroid differentiation regulator 1 (ERDR1) is a recently identified cytokine widely expressed in many human tissues, localized in the inner part of the cytoplasmic membrane, and released through vesicles under stressful conditions (Houh et al., 2016). ERDR1 is negatively regulated by proinflammatory IL-18 and suppressed by both TLR-2 and the myeloid differentiation factor 88 (MyD88) pathways (Rodrigues-Braz et al., 2021). In this regard, Kim et al. evidenced that ERDR1 was reduced while IL-18 was increased in rosacea patients compared to healthy controls. These researchers employed a murine model to support the hypothesis of the participation of ERDR1 in the pathogenesis of rosacea. For this reason, they intradermally injected LL-37 into mice inducing the typical signs of rosacea but treatment with recombinant ERDR1 significantly reduced erythema and leukocyte infiltration (including CD4 and CD8 T-cells) (Kim M. et al., 2015).

The inflammasome is a caspase-1 activating multiprotein complex involving active IL-1β release and consequent stimulation of the interleukin 1 receptor (IL-1R) in many cells, with additional neutrophilic infiltration (Tang and Zhou, 2020). Recent studies have revealed that the nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) family pyrin domain containing 3 (NLRP3) inflammasome activation plays a crucial role in LL-37-induced skin inflammation and rosacea pathogenesis (Yoon et al., 2021). Furthermore, it has been observed that UV irradiation increases inflammasome processing and a subsequent release of IL-1β. Thus, activation of the P2X purinoceptor 7 of keratinocytes by UV radiation and LL-37 enhances inflammasome activation. Therefore, LL-37 can modulate the proinflammatory effects of UV radiation contributing to increased susceptibility to sun exposure in rosacea patients (Salzer et al., 2014).

Recent studies have revealed the disruption of the mammalian target of rapamycin (mTOR) pathway in a variety of skin diseases (Karagianni et al., 2022). mTOR is a serine/threonine protein kinase involved in the coordination of a variety of signals regulating many fundamental cellular processes such as cell growth and differentiation, being crucial in skin homeostasis and shaping an appropriate epidermal barrier (Ding et al., 2016). Recently, Deng et al. reported that mTORC1 pathway is hyperactivated in rosacea (Deng et al., 2021). Transcriptional signatures and a cytoplasmic overexpression of the phosphorylated form of the S6 downstream molecule of mTORC1 in both epidermal and infiltrating cells, were found in facial biopsies from rosacea patients compared to healthy controls. Then, using a LL37-induced rosacea-like mouse model, both genetic ablation of mTORC1 and its pharmacological inhibition stopped the development of rosacea. The authors reported a positive correlation between epidermal activation of mTORC1 pathway and the severity of rosacea patients, revealing a mechanism linking dysregulation of the innate immune system and inflammatory response in this disease.

Adaptive immunity is also dysregulated in rosacea patients. The involvement of the adaptive immune system in the pathogenesis of rosacea is less well understood than relevance of the innate immune system. The T-cell response in rosacea is dominated by Th1/Th17 cells as evidenced by significantly increased interferon γ (IFN-γ) or IL-17. Macrophages and mast cells are increased in all subtypes of rosacea, whereas neutrophils reach a maximum in PPR (Buhl et al., 2015).

Regarding B-cell-mediated response, Mylonas et al. have recently published that an overexpression of type I IFN in rosacea flare-ups correlates with the accumulation of plasmacytoid dendritic cells (pDCs) in the dermal infiltrate of skin lesions. In addition, this study showed that commensal skin bacteria are necessary for pDCs activation and type I IFN production, but in rosacea patients dysbiotic bacteria and AMPs increase this capacity. Moreover, cleaved fragments of LL-37 cause infiltration of pDCs into the skin, which are activated to produce high quantities of type I IFN inducing a strong immune response with increased expression of Th17/Th22 cytokines (Mylonas et al., 2023).

2.2 Pathophysiology of rosacea: a vascular and neurovascular point of view

Other important mechanism implicated in the pathogenesis of rosacea is the neurovascular hyperreactivity. An overexpression of some types of transient receptor potential (TRP) cation channels is found in several neuronal and non-neuronal cells of patients with rosacea (Sulk et al., 2012). The TRP channels are divided into several subfamilies (each subtype of TRP channels has different functions) and are localized in both sensory nerves and other non-neuronal cells such as mast cells, dendritic cells, endothelial cells, or keratinocytes, participating in nociceptive and neurogenic inflammatory processes (Rodrigues-Braz et al., 2021). Physical (temperature changes) or chemical stimuli (alcohol, spicy foods) activate these TRP channels triggering the secretion of vasoactive neuropeptides such as substance P (SP), vasoactive intestinal peptide (VIP) and calcitonin gen-related peptide (CGRP) (Marson and Baldwin, 2020). SP also induces mast cell degranulation expanding this neurogenic inflammation (Choi and Di Nardo, 2018). These mechanisms are related to the presence of telangiectasia and sustained flushing observed in rosacea patients in such a way that affected rosacea skin has a significantly lower threshold for heat and chemicals compared to non-affected skin.

Angiogenesis, persistent vascular and lymphatic dilation, and increased vascular permeability are also involved in the pathophysiology of rosacea. In skin biopsies of rosacea patients, the vascular endothelial growth factor (VEGF) expression is increased in epidermal and immune-infiltrating cells (Smith et al., 2007), being a critical regulator of angiogenesis. Moreover, LL-37 can increase VEGF levels in keratinocytes (Apte et al., 2019). In this regard, Chen et al. by means of a LL-37-induced rosacea-like murine model, showed that intraperitoneal thalidomide injection significantly alleviated erythema and reduced inflammatory cell infiltration, microvessel density and VEGF expression, in dermis (Chen et al., 2019). On the other hand, LL-37 can produce proangiogenic effects by activating the formyl peptide type 1 (FPR1) and the epidermal growth factor receptor (EGFR) in epithelial cells (Kajiya et al., 2017). It has also been reported an increased expression of the vascular cell adhesion molecule-1 (VCAM-1), the intracellular adhesion molecule-1 (ICAM-1) and E-Selectin in conjunction with high expression of LL-37, in facial skin biopsies of rosacea patients (Kulkarni et al., 2020). In addition, mast cell activation and recruitment on skin lesions as well promotes angiogenesis through the secretion of proangiogenic substances such as VEFG or fibroblast growth factor (FGF) (Shaik-Dasthagirisaheb et al., 2013).

2.3 Pathophysiology of rosacea: a genetic point of view

Some information indicates that there is a strong genetic predisposition and heritability of rosacea. Firstly, a family history is observed in more than one third of rosacea patients. Secondly, there is a high incidence of rosacea in certain populations such as Celtic and northern European descendants (Awosika and Oussedik, 2018). Furthermore, homozygous twins have higher NRS scores than heterozygous twins, and it has been estimated that the genetic contribution to NRS is close to 50% (Aldrich et al., 2015).

Moreover, an association of rosacea with some autoimmune diseases has been established (Egeberg et al., 2016). Interestingly, there are some shared associations between genes encoding human leukocyte antigen (HLA) variants and diseases such as celiac disease, type 1 diabetes, multiple sclerosis, inflammatory bowel disease, sarcoidosis, etc. (Awosika and Oussedik, 2018).

Nevertheless, the specific role of genetic in the development of rosacea is not fully elucidated, although in recent years several closely related associations with its pathophysiology have been evidenced. In this regard, Chang et al. conducted a genome-wide association study (GWAS) of 2,618 rosacea cases and 20,334 controls and identified one significant single-nucleotide polymorphism (SNP) associated with rosacea (Chang et al., 2015). This rosacea-associated intergenic SNP *rs763035* is positioned between *HLA-DRA* and *BTNL2* genes. Immunohistochemical skin analysis in PPR patients from this cohort reported the presence of HLA-DRA in epidermal Langerhans cells, BTNL2 in keratinocytes, and both in perifollicular infiltrates and endothelial cells. In addition, 3 HLA alleles such as *HLA-DRB1*03:01*, *HLA-DQB1*02:01*, and *HLA-DQA1*05:01*, were significantly associated with rosacea. These data could support the relevance of antigen presentation such as those from microorganisms in the pathophysiology of rosacea. Recently, Aponte et al. published other GWAS with 73,265 individuals of 97% European ancestry who self-reported rosacea (Aponte et al., 2018). Seven loci were identified including 2 related with skin and pigmentation phenotypes, 2 with inflammation 1 with both phenotypic categories, and 2 intergenic loci that were *a priori* unrelated to the pathophysiology of rosacea. Helfrich et al. in a case–control observational study of facial biopsies gene expression of ETR patients, revealed that some genes were overexpressed, being significantly remarkable those related with neuropeptides, mast cells and inflammation, matrix remodeling and AMP processing (Helfrich et al., 2015). Other case–control studies have identified some more SNPs in rosacea-associated genes providing evidence for the contribution of a genetic predisposition to the pathophysiology of rosacea (Yazici et al., 2006; van Steensel et al., 2008; Karpouzis et al., 2015; Akdogan et al., 2019; Hayran et al., 2019).

2.4 Pathophysiology of rosacea: a microbiological point of view

2.4.1 Skin microbiota and pathophysiology of rosacea

Several microbes located on the skin have been associated with the pathogenesis of rosacea. The composition, stability and functionality of the cutaneous microbiota depends on the interactions between skin microorganisms and the conditions provided by the host

(Byrd et al., 2018). The skin microbiota can prevent colonization by pathogens but in certain situations even beneficial or commensal bacteria can become pathogenic. This is noticed in many skin diseases (Sánchez-Pellicer et al., 2022) including rosacea. In this regard, Anna D. Holmes in 2013, based on these principles, established a multi-step model to explain the influence of the skin microbiota as a major player on the onset and progression of rosacea (Holmes, 2013). According to this model, firstly, hyperreactivity of TLRs or decreased tolerance to PAMPs from commensal or pathogenic bacteria, not activating under normal conditions, would trigger inflammatory pathways. This initial inflammation would modify the skin physiology and impact on the skin microbiota which will be reflected with the increased load of *Demodex* mites among other associations. This modification of the skin microbiota would affect the innate immunity which will exacerbate the whole inflammatory process contributing to the development of papulopustular lesions. Once a stable status has been reached between skin microbiota and innate immunity, a new imbalance would lead to the cyclical nature of rosacea. However, 10 years after the presentation of this model, knowledge of the pathophysiology of rosacea has increased, although it is not fully elucidated. In other words, it is not yet fully established whether these microorganisms are triggering factors of rosacea or whether they appear as a consequence of rosacea. This is a key issue still unresolved nowadays.

Demodex mites are recognized as commensal present in a diverse spectrum of host animals, being normal denizens of hair follicles and sebaceous glands because sebum is its main source of feeding. In contrast to other mites such as *Dermatophagoides*, they are both obligate commensals and host-specific. Characteristic *Demodex* species in humans are *Demodex folliculorum* and *Demodex brevis* (Foley et al., 2021). *Demodex* mites do not typically cause dermatological problems unless they reach a high load and/or penetrate the dermis. However, a strong association has been observed between density of *Demodex* mites and incidence of rosacea. A meta-analysis published in 2017 of case–control studies revealed that prevalence of *Demodex* colonization was 70.4% in rosacea patients vs. 31.8% in healthy controls, and the mean density was 71.0 mites/cm^2 in rosacea patients vs. 8.7 mites/cm^2 in healthy controls (Chang and Huang, 2017). Furthermore, in this meta-analysis, both ETR and PPR patients showed significantly higher *Demodex* density than healthy controls, although in PPR group tended to be greater than in ETR. The authors concluded that although this data cannot demonstrate a cause-effect relationship between *Demodex* mites and rosacea, there is an association suggesting mites could play a key pathogenic role. However, the authors stated as a limitation the great variability between studies at the level of different sampling, examination methods, and control groups.

Some research has shown that *Demodex* mites may itself contribute to the early inflammatory process in rosacea patients. On this issue, *Demodex* mites stimulate TLR-2 consequently increasing production of proinflammatory cytokines and playing a role in the continuum of rosacea pathogenesis (Lacey et al., 2018). In addition, mechanical blockage of the pilosebaceous unit due to *Demodex* overgrowth would also affect the skin barrier function and cause tissue damage (Moran et al., 2017). In contrast, there are some immune tolerance mechanisms that could explain a cutaneous proliferation of *Demodex* mites in rosacea patients. As we have mentioned previously, VEGF expression is increased in epidermal and immune-infiltrating cells such as lymphocytes, macrophages, and plasma cells (Smith et al., 2007). VEGF, due to its immunosuppressive properties, could induce T-cell proliferation and through collaboration with tolerogenic dendritic cells, promote the initial spread of *Demodex* mites (Forton, 2020) in a similar way that enhances the immune escape of tumor cells (Bourhis et al., 2021). Moreover, *Demodex* expresses the Thomsen-nouveau antigen (Tn Ag) (Kanitakis et al., 1997) which interacts with the macrophage galactose-type lectin (MGL) receptor of dendritic cells inducing their tolerance (Zaal et al., 2020). Thus, the inflammatory reaction could be insufficient to eradicate *Demodex* mites as some of the infiltrating T-cells have become dysfunctional, so that polymorphisms in dendritic cells would explain the different susceptibility to *Demodex* antigens (Forton, 2020). On the other hand, a recent meta-analysis evaluating the efficacy of different anti-*Demodex* treatments concluded that topical and systemic ivermectin, topical ivermectin-metronidazole, and topical tea tree oil, are promising anti-*Demodex* interventions (Li et al., 2023). In addition, topical ivermectin 1% treatment for 12 weeks significantly decreased *Demodex* density and downregulated IL-8, LL-37, TLR-4, human β-defensin 3 (HBD3), and tumor necrosis factor α (TNF-α) gene expression, implicating anti-inflammatory effects along improving clinical course of rosacea (Schaller et al., 2017b). Therefore, the role of *Demodex* mites in the pathophysiology of rosacea is complex and partially unknown, remaining unanswered questions about their immunostimulatory and immunotolerant activity.

Bacillus oleronius (the current name is *Heyndrickxia oleronia*) is a gram-negative bacterium that was first isolated from a *D. folliculorum* mite extracted from the face of a PPR patient. Remarkably, *B. oleronius* presented antigens that significantly stimulated the proliferation of peripheral blood mononuclear cells to a greater degree in rosacea patients than in controls (Lacey et al., 2007). Further studies have confirmed the immunoreactivity of rosacea patients to 62- and 83-kDa proteins of *B. oleronius* (O'Reilly et al., 2012; Jarmuda et al., 2014). Moreover, McMahon et al. demonstrated how *B. oleronius* proteins can induce neutrophil recruitment through activation of the inositol trisphosphate (IP3) pathway with production of proinflammatory cytokines (McMahon et al., 2016). Maher et al. showed that typical increased skin temperature of rosacea patients modified growth and protein pattern of *B. oleronius* leading to a greater production of immunoreactive proteins (Maher et al., 2018). Therefore, there is evidence that bacteria provided by *Demodex* mites can aggravate the established inflammatory response in rosacea. However, when Murillo et al. investigated the *Demodex* microbiota of rosacea patients by a culture-independent method did not identify *B. oleronius* in facial skin samples (Murillo et al., 2014a). Recently, Mylonas et al. found that *B. oleronius* amplifies type I IFN production by pDCs compared with other skin commensal bacteria. Nevertheless, the presence of *B. oleronius* was itself insufficient and a previous bacterial clearance by cathelicidin peptides was required, being specific microbial DNA the really trigger (Mylonas et al., 2023).

A recent study has revealed that *Corynebacterium kroppenstedtii* lives in mutualistic symbiosis with *D. folliculorum* and viable form of this bacterium seem to be an obligatory criterion for viability of the host (Clanner-Engelshofen et al., 2020). Rainer et al. found that *C. kroppenstedtii* was among the most abundant bacteria in rosacea subjects between 40 and 49 years, especially in patients with combined ETR and PPR, compared to an absence in their matched controls (Rainer et al., 2020). *C. kroppenstedtii* has been occasionally associated

with human infections, mainly breast abscesses and granulomatous mastitis, but more studies will be necessary to clarify its real role in these diseases (Tauch et al., 2016). All these findings suggest that an effective antibiotic treatment against this bacterium could reduce the burden of *Demodex* mites and thus improve the clinical management of rosacea patients.

Staphylococcus epidermidis, a common skin inhabitant with beneficial effects (Byrd et al., 2018), was isolated in pure culture from pustular lesions of rosacea patients in contrast to the absence of growth from unaffected skin of these patients (Whitfeld et al., 2011). A few years earlier Dahl et al. published a study showing that *S. epidermidis* from facial skin of PPR patients secreted more proteins and generally more of each protein at 37°C compared with 30°C (Dahl et al., 2004). This data suggested that *S. epidermidis* would behave in a particular way due to changing skin conditions which are not present in patients not affected by rosacea, generating virulence factors. An extrapolation may be made to other bacteria which could modify its characteristics and contribute to the pathogenesis of rosacea under the conditions of a sick skin.

Given the similarity between acne vulgaris and some rosacea phenotypes, the role of *Cutibacterium acnes* in the pathophysiology of rosacea has been explored and suspected. *C. acnes* is a ubiquitous bacterium in healthy human skin being predominant in sebaceous regions, with a key role in cutaneous homeostasis, even acting in the prevention of pathogens colonization (Byrd et al., 2018; Sánchez-Pellicer et al., 2022). In general, *C. acnes* strains more associated with acne induce a more powerful inflammatory response than those less associated strains (Sánchez-Pellicer et al., 2022). Regarding rosacea, Jahns et al. investigated the presence of *C. acnes* in skin biopsies from PPR patients through an immunofluorescence assay (staining with a *C. acnes*-specific monoclonal antibody QUBPa3) (Jahns et al., 2012). In this study, skin biopsies from 82 rosacea patients and 25 controls were analyzed and only in 8.5% of rosacea patients were detected *C. acnes*. With these findings, the authors stated that *C. acnes* is unlikely to play a major role in the pathogenesis of rosacea. On the other hand, some researchers have hypothesized that removal of *C. acnes* from the skin microbiota is really what could contribute to the pathogenesis of rosacea (Marson et al., 2022). This is based on some recent studies using NGS of *16S rRNA* bacterial gene demonstrating a reduction of *C. acnes* compared to controls (Wang et al., 2020). However, Rainer et al. reported that *C. acnes* was the most representative bacterium in both rosacea patients and controls but was decreased in rosacea male patients compared to male controls (Rainer et al., 2020). Therefore, although *C. acnes* could apparently have a protective role against rosacea, some studies have reported contradictory results, and no causal relationship has been fully established. Some researchers have also proposed that like what occurs in acne (Sánchez-Pellicer et al., 2022), the specific pattern of *C. acnes* strains found in rosacea patients would be the fact particularly relevant in relation to rosacea pathophysiology (Thompson et al., 2021).

Systemic antibiotics have demonstrated efficacy in management of rosacea, specially in PPR patients (Xiao et al., 2023). However, the specific effect of these treatments on the cutaneous microbiota or on bacteria related to the pathophysiology of rosacea has barely been studied (Woo et al., 2020). There is a controversy in a practical order over the use of antimicrobial doses (50–200 mg) vs. anti-inflammatory doses (40 mg) of tetracyclines. However, several guidelines emphasize that it is not appropriate to use antimicrobial doses of antibiotics for the treatment of rosacea, as the administration of these drugs should only have an anti-inflammatory effect, and this effect is already obtained with doses as low as 40 mg of doxycycline (Salleras et al., 2019). This dose provides subantimicrobial levels of doxycycline, reducing the inflammatory response in rosacea patients without producing drug concentrations required to treat infections. Doxycycline can reduce the levels of reactive oxygen species (ROS) generated by neutrophils, can inhibit the expression of nitric oxide synthase, and can suppress the release of MMPs and proinflammatory cytokines (McKeage and Deeks, 2010).

2.4.2 Gut microbiota and pathophysiology of rosacea

The human gastrointestinal tract contains over 100 trillion bacteria, the majority inhabiting the large intestine and forming the gut microbiota. These bacteria are involved in numerous metabolic reactions that substantially influence host physiology (Gilbert et al., 2018). The gut microbiota composition is mainly modulated to a greater degree by environmental or lifestyle factors such as diet or exposure to antibiotics. It also depends on age, sex, stress, diseases, and host-related genetic factors (Rothschild et al., 2018). The gut microbiota composition is stable in healthy adults and comprises a highly adaptive microbial community that constitutes a dynamic ecological balance (Lozupone et al., 2012). However, the gut microbiota could be exposed to disturbances altering this dynamic balance. A key characteristic of gut microbiota is the resilience so that there is a strong tendency to maintain its structure, meaning that it can continue being stable after a phase of modification and further recovery (Sommer et al., 2017). When this modifying factor is sustained and/or very powerful, so that it exceeds the resilience of the gut microbiota, this stable state disappears and the gut microbiota adapts to an alternative state, which could be dysbiotic.

The gut microbiota is closely involved with the immune system. Gut commensal bacteria act as regulators in the processes of immune tolerance (Takiishi et al., 2017). In fact, about 70% of lymphocytes are found in the gut-associated lymphoid tissue (GALT). Body areas colonized by microorganisms present the highest number of immune cells. Thus, changes in composition and diversity of the gut microbiota can lead to immunological and inflammatory disturbances in organs distant from the gut (Belkaid and Naik, 2013). In recent years, the concept of the skin-gut axis has been proposed to understand the pathophysiology of several skin chronic inflammatory diseases (O'Neill et al., 2016). The skin-gut axis describes how skin health is influenced by gastrointestinal health through the involvement of the immune system, metabolic-hormonal pathways, and the nervous system (Mahmud et al., 2022). The gut microbiota impacts on immunity which is recognized as the key regulator of the gut-skin axis and a gut dysbiosis impairs the balance of the immune system (Salem et al., 2018). While the exact mechanisms of the functionality of the gut-skin axis have not been fully established, there is growing evidence of the beneficial effects of probiotics in inflammatory skin diseases (Navarro-López et al., 2018, 2019; Sánchez-Pellicer et al., 2022) and this strikingly suggests that there is a complex relationship between the gut and the skin. Additional evidence for the relevance of the skin-gut axis is that many skin diseases co-exist together with non-cutaneous conditions such as gastrointestinal diseases (Wang and Chi, 2021; Thye et al., 2022).

The exact mechanisms of how the gut microbiota is linked to the onset and development of rosacea have not been fully established. By analogy with other immune-based inflammatory diseases such as atopic dermatitis, psoriasis, or acne vulgaris and with evidence of the existence of the gut–skin axis, it is likely that the main mechanism participates in the inflammatory immune response. A dysbiosis can lead to a compromised intestinal mucosal layer and impaired epithelial tight junctions, resulting in a worsening of the intestinal barrier function with translocation of bacteria and/or harmful compounds of bacterial origin (such as toxins or fragments of bacterial elements stimulating the immune system) from the gut into the bloodstream (Kinashi and Hase, 2021). A healthy gut microbiota maintains the integrity of the intestinal barrier by transforming complex polysaccharides into short-chain fatty acids (SCFA) (Koh et al., 2016). In addition, butyrate, a major SCFA, exerts a potent anti-inflammatory effect as it suppresses immune responses by inhibiting proliferation, migration, adhesion, and cytokine production by inflammatory cells (Salem et al., 2018). Enhanced PAMPs in the bloodstream and decreased butyrate of bacterial origin could also imply a hyperresponsiveness of B-cells and impaired differentiation of T-cells (Mahmud et al., 2022). Dysbiotic bacteria and/or harmful bacterial compounds along with altered immune cells, can reach the skin from bloodstream and impact on cutaneous physiology, pathology, and immune response (De Pessemier et al., 2021). As the pathophysiology of rosacea involves activation of the skin immune and nervous systems in response to physical, chemical, or biological triggers, these gut dysbiosis-driven changes could lead to disease progression, increased severity, flare-ups, or sustained symptomatology.

The coexistence of rosacea and gastrointestinal disorders has been documented. This supports the relationship between the gut and the skin in the pathophysiology of this disease. Egeberg et al. in 2016 published a Danish nationwide cohort study with 49,475 rosacea patients and 4,312,213 general population controls, investigating the association between rosacea and celiac disease, Crohn's disease, ulcerative colitis, *Helicobacter pylori* infection, small intestinal bacterial overgrowth (SIBO), and irritable bowel syndrome (Egeberg et al., 2016). The baseline prevalence of all these gastrointestinal diseases was significantly higher in patients with rosacea compared to control subjects. However, through a 5-year follow-up survival analysis, adjusted hazard ratios did not reveal significant associations between rosacea and *H. pylori* infection and SIBO. Therefore, this large cohort study reported an increased prevalence of *H. pylori* infection and SIBO in patients with rosacea, whereas the risk of new onset of *H. pylori* infection and SIBO was not increased in rosacea patients. A singular question would be whether patients treated with antibiotics for SIBO or *H. pylori* infection will improve the symptomatology of rosacea. In this regard, a 3-year follow-up study evaluating the role of SIBO in the pathophysiology of rosacea revealed that SIBO treatment with rifaximin also led to clinical remission of rosacea in all patients, and then it persisted in the majority throughout follow-up period (Drago et al., 2016). Furthermore, this study revealed that the risk of SIBO is significantly higher in PPR than in ETR. Remission of rosacea concomitant to SIBO treatment has been evidenced in other studies (Wang and Chi, 2021). The subjacent mechanism relating SIBO to rosacea has not been clarified. Nevertheless, bacterial invasion in the small intestine leads several pathological consequences such as direct mucosal injury, toxins, malabsorption, decreased brush border enzyme activity, excessive H_2 and CH_4, among others (Bushyhead and Quigley, 2022). On the other hand, Jørgensen et al. published in 2017 a meta-analysis comprising 928 rosacea patients and 1,527 controls, highlighting a significant association between *H. pylori* infection only if the diagnostic was restricted to breath test. In addition, the effect of eradication treatment on rosacea symptoms was not significant (Jørgensen et al., 2017). Unlike SIBO, a connection between *H. pylori* and rosacea is apparently better established. *H. pylori* infection can trigger a cytotoxic reaction inducing release of TNF-α and IL-8 due to the factor virulence cytotoxin-associated gene A (CagA), aggravating the inflammatory reaction involved in the pathophysiology of rosacea. Moreover, *H. pylori* can impact on skin conditions by increasing N_2O concentration leading to vasodilatation and inflammation (Yang, 2018).

3 Analysis of the skin microbiota in rosacea patients

Studies characterizing the skin microbiota in rosacea patients are scarce and relatively recent (Table 1). New culture-independent techniques based on NGS of the *16S rRNA* gene have allowed an increasingly comprehensive characterization of the microbiome (Gilbert et al., 2018). In the past, skin microorganisms were characterized by culture methods, but these underestimated the complete diversity of the cutaneous microbiota. Therefore, to overcome the limitations of microbiological culture and to understand the full diversity of the skin microbiota, sequencing methods have been applied. Let us review the research on the topic to date.

Zaidi et al. published in 2018 a study involving 60 twins over 18 years of age (mainly monozygotic), of which 18 presented rosacea (32 participants matched) (Zaidi et al., 2018). The authors analyzed the skin microbiota from bilateral malar cheeks using Sebutape® strips (affected skin for cases and unaffected skin for controls). This twin design provided genetic, and in many cases environmental, control and an appropriate matching. No significant difference was detected in the α-diversity Shannon index between rosacea affected and non-rosacea affected monozygotic twin pairs. Then, the authors performed a correlation analysis between the NRS score and the Shannon index of matched twins, showing a negative association and suggesting that rosacea severity negatively affects bacterial diversity, but without statistical significance. A principal coordinate analysis (PCoA) based on weighted and unweighted UniFrac or Bray-Curtis distances did not demonstrate a significant separate clustering between rosacea patients and healthy controls, although monozygotic twins presented a more similar skin microbiota than dizygotic twins. Furthermore, no significant difference was observed in the relative abundance of the predominant phylum in subjects with and without rosacea, which were in descending sequence Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes. Interestingly, a univariate random effect Poisson regression (REPR) showed a significant association between NRS score and *Gordonia*, *Blautia*, *Chryseobacterium*, *Wautersiella* and *Geobacillus* genera. Multivariate REPR revealed that *Gordonia* and *Geobacillus* and age range 30–60 years were significantly predictive of NRS score. These results support the hypothesis that rosacea severity is related and linked to skin microbiota.

TABLE 1 Main studies including skin microbiota data in rosacea patients.

Study	Methodology and study population	Key results
Zaidi et al. (2018)	• 60 twins (32 without rosacea, 18 with rosacea, 32 matched) • Affected (cases) an unaffected (controls) skin from cheeks sampled using Sebutape® strips	• No significant difference was observed regarding α-diversity between rosacea and non-rosacea monozygotic twin pairs • PCoA did not demonstrate a separate clustering between rosacea patients and healthy controls • No significant difference was observed in relative abundance of any predominant phylum between rosacea patients and healthy controls • *Gordonia* and *Geobacillus* genera and age range "30–60 years" were significantly predictive of NRS score
Rainer et al. (2020)	• Case–control study • 19 individuals with ETR, PPR or both, and 19 age- and sex-matched controls • Skin from nose and cheeks using sterile swabs	• Greater richness in rosacea patients vs. paired controls without statistically significant degree • Analysis of similarity did not show clustering between ETR and PPR patients and matched controls • *Cutibacterium acnes*, majority species in both rosacea patients and controls • *Corynebacterium kroppenstedtii* increased in rosacea patients mainly with combined ETR and PPR
Thompson et al. (2020)	• Data analysis extension of Rainer 2020 study	• Significant increase in *Campylobacter ureolyticus* and *Prevotella intermedia* and a depletion in *Acinetobacter* PPR patients compared to controls
Thompson et al. (2021)	• Case–control study • 19 individuals with ETR, PPR or both, and 19 age- and sex-matched controls (same rosacea cohort as Rainier 2020 study) • 8 acne patients and 8 age- and sex-matched controls. Skin from nose and cheeks using sterile swabs	• Significantly higher α-diversity in acne patients than in rosacea patients • No significant difference was observed regarding α-diversity between cases and controls of both conditions • Different clustering in PCoA plot between cases and controls for acne and cases and controls for rosacea • Proteobacteria, majority phylum in acne patients • Actinobacteria, majority phylum in rosacea patients • *Serratia marcescens* and *Cutibacterium acnes* were increased in rosacea patients vs. acne patients • *Cutibacterium acnes* abundance relative in PPR patients was like that in acne patients
Woo et al. (2020)	• Rosacea patients with IGA 3 and 4 • Skin from cheeks using sterile swabs • Skin microbiota analysis before and after 6 weeks doxycycline oral treatment	• α-diversity did not change regarding age (older and younger than 60 years), IGA, before and after doxycycline treatment • Weak clustering non-significant based on analysis of similarities both per patient and per treatment • *Weissella confusa* increased significantly after doxycycline treatment • *Cutibacterium acnes* presented a significantly higher relative abundance in IGA 3 patients • *Snodgrassella alvi* presented a significantly higher relative abundance in IGA 4 patients
Wang et al. (2020)	• Case–control study • 21 ETR, 15 PPR and 22 healthy controls • Skin fungal and bacterial communities' analysis • Skin from cheeks using sterile swabs	• α-diversity increased in PPR patients compared to healthy controls • ETR patients presented a higher relative abundance of Firmicutes phylum compared to healthy controls • PPR patients presented a lower relative abundance of Actinobacteria phylum compared to healthy controls • *Cutibacterium* was significantly decreased in both ETR and PPR patients • *Staphylococcus* was increased in ETR patients • *Streptococcus* was increased PPR patients • Significant changes were not observed in the rosacea-associated fungal microbiome • PCoA did not demonstrate a separate clustering of fungal microbiome and bacterial microbiota between rosacea patients and healthy controls
Murillo et al. (2014a)	• *Demodex* microbiota • 15 ETR, 15 PPR and 17 sex and age-matched healthy controls • Skin biopsies of alar crease	• Actinobacteria, dominant phylum in ETR patients and controls but greatly diminished in PPR patients • Proteobacteria and Firmicutes, increased in PPR patients • *Duganella zoogloeoides*, the most represented species in ETR patients • *Acinetobacter pitii*, the most represented species in PPR patients • Pathogens such as *Bartonella*, Haemophilus or *Escherichia* were only observed in rosacea patients

Rainer et al. presented in 2020 a case–control study with 19 adult subjects diagnosed with ETR, PPR, or both, age and sex-matched with 19 controls without rosacea (Rainer et al., 2020). These researchers evaluated the cutaneous microbiota of the nose and cheeks using sterile foam-tipped swabs. Skin microbiota with greater richness was evidenced in rosacea patients vs. paired controls by means of a phylogenetic diversity whole tree metric obtained from rarefaction curves, but without statistically significant degree. Analysis of similarity using weighted UniFrac distances did not show any clustering between ETR and PPR skin samples vs. their matched controls. Concerning the abundance of majority species, *C. acnes* was the most representative in both rosacea patients and controls. However, although *C. acnes* was increased in male controls (57%) compared to female controls (30%), the relative abundance was similar between male (24%) and female (28%) rosacea patients. *C. kroppenstedtii* was the second most abundant species in rosacea patients, reaching 6% in subjects between 40 and 49 years as compared to the virtual absence in their matched controls. Among the different rosacea subtypes, *C. kroppenstedtii* was highly increased in patients with combined ETR and PPR (19%) and was 5 and 1% in patients with PPR and ETR, respectively. Therefore, only remarkable differences between different rosacea subtypes and controls were detected at species-specific level. Thompson et al. published also in 2020 an extension of the results of this study highlighting other interesting species-level differences between study groups (Thompson et al., 2020). It was emphasized a significant increase in *Campylobacter ureolyticus* and *Prevotella intermedia* and a depletion in *Acinetobacter* in the skin microbiota of PPR patients compared to controls. These associations could be responsible for a relationship between rosacea and its comorbidities (Haber and El Gemayel, 2018).

This last researcher group published another recent microbiome study in acne and rosacea patients (Thompson et al., 2021). These skin conditions follow different clinical courses with some similar clinical manifestations, suggesting there are fundamental pathophysiological differences. The authors considered whether the skin microbiota (samples from bilateral cheeks and nose) could explain such differences and conducted a case–control study with 8 acne patients matched to 8 controls and 19 rosacea patients matched to 19 controls [same rosacea cohort as (Rainer et al., 2020)]. Notably, acne population was younger and more racially diverse than rosacea population. The Shannon index revealed significantly higher α-diversity in acne patients than in rosacea patients. However, α-diversity was similar between cases and controls for both conditions. Using a weighted UniFrac distance analysis, a significant difference was observed between all study groups, but even when examining at the PCoA plots, clustering was apparent between cases and controls for acne and cases and controls for rosacea. Proteobacteria was the most abundant phylum in acne patients and was significantly increased compared to rosacea patients. Actinobacteria was the most abundant phylum in rosacea patients and was significantly increased compared to acne patients. *Serratia marcescens* and *C. acnes* were both increased in patients with rosacea vs. patients with acne. The authors suggested that the different relative abundance of *C. acnes* in the study groups could reflect a specific pattern of *C. acnes* strains. For example, the relative abundance of *C. acnes* in rosacea patients with inflammatory papules and pustules was comparable to that in acne patients, but lower than in rosacea patients without inflammatory papules and pustules. This is of interest as rosacea with inflammatory papules and pustules are clinically close to acne.

By means of a different approach regarding these studies, Woo et al. reported in 2020 a study with 12 rosacea patients with severity scores 3 and 4 using the investigator's global assessment (IGA) grading scale (Woo et al., 2020). The skin microbiota from their cheek before and after an oral treatment with doxycycline for 6 weeks was examined. No changes in α-diversity indices were observed regarding age (older and younger than 60 years) or rosacea severity before and after treatment with doxycycline. Analysis of similarities based on weighted UniFrac distance evidenced a weak clustering of samples per patient and per treatment (both not significant) suggesting interindividual variability of the skin microbiota and an associated resilience. The most abundant species on the skin of rosacea patients before treatment with doxycycline were *Staphylococcus epidermidis* (28%), *C. acnes* (13%), *Pseudomonas koreensis* (8%), *Actinetobacter haemolyticus* (7%) and *Snodgrassella alvi* (6%). The most abundant species on the skin of rosacea patients after treatment with doxycycline were *S. epidermidis* (22%), *Stenotrophomonas rhizophila* (8%), *C. acnes* (7%) and *Corynebacterium tuberculostearicum* (7%). Relative abundance of *Weissella confusa* increased significantly after doxycycline treatment. Regarding severity, *C. acnes* exhibited a significantly higher relative abundance in IGA 3 patients while *S. alvi* in IGA 4 patients. This study showed that the cutaneous microbiota in rosacea patients had some specific characteristics depending on age and severity and, importantly, is modified by a systemic antibiotic treatment.

Another case–control study was that of Wang et al. characterizing the cutaneous fungal community in rosacea patients in addition to the cutaneous bacterial ecosystem (Wang et al., 2020). Twenty-one ETR patients, 15 PPR patients, and 22 healthy subjects (50 women and 8 men between 18 and 64 years) were included. Skin swabs were collected from both cheeks for sequencing and analysis of *16S rRNA* and *ITS1* amplicons. Regarding bacterial microbiota, ETR and PPR patients presented a higher and lower relative abundance of Firmicutes and Actinobacteria compared to healthy controls, respectively. *Cutibacterium* was the dominant genus in healthy controls and its relative abundance was significantly decreased in patients with ETR and PPR. *Staphylococcus* and *Streptococcus* showed different behavior depending on the rosacea subtype, with *Staphylococcus* increasing in patients with ETR and *Streptococcus* increasing in patients with PPR. However, no significant difference was observed based on mild, moderate, or severe forms of rosacea. Moreover, no significant changes were observed in the fungal microbiome associated with rosacea, being dominant *Malassezia* and *Alternaria* genera. Likewise, an increase in the Shannon index was observed in PPR patients compared to healthy controls, but no difference was evidenced at mycobiome level. PCoA based on weighted UniFrac distance of bacterial and fungal microbiome also did not demonstrate a different clustering between rosacea patients and controls.

As mentioned above, subjects suffering rosacea present a significantly higher prevalence of the degree of *Demodex* mite infestation and this could play a role in the rosacea pathophysiology. In this way, Murillo et al. characterized the specific microbiota of *Demodex* mites in 15 ERT subjects, 15 PPR subjects and 17 sex and age-matched healthy controls (Murillo et al., 2014a). Notably, this was the first study using a culture-independent method (*16S rRNA* sequencing) for analysis of the microbiota of *Demodex* mites from

skin biopsies (alar crease). Phylum composition was reported to be significantly different in PPR patients compared to ETR patients and healthy controls. Actinobacteria was the dominant phylum in ETR patients and controls but was greatly diminished in PPR patients. In addition, Proteobacteria and Firmicutes increased in PPR patients compared to ETR patients and controls. *C. acnes*, *S. epidermidis*, *C. kroppenstedtii*, *Streptococcus mitis*, *Propionibacterium granulosum* and *S. alvi* were the 6 species shared by the 3 study groups. *Duganella zoogloeoides* and *Acinetobacter pitii* were the most represented species in the *Demodex*-specific microbiota of ETR and PPR patients, respectively. Interestingly, pathogens such as *Bartonella*, *Haemophilus* or *Escherichia* were only observed in rosacea patients. In fact, *Bartonella quintana* which is known to causes trench fever and chronic bacteremia, endocarditis, and bacillary angiomatosis, was detected in one subject of this rosacea cohort (Murillo et al., 2014b). The authors concluded that the mite microbiota in rosacea patients could differ according to the host status, although only a limited number of mites were analyzed in healthy controls compared to rosacea patients due to a lower density of *Demodex*.

4 Analysis of the gut microbiota in rosacea patients

Studies characterizing the gut microbiota in rosacea patients are also very scarce, very recent, with small sample sizes and all based on NGS of the *16S rRNA* gene. Therefore, conclusions are particularly difficult to achieve. According to the current knowledge of the gut-skin axis and considering the potential relevance of modulating the intestinal microbiota as a therapeutic target for rosacea, it is imperative that further research will be conduct in this area.

Nam et al. reported in 2018 a study to establish relationships between the gut microbiota of 12 rosacea patients (50% ETR, 17% PPR and the remainder were of unknown subtype) and 251 healthy controls, all females (Nam et al., 2018). These researchers did not find significant differences at α- and β-diversity level between rosacea and rosacea-free groups. However, significant differences were identified at genera level. *Acidaminococcus* and *Megasphaera* were more abundant and Peptococcaceae family unknown genus and *Methanobrevibacter* were relatively lacking in rosacea subjects compared to healthy controls. Furthermore, after an adjustment by some variables (age, body mass index, diabetes type 2, gastric polyps, colon cancer) *Acidaminococcus*, *Megasphaera*, and Lactobacillales were significantly increased in rosacea patients compared to rosacea-free subjects, as Peptococcaceae, *Methanobrevibacter*, *Slackia*, *Coprobacillus*, *Citrobacter*, and *Desulfovibrio* were significantly decreased.

Chen et al. in 2021, published another similar study although obtaining quite different results (Chen et al., 2021). They compared the gut microbiota of 11 patients with rosacea and 110 age- and sex-matched healthy controls. Most of the rosacea patients were female, but mean age (53 years) and ETR patients (50%) was higher than in Nam 2018 study (Nam et al., 2018). A decrease in richness but not in α-diversity was observed in rosacea patients. The PCoA based on the unweighted UniFrac distance showed a different clustering between both study groups, suggesting a dissimilar gut microbial structure. In addition, the inclusion of covariates such as alcohol, tea or yogurt consumption, tobacco, exercise, vegetarianism, or rosacea subtype did not affect the profile of the gut microbiota structure in PCoA. Although both groups presented a gut microbiota dominated by Bacteroidetes, Firmicutes, and Proteobacteria, rosacea patients presented a higher abundance of *Bacteroides* and *Fusobacterium*, and a lower abundance of *Prevotella* and *Sutterella* than the controls. Using a linear discriminant analysis effect size (LEfSe), increases of *Rhabdochlamydia*, *Bifidodbacterium*, *Sarcina*, and *Ruminococcus*, and decreases of *Lactobacillus*, *Megasphaera*, *Acidaminococcus*, *Haemophilus*, *Roseburia*, *Clostridium*, and *Citrobacter,* were identified as characteristics of rosacea patients. The authors went one step further and investigated the functional profile of the samples through a phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis. Gene families implicated in arylsulfatase A related enzymes, glycosiltransferease, and cobalamin transport were more abundant in rosacea patients. However, ABC-type sugar and amino acid transport system related enzymes, chemotaxis and transcription related enzymes were less abundant in rosacea patients.

The most recently published study is that of Moreno-Arrones et al. evaluating the gut microbiota of 15 PPR patients (mean age 36 years, 80% females) and 15 controls (mean age 39 years, 33% females) (Moreno-Arrones et al., 2021). The CHAO1 richness index was increased in PPR patients. A canonical correspondence analysis (CCA) showed a significant different clustering between cases and controls. By means of a LEfSe analysis was identified a decrease of *Prevotella copri*. Moreover, an increase of Bacteroidales order, Syntrophomonadaceae and Lachnospiraceae families, *Anaerovorax* and *Tyzzerella* genera, and *Akkermansia muciniphila* and *Parabacteroides distasonis* species, was established as compositionally characteristic of PPR status.

5 Probiotics as a therapeutic target in rosacea patients

The modulation of the skin and gut microbiota due to its potential influence on the pathogenesis of rosacea could be an interesting therapeutic target. As the international scientific association for probiotics and prebiotics (ISAPP) stated, probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). Randomized clinical trials have shown beneficial results in the clinical course of inflammatory skin diseases such as atopic dermatitis (Navarro-López et al., 2018), psoriasis (Navarro-López et al., 2019), or acne vulgaris (Jung et al., 2013). However, there is a general lack of clinical and preclinical evidence regarding probiotics and rosacea. The clinical development of symptoms of rosacea patients, despite the current therapeutic arsenal, is not always appropriate and many times these patients show a maintained symptomatology with frequent relapses. There is still an ongoing need for more efficacious treatments (van Zuuren et al., 2021).

Manzhalii et al. conducted an open-label, randomized clinical trial in 57 patients with erythema and papulopustular lesions, of which 36% were patients with PPR (the remaining 22 and 57% were, respectively, diagnosed with acne and seborrheic dermatitis) (Manzhalii et al., 2016). The patients were divided into 2 groups and one of them was treated with standard topical therapy consisting of tetracyclines, corticosteroids and retinoids. The other group was

treated with the same topical therapy plus oral administration of the probiotic strain *Escherichia coli* Nissle 1917. After 1 month of follow-up, 32% patients of probiotic group showed recovery and 57% significant amelioration, compared to 17% patients of control group showing recovery and 39% significant amelioration. Furthermore, patients treated with the probiotic evidenced an increase in the quality-of-life questionnaire score. Post-treatment stool culture indicated that therapy with *E. coli* Nissle 1917 caused an increased growth of *Lactobacillus* and *Bifidobacterium*, and a reduction of *Staphylococcus*, yeasts, *Bacteroides*, *Proteus*, *Citrobacter*, and *Klebsiella*. Therefore, Nissle strain improved the clinical progression of these patients with a substantial modification of the gut microbiota.

Buianova et al. published in 2018 other randomized clinical trial with 60 rosacea patients as a short communication (Buianova et al., 2018). These study subjects were separated in 2 groups. Thirty rosacea patients were treated 1 week with oral antibiotics, vitamins, antihistamine, and topical permethrin. The remaining 30 rosacea patients were added a mixture containing a *Bifidobacterium* strain 5×10^7 CFU 3 times per day and polyoxidonium (immunomodulator) for 3 weeks. In the probiotic group 57% of patients experienced complete clinical remission compared to 28% in the control group. In addition, a stool culture at the end of the treatment period indicated an increase in *Lactobacillus* and *Bifidobacterium* burden in rosacea patients of the probiotic group.

A case report illustrated the efficacy of an oral antibiotic and probiotic combined therapy in a patient with scalp rosacea (Fortuna et al., 2016). This patient presented papules and pustules located on face and scalp with an intermittent erythema and burning sensation along with blepharitis and conjunctivitis. The patient was treated for 8 weeks with 40 mg of doxycycline per day and a probiotic mixture composed of *Bifidobacterium breve* BR03 *and Lactobacillus salivarius* LS01 10^9 CFU 2 times per day. After 8 weeks, antibiotic treatment was stopped but the probiotic continued. The patient significantly improved both cutaneous and ocular symptoms and after 6 months did not present any relapse.

Another therapeutic option beyond oral probiotics are topical probiotics. Topical application of probiotic bacteria could improve the natural barrier of the skin by exerting a direct effect at the site of application. However, in general, there have been few clinical trials evaluating the efficacy of topically applied probiotics (Habeebuddin et al., 2022). Regarding rosacea, a recent clinical trial has explored the efficacy of the product M89PF containing Vichy volcanic mineralizing water, probiotic fractions of *Vitreoscilla filiformis*, hyaluronic acid, niacinamide, and tocopherol (Berardesca et al., 2023). *V. filiformis* extract topically applied presents several interesting properties such as optimizing cell immunity, protecting against pathogen skin bacteria, and improving skin barrier function (Gueniche et al., 2021). In this clinical trial, 20 rosacea patients were randomly assigned to receive M89FP or non-medical cosmetic standard skin care over every half-face side for 30 days. M89FP therapy significantly enhanced skin hydration as reduction of the transepidermal water loss (TEWL), decreased *Demodex* density, improved erythema (measured by chromameter), and improved self-perception of skin erythema, tightness, and dryness.

Some studies have found associations between certain bacterial skin colonization profiles and impairment of cutaneous barrier function specifically in patients with rosacea (Yuan et al., 2020). Therefore, an altered skin barrier could promote the overgrowth of key bacteria on the skin aggravating the symptomatology of rosacea. In this regard, probiotics contributing to restore the natural skin barrier could improve the clinical course of rosacea. Let us review some probiotic strains with beneficial effects on the skin barrier function. *Lactobacillus paracasei* CNCM-I 2116 was able to induce a faster function barrier recovery after impairment with sodium lauryl sulfate using an *ex vivo* skin organ culture (Gueniche et al., 2010). In addition, 3 weeks of high doses of this strain significantly reduced the TEWL in a murine model of sensitized skin with dinitrochlorobenzene (Philippe et al., 2011). A randomized, double-blind, placebo-controlled, clinical trial also demonstrated that supplementation for 2 months with this strain decreased skin sensitivity and increased skin barrier recovery (Gueniche et al., 2014). In a reconstructed human epidermis model, a lysate of *Lactobacillus reuteri* DSM 17938 enhanced laminin A/B levels which are important extracellular matrix proteins, suggesting a beneficial effect on skin barrier (Khmaladze et al., 2019). A randomized, double-blind, placebo-controlled clinical trial demonstrated that oral intake of *Lactobacillus plantarum* HY7714 at 10^{10} CFU per day for 12 weeks, suppressed the TEWL in facial and forearm skin along with an increase in skin water content (Lee et al., 2015). The same research group demonstrated in an observational study that healthy volunteers who received this probiotic strain developed changes in their gut microbiota with an increase of *Bifidobacterium* and a decrease of Proteobacteria, along with a decrease in MMP-2, MMP-9, zonulin, and calprotectin plasma levels, all of which are related to skin and intestinal permeability (Nam et al., 2020). In addition, RNA-seq analysis showed increased expression of genes related to the integrity of the intestinal barrier. Furthermore, oral treatment with *L. plantarum* HY7714 at 10^9 CFU per day for 8 weeks in hairless mice, decreased UV-induced epidermal thickness and suppressed the TEWL (Ra et al., 2014). Using an *in vitro* model, the differentiation and proliferation of keratinocytes was enhanced by means of a product composed by the plant *Scutellaria baicalensis* fermented with a strain of *L. plantarum* (Lee, 2019). A clinical trial with healthy volunteers supplemented with candies containing 2.1% *L. plantarum* lysates vs. candies not containing bacterial lysates for 8 weeks, showed a significant decrease in the TEWL and increase of skin hydration in face and forearm of the experimental-candy subjects (Kim H. et al., 2015). Other randomized, double-blind, placebo-controlled, clinical trial in healthy female volunteers revealed that treatment with heat-killed *Lactobacillus casei* subsp. *casei* 327 at 10^{11} CFU per day decreased the TEWL (Saito et al., 2017). Finally, drinking of *Lactobacillus helveticus*-fermented milk whey for 5 weeks significantly lowered the TEWL in hairless mice with sodium lauryl sulfate-induced dermatitis (Baba et al., 2010).

6 Conclusion

Rosacea is a multifactorial disease which causes a relevant deterioration in the quality of life of the patients. The pathophysiology of rosacea is becoming increasingly well understood, but the role of the skin and gut microbiota as well as certain bacteria and other specific microorganisms must be clarified. The impact of the gut-skin axis on rosacea has been little explored, in contrast to other inflammatory skin diseases such as atopic dermatitis or psoriasis. The clinical progression of patients with rosacea, despite the current available therapies approved by medicine agencies, is not always

adequate and in frequent cases these patients will have a sustained symptomatology with frequent flare-ups. It is therefore imperative to explore more effective and safe treatments or therapeutic schedules for rosacea. Introduction and consolidation of new culture-independent techniques based on NGS of the *16S rRNA* gene in recent years has enabled to obtain unprecedented information about the microbiome. Studies characterizing the skin microbiota using this methodology in rosacea patients are scarce and recent. Similarity analyses of cutaneous microbiota between rosacea cases and healthy controls, or between affected and unaffected skin have provided contradictory results (Zaidi et al., 2018; Rainer et al., 2020; Wang et al., 2020; Woo et al., 2020; Thompson et al., 2021). Several differences between rosacea subtypes and controls have been detected at species level and these associations could be responsible for a relationship between rosacea and its comorbidities (Rainer et al., 2020; Thompson et al., 2020). Moreover, rosacea severity is related to changes in skin microbiota (Zaidi et al., 2018; Woo et al., 2020). On the other hand, studies characterizing the gut microbiota of rosacea patients based on NGS are also scarce. In this regard, significant differences have been consistently identified at genera level between rosacea patients and rosacea-free individuals (Nam et al., 2018; Chen et al., 2021; Moreno-Arrones et al., 2021). All these findings at skin and gut microbiota level reinforce the role of the skin-gut axis in the pathophysiology of rosacea. At this point and at this moment, oral probiotics, or even topical probiotics (mainly postbiotics) would come into play. However, we identify a deficiency of preclinical and human clinical trial evidence on the efficacy of these products in rosacea patients. In this narrative review we have established the basics and compiled the main directions of current knowledge to understand the mechanisms by which the microbiome influences the pathogenesis of rosacea, and how modulation of the skin and gut microbiota could benefit these patients.

Author contributions

PS-P: Writing – original draft, Writing – review & editing. CE-M: Writing – review & editing. JG-G: Writing – review & editing. ML-V: Writing – review & editing. LN-M: Writing – review & editing. EN-D: Writing – review & editing. JA-S: Writing – review & editing. VN-L: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 02 February 2024
DOI 10.3389/fmicb.2024.1326339

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OPEN ACCESS

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RECEIVED 24 October 2023
ACCEPTED 18 January 2024
PUBLISHED 02 February 2024

CITATION
Wu Y, Wang X, Wu W and Yang J (2024) Mendelian randomization analysis reveals an independent causal relationship between four gut microbes and acne vulgaris.
Front. Microbiol. 15:1326339.
doi: 10.3389/fmicb.2024.1326339

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Mendelian randomization analysis reveals an independent causal relationship between four gut microbes and acne vulgaris

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Background: Numerous studies have suggested a correlation between gut microbiota and acne vulgaris; however, no specific causal link has been explored.

Materials and methods: To investigate the possible causal relationship between gut microbiota and acne vulgaris, this study employed a large-scale genome-wide association study (GWAS) summary statistic. Initially, a two-sample Mendelian randomization (MR) analysis was utilized to identify the specific gut microflora responsible for acne vulgaris. We used the Inverse Variance Weighted (IVW) method as the main MR analysis method. Additionally, we assessed heterogeneity and horizontal pleiotropy, while also examining the potential influence of individual single-nucleotide polymorphisms (SNPs) on the analysis results. In order to eliminate gut microbiota with reverse causal associations, we conducted reverse MR analysis. Multivariate Mendelian randomization analysis (MVMR) was then employed to verify the independence of the causal associations. Finally, we performed SNP annotation on the instrumental variables of independent gut microbiota and acne vulgaris to determine the genes where these genetic variations are located. We also explored the biological functions of these genes through enrichment analysis.

Result: The IVW method of forward MR identified nine gut microbes with a causal relationship with acne vulgaris ($p < 0.05$). The findings from the sensitivity analysis demonstrate the absence of heterogeneity or horizontal pleiotropy, and leave-one-out analysis indicates that the results are not driven by a single SNP. Additionally, the Reverse MR analysis excluded two reverse-correlated pathogenic gut microbes. And then, MVMR was used to analyze seven gut microbes, and it was found that Cyanobacterium and Family XIII were risk factors for acne vulgaris, while Ruminococcus1 and Ruminiclostridium5 were protective factors for acne vulgaris. After conducting biological annotation, we identified six genes (PLA2G4A, FADS2, TIMP17, ADAMTS9, ZC3H3, and CPSF4L) that may be associated with the pathogenic gut microbiota of acne vulgaris patients. The enrichment analysis results indicate that PLA2G4A/FADS2 is associated with fatty acid metabolism pathways.

Conclusion: Our study found independent causal relationships between four gut microbes and acne vulgaris, and revealed a genetic association between acne vulgaris patients and gut microbiota. Consider preventing and treating acne vulgaris by interfering with the relative content of these four gut microbes.

KEYWORDS

acne vulgaris, gut microbiota, Mendelian randomization analysis, short-chain fatty acid, inflammation

1 Introduction

Acne vulgaris is a very common chronic inflammatory disease of the skin, manifested which is not only prone to occur during adolescence, but also persists in some adult patients (Kutlu et al., 2023). According to statistics, acne vulgaris has become the eighth most prevalent disease worldwide, affecting approximately 9% of the global population (Tan and Bhate, 2015). The pathogenesis of acne is not yet clear, but it is currently considered to be related to hyperactive sebaceous gland activity, aberrant keratinization of hair follicles and sebaceous glands, perturbations in the diversity of skin microbiota, inflammatory mechanisms, and organism immunity (Dréno, 2017; Dagnelie et al., 2022). Some acne vulgaris cases may be related to diet, hormones, genetics, cosmetics, medication, and emotions. The persistent inflammation caused by acne vulgaris can result in excessive pigmentation and the formation of scars after inflammation, which can have severe consequences for adults. This can affect the mental health of patients, leading to feelings of inferiority, depression, and even social isolation. Therefore, it is crucial for us to comprehend the potential factors that contribute to acne vulgaris and investigate its causes in order to generate fresh approaches for its treatment.

The microbiome of the digestive system, known as the gut microbiota, is a complex and constantly evolving community of microorganisms. It is related to the dynamics of human immune cells (Schluter et al., 2020). Dysregulation of the gut microbiota can lead to metabolic disorders of microorganisms in the intestines, which can in turn impact immune function. This indicates that gut microbiota can drive the immune system to exert regulatory effects. Recent studies have revealed that the impact of the gut microbiota extends beyond the gastrointestinal system, affecting the brain and skin as well. This has given rise to the concepts of the gut-brain axis and the gut-skin axis (Salem et al., 2018; Agirman et al., 2021). In the context of the gut-brain axis, research has found that regulating the gut and central nervous system (CNS), with a focus on their immune regulatory effects, can simultaneously affect inflammation in the gut, body, and brain (Ortega et al., 2023). Scientific research has identified a connection between imbalances in the gut microbiota and imbalances in the skin microbiota (Mahmud et al., 2022). Alterations in the gut microbiota have been linked to the development of inflammatory skin conditions (Polkowska-Pruszyńska et al., 2020). While some evidence suggests a correlation between the gut microbiota and acne vulgaris (Siddiqui et al., 2022), further investigation is needed to determine the exact nature of this relationship.

Mendelian randomization (MR) is a statistical method used to study the causal relationship between phenotype (exposure) and disease (outcome). It follows Mendel's law of genetics, which states that allele genes are randomly distributed. MR analysis uses genetic variations related to the exposure as instrumental variables to infer the causal relationship with the outcome, thereby greatly reducing confounding and reverse causal associations (Sanderson et al., 2019). Multiple variable Mendelian randomization (MVMR) is the latest extension of MR, primarily using multiple potentially correlated exposures to assess the independent causal effects of each exposure on the outcome. MVMR is more robust when there are confounding factors between multiple exposures (Sanderson, 2021).

In this study, we used two samples MR analysis and MVMR analysis to investigate whether there is an independent causal relationship between different gut microbes and acne vulgaris. If it can be proven that a certain gut microbiota influences the occurrence and development of acne vulgaris, then intervention measures can be taken on the gut microbiota to effectively treat acne vulgaris.

2 Methods and materials

2.1 Study design

The main goal of this study is to investigate the causal relationship between gut microbiota and acne vulgaris. This study utilizes gut microbiota as an exposure factor and selects single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) based on a threshold of $p < 1 \times 10^{-5}$. We conducted forward MR analysis using acne vulgaris as the outcome. Then, we perform reverse MR analysis using gut microbiota as the outcome. Finally, we used MVMR analysis to study the independent causal effects of different intestinal microbial clusters on acne vulgaris.

In this study, MR analysis must meet three main assumptions: (1) Relevance assumption: IVs are strongly associated with exposure and are independent of each other; (2) Exclusive assumption: IVs are irrelevant to the outcome; and (3) Independence assumption: IVs do not relate to the confusion factor (Birney, 2022) (Figure 1).

2.2 Data sources

Data for gut microbiota was obtained from a large-scale Genome-Wide Association Study (GWAS) (Kurilshikov et al., 2021). This study involved multiple cohorts, including 24 cohorts with a total of 18,340 individuals. Each cohort included only taxa that were present in more than 10% of the samples, resulting in a total of 211 taxa (131 genera, 35 families, 20 orders, 16 classes, 9 phyla). The included cohort adjusts the coherence variables for both gender and age in the calculation.

Acne vulgaris data are derived from GWAS studies and meta-analyses in European cohorts of EstBB, FinnGen and Lifelines, including 34,422 acne patients and 364,991 normal controls (Teder-Laving et al., 2023).

2.3 Selection of IVs

As the majority of gut microbiota do not have significant SNPs under the traditional threshold of $p < 5 \times 10^{-8}$, a relatively lenient statistical threshold ($p < 1 \times 10^{-5}$) is used in forward MR analysis to screen for SNPs related to the exposure (Feng et al., 2022; Liu et al., 2022). To meet MR's assumption 1, linkage disequilibrium analysis was conducted using European reference data from the 1,000 Genomes project. Set the linkage disequilibrium related coefficient to $r^2 < 0.01$, and the window size to 1,000 kb, to rule out genetic variations with linkage disequilibrium. Next, SNPs associated with exposure were extracted from the outcome. SNPs strongly correlated with the outcome ($p < 1 \times 10^{-5}$) were excluded and performed harmonization to eliminate SNPs with palindromic sequence and allele inconsistency (Zhang et al., 2021). Finally, we also computed the F statistics for each SNP to eliminate genetic variations with weak statistical power ($F < 10$) (Liu et al., 2022). The SNPs obtained above were used as IVs to study the effects of gut microbiota on acne vulgaris.

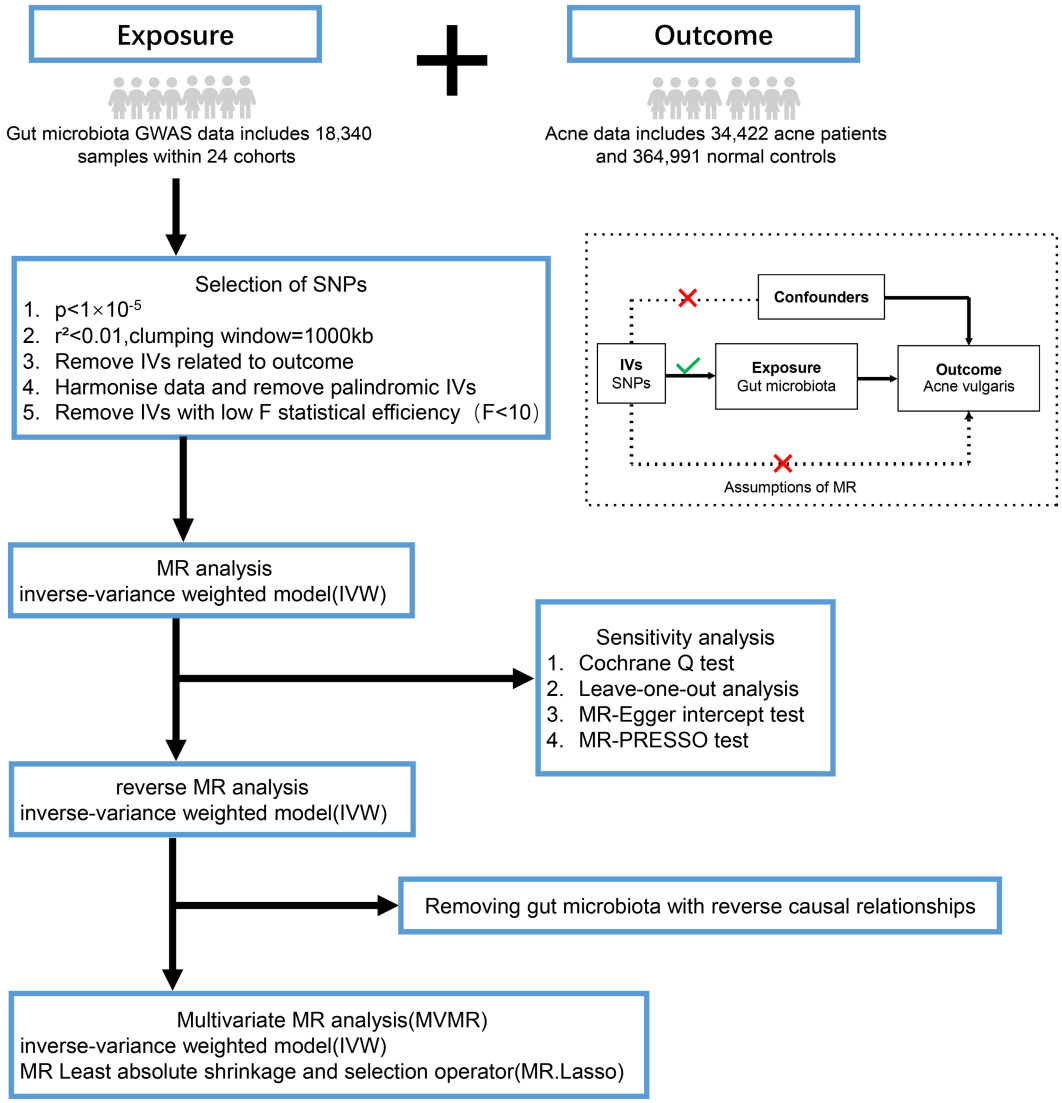


FIGURE 1
Overview of the analysis process of the causal relationship between the gut microbiota and acne vulgaris through MR analysis and MVMR analysis. GWAS, genome-wide association study; SNPs, single nucleotide polymorphisms; MR-PRESSO, mendelian randomization of pleiotropy residuals and outliers.

2.4 Forward MR analysis

We primarily utilized the Inverse Variance Weighted (IVW) method for conducting forward MR analysis. The IVW method is considered an ideal estimation method, as it can minimize the impact of confounding variables and provide unbiased estimates in the absence of horizontal pleiotropy (Bowden et al., 2017). Subsequently, we conducted a sensitivity analysis. We used Cochran's Q test for heterogeneity analysis, where a p-value less than 0.05 is suggestive of possible heterogeneity in the IVs (Xiang et al., 2021). If heterogeneity was observed, the MR effect could be estimated through a direct random effects model. A leave-one-out sensitivity analysis was performed by sequentially removing each SNP and evaluating whether there were statistical differences in the result. If the results hardly change before and after removing single SNP, it suggests that single SNP may not have a significant impact on effect estimation. Additionally, the MR Egger method was implemented to test for horizontal pleiotropy. If an intercept term was identified in the MR-Egger intercept analysis with a p value for the intercept less than 0.05, then the study findings were deemed to have significant horizontal pleiotropy. The global test of Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) is primarily utilized to evaluate horizontal pleiotropy and outliers.

2.5 Reverse MR analysis

To assess whether acne vulgaris has a causal impact on the gut microbiota, we employed a genome-wide significance threshold ($p < 5 \times 10^{-8}$) to screen for SNPs associated with acne vulgaris. These SNPs were used as IVs in reverse MR analysis, with acne vulgaris as the exposure and the gut microbiota as the outcome. The statistical

methods used in reverse MR analysis were the same as those used in forward MR analysis.

2.6 MVMR analysis

Considering the possible interactions between gut microbiota, which may interfere with the results of univariate Mendelian randomization analysis, we proceeded with MVMR analysis. MVMR analysis aims to correct for the interactions between exposures by combining multiple exposures that may interact with each other (Sanderson, 2021).This analysis examines whether the influence of each significant gut microbiota on acne vulgaris, as identified in the univariate MR analysis, is independent.

In the bidirectional MR analysis, we identified several gut microbes that have an impact on acne vulgaris. These gut microbes were used as exposures to explore their relationship with the outcome. We selected IVs using a statistical threshold of $p < 1 \times 10^{-5}$ and removed SNPs in linkage disequilibrium (LD, $r^2 > 0.01$, window size of 1,000 kb). We also excluded SNPs related to the outcome from the IVs. Additionally, we eliminated palindromic SNPs and SNPs with inconsistent alleles.

In MVMR analysis, we employ Least absolute shrinkage and selection operator (LASSO) regression to eliminate highly collinear exposures. Then, we utilize multivariable IVW as the primary method for conducting MVMR analysis, with MR LASSO being used to complement the IVW results. To enhance the robustness of causal relationships, we also perform sensitivity analysis using the MR-PRESSO method.

2.7 Annotation of biology

We selected IVs for significant gut microbiota from MVMR and subsequently linked these SNPs to specific genes. IVs of acne vulgaris disorders were also mapped onto genes. We then used version 12.0 of STRING to identify protein–protein interaction (PPI) networks of mapped genes that had independent causal relationships between gut microbiota and acne vulgaris, and identified core genes. Finally, we performed GO enrichment analysis on these core genes.

2.8 Data analysis

All statistical analyses were performed in R, version 4.3.1. Univariate Mendelian randomization analyses were primarily conducted using the "TwoSampleMR" software package (version 0.5.7), while multivariate Mendelian randomization analyses were primarily carried out with the "MendelianRandomization" software package. MR-PRESSO tests were performed using the "MRPRESSO" package. GO enrichment analysis was performed using software packages such as "clusterProfiler" and "org.Hs.eg.db."

3 Result

3.1 Selection of IVs

In this study, the exposed data came from 211 gut microbes, including five biological classifications: phyla, class, order, family, and genus. The outcome data were collected from 34,422 acne patients and 364,991 normal people. In the exposed data processing, 2,129 SNPs were selected as IVs after removing the linkage disequilibrium and deleting the SNPs associated with the outcome according to the threshold of $p < 1 \times 10^{-5}$. We collected information about SNPs, including effector alleles, the effect of SNP on phenotype (β), effect allele frequency (EAF), standard error of β value (SE), and *p*-values. In addition, we also calculated the *F*-value for each SNP, and the F-statistic value for the SNPs used as IVs was greater than 10, further indicating the robustness of the IVs we used.

3.2 Forward MR analysis

After setting significant difference criteria ($p < 0.05$) based on the IVW method, we found that nine gut microbes have a causal effect on acne vulgaris, including one phylum, two families, and six genera (Supplementary Table S1).

IVW analysis showed that Family XIII [odds ratio (OR) = 1.24, 95% confidence interval (CI): 1.06 ~ 1.44, $p = 0.006$], Oxalobacteraceae (OR = 1.08, 95%CI: 1.01 ~ 1.14, $p = 0.012$), Cyanobacteria (OR = 1.09, 95%CI: 1.00 ~ 1.18, $p = 0.041$), Coprococcus3 (OR = 1.13, 95%CI: 1.00 ~ 1.27, $p = 0.045$) and Oxalobacter (OR = 1.06, 95%CI: 1.00 ~ 1.12, $p = 0.048$) could increase the risk of acne vulgaris. Ruminococcus1 (OR = 0.86, 95%CI: 0.77 ~ 0.97, $p = 0.012$), Ruminiclostridium5 (OR = 0.87, 95%CI: 0.77 ~ 0.98, $p = 0.023$), *Eubacterium hallii* group (OR = 0.91, 95%CI: 0.84 ~ 0.99, $p = 0.023$) and Fusicatenibacter (OR = 0.88, 95%CI: 0.81 ~ 0.99, $p = 0.040$) were the protective factors of acne vulgaris (Figure 2).

The Cochran's Q test for IVW showed no heterogeneity among IVs (Table 1). Leave-one-out analysis did not detect any abnormal SNPs (Supplementary Figure S1). The MR-Egger regression intercept ($p > 0.05$) and the results of the MR-PRESSO global test ($p > 0.05$) both indicate the absence of horizontal pleiotropy (Table 1).

3.3 Reverse MR analysis

In the reverse MR Study, 25 SNPs were selected as IVs for acne vulgaris, and all had F-statistics greater than 10.

The IVW analysis results revealed that acne vulgaris did not have a causal impact on Cyanobacteria, Family XIII, Ruminococcus1, Ruminiclostridium5, Fusicatenibacter, Coprococcus3, and Oxalobacter in the gut microbiota. However, it did have a causal effect on Oxalobacteraceae and the *Eubacterium hallii* group (Figure 3). The Cochran's Q test, MR-Egger, and MR-PRESSO global tests showed no significant heterogeneity and horizontal pleiotropy (Supplementary Table S2).

3.4 MVMR analysis

Based on the findings acquired from the bidirectional analysis of MR, it has been determined that seven gut microbes have a causal relationship with acne vulgaris. Following a series of quality control and employing a relatively lenient criterion ($p < 1 \times 10^{-5}$), a total of 67 SNPs were chosen from these seven gut microbes to serve as IVs for the multivariate MR analysis.

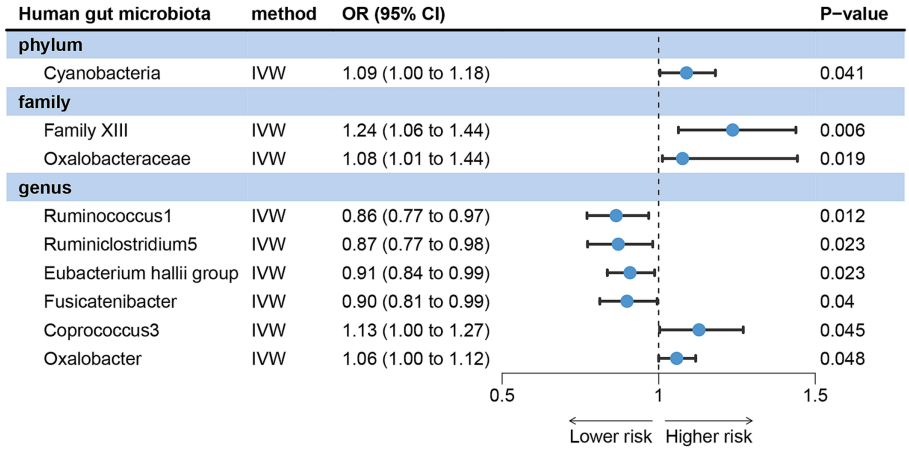


FIGURE 2
Forward MR analysis revealed potential causal relationships between gut microbiota with acne vulgaris risk. IVW, inverse variance weighted; OR, odds ratio; CI, confidence interval.

TABLE 1 Heterogeneity and pleiotropy for forward Mendelian analysis.

Exposure: human gut microbiota	NSNPs	Outcome	Heterogeneity test			Pleiotropy test	
			Method	Cochran's Q	*p*-value	Egger intercept (*p*-value)	MR PRESSO global test (*p*-value)
Phylum							
Cyanobacteria	8	Acne	IVW	4.734	0.692	0.951	0.664
Family							
Family XIII	6	Acne	IVW	4.512	0.478	0.402	0.524
Oxalobacteraceae	14	Acne	IVW	17.883	0.162	0.906	0.189
Genus							
Ruminococcus1	8	Acne	IVW	3.188	0.867	0.619	0.890
Ruminiclostridium5	10	Acne	IVW	7.036	0.633	0.984	
Eubacterium hallii group	13	Acne	IVW	9.692	0.643	0.272	0.661
Fusicatenibacter	19	Acne	IVW	25.108	0.122	0.142	0.122
Coprococcus3	9	Acne	IVW	4.466	0.813	0.434	0.828
Oxalobacter	11	Acne	IVW	7.197	0.707	0.617	0.717

IVW, inverse variance weighted; MR PRESSO, MR pleiotropy residual sum and outlier.

IVW analysis method showed that Cyanobacteria, Family XIII, Ruminiclostridium5 and Ruminococcus1 could have a direct causal effect on acne independently of other gut microbes. After adjusting for these four gut microbes, we observed that the direction of the causal effect was consistent with that of the forward MR. The positive association was discovered between Cyanobacteria (OR = 1.14, 95% CI: 1.05 ~ 1.23, $p = 0.001$) and Family XIII (OR = 1.18, 95% CI: 1.03 ~ 1.36, $p = 0.016$) with the risk of acne vulgaris. On the other hand, Ruminiclostridium5 (OR = 0.85, 95% CI: 0.72 ~ 0.97, $p = 0.014$) and Ruminococcus1 (OR = 0.87, 95% CI: 0.78 ~ 0.98, $p = 0.024$) were negatively associated with the risk of acne vulgaris (Figure 4).

In MVMR analysis, the effects of Oxalobacter, Fusicatenibacter, and Coprococcus3 on acne vulgaris became less significant, indicating that these three gut microbes may be influenced by other microbiota rather than independent influencing factors. MR-LASSO regression analysis gave similar and significant estimates (Figure 4). Sensitivity analysis showed no pleiotropy and outliers (Supplementary material S1).

3.5 Annotation of biology

Four gut microbes with independent causal relationships were identified through MVMR. We found that these IVs from four gut microbes could mapped to 16 genes after a series of quality controls (Supplementary material S2). Mapping the IVs from acne vulgaris to 13 genes (Supplementary material S2). After conducting a PPI network analysis using the STRING, these 29 genes were found to form three interconnected networks (Figure 5), and identified six core genes (PLA2G4A, FADS2, TIMP3, ADAMTS9, ZC3H3, and CPSF4L). GO enrichment analysis and function annotation showed that

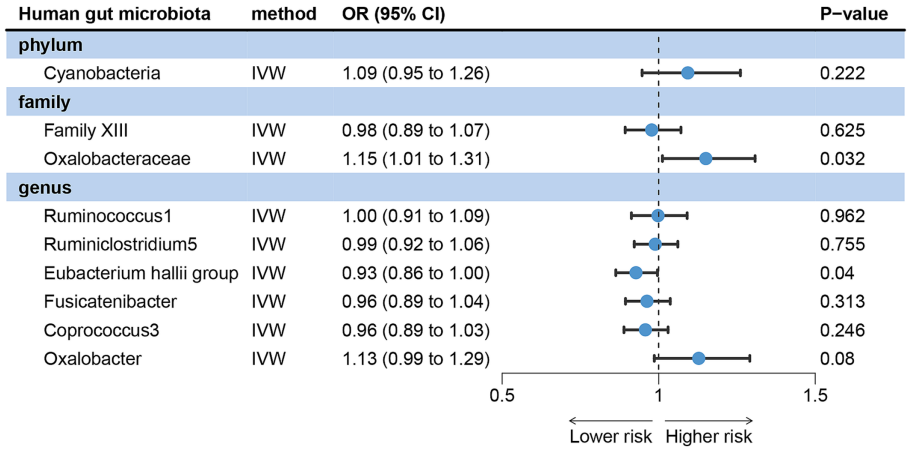


FIGURE 3
Reverse MR analysis revealed potential causal relationships between acne vulgaris and the gut microbiota.

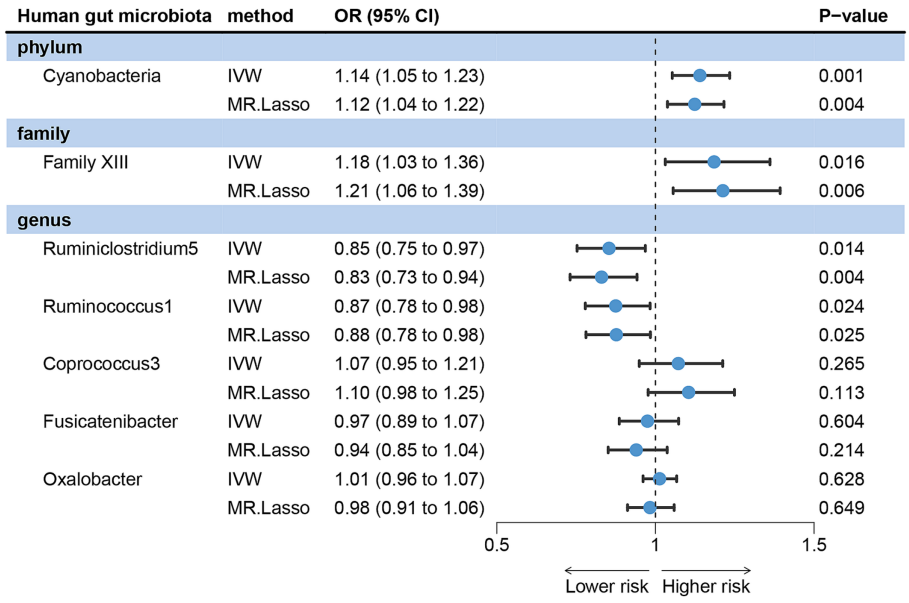


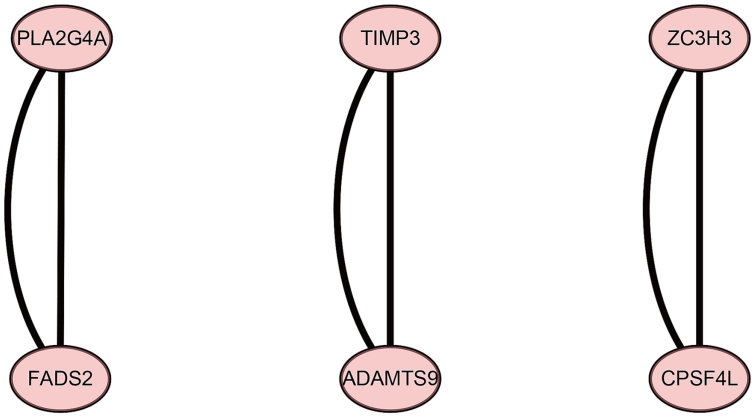
FIGURE 4
Forest plots showing independent causal associations between gut microbiota and acne vulgaris.

PLA2G4A/FADS2 was enriched in fatty acid metabolism, TIMP3/ADAMTS9 was associated with inflammation, and ZC3H3/CPSF4L was enriched in mRNA processing pathway.

4 Discussion

For this study, we gathered extensive GWAS data pertaining to acne vulgaris and gut microbiota. Utilizing bidirectional MR analysis and MVMR analysis, we sought to investigate the causal impacts of 211 gut microbes on acne vulgaris. Notably, this is also the first time that MR analysis has been used to study the relationship between acne vulgaris and gut microbiota. Forward MR results showed that Family XIII, Oxalobacteraceae, Cyanobacteria, Coprococcus3 and Oxalobacter were risk factors for acne vulgaris. Ruminococcus1, Ruminiclostridium5, *Eubacterium hallii* group and Fusicatenibacter were the protective factors for acne vulgaris. However, after reverse MR Analysis, it was found that acne vulgaris had a causal effect on Oxalobacteraceae and *Eubacterium hallii* group. Therefore, we considered that Oxalobacteraceae and *Eubacterium hallii* group might interact with acne vulgaris. The comprehensive two-sample Mendelian analysis results indicated that seven gut microbes have a causal effect on acne vulgaris. To avoid the interference of the presence of confounding factors, we then performed MVMR analysis of seven gut microbes. According to the results of MVMR analysis, Cyanobacteria, Family XIII, Ruminiclostridium5, and Ruminococcus1 have the ability to directly influence acne vulgaris, independent of other gut microbes.

The gut microbial flora colonizes the human gut at birth, and with time and age, the various microorganisms interact to balance



PLA2G4A/FADS2: Omega-3/omega-6 fatty acid synthesis (Lipid Metabolism);
TIMP3/ADAMTS9: Metalooroteinase and metalooroteinase inhibitor (Inflammation);
ZC3H3/CPSF4L: mRNA cleavage and polyadenylation specificity factor complex。

FIGURE 5
Protein–protein interaction (PPI) network between four gut microbes and acne vulgaris.

and eventually become in a symbiotic state, which plays an immune role. If the diversity of gut microbiota changes, it can lead to the disruption of mucosal immune tolerance, which in turn affects skin health. When stimulated, a type of innate lymphoid cells (ILCs) located in epithelial cells can be activated to produce cytokines, thereby playing a defensive or pathogenic role in inflammation. Epithelial cells also express various pattern recognition receptors (PRRs), including Toll like receptors (TLRs). Under anti-inflammatory stimulation, it also produces chemokines for bone marrow cells and lymphocytes, thereby mobilizing the immune system (Shi et al., 2017). Acne vulgaris is a disease characterized by skin inflammation. Research has found that *Propionibacterium acnes* induces acne inflammation mainly through a member of TLRs-TLR2. Recently, it has been discovered that there is a complex interaction between the gut and skin, namely the gut dermis axis, which plays a crucial role in the inflammatory immune response (Salem et al., 2018). Studies have shown that skin inflammation may be caused by small changes in a certain bacterial species in the gut microbiome (Ainonen et al., 2022), and experiments have shown that the gut microbiome affects the development of acne through the mTOR pathway (Noureldein and Eid, 2018). According to numerous studies in recent years, the mTOR cascade can respond to many stimuli to regulate signaling pathways and important cellular biology functions, such as growth, survival, proliferation, and cell senescence, which are dysregulated in a variety of diseases (Jia et al., 2023; Xia et al., 2023). In addition, some studies have shown that acne vulgaris can increase endotoxemia and intestinal permeability, thereby altering the intestinal barrier and causing the imbalance of intestinal microbiota (Thompson et al., 2020). This was also demonstrated in a study in which acne vulgaris patients had distinct gut microbiome components (Deng et al., 2018). So, we considered that there might be a reciprocal relationship between acne vulgaris and gut microbiota. This hypothesis can be used to explain the significance of Oxalobacteraceae and *Eubacterium hallii* group in bidirectional MR Analysis, indicating that Oxalobacteraceae and *Eubacterium hallii* group interact with acne vulgaris.

There may be interactions between gut microbiota, and we hope to find microbiota that act independently on the occurrence and development of acne vulgaris. Through the analysis of MVMR, we have discovered that four gut microbes have independent causal effects on acne vulgaris. Cyanobacteria and Family XIII are risk factors for acne vulgaris, while Ruminiclostridium5 and Ruminococcus1 are protective factors for acne vulgaris.

Cyanobacteria is a large group in the gut microbiome and includes oxyphotosynthetic cyanobacteria and non-photosynthetic cyanobacteria-like black algae, which were recently identified in the human gut (Soo et al., 2014). Lipopolysaccharide (LPS) from the outer membrane of cyanobacteria has a unique structure and can trigger TLR4 to induce immune response (Hu and Rzymski, 2022). TLR4, a member of the TLR family, plays a crucial role in recognizing the pathogen-associated molecular pattern (PAMP) and damp-associated molecular pattern (DAMP) of microorganisms and native molecules. The activation of TLR4 in the MyD88 pathway involves the binding of TIRAP to the TIR domain of TLR4, leading to the activation of the IκB kinase complex. Consequently, the transcription of inflammatory genes is initiated, thereby contributing to the development of inflammation (Burns and Yusuf, 2014). It is noteworthy that the excessive activation of TLR4 has been associated with autoimmune and inflammatory diseases (Kondo et al., 2012). Additionally, the LPS derived from cyanobacteria has been implicated in various human diseases such as skin diseases, gastrointestinal diseases, respiratory diseases, and allergic diseases (Durai et al., 2015). Studies have identified a correlation between increased abundance of cyanobacteria in the gut and several diseases, including precancerous lesions of colorectal cancer, human norovirus infection, irritable bowel syndrome, allergic rhinitis, and lung cancer (Sarangi et al., 2017; Zhang et al., 2018; Zhu et al., 2020; Chumpitazi et al., 2021; Xiong et al., 2021). Similar to the results in the current study, we found for the first time that Cyanobacteria were associated with the development of acne vulgaris. Cyanobacteria is a risk factor for acne vulgaris, indicating that Cyanobacteria may be involved in regulating the progression of acne vulgaris through the stimulation of LPS.

We found that there are few studies on Family XIII, and the definition of Family XIII is not very detailed. In a study, it was observed that there is an increase in the abundance of Family XIII in patients with multiple sclerosis (Galluzzo et al., 2021). Multiple sclerosis is a chronic demyelinating inflammatory disease of the central nervous system. Therefore, we considered that Family XIII of gut microbiota might be involved in some inflammatory reactions in human body. This is the first time we have discovered the correlation between Family XIII and acne vulgaris. The specific mechanism is yet to be explored.

Certain research has discovered that there is a diminished abundance of Ruminococcus in inflammatory conditions like psoriasis, allergic diseases, and inflammatory bowel diseases. A study showed a significant reduction in the family Ruminococcaceae in patients with acne vulgaris and suggested that disruption of gut microbes may contribute to early inflammation in acne vulgaris (Deng et al., 2018). Ruminococcus belongs to the Ruminococcaceae (Molinero et al., 2021). This is consistent with our conclusion that the pathogenesis of acne vulgaris may be caused by the reduction of Ruminococcus1 in gut microbiota. The research on the gut microbiota and sex hormones has revealed that the gut microbiota can influence the levels of sex hormones, thereby affecting the immune development of the host (Korpela et al., 2021). Testosterone is an important male hormone in the human body, and Ruminococcus has a negative correlation with the increase of testosterone levels in healthy women (d'Afflitto et al., 2022). Androgens can induce excessive secretion of lipid in sebaceous glands and have proinflammatory effects on cortical cells. Current studies have suggested that acne vulgaris is an androgen-dependent inflammatory disease of sebaceous glands. *In vitro* experiments have also proved that androgens can promote the formation of acne vulgaris and promote the biosynthesis of growth factors in dermal fibroblasts (Hu et al., 2021). In the treatment of acne vulgaris, drugs that inhibit androgen receptor signaling are also currently selected (Rao et al., 2021). This led us to hypothesize that the reduction of Ruminococcus1 may increase the level of testosterone in the body, and then lead to the occurrence of acne vulgaris.

Ruminiclostridium5 is a beneficial bacterium that belongs to the phylum Firmicutes and it was first reported to be associated with acne vulgaris. It has been observed that this bacterium plays a role in the production of short-chain fatty acids (SCFAs). Research has indicated that SCFAs have a protective effect against inflammatory diseases such as colitis, arthritis, and allergies (Kim et al., 2014), playing an important role in maintaining immune homeostasis (Wang et al., 2023). The bacterium is capable of degrading polysaccharides through self-secretion, resulting in the production of multi-enzyme complexes. These complexes then generate short-chain fatty acids like butyrate and acetate, which contribute to the anti-inflammatory effects of Ruminiclostridium5 (Yuan et al., 2022). Especially butyrate salts, it can maintain the integrity of mucous membranes, prevent the symbiotic expansion of potential pathogenic bacteria in the intestines, inhibit the expression of destructive cytokines, and regulate immunity and inflammation (Xiao et al., 2020). This could be the mechanism by which Ruminiclostridium5 reduces the risk of acne vulgaris. Taken together with our MR Findings suggesting a causal relationship between Ruminiclostridium5 and acne vulgaris is plausible, we can achieve acne vulgaris prevention and control by increasing the abundance of Ruminiclostridium5 in various ways.

The biological annotation analysis indicates that we have identified 3 pairs of associated genes between gut microbiota and acne vulgaris, including PLA2G4A/FADS2, TIMP3/ADAMTS9 and ZC3H3/CPSF4L. PLA2G4A is a member of the cytosolic phospholipase A2 family, which generates arachidonic acid derivatives and is upregulated in atopic and allergic environments (Mouchlis and Dennis, 2019). Numerous research studies have provided evidence that infection caused by *Staphylococcus aureus* enhances the activity of PLA2G4A (Hardman et al., 2017). FADS2 is a gene associated with lipid metabolism, upregulation of its expression can induce pro-inflammatory sebaceous gland activity (Zouboulis and Angres, 2021). Recent genome-wide association studies and meta-analysis have shown that FADS2 is a risk gene for acne vulgaris (Teder-Laving et al., 2023). TIMP-3 is a secreted protein that has a broad inhibitory effect on matrix metalloproteinases (MMPs). Research has shown that TIMP-3 inhibits the expression of inflammatory cytokines upregulated by ultraviolet radiation in human keratinocytes (Park et al., 2018). ADAMTS9 is a member of the protein family that encodes ADAMTS (a disintegrin and metalloproteinase with a platelet reactive protein motif). In a study on polycystic ovary syndrome, it was found that the level of IL-17A in patients was negatively correlated with ADAMTS9 (Karakose et al., 2016). At present, there are few studies on the ZC3H3 and CPSF4L genes. Some studies have found that ZC3H3 is involved in mediating nuclear RNA decay (Silla et al., 2020), and CPSF4L may be associated with obesity (Dhana et al., 2018). Currently, research indicates that the key mechanisms involved in the development of acne vulgaris include increased sebum production and changes in the composition of sebum fatty acids (Moradi Tuchayi et al., 2015). These findings align with the outcomes derived from GO enrichment analysis. In particular, the fatty acid metabolism pathway shows enrichment of PLA2G4A/FADS2, which further supports the correlation between four gut microbes and acne vulgaris.

The primary advantage of this investigation resides in the fact that the utilization of the MR Approach lessened confounding variables and the obstruction of reverse causality on the outcomes, which may be more persuasive than customary observational investigations. However, several limitations should be acknowledged. First of all, the data in this study were from people of European ancestry, and the situation of other ethnic groups is not clear. Next, the absence of sex and age statistics hindered the possibility of conducting further subgroup analyses. Third, we did not consider multiple testing given that multiple testing correction may be too conservative and may omit potential gut microbiota causally related to acne vulgaris. Fourthly, due to data limitations, our study cannot confirm whether the gut microbiota, which is causally associated with acne, is affected by antibiotic.

5 Conclusion

This study has discovered four gut microbes that have a causal relationship with acne vulgaris, three of which (Cyanobacteria, Family XIII, Ruminiclostridium5) have not been reported in previous studies, which allows us to further investigate the affected mechanisms of these three gut microbes in the future. We also identified six genes associated with the gut microbiota and acne vulgaris, revealing a genetic association between acne patients and the gut microbiota. At present, the use of probiotics to change the intestinal flora in the treatment of acne vulgaris is becoming more and more popular, which

also makes us wonder whether we can change the relative abundance of these four gut microbes in the human body to play a role in the treatment or the intervention of acne vulgaris.

Data availability statement

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

Author contributions

YW: Writing – original draft, Conceptualization, Data curation, Formal analysis, Methodology, Resources, Visualization, Writing – review & editing. XW: Writing – original draft, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. WW: Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation. JY: Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by grants from the National Natural Science Foundation of China (81760565 and 82160600) and Youth Top Talent project of High-level talent development support program of Yunnan Province (YNWRQNBJ-2020-239).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1326339/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 21 March 2024
DOI 10.3389/fmicb.2024.1345717

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OPEN ACCESS

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RECEIVED 28 November 2023
ACCEPTED 11 March 2024
PUBLISHED 21 March 2024

CITATION
Xue K, Zhang G, Li Z, Zeng X, Li Z, Wang F, Zhang X, Lin C and Mao C (2024) Dissecting the association between gut microbiota and hypertrophic scarring: a bidirectional Mendelian randomization study.
Front. Microbiol. 15:1345717.
doi: 10.3389/fmicb.2024.1345717

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Dissecting the association between gut microbiota and hypertrophic scarring: a bidirectional Mendelian randomization study

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Hypertrophic scars affect a significant number of individuals annually, giving rise to both cosmetic concerns and functional impairments. Prior research has established that an imbalance in the composition of gut microbes, termed microbial dysbiosis, can initiate the progression of various diseases through the intricate interplay between gut microbiota and the host. However, the precise nature of the causal link between gut microbiota and hypertrophic scarring remains uncertain. In this study, after compiling summary data from genome-wide association studies (GWAS) involving 418 instances of gut microbiota and hypertrophic scarring, we conducted a bidirectional Mendelian randomization (MR) to investigate the potential existence of a causal relationship between gut microbiota and the development of hypertrophic scar and to discern the directionality of causation. By utilizing MR analysis, we identified seven causal associations between gut microbiome and hypertrophic scarring, involving one positive and six negative causal directions. Among them, *Intestinimonas*, *Ruminococcus2*, *Barnesiella*, *Dorea*, *Desulfovibrio piger*, and *Ruminococcus torques* act as protective factors against hypertrophic scarring, while *Eubacterium rectale* suggests a potential role as a risk factor for hypertrophic scars. Additionally, sensitivity analyses of these results revealed no indications of heterogeneity or pleiotropy. The findings of our MR study suggest a potential causative link between gut microbiota and hypertrophic scarring, opening up new ways for future mechanistic research and the exploration of nanobiotechnology therapies for skin disorders.

KEYWORDS

gut microbiota, hypertrophic scar, *Firmicutes*, Mendelian randomization, GWAS

1 Introduction

With an estimated incidence reaching up to 70%, hypertrophic scar stands as one of the prevalent complications in burn patients, characterized by abnormal, elevated, and thickened tissue growth at the site of healed skin lesions (Bombaro et al., 2003). Various factors contribute to the development and progression of hypertrophic scar, with local risk variables such as

wound or scar stress, systemic factors like hypertension, genetic factors including single-nucleotide polymorphisms, and lifestyle choices all playing a role. To address hypertrophic scar volume and alleviate discomfort and itch of patients, a suitable approach is crucial. Clinical available interventions include surgery, radiotherapy, and conservative treatments such as gel sheets, tape fixation, and the application of topical or injectable external medications, should be utilized on an individual basis (Ogawa, 2022), as their improper use can potentially lead to adverse effects such as skin shrinkage, telangiectasia, pigmentation issues, skin ulcers, and other imperfections (Lee and Jang, 2018). The impact of hypertrophic scars places a considerable psychological and financial burden on affected patients. Consequently, it is imperative to delve into a comprehensive understanding of the modifiable risk factors and the potential ramifications associated with hypertrophic scarring.

The human gut microbiota is characterized by a rich diversity of bacteria, comprising up to 1,000 different microbial species (Li et al., 2022). This intricate microbial community plays a pivotal role in a myriad of physiological functions, exerting a profound influence on human health. Dysbiosis of the gut microbiota, including both compositional and functional imbalances, is associated with a spectrum of diseases ranging from local gastrointestinal conditions to neurological, metabolic, hepatic, and cardiovascular diseases (Lynch and Pedersen, 2016). Actually, due to the heterogeneity in experimental procedures and study designs, it is currently challenging to identify distinct microbiome signatures for most human diseases, including some intestinal diseases such as pediatric celiac disease (where environmental factors during childhood may not be as significant as in adulthood; Abdukhakimova et al., 2021). However, certain specific bacteria within the microbiota may warrant further investigation to understand their potential applications as probiotic therapies, diagnostic tools, or prognostic biomarkers. The emerging concept of the gut–skin axis outlines the interaction between gut microbiota and skin (Saarialho-Kere, 2004; Salem et al., 2018; Mahmud et al., 2022). More specifically, the health and balance of gut microbiota can influence the condition and function of skin, and vice versa. The effects induced by microbiota on specific inflammatory skin disorders are thought to originate from factors such as a compromised intestinal barrier, elevated levels of inflammatory mediators, and the release of metabolites by microbes (Mahmud et al., 2022). Nevertheless, the presence of a causal relationship between gut microbiota and hypertrophic scarring remains uncertain. Therefore, it is crucial to thoroughly explore this potential causal link.

Mendelian randomization (MR) is a statistical technique employed to assess causal relationships between a risk factor or biomarker (exposure) and a disease (outcome) by leveraging genetic variations as instrumental variables. The fundamental principle underlying MR is to utilize the random assortment of genetic material during meiosis, the process of gamete formation, to simulate a randomized controlled trial and draw inferences regarding causation (Burgess et al., 2015). MR presents distinct advantages over conventional observational research by providing more robust evidence for causal relationships. This strength arises from the fact that genetic variations are established at conception, thereby minimizing susceptibility to confounding or reverse causation (Abdellaoui et al., 2023).

In this work, we aim to explore the causal connections between gut microbiota and hypertrophic scarring and identify specific gut microbiota through a bidirectional MR analysis. The findings of this study may provide valuable insights for future research on the genetic underpinnings and biological therapies related to the intricate features associated with gut microbiota and hypertrophic scar.

2 Materials and methods

2.1 Study design and ethics statement

Figure 1A provides a concise overview of the fundamental analysis flow. Employing genome-wide association study (GWAS) summary statistics, we performed a bidirectional two-sample MR to investigate the causal link between gut microbiota (exposure) and hypertrophic scarring (outcome). The MR design hinges on three critical assumptions. Firstly, the genetic variants should demonstrate a reliable association with the exposure. Secondly, the genetic variants should not be correlated with any confounders affecting the relationship between the exposure and the outcome. Lastly, these genetic variants must link to the outcome exclusively through the exposure.

Our analyses utilized publicly available GWAS data, eliminating the need for ethics committee approval.

2.2 Data sources

The GWAS summary data for 418 gut microbiotas were obtained from the MiBioGen consortium (Kurilshikov et al., 2021) and the Dutch Microbiome Project (Lopera-Maya et al., 2022). The MiBioGen consortium meticulously curated and examined genome-wide genotypes in conjunction with 16S fecal microbiome data sourced from a vast pool of 18,340 European populations. Additionally, the Dutch Microbiome Project undertook a comprehensive genome-wide association study encompassing 207 taxa and 205 pathways, depicting the intricate landscape of microbial composition and function within a substantial cohort comprising 7,738 participants.

The summary data for the GWAS on hypertrophic scar were sourced from the FinnGen consortium (L12_HYPETROPHICSCAR). This dataset comprised a vast array of information, encompassing 16,380,443 single-nucleotide polymorphisms (SNPs) and a sample size of 208,248 European populations.

All GWAS data used in this study are available in the IEU Open GWAS Project.

2.3 Instrumental variable selection

To fortify data robustness and maintain result accuracy, we selected SNPs associated with gut microbiota by using a reasonably comprehensive threshold ($p < 1 \times 10^{-5}$). This approach aligns with established practices from previous studies, reinforcing the reliability of our candidate SNPs selection (Li Y et al., 2023; Li N et al., 2023; Wang et al., 2023; Zhang Y et al., 2023). Subsequently, we conducted a linkage disequilibrium analysis using PLINK software (v1.9)[1] to

1 http://zzz.bwh.harvard.edu/plink/

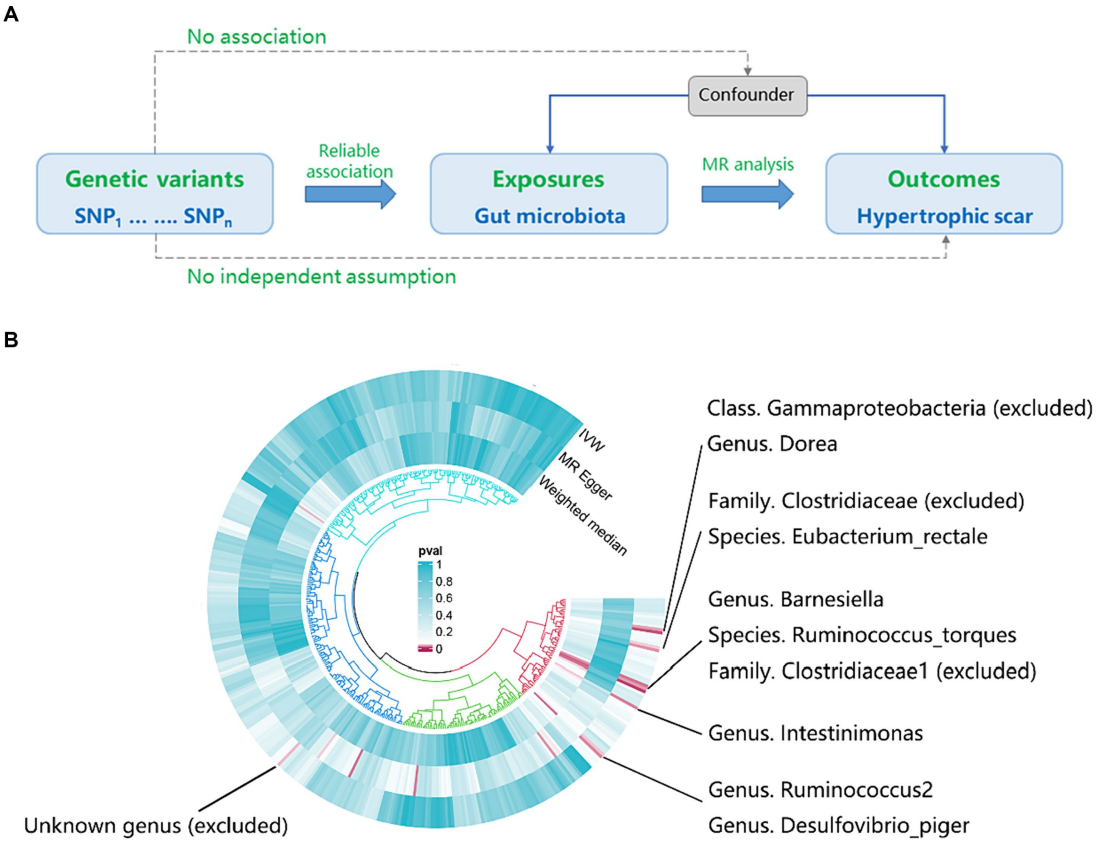

FIGURE 1
The workflow and initial results of a two-sample Mendelian Randomization (MR). **(A)** Fundamental analysis flow for MR; **(B)** Identified gut microbiota associated with hypertrophic scarring through initial MR analysis ($p_{IVW} < 0.05$), and the exclusions of unknown genera and gut microbiota that did not meet the specified criteria of instrumental variable selection.

clump SNPs with a linkage disequilibrium distance exceeding 10,000 kb and an r^2 less than 0.001. PLINK is a powerful and versatile command-line toolset designed for the analysis of genetic data. The CLUMP function is a specific feature within PLINK that is often used for post-GWAS analysis. It is used for clumping together genetic variants based on linkage disequilibrium patterns, helping identify a subset of independent genetic variants that capture the association with the exposure variable. To assess the robustness of our chosen instrumental variables, we employed the F statistic, with a threshold of over 10 being commonly considered indicative of strong instrumental variables, thereby ensuring the reliability of our evaluation (Pierce et al., 2011; Brion et al., 2013). The F-statistic, often used in statistical hypothesis testing, has various applications across different fields. In an MR analysis, it is used to evaluate whether the genetic instrument is strong enough to provide reliable causal inference. A higher F-statistic indicates a stronger instrument. Moreover, our analysis was constrained to results derived from a minimum of three shared SNPs (Li FF et al., 2023).

2.4 Statistical analysis

This study primarily employed the inverse-variance weighted (IVW) method to estimate associations between gut microbiota and hypertrophic scarring. The IVW method combines these individual variant-exposure and variant-outcome associations to obtain an overall estimate of the causal effect. It assumes that all genetic variants are valid instruments and that there is no horizontal pleiotropy (i.e., the genetic variants only affect the outcome through their impact on the exposure). While the IVW method is widely used, researchers should also consider sensitivity analyses and other methods, such as weighted median and MR-Egger regression, to assess the robustness of results and detect potential violations of assumptions. Therefore, results from MR were deemed meaningful if the IVW method identified a significant association ($p < 0.05$), and concordantly, two additional methods, MR-Egger regression and weighted median (WM), also indicated effects in the same direction (Li FF et al., 2023).

Sensitivity analyses were evaluated through leave-one-out analysis and the Q-test of both MR Egger and IVW methods (Yang et al., 2023), and directional horizontal pleiotropy was examined using the Egger intercept calculation (Cui and Tian, 2021). Leave-one-out analysis is a technique used to assess the robustness and influence of individual data points in statistical models, systematically removing one genetic instrument at a time and re-evaluating the causal estimates. The Q-test is a statistical test used in MR to assess the presence of heterogeneity among the causal estimates obtained from individual genetic instruments. This test is particularly applied in the IVW and MR Egger methods. Additionally, directional horizontal pleiotropy refers to a situation where genetic variants used as instruments have pleiotropic effects on the outcome, and there is a

systematic directional bias. The MR Egger method is designed to address situations where there is directional horizontal pleiotropy by allowing for an intercept in the regression model.

Furthermore, a reverse model was implemented to estimate the effect of hypertrophic scarring on gut microbiota. This analysis aimed to explore the potential existence of a reverse-direction causal relationship, assessing whether the exposure is positioned upstream of the outcome (Li W et al., 2023). Reverse MR is a relatively less common approach compared to standard MR analyses, but it can provide insights into the potential causal relationships between outcomes and exposures, especially in situations where conventional study designs may face challenges.

We performed all statistical analyses using R (version 4.2.2) and TwoSampleMR package (version 0.5.7). The detail analysis process and script have been uploaded to GitHub.[2]

3 Results

3.1 Causal effects of gut microbiota on hypertrophic scarring

According to the results provided in Figure 1B (IVW: $p < 0.05$), 11 gut microbiota were identified as being associated with hypertrophic scarring. Following a rigorous screening process, exclusions were made for the unknown genus and gut microbiota that did not meet the specified criteria of instrumental variable selection, as detailed in Supplementary Table S4. As a result, we pinpointed five genera and two species demonstrating associations with hypertrophic scar development (Figure 2). Regarding the genus level, the IVW analysis disclosed that *Intestinimonas* (odds ratio (OR) = 0.62, 95% confidence interval (CI) = 0.41–0.93, $p = 0.020$), *Ruminococcus2* (OR = 0.62, 95% CI = 0.39–0.97, $p = 0.036$), *Barnesiella* (OR = 0.70, 95% CI = 0.51–0.96, $p = 0.027$), *Dorea* (OR = 0.50, 95% CI = 0.30–0.84, $p = 0.009$), and *Desulfovibrio piger* (OR = 0.66, 95% CI = 0.46–0.94, $p = 0.021$) were inversely associated with hypertrophic scarring. At the species level, a positive correlation was observed between *Eubacterium rectale* (OR = 1.69, 95% CI = 1.07–2.66, $p = 0.024$) and hypertrophic scarring, while *Ruminococcus torques* (OR = 0.55, 95% CI = 0.32–0.93, $p = 0.025$) exhibited a negative correlation with hypertrophic scarring. Similarly, analyses employing MR-Egger regression and weighted median (WM) showed that the slope of each line corresponds to the estimated MR effect for each method, with a negative slope indicating a negative correlation and vice versa. The results of MR-Egger and WM methods consistently demonstrated effects in the same direction as the IVW analysis (Figure 3A), suggesting consistency in the results across different MR methods and increasing the robustness of our conclusions. For a comprehensive overview of the results, please refer to the Supplementary Tables S1–S3. Consequently, these findings collectively affirmed a causal link between specific gut microbiota and the incidence of hypertrophic scar.

3.2 Sensitivity analysis

To reinforce the purported causal relationships between gut microbiota and hypertrophic scarring, an array of sensitivity analyses was conducted. Heterogeneity was evaluated through leave-one-out analysis and the Q-test of both MR Egger and IVW methods. Leave-one-out plots are a diagnostic tool to assess the influence of individual data points in statistical analyses, and they provide insights into how the removal of specific observations affects the overall results. Each black point represents the application of the IVW algorithm to estimate the causal effect of gut microbiota on hypertrophic scarring, excluding specific variants from the analysis. The red point represents the IVW estimate using all SNPs. Firstly, the leave-one-out analysis revealed no outliers (Figure 3B), and the heterogeneity test confirmed the absence of significant heterogeneities for both IVW and MR Egger models ($p > 0.05$; Supplementary Table S5). Additionally, the Egger intercept, closely approximating zero with $p > 0.05$, indicated no evidence of directional horizontal pleiotropy effects (Supplementary Table S5). In summary, these sensitivity analyses provided robust confirmation of the reliability of our suggested causal effects in the MR results.

3.3 Reverse MR analysis

In reverse causality, hypertrophic scarring is used as exposure to validate gut microbiota outcome. The reverse MR analysis did not unveil any potential causality between hypertrophic scarring and the aforementioned bacterial taxa, as indicated by $p > 0.05$ or SNPs <3 (Supplementary Tables S6–S8). The findings indicate that there is no reverse-direction causal relationship between hypertrophic scarring and gut microbiota.

4 Discussion

Hypertrophic scar, as a fibroproliferative condition in the reticular dermis layer, is characterized by persistent inflammation, heightened angiogenesis, and excessive collagen deposition (Berman et al., 2017; Ogawa, 2017). Nutritional deficiencies, systemic diseases like diabetes, and autoimmune disorders involving chronic inflammation not only impede the intricate cascade of healing but also foster an environment conducive to the aberrant proliferation of fibroblasts, thereby contributing to the development of hypertrophic scars (Ogawa, 2022; Faour et al., 2023). In fact, the relationship between gut microbiota and skin-associated disorders has been a central focus of extensive investigation in recent years, encompassing conditions like hidradenitis suppurativa, rosacea, acne vulgaris, and atopic dermatitis (Mahmud et al., 2022). Our study unveiled the broadly applicable microbial signatures associated with hypertrophic scarring through performing a bidirectional two-sample MR analysis. Seven causal associations between gut microbiome and hypertrophic scarring were identified, involving *Intestinimonas*, *Ruminococcus2*, *Barnesiella*, *Dorea*, *Desulfovibrio piger*, *Eubacterium rectale*, and *Ruminococcus torques*.

The human gut microbiota is composed of four main phyla, which are collectively known as *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Arumugam et al., 2011). Genus *Barnesiella* from phyla

2 https://github.com/WMU-kk/MR-GM-HS

Bacterial traits	Method	NSNP	OR	95%CI	pvalue
Genus					
Intestinimonas	IVW	16	0.62	0.41-0.93	0.020
	MR Egger		0.56	0.18-1.73	0.333
	WM		0.57	0.33-0.97	0.038
Ruminococcus2	IVW	15	0.62	0.39-0.97	0.036
	MR Egger		0.32	0.11-0.91	0.053
	WM		0.64	0.34-1.18	0.154
Barnesiella	IVW	14	0.70	0.51-0.96	0.027
	MR Egger		0.76	0.25-2.30	0.632
	WM		0.66	0.42-1.02	0.062
Dorea	IVW	6	0.51	0.30-0.84	0.009
	MR Egger		0.37	0.01-19.72	0.651
	WM		0.57	0.30-1.06	0.076
Desulfovibrio_piger	IVW	6	0.66	0.46-0.94	0.021
	MR Egger		0.32	0.10-1.04	0.131
	WM		0.60	0.39-0.91	0.017
Species					
Eubacterium_rectale	IVW	9	1.69	1.07-2.66	0.024
	MR Egger		1.33	0.12-15.25	0.826
	WM		1.57	0.88-2.81	0.127
Ruminococcus_torques	IVW	7	0.55	0.32-0.93	0.025
	MR Egger		0.87	0.04-17.52	0.929
	WM		0.52	0.30-0.92	0.025

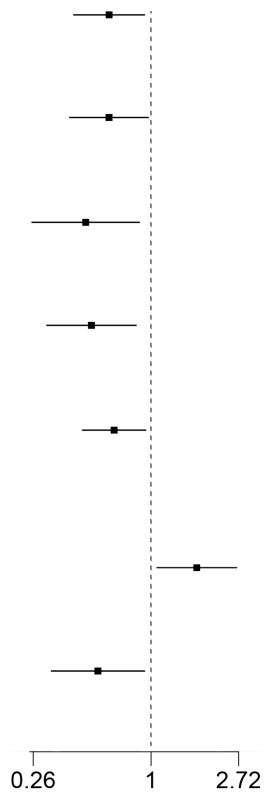

FIGURE 2
Forest plots show causal-effect estimates of gut microbiota and hypertrophic scarring. IVW, inverse-variance weighted; WM, weighted median; NSNP, the number of SNP; OR, odds ratio; CI, confidence interval.

Bacteroidetes and genus *Desulfovibrio piger* from phyla *Proteobacteria* exhibited correlations with various immunoregulatory cells, indicating their potential to create a gut environment less susceptible to inflammation (Loubinoux et al., 2002; Weiss et al., 2014). Moreover, in a previous investigation utilizing 16S rRNA gene sequencing, participants with pathological scars exhibited a relative higher abundance of *Firmicutes* (Li M et al., 2023). Similarly, we observed that specific gut microbiota from phyla *Firmicutes* act as protective factors against hypertrophic scarring, including *Intestinimonas*, *Ruminococcus2*, *Dorea*, and *Ruminococcus torques*. Regarding the modified species, *Ruminococcus torques* was reported to be associated with the alleviation of inflammation through regulating bile acid compositions (Zhang M et al., 2023). At the genus level, both *Intestinimonas* and *Ruminococcus2* are butyric acid-producing bacteria, deemed potential probiotics for alleviating and treating inflammatory diseases due to their capacity to regulate T regulatory cells and inhibit Histone Deacetylase (HDAC) (Gao et al., 2023). Additionally, it has been revealed that *Dorea*, a commensal bacterium, functions as immune sentinels within tissues (Zhang et al., 2023b). Moreover, *Ruminococcus* have also been reported to possess the ability to ferment glucose, xylose, and indigestible dietary fiber (Crost et al., 2013; Ghanbari Maman et al., 2020), obtaining energy and nutrients from otherwise complex and difficult-to-digest substrates. Overall, the deficiency of the aforementioned six gut microbiota may contribute to the occurrence of inflammation, representing a potential factor in the onset of hypertrophic scars. On the other hand, the findings concerning the species *Eubacterium rectale* from phylum *Firmicutes* suggested a potential role as a risk factor for hypertrophic scars. This aligns with a prior study indicating that *Eubacterium rectale* contributes to promoting colitis by activating the transcription factor NF-κB (Wang et al., 2021). Nevertheless, definitive evidence is necessary to confirm how *Eubacterium rectale* elevates the risk of hypertrophic scarring, given its role as a butyrate-producing flora that generally provides advantages in specific disorders (Lu et al., 2022). To sum up, our study suggested that the mentioned gut microbiota may play a crucial role in the development of hypertrophic scars by modulating the systemic inflammatory response.

In this study, MR analysis was employed to establish the causal relationship between gut microbiota and the development of hypertrophic scar. This approach helped eliminate the impact of confounding variables and minimized the potential for reverse causation, thereby enhancing the ability to infer the causality. Notably, compared to other research (Li Y et al., 2023; Xia et al., 2023; Yang et al., 2023) (211 gut microbiota from MiBioGen consortium), a larger dataset (418 gut microbiota) was employed here through combing GWAS summary statistics from MiBioGen consortium and Dutch Microbiome Project. As a result, a higher statistical power can be achieved and is better at detecting smaller causal effects, providing a more accurate reflection of the likely range of the true causal effect. Furthermore, we identified seven

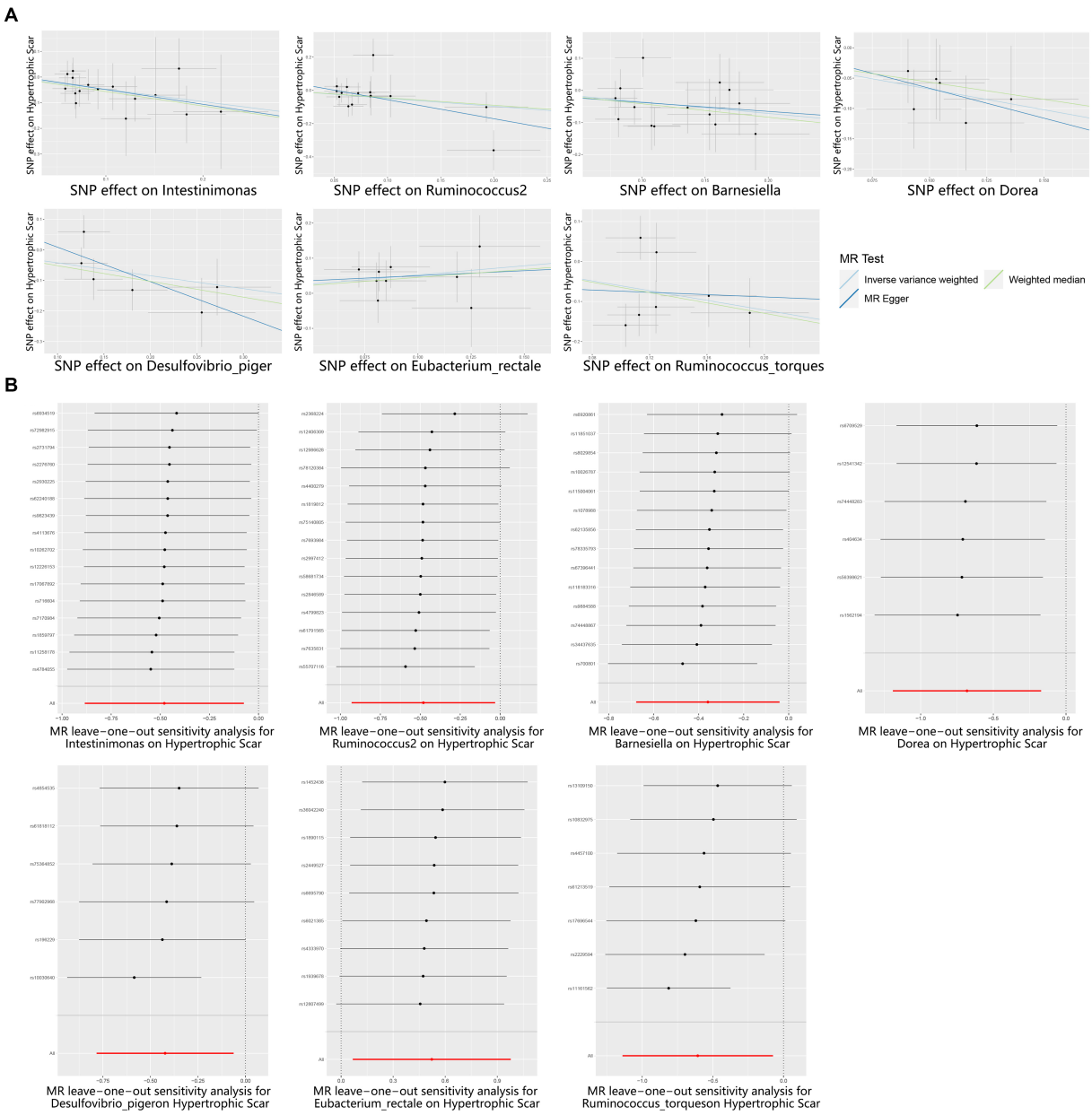

FIGURE 3
Scatter and Leave-one-out plots for the casual association between gut microbiota and hypertrophic scarring. **(A)** Scatter plots for the causal association between gut microbiota and hypertrophic scarring, and the slope of each line corresponds to the estimated MR effect for each method. **(B)** Leave-one-out plots for the causal association between gut microbiota and hypertrophic scarring. Each black point represents the application of the IVW MR algorithm to estimate the causal effect of gut microbiota on hypertrophic scarring, and the red point represents the IVW estimate using all SNPs.

crucial gut microbiotas, from which extracellular vesicles should need further investigation to be a potential nanobiotechnology therapy for hypertrophic scars and wound healing (Han et al., 2023). However, there are several limitations in this study. One notable aspect is that the GWAS summary statistics predominantly stem from European populations, which may affect the generalizability of the findings to other ethnic groups. Besides, while MR analysis demonstrated statistical causality and supported a link between gut microbiota and hypertrophic scarring, further functional experimental research is necessary to validate these results and clarify plausible genetic pathways.

The potential association between a distinct microbiome environment and a disease impacting a disparate physiological tract may be considered plausible. For instance, oral dysbiosis has been linked to an increased risk of cardiovascular diseases. Bacteria associated with periodontal disease can enter the bloodstream, potentially contributing to inflammation and atherosclerosis in blood vessels (Leishman et al., 2010; Teles and Wang, 2011). Besides, emerging research suggested a connection between the gut microbiome and neurodegenerative conditions like Parkinson's disease. Changes in the gut microbiome composition might influence the development and progression of neurodegenerative diseases

through the gut-brain axis (Zhu et al., 2021; Zhang et al., 2023a). From a similar perspective, this study analyzed the impact of the non-cutaneous microbial environment on hypertrophic scarring. However, the evidence about this microbiome influence is still unclear or inconsistent. On the contrary, the evidence supporting the influence of local microbiota on the skin is continually growing. Changes in the skin microbiome have been implicated in various skin conditions, such as seborrheic dermatitis and acne (Ferček et al., 2021). It has also been found that the dysbiosis of the microbiota occurring in hypertrophic scars is primarily associated with *S. aureus* colonization (Yu et al., 2023). Currently, there is a lack of research on the influence of gut microbiota on skin microbiota and the impact of skin microbiota on hypertrophic scars. Further research is needed to explore these aspects.

5 Conclusion

In this study, we conducted a comprehensive assessment of the causal connections between gut microbiota and the development of hypertrophic scar. Through MR analysis, six bacterial taxa were identified as protective factors, while one was identified as a risk factor for hypertrophic scar. In particular, the reverse MR study failed to demonstrate a reverse causal relationship between gut microbiota and hypertrophic scarring. Our study contributed additional supportive evidence and valuable insights into the causal relationship between gut microbiota and the development of hypertrophic scar, providing avenues for mechanistic exploration and the identification of potential therapeutic targets.

Data availability statement

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

Author contributions

KX: Conceptualization, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. GZ: Data curation, Formal analysis, Investigation, Resources, Writing – original draft. ZihL: Data curation, Formal analysis, Investigation, Resources, Writing – original draft. XZe: Data curation, Formal analysis, Investigation, Resources, Writing – review & editing. ZiL: Visualization, Writing – review & editing. FW: Visualization, Writing – review & editing. XZh: Visualization, Writing – review & editing. CL: Supervision, Validation, Visualization, Writing – review & editing. CM: Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We sincerely thank for the financial support of Zhejiang Provincial Basic Public Welfare Research Program of China (grant no. LTGY23H150004, LY22H150004, LGD22H070001), Key Science and Technology Program of Zhejiang Province (grant no. 2023C0317), Medical Health Science and Technology Project of Zhejiang Province (grant no. 2023KY146), Wenzhou Science and Technology Bureau Project of China (grant no. Y2023100).

Acknowledgments

We express our sincere appreciation to the two large GWAS databases, MiBioGen and the FinnGen consortium, for generously sharing research results data. We are also deeply grateful to all participants and investigators involved in the FinnGen and MiBioGen studies.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1345717/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 11 April 2024
DOI 10.3389/fmicb.2024.1383656

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OPEN ACCESS

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RECEIVED 07 February 2024
ACCEPTED 27 March 2024
PUBLISHED 11 April 2024

CITATION
Li M, Kopylova E, Mao J, Namkoong J, Sanders J and Wu J (2024) Microbiome and lipidomic analysis reveal the interplay between skin bacteria and lipids in a cohort study.
Front. Microbiol. 15:1383656.
doi: 10.3389/fmicb.2024.1383656

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Microbiome and lipidomic analysis reveal the interplay between skin bacteria and lipids in a cohort study

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Human skin acts as a protective barrier between the body and the external environment. Skin microbiome and intercellular lipids in the stratum corneum (SC) are essential for maintaining skin barrier function. However, the interplay between skin bacteria and the lipids is not fully understood. In this study, we characterized the skin microbiome and SC lipid profiles from the forearm and face in a cohort of 57 healthy participants. 16S rRNA gene sequencing showed the skin microbial composition is significantly different between body locations and genders. Female forearm samples have the highest microbial diversity. The relative abundance of *Staphylococcus hominis, Micrococcus luteus, Corynebacterium tuberculostearicum, Finegoldia magna,* and *Moraxellaceae* sp. are significantly higher in the forearm than the face. The predictive functional analysis of 16S rRNA gene sequencing by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) and ANCOM-BC showed different bacterial metabolic pathway profiles between body locations or genders, and identified 271 differential pathways, including arginine and polyamine biosynthesis, chorismate biosynthesis pathways, which are more abundant in the female forearm, and sulfur oxidation pathway, which is more abundant in the male face. The SC lipid profiles differ between the body locations as well. Total free fatty acids (FFA), cholesterol sulfate and sphingosine are more abundant in the face. Dihydro-/6-hydroxy/phyto-ceramides are more abundant in the forearm. The correlation analysis of 16S rRNA gene sequencing and lipids revealed novel interplay between the bacteria and skin lipids. Shannon entropy and *S. hominis* negatively correlated with FFA, cholesterol sulfate and sphingosine; while positively correlated with dihydro-/6-hydroxy/phyto-ceramides. The correlation of predictive pathway profiles and lipids identified pathways involved in amino acids metabolism, carbohydrates degradation, aromatic compounds metabolism and fatty acid degradation metabolism are positively correlated with dihydro-/6-hydroxy/phyto-ceramides and negatively correlated with FFA, cholesterol sulfate and sphingosine. This study provides insights on the potential correlation between skin microbiome and lipids.

KEYWORDS

skin microbiome, skin lipids, lipidomics, microbiome–lipid interaction, 16S rRNA sequencing

Introduction

The skin is the largest organ of the human body and acts as a protective barrier between the body and the external environment. The intercellular lipids in the outermost layer of the skin, the stratum corneum (SC), are one of the fundamental components to maintain the skin barrier function (Knox and O'Boyle, 2021). These lipids suppress excessive water and electrolyte loss and prevent the compounds from the environment permeating into the epidermis and the dermis, and thereby provoke an immune response (van Smeden and Bouwstra, 2016). The composition of the skin lipid matrix is dominated by three classes: ceramides, cholesterol, and free fatty acids (FFAs) (Knox and O'Boyle, 2021). Ceramides are the most common constituent, accounting for 40–50% of the total intercellular lipids (Choi and Maibach, 2005). Depending on the type of sphingosine and the type of fatty acid bound together, there are 12 different subclasses of ceramides identified in human SC (Murphy et al, 2022). Among these ceramides subclasses, ceramide esterified omega fatty acids (EOS), phytoceramide saturated fatty acids (NP), phytoceramide alpha-hydroxy fatty acids (AP), also called ceramides 1, 3, and 6-II respectively, are considered essential ceramides that excel in supporting skin health by preserving the integrity of the lamellar layer (Coderch et al., 2003; Huey-Chun and Chang, 2008). They are also widely used in a variety of skincare products. The distribution and composition of the skin lipids vary across different body locations (Starr et al., 2016), and are influenced by age, gender and seasonal variations (Rogers et al., 1996; Starr et al., 2016; Choe et al., 2018). Studies have demonstrated that alterations in the SC lipid composition can lead to impaired skin barrier functions, giving rise to skin disorders such as psoriasis and atopic dermatitis (Pietrzak et al., 2010; Emmert et al., 2021). Therefore, understanding the composition of skin lipids profile and the impact of host and external factors on it is critical for skin health.

The SC is also colonized by a variety of living microorganisms, called the skin microbiome, which is essential for maintaining skin barrier function. The microbes form an invisible ecosystem that protects the skin from opportunistic pathogens, contributes to the production of essential nutrients and educates the immune system to ensure human health (Grice, 2015; Byrd et al., 2018). The skin microbial composition highly depends on the topographic locations of the human body and varies by age and gender (Grice et al., 2008). The skin barrier and microbiome have a symbiotic relationship, influencing one another through physical, chemical, and immunological interactions. The skin microbiome can secrete the components that make up the lipid structure. For instance, *Staphylococcus epidermidis* produces a sphingomyelinase that acquires essential nutrients for the bacteria and indirectly assists the host in producing ceramides to help build the skin lipids (Zheng et al., 2022). Meanwhile, epidermal lipids can serve as a nutrient source for the skin microbiome (Tchoupa et al., 2023). Pathogenic microorganisms are also directly inhibited by some lipids. For example, sapienic acid from the SC can effectively inhibit pathogenic *S. aureus* (Moran et al., 2017). Therefore, the cross-talk between the skin microbiome and lipids is very important to maintain skin barrier function, however, these interactions are not fully understood.

In this study, we characterized the skin microbiome and SC lipids profiles from the forearm and face in a cohort of 57 healthy participants using 16S rRNA gene sequencing and lipidomic analysis. The objective was to understand the impact of host factors such as age, gender, skin type and body location on skin microbiome and lipid profile, and to explore the interaction of skin bacteria and lipids. We also explored the mechanism of the relationship by using predictive functional analysis.

Materials and methods

Study design and sample collection

The study was approved by Institutional review board of Concordia Clinical Research, Inc. (IBR Committee No. 188Z) (Cedar knolls, NJ, USA). A total of 57 healthy participants from Piscataway, New Jersey, USA were recruited. The study design was illustrated in Figure 1 (Gomes et al., 2023). The informed consent was signed by each participant. Prior to sampling, all participants were provided with a questionnaire in which they were asked for age, gender and skin type (oily, dry, normal). Participants were instructed not to take a shower or wash their face on the morning of the sample collection day. They were also instructed not to apply any products on the face and both forearms, including but not limited to soaps, shower gels, lotions, creams, oils, sunscreen and makeup.

One skin swab was collected from one forearm and cheek using a swabbing technique for microbiome analysis. A 5x5cm area of the skin was sampled by swabbing the skin for 30 s with a sterile flock swab, which was dipped into an aliquot of phosphate buffered saline (PBS). The lateral edge of the swab was rubbed across the entire defined area while being rotated between the thumb and forefinger for 30 s. More specifically, the rotating swab was rubbed back and forth in a cross-wise manner in the defined area in the same fashion for each participant to maintain consistency. After 30 s, the head of each swab was placed into a sterile microcentrifuge tube and aseptically cut from the breakpoint of the handle before closing the tube lid. All the samples were frozen at −80°C until further analysis.

Tape stripping was done on the surface of one forearm and forehead using D-Squame standard sampling disc, 22 mm in diameter (Clinical & Derm, LLC, Dallas, Texas) for lipidomic analysis. The tape was applied to the skin surface and briefly pressed with a standardized pressure pen of 225 g/cm2 (D-Squame pressure instrument D500, Clinical & Derm, LLC). On the same spot, a total of 4 consecutive tapes were collected. Each collected tape was placed in a storage card (D-Squame standard storage card D120, Clinical & Derm, LLC) and stored in −80°C until the analysis.

16S rRNA gene sequencing

V1-3 hypervariable region of 16S rRNA gene sequencing was conducted by RTL Genomics (Lubbock, Texas) as previously described (Li et al., 2023). DNA was extracted via KingFisher FLEX instrument (ThermoFisher Scientific, Inc., Waltham, Massachusetts) and using Zymo ZR-96 magbead kit (Zymo Research, Irvine, California) following manufacturer's instructions. The extraction protocol was modified to include a mechanical lysis step with a Qiagen TissueLyser. V1-3 region of 16S rRNA gene was amplified for sequencing in two-step, independent reactions using HotStar Taq Master Mix Kit (Qiagen) with 28F-519R primers (28F: 5"GAG TTT

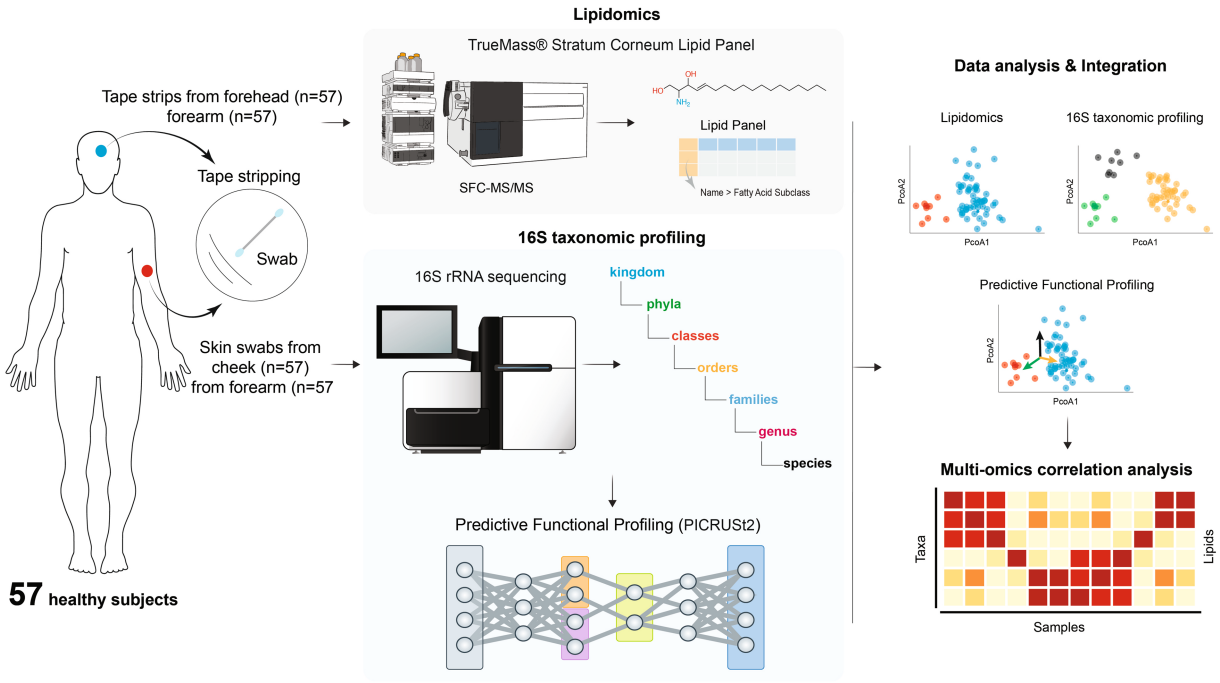

FIGURE 1
Overview of the study design.

GAT CNT GGC TCA G 3″; 519R: 5″ GTN TTA CNG CGG CKG CTG 3″). PCR amplification included 0.5 µl of 5 µM forward primer, 0.5 µl of 5 mM reverse primer, 5 µl of DNA template, and 14 µl of Taq Master Mix. To encourage amplification in low biomass samples, 2 µl BSA and 2 µl $MgCl_2$ were added to reactions. The negative control was a reaction mixture with no template DNA. PCR reaction conditions included initial denaturation at 95°C for 5 min, then 10 cycles of 94°C for 30 s, 50°C for 90 s (+0.5°C per cycle), 72°C for 1 min, followed by 25 cycles of 94°C for 30 s, 54°C for 90 s, 72°C for 1 min, and finally, one cycle of 72°C for 10 min and 4°C hold. Barcoding PCR reaction conditions included initial denaturation at 95°C for 5 min, then 10 cycles of 94°C for 30 s, 54°C for 40 s, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold. Amplification products were visualized with eGels (Life Technologies). Products were then pooled equimolar and each pool was size selected in two rounds using SPRIselect beads (BeckmanCoulter) in a 0.75 ratio for both rounds. Size selected pools were then quantified using Qubit 4 fluorometer (Life Technologies) and loaded on an Illumina MiSeq 2 × 300 flow cell at 10 pM for sequencing.

Skin lipid analysis

Stratum corneum lipids were analyzed by Metabolon Inc. (Morrisville, North Carolina). Total free fatty acids, cholesterol, cholesterol sulfate and ceramides were measured by SFC-MS/MS using TrueMass® Stratum Corneum Lipid Panel (Emmert et al., 2021). Tissue samples were extracted with hexane after addition of a known amount of surrogate standard solution consisting of stable-labeled forms of ceramides, fatty acids, cholesterol and cholesterol sulfate. The organic extracts were combined and evaporated to dryness. The dried extract was reconstituted, and an aliquot was analyzed on a Waters UPC2/Sciex QTrap 5,500 mass spectrometer SFC-MS/MS system in MRM mode using characteristic parent-fragment mass transitions for each analyte trace. The quantitation of the individual lipid species was based on a single-point calibration using a surrogate standard. Concentrations were determined by peak area comparisons of the individual lipid species with the peak areas of their corresponding surrogate standards for which concentrations are known. Concentrations were given in pmol/tape for individual analytes as well as each lipid class. Additionally, the percentage composition of 10 individual ceramide subtypes is listed for each sample (Table 1), including ceramide alpha-hydroxy fatty acids (AS), ceramide esterified omega fatty acids (EOS), ceramide saturated fatty acids (NS), dihydroceramide alpha-hydroxy fatty acids (ADS), 6-hydroxyceramide alpha-hydroxy fatty acids (AH), phytoceramide alpha-hydroxy fatty acids (AP), 6-hydroxyceramide esterified omega fatty acids (EOH), dihydroceramide saturated fatty acids (NDS), 6-hydroxyceramide saturated fatty acids (NH), phytoceramide saturated fatty acids (NP).

Data analysis

16S rRNA gene sequencing data analysis

Raw FASTQ sequencing data (forward and reverse reads) was imported into QIIME2 (version 2022.2.0) (Bolyen et al., 2019). Quality control analysis identified lower quality regions in the first 20 nucleotides (primers) and, notably, in the last 20 nucleotides for the forward reads and the last 40 nucleotides for the reverse reads (as Phred read quality scores dropped below 20). To ensure sufficient overlap between the forward and reverse reads, the primers and the final 20 nucleotides were trimmed from both forward and reverse

TABLE 1 Ceramides subtypes measured by TrueMass® Stratum Corneum Lipid Panel in this study.

Ceramides class		Sphingosine base
Ceramides	Ceramide alpha-hydroxy fatty acids (AS)	Sphingosine
	Ceramide esterified omega fatty acids (EOS)	
	Ceramide saturated fatty acids (NS)	
Dihydroceramide	Alpha-hydroxy-dihydrosphingosine (ADS)	Dihydrosphingosine
	Non-hydroxy-dihydrosphingosine (NDS)	
6-Hydroxyceramide	6-hydroxyceramide alpha-hydroxy fatty acids (AH)	6-Hydroxysphingosine
	6-hydroxyceramide esterified omega fatty acids (EOH)	
	6-hydroxyceramide saturated fatty acids (NH)	
Phytoceramide	Phytoceramide alpha-hydroxy fatty acids (AP)	Phytosphingosine
	Phytoceramide saturated fatty acids (NP)	

reads. The DADA2 (Callahan et al., 2016) plugin in QIIME2 was used to generate an ASV feature table with 6,301 ASVs (*qiime dada denoise-paired* command, with trimming of leading and trailing low quality nucleotides). A phylogenetic tree was built from the ASV sequencing using mafft (Katoh and Standley, 2013) and fasttree (Price et al., 2010) (*qiime phylogeny align-to-tree-mafft-fasttree* command). The resulting phylogenetic tree served for unweighted UniFrac (Lozupone and Knight, 2005) diversity metrics computation. Alpha diversity was explored as a function of sampling depth, and a rarefaction depth of 3,940 was selected for core diversity analyses because this was the highest sampling depth at which all 114 samples were retained, and Shannon alpha diversity appeared to level off after sampling depth of 2000 (*qiime diversity alpha-rarefaction* command). Alpha and beta diversity were computed using core diversity analysis (*qiime diversity core-metrics-phylogenetic*). Group significance analysis (Kruskal-Wallis for alpha diversity and PERMANOVA (Anderson, 2001) for beta diversity) was also computed (*qiime diversity alpha-group-significance* and *qiime beta-group-significance* commands). To assign taxonomy to the ASV sequences, we trained our own taxonomic classifier. First, the pre-formatted Silva 138 SSURef NR99 full-length sequences and taxonomy database (Quast et al., 2013; Robeson et al., 2021) were downloaded from QIIME2 data resources. Then, 550 nucleotides spanning the V1-3 hypervariable region of 16S rRNA gene were extracted from the full-length sequences (*qiime feature-classifier extract-reads* command with 28F-519R primers). A Naive Bayes classifier was trained on these extracted regions and the model then applied to classifying the ASVs (*qiime feature-classifier classify-sklearn* command). For species-specific analyses, the ASV feature table was collapsed to species level (*qiime taxa collapse* command) and converted to relative abundance (*qiime feature-table relative-frequency* command). Differential abundance analysis was run using ALDEx2 (v1.3.2) (Fernandes et al., 2014) with Benjamini–Hochberg correction and ANCOM-BC (Lin and Peddada, 2020) with Holm–Bonferroni correction using the species-level feature table. Differential abundance analysis for Shannon entropy was performed using pairwise Kruskal–Wallis with Benjamini–Hochberg correction.

Predictive functional analysis by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2)

The ASV feature table and sequences were input into the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) plugin in QIIME2 (*qiime picrust2 full-pipeline* command) (Janssen et al., 2018; Douglas et al., 2020) to generate predicted MetaCyc pathways. PICRUSt2 performs phylogenetic placement of the ASVs into a reference tree and then estimates the functional potential of the ASVs based on the known functions associated with the reference organisms in the tree. The default reference tree was used. Six of the 6,301 ASVs aligned poorly to the references and were excluded from downstream analysis. A total of 410 pathways were predicted. The Bray-Curtis distance metric was used for beta diversity analysis. Differential pathway abundance analysis was run using ANCOM-BC.

Lipids data analysis

The Euclidean distance metric was selected for beta diversity analysis due to the nature of the data, which consists of absolute concentration of lipids.

Integration and correlation analysis

Species-lipids, species-pathway and pathway-lipids correlation analysis was performed using Hierarchical All-against-All association testing HAllA (version 0.8.20) (Ghazi et al., 2022) using Spearman correlation and Benjamini–Hochberg false-discovery rate correction (q-value). Complementary to univariate analysis performed using ANCOM-BC and ALDEx2, supervised multivariate analysis using Random Forest was applied to gain insights into the predictive capabilities of the species microbiome and lipids with regard to location and gender group classification. The Boruta package (v.8.0.0) (Kursa and Rudnicki, 2010) was used for feature selection, with a maximum of 10,000 runs. Subsequently, a Random Forest model was constructed using the selected features, leading to an out-of-bag (OOB) error rate of 14.91% based on 1,000 trees, as depicted in Supplementary Figure S1. A multidimensional scaling (MDS) plot was generated using the proximity matrix of the Random Forest model (rescaled to the range of [−1, 1] to standardize the axes and facilitate a clearer visualization of the correlation between lipids, 16S and pathway variables and the ordination axes) and PICRUSt2 predicted pathways, together with Random Forest selected features driving the separation, were fitted on the MDS plot using the envfit function from the Vegan package, see Supplementary Figure S2A. The contribution of different species to selected PICRUSt2 predicted pathways is shown in Supplementary Figure S2B. The statistical method adonis2 from the Vegan R package (v.2.6-4) was used to

assess the significance of variation in location and gender explained by 16S species (unweighted unifrac), PICRUSt2 predicted pathways (Bray–Curtis) and lipids (Euclidian), see Figure 2. Pairwise PERMANOVA analysis with Holm–Bonferroni correction was performed using the pairwise.adonis function (based on a loop using adonis2) (Arbizu, 2020).

Results

Skin microbial composition and predictive functional metagenomic profiles vary by body sites and gender

Alpha diversity (Shannon entropy) of the skin microbiome was significantly different between face and forearm samples, as well as between male and female samples (Figure 3). Female forearm samples have the highest microbial diversity, and male face samples have the lowest diversity. No significant change in alpha diversity was observed for skin type or age group (Supplementary Figure S3).

Beta diversity of the skin microbiome (PCoA analysis using unweighted unifrac distance) showed significant separation of the samples by body location (PC1, 25% explained variance) and gender (PC2, 5% explained variance) (Figure 2A). Pairwise PERMANOVA results showed q-value = 0.006 for all pairwise comparisons between location or gender. Discriminating bacteria between body location or gender are shown in Supplementary Figure S4. For instance, *Staphylococcus hominis* (q-value < 0.001), *Corynebacterium tuberculostearicum* (q-value <0.001), *Micrococcus luteus* (q-value = 0.003), *Finegoldia magna* (q-value < 0.001) and *Moraxellaceae* sp. (q-value = 0.002) are more location specific. The relative abundance of these species were significantly higher in the forearm than the face. While *Streptococcus* sp. and *S. epidermidis* are more gender specific (ANCOM-BC location q-value >0.05 for both and gender q-value = 0.013 and q-value = 0.047, respectively). The relative abundance of *Streptococcus* sp. is higher in females than males, and *S. epidermidis* is higher in males than females especially in face samples.

To further investigate the functional potentials of the skin microbiome in this cohort, PICRUSt2 analysis was performed to predict MetaCyc metabolic pathways using 16S rRNA gene ASV data. In contrast to the skin microbial composition profiles, the PCoA plot for predicted functional profiles (Figure 2B) showed significant separation of the samples only for male face and forearm (q-value = 0.09) and male face and female forearm (q-value = 0.09). ANCOM-BC analysis identified 271 metabolic pathways (q-value <= 0.05) showing differential abundance between location or gender. The differential pathways are listed in Supplementary Table S1. To investigate the relationship between differentially abundant predicted pathways and the measured species and lipids, a Random Forest model was built on skin lipids and microbiome species data and a multidimensional scaling (MDS) plot generated using sample proximities (Supplementary Figures S1, S2). Differentially abundant predicted pathways were fitted onto the MDS plot and illustrated pathway distribution across sample groups. This analysis revealed stronger correlation of pathways to the forearm, especially in females, such as chorismate metabolism (ALL-CHORISMATE-PWY, $r^2 = 0.28$), arginine and polyamine biosynthesis (ARG + POLYAMINE-SYN, $r^2 = 0.46$), polyamine biosynthesis I (POLYAMSYN-PWY, $r^2 = 0.45$) and polyamine biosynthesis II (POLYAMINSYN3-PWY, $r^2 = 0.44$). Pathways exhibited a stronger correlation to forearm samples, with an average $r^2 = 0.14$ for pathways having MDS1 scores less than 0, aligning with the observed higher Shannon entropy in the forearm samples as compared to the face. In contrast, the average $r^2 = 0.07$ for pathways having MDS1 scores greater than 0. Of the 1,411 species in the full 16S feature table, *Streptococcus* spp. had the highest number of ASVs which were mapped to either of these four pathways (by parsing the PICRUSt2 stratified output abundance table), suggesting a potential functional contribution of *Streptococcus* spp. within these metabolic pathways.

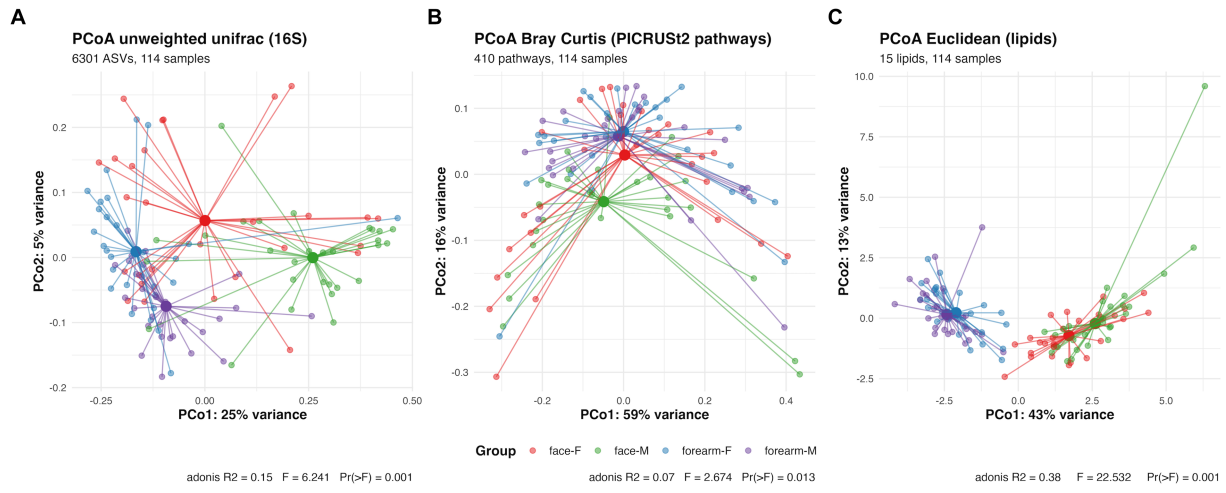

FIGURE 2
PCoA plot and PERMANOVA analysis of skin microbial composition characterized by **(A)** 16S rRNA gene sequencing **(B)** predictive metabolic pathway profiles analyzed by PICRUSt2 using 16S rRNA gene sequencing data **(C)** skin lipidomic profiles characterized by SFC-MS/MS. F, female; M, male.

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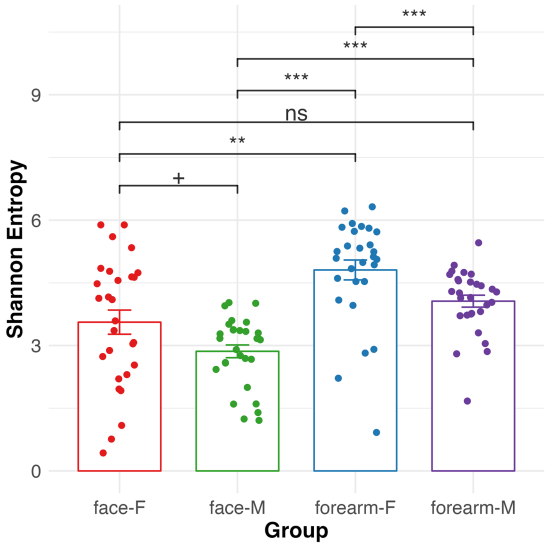

FIGURE 3
Alpha diversity of skin microbiome in different body sites and gender characterized by 16S rRNA gene sequencing. Kruskal–Wallis pairwise *q*-values represent '***' 0–0.001, '**' 0.001–0.01, '*' 0.01–0.05, '+' 0.05–0.1. F, female; M, male.

Skin lipid profile differs between body sites

Total FFA, ceramides, cholesterol and cholesterol sulfate in the skin surface were quantified and the percentages of each ceramides subtypes were measured by SFC-MS/MS (Table 1). The PCoA plot of the lipid profiles using a Euclidean distance matrix showed significant separation between the body locations (Figure 2C). Females and males have different lipids profiles in the face samples (pairwise adonis *q*-value = 0.01), not in the forearms (pairwise adonis *q*-value = 0.09). No significant differences were observed in age and skin type groups (data not shown). Total FFA, cholesterol sulfate, sphingosine (AS and NS) are more abundant in the face, while Dihydro-/6-Hydroxy/phyto-Ceramides (NH, NP, AH, AP) are more abundant in the forearm (Supplementary Figure S5).

Correlations between skin microbiome and lipid profiles

To explore the relationship of the skin microbial composition and lipids profiles, HAllA analysis was performed. Eighty-two species clusters were identified to have significant correlation with Shannon diversity and/or lipids (Supplementary Table S2). The most significant bacteria cluster including *S. hominis, Corynebacterium* sp., *Corynebacterium tuberculostearicum, Finegoldia magna* had positive correlation with Shannon diversity, dihydro- (ADS)/6-hydroxy (AH, NH)/phyto-cCeramides (AP, NP), and negative correlation with FFA, cholesterol sulfate, sphingosine based ceramides (AS, NS). A few other bacteria, for instance, *Pseudomonas* sp., *Moraxellaceae* sp. and *Roseomonas* sp. and *Brevibacterium casei* had similar correlation patterns as Cluster 1. In contrast, *C. acnes* tended to have inverse correlation patterns as compared to the clusters described above. They are positively correlated with FFA, cholesterol sulfate, sphingosine based ceramides, and negatively correlated with dihydro−/6-hydroxy/phyto-ceramides. The relationship among Shannon entropy, representative bacteria clusters and lipids was illustrated in Figure 4.

To further explore the potential mechanism of the correlation between the species and lipids, HAIIA analysis was run using the predictive metabolic pathways generated by PICRUSt2 and lipids. Hundred and forty-seven pathway clusters of species were identified to have significant correlation with lipids (Supplementary Table S3). The most representative pathway cluster is composed of 9 lipids and 226 pathways, which are involved in amino acids metabolism, carbohydrates degradation, and aromatic compounds metabolism. This cluster is positively correlated with ADS, NP, AP, AH, NH, while negatively correlated with FFA, cholesterol sulfate, AS, NS. Many of the pathways in this cluster have positive correlation to *S. hominis*, *Corynebacterium tuberculostearicum and Corynebacterium* sp. (Figure 4); all species are more abundant on the forearm.

Additionally, we performed HAIIA analysis to explore the correlation between bacteria species and predictive metabolic pathways, especially lipid related pathways. We observed lipid related pathways that are significantly correlated with certain bacteria (Supplementary Table S4). For instance, *S. hominis, C. tuberculostearicum, Micrococcus luteus, Finegoldia magna*, and *Moraxellaceae* sp. are more location specific and had significant positive correlations with fatty acid degradation pathway (FAO-PWY: fatty acid β-oxidation I (generic); PWY-7094: fatty acid salvage). *S. epidermidis* and family Neisseriaceae had significant correlations (q-value <= 0.05) with fatty acid synthesis pathways including super pathway of fatty acid biosynthesis initiation (FASYN-INITIAL-PWY) and fatty acid elongation -- saturated pathways (FASYN-ELONG-PWY). The correlations of representative predicted metabolic pathways and bacteria/lipids are illustrated in Figure 4. The correlations of all predicted pathways having a significant q-value and bacteria/lipids are illustrated in Supplementary Figure S6.

Discussion

Skin microbial composition varies by the body locations and is highly influenced by age, gender and skin types etc. (Grice et al., 2008; Ross et al., 2017; Li et al., 2019; Boxberger et al., 2021). Our findings are consistent with other studies showing significant differences in skin microbial composition between body locations and gender. The female forearm has the highest microbial diversity (Figure 3) and certain bacteria were identified as either location specific, such as *S. hominis, M. luteus, C. tuberculostearicum, F. magna* and *Moraxellaceae* sp.; or gender specific, e.g., *Streptococcus* sp. and *S. epidermidis* (Supplementary Figure S4). More interestingly we found that predicted bacteria metabolic pathway profiles differed by body location and gender as well. Predicted pathways involved in arginine, polyamine, and chorismate biosynthesis and fatty acid degradation are more abundant in the female forearm, and sulfur oxidation and fatty acid biosynthesis pathways are more abundant in male face. The differences in skin microbial composition and metabolism might be due to the difference in sweat or sebum

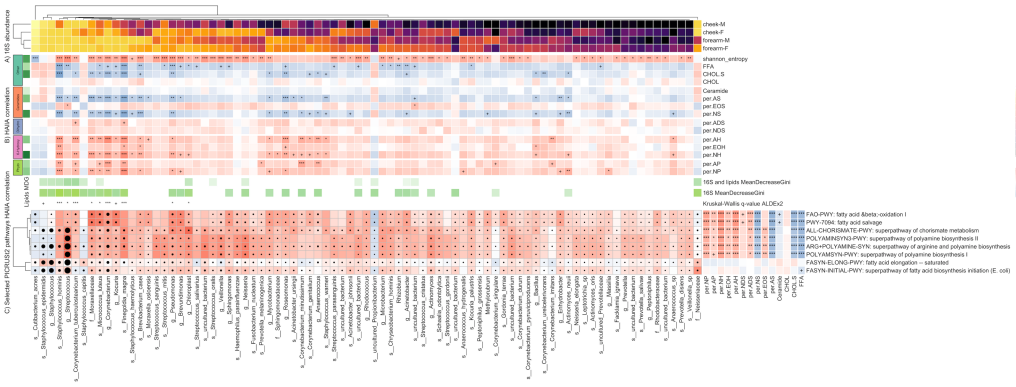

FIGURE 4
Heatmap illustrating the relationship between 16S species, representative predictive metabolic pathways and lipids. Columns represent 84 species discriminating between location and gender. The discriminating species were selected as the union of (i) species having at least one significant (q-value <= 0.05) HAllA correlation to lipids or Shannon entropy (ii) species selected using the Boruta feature selection algorithm for the Random Forest model using only species as input, and (iii) species selected using the Boruta feature selection algorithm for the Random Forest model using species and lipids as input. The MeanDecreaseGini score represents feature importance in the Random Forest classification model. Benjamini-Hochberg q-values (HAllA correlations) and Kruskal–Wallis q-values (ALDEx2 differential abundance) represent '***' 0–0.001, '**' 0.001–0.01, '*' 0.01–0.05, '+' 0.05–0.1. **(A)** Relative abundance of 16S species per location-gender group **(B)** HAllA correlation of 16S species to lipids. Lipids are grouped by their Sphingoid Base (Ceramides, Dihydro-ceramides, 6-Hydroxy-ceramides, Phyto-ceramides) or Other. **(C)** Rows represent 8 PICRUSt2 predicted pathways, amino-acid and lipid-related, discriminating between location or gender (ANCOM-BC q-value <= 0.05). Cells are colored by HAllA correlation between 16S species and predicted pathways. Black circles within cells are scaled by the number of ASVs contributing to PICRUSt2 predicted pathways. HAllA correlation between discriminant predicted pathways and lipids shown to the right. F, female; M, male.

production, cosmetics application, skin pH, thickness, or hormone production which may favor the growth or activity of specific bacteria in a certain body location or gender (Grice and Segre, 2011; Ross et al., 2017). The bacterial species statistically enriched according to body location and gender show phylogenetic conservation of genes involved in specific metabolic pathways suggests that they may be involved in these metabolisms in the skin. For instance, *S. hominis, M. luteus, C. tuberculostearicum, F. magna* and *Moraxellaceae* sp. may be involved in arginine/polyamine biosynthesis, chorismate biosynthesis and fatty acid degradation, while *Streptococcus* sp. and *S. epidermidis* may be involved in sulfur oxidation and fatty acid biosynthesis metabolism. Further investigation into the actual genomic content of these organisms, and their expression *in situ*, will be necessary to establish these relationships.

The composition and distribution of skin lipids also vary by the body sites (Ludovici et al., 2018). Different ceramides subclasses are also distributed differently. In line with the previous study showing the relative abundance of NS is higher in forehead than arm in healthy and atopic skin, and NH is higher in arm than forehead (Emmert et al., 2021), we observed that total FFA, cholesterol sulfate, sphingosine (AS and NS) are more abundant in the face, while dihydro−/6-hydroxy/phyto-ceramides (NH, NP, AH, AP) are more abundant in the forearm. It may be due to the different sebaceous gland density and secretion in different body sites (Ludovici et al., 2018).

Age and skin type also impact the skin microbiome composition and/or lipid profiles (Rogers et al., 1996; Grice et al., 2008). However, in this cohort, we did not observe significant differences in skin microbiome or lipids profiles between skin types and age groups. This study relied on self-reported skin type, so the lack of significance may be due to incorrect assessments. The low number of participants in each age group would also reduce our ability to identify significant differences.

The novel discovery from this study is we observed a unique pattern of the interaction between skin bacteria and lipids. For instance, *S. hominis, Staphylococcus* sp., *C. tuberculostearicum, F. magna* are positively co-correlated with Shannon diversity and skin lipids especially the essential ceramides (AP, NP), indicating a potential functional ecotype where these bacteria are replying on and/or involving in production of a particular skin ceramides profile. *S. hominis* is the second most frequently isolated coagulase-negative staphylococci (CoNS) from healthy skin (Kloos and Schleifer, 1975; Becker et al., 2014). Researchers have shown that *S. hominis* is a protective CoNS preventing pathogenic *S. aureus* from colonizing or infecting the skin (Severn et al., 2022). The correlation between *S. hominis* and skin ceramides suggested that *S. hominis* could be a beneficial bacteria to help build the skin ceramides, similar to its closely related species *S. epidermidis*, which has been shown to produce ceramides to maintain skin barrier (Zheng et al., 2022). Therefore, we hypothesize that *S. hominis* and other bacteria in the same clusters could be beneficial to enhance skin barrier function.

We further conducted PICRUSt2 analysis to predict the functional metabolic pathways of the skin microbiome using characterized 16S rRNA gene sequences and correlated the pathways to bacteria and the lipids. The correlation between skin lipids, skin bacteria and representative predicted metabolic pathways is illustrated in Figure 4. We did not observe any ceramides synthesis pathways that are correlated with *S. hominis, C. tuberculostearicum, Micrococcus luteus, Finegoldia magna, Moraxellaceae* sp. In contrast, these bacteria had significant positive correlations with fatty acid oxidation and salvage pathways, indicating that these bacteria may be also involved in long chain fatty acid degradation metabolism. While *S. epidermidis* and bacteria from family Neisseriaceae had significant correlations with fatty acid elongation and initiation synthesis, indicating that

these bacteria may be involved in fatty acid biosynthesis.. However, the hypothesis was generated based on computational correlation analysis. Future *in situ* studies are needed to further explore the mechanism of the relationship between the bacteria and skin lipids.

In summary, we investigated the impact of body location and gender on skin microbial composition, functional metabolic pathway profiles and skin lipids profiles, and revealed unique patterns of interactions between skin bacteria and lipids. This study provides valuable insights on the relationship of skin microbiome and lipids, and gains a deeper understanding of how skin microbiome shapes and is being shaped by skin lipids.

Data availability statement

The 16S rRNA sequencing data is publicly available at https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1073917.

Ethics statement

The studies involving humans were approved by Concordia Institutional Review Board (IRB) 7 East Frederick Place Ceadr Knolls, New Jersey 07927 Telephone: (973)734-0734. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ML: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. EK: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. JM: Funding acquisition, Supervision, Writing – review & editing. JN: Data curation, Formal analysis, Methodology, Writing – review & editing. JS: Formal analysis, Methodology, Writing – review & editing. JW: Conceptualization, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This project was funded by Colgate-Palmolive.

Acknowledgments

The authors would like to thank Amnon Amir for valuable discussions for ASV construction analysis using QIIME2 and DADA2. The authors also thank Paulo Portal Gomes for making the Figure 1.

Conflict of interest

ML, JN, JW, and JM are employed by Colgate-Palmolive. The authors declare that this study received funding from Colgate-Palmolive. The funder had the following involvement in the study: Conceptualization, Funding acquisition, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. JS and EK are consultants of Clarity Genomics, Inc. and were compensated by Colgate-Palmolive for their contributions to the data analysis.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1383656/full#supplementary-material

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frontiers | Frontiers in Microbiology

TYPE Systematic Review
PUBLISHED 28 May 2024
DOI 10.3389/fmicb.2024.1319654

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OPEN ACCESS

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RECEIVED 11 October 2023
ACCEPTED 01 May 2024
PUBLISHED 28 May 2024

CITATION
Zhao M, Wen X, Liu R and Xu K (2024) Microbial dysbiosis in systemic lupus erythematosus: a scientometric study. *Front. Microbiol.* 15:1319654. doi: 10.3389/fmicb.2024.1319654

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Microbial dysbiosis in systemic lupus erythematosus: a scientometric study

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Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. Mounting evidence suggests microbiota dysbiosis augment autoimmune response. This study aims to provide a systematic overview of this research field in SLE through a bibliometric analysis.

Methods: We conducted a comprehensive search and retrieval of literature related to microbial researches in SLE from the Web of Science Core Collection (WOSCC) database. The retrieved articles were subjected to bibliometric analysis using VOSviewer and Bibliometricx to explore annual publication output, collaborative patterns, research hotspots, current research status, and emerging trends.

Results: In this study, we conducted a comprehensive analysis of 218 research articles and 118 review articles. The quantity of publications rises annually, notably surging in 2015 and 2018. The United States and China emerged as the leading contributors in microbial research of SLE. Mashhad University of Medical Sciences had the highest publication outputs among the institutions. Frontiers in Immunology published the most papers. Luo XM and Margolles A were the most prolific and highly cited contributors among individual authors. Microbial research in SLE primarily focused on changes in microbial composition, particularly gut microbiota, as well as the mechanisms and practical applications in SLE. Recent trends emphasize "metabolites," "metabolomics," "fatty acids," "T cells," "*lactobacillus*," and "dietary supplementation," indicating a growing emphasis on microbial metabolism and interventions in SLE.

Conclusion: This study provides a thorough analysis of the research landscape concerning microbiota in SLE. The microbial research in SLE mainly focused on three aspects: microbial dysbiosis, mechanism studies and translational studies (microbiota-based therapeutics). It identifies current research trends and focal points, offering valuable guidance for scholars in the field.

KEYWORDS

systemic lupus erythematosus, autoimmune disease, microbiota, bibliometric analysis, VOSviewer

1 Introduction

Systemic lupus erythematosus (SLE) is a chronic, systemic autoimmune disorder characterized by abnormal activation of T and B lymphocytes, production of autoantibodies, complement system activation, and the formation of immune complexes (Zucchi et al., 2022). Its primary clinical characteristics encompass the involvement of multiple systems and organs, along with recurrent relapses and remissions (Kiriakidou and Ching, 2020). Individuals afflicted with SLE present a wide array of symptoms and disease progression. Predominant manifestations include fever, fatigue, malar rash, photosensitivity, myalgia or arthralgia, arthritis, and renal complications (Goldblatt and O'Neill, 2013). Furthermore, SLE patients exhibit an elevated susceptibility to atherosclerosis, thrombosis, arteritis, embolism, and vascular spasm. The gravest consequences of SLE are infections and severe multisystemic damage, particularly affecting the nervous system and kidneys (Gordon et al., 2018). SLE is strikingly dominated by women of childbearing age, with the ratio of women to men being 10:1. The prevalence and severity of SLE exhibit considerable variation across different regions and ethnic backgrounds (Fanouriakis et al., 2021).

The precise etiology of SLE remains incompletely elucidated. The pathogenesis of SLE may be attributed to genetic, hormonal, and environmental factors, including infections, medications, and exposure to UVA light (Barbhaiya and Costenbader, 2016). With the rapid advancement of next-generation sequencing technology, our understanding of microorganisms has deepened. The human body harbors approximately 10^14 microorganism species. The human microbiota includes bacteria, fungi, viruses, archaea, and protozoa, bacteria being the predominant microorganisms, which is mainly composed of four phyla (*Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*) (Tong et al., 2020). Studies of microbiota dysregulation have highlighted dysbiosis as a significant internal environmental factor associated with SLE. Apperloo-Renkema et al. (1994) initially demonstrated that modifications in the composition of intestinal microbiota could induce SLE in animal models. This phenomenon is likely attributed to a compromised defense mechanism of indigenous gut microbes against exogenous bacteria (Apperloo-Renkema et al., 1994). Persistent antigenic stimulation could instigate alterations in the intestinal microecology, resulting in immune system dysregulation. Consequently, the immune system may erroneously target self-tissues through the production of antibodies or sensitized lymphocytes. Amplified inflammatory responses further exacerbate the production of these antibodies, culminating in a spectrum of clinical manifestations and subsequent complications associated with SLE (Zhang and Reichlin, 2008).

In recent years, the role of microbiota in the pathogenesis of SLE has garnered widespread attention, resulting in an exponential increase in related publications. While there have been review and research articles on microorganisms and SLE, a lack of trend analysis in this research area is evident. Bibliometrics is a multidisciplinary science that utilizes mathematical and statistical methods to quantitatively analyze all forms of knowledge, thereby reflecting the knowledge structure and developmental characteristics of a scientific field. Bibliometric analysis is advantageous in identifying and describing subtle differences and evolutions within a scientific domain. It has already been extensively applied in fields such as economics (Peng et al., 2023), management (de Sousa, 2021), information science (Wang et al., 2023), energy and the environment (Ola et al., 2023). Consequently, this paper employs bibliometric methods to systematically analyze the research landscape of microorganisms and SLE in the medical field, to provide historical context and predict the current hot topics and emerging areas within this field.

2 Materials and methods

2.1 Data sources and search strategy

In this study, we utilized the Web of Science Core Collection (WoSCC) database to retrieve relevant literature on microorganisms and SLE published between 1991 and 2022. The WoSCC is a collection of high-quality academic resources available on the Web of Science™ platform, commonly used for literature retrieval, journal selection, research evaluation, and bibliometric analysis. To ensure minimal bias resulting from database updates, literature search and retrieval were conducted on the same day. The search strategy included synonymous terms for microorganisms and SLE, with Boolean logic set as (TS = (microbiome OR microbiota OR microbe OR microorganism OR flora OR microflora OR germ OR bacteri* OR Microbial metabolism OR Dietary Supplements OR probiotics OR Prebiotics OR Synbiotics OR Fecal Microbiota Transplantation) AND TS = (systemic lupus erythematosus) AND LA = (English)). The above-mentioned keywords were selected from the Medical Subject Headings (MeSH) provided by the National Library of Medicine (NLM)/PubMed. The data were exported as plain text files, which included full records and cited references. The article types were set as "Research Article" and "Review Article" to assess the trends and hot topics in research on microorganisms and SLE. Initially, a total of 2038 articles were retrieved through the search. However, after manually excluding irrelevant content related to the research topic, 336 relevant articles remained. The exclusion of irrelevant articles was conducted based on careful examination and consideration of the research focus. Only articles that were directly related to the study of microorganisms and SLE were included in the final set of results. Articles selection process was presented in a PRISMA flowchart (Figure 1).

2.2 Bibliometrics analysis and data visualization

VOSviewer is a software tool that creates visualizations of network data, enabling the exploration and analysis of these visualizations (van and Waltman, 2010). In this study, VOSviewer (v1.6.18.0) was utilized to generate co-occurrence networks, temporal maps, and density maps of keywords based on the research literature on microorganisms and SLE from the WoSCC database. Additionally, national and institutional collaboration networks were visualized. Microsoft Excel 2021 was employed to analyze publication trends, while the bibliometrix (Aria and Cuccurullo, 2017) was used to analyze other information such as authorship, journals, and countries' publication outputs. These

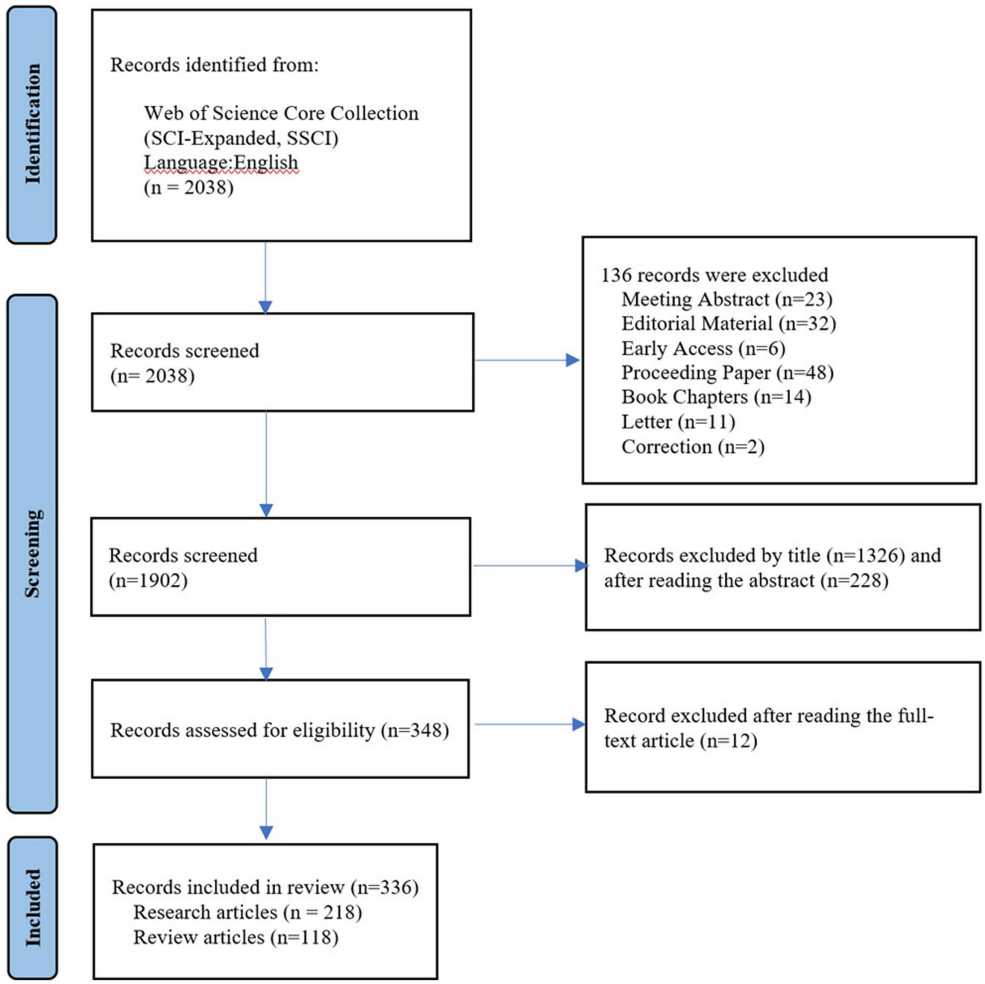

FIGURE 1
The study PRISMA flow diagram.

methods were employed to identify the hot topics and research trends in this field, providing valuable insights and references for future studies.

3 Results

3.1 Quantities and trend of publications output

Publication output is an important indicator for assessing the research hotspot and the progress made in the field. In this study, a total of 336 articles related to the microbial research in SLE were included, consisting of 218 Research articles and 118 Review articles (Figure 2). The annual publication output of microbial research in SLE is depicted in Figure 2. It showed that the earliest Research articles and the first Review article were published in 1991. From 1991 to 2014, the annual production was relatively stable (less than 10 articles), with minor fluctuations. From 2015 to 2021, the publication output significantly increased to 47 articles, though there was a sharp decline in 2018. It suggested that the microbial research in SLE has gained increasing attention.

3.2 Analysis of countries/regions and affiliations

The publications on microbial research in SLE are contributed by 34 countries/regions. Table 1 displayed the top 10 countries in the number of publications. The United States ranked first, accounting for 37.80% (127/336) of the total publications, followed by China with 21.13% (71/336). Italy and Spain also made substantial contributions. The proportion of Multiple Country Publications (MCP) reflects the level of international collaboration and academic exchange. It showed that most countries/regions, conducted academic researches by themselves, but there was also international collaboration to a certain degree. The publication numbers did not correlate to international collaboration in terms of author collaborations (Figure 3A). Notably, more than half of Israeli publications resulted from international collaborations, though its publication number ranked sixth. The cooperation network of the countries/regions illustrated that international collaboration was mainly carried out by countries/regions actively involved in this field (Figure 3B). The collaboration between China and the United States was the most prominent. When calculating the average citations per article (total citations/total number of publications) for each country, the top three countries were Germany

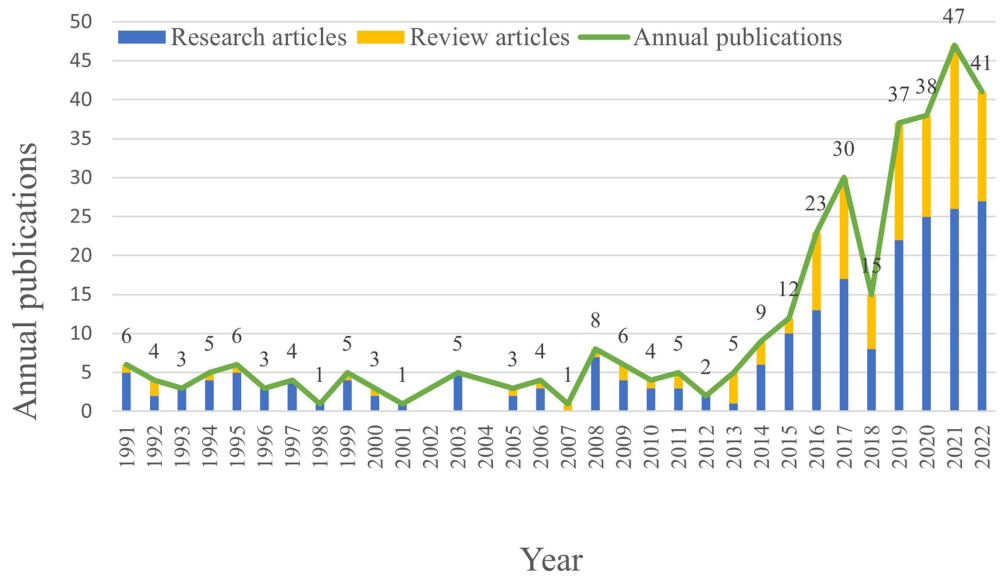

FIGURE 2
Quantities of publications output of the microbial research in SLE from 1991 to 2022.

TABLE 1 The top 10 countries/regions in the microbial research of SLE.

Country	Number of publications	Total citations	Average article citations
USA	127	4,927	46.00
China	71	1,379	21.20
Italy	26	487	24.40
Spain	21	929	51.60
Japan	17	129	9.20
Israel	16	470	36.20
Brazil	15	354	35.40
Germany	13	344	57.30
Netherlands	12	355	39.40
France	12	38	9.50
England	10	175	29.20

Average article citations = total citations/total number of publications.

(57.30 citations), Spain (51.60 citations), and the United States (46.00 citations) with more than 40 citations, while the average citations of France and Japan were less 10. Among the top 10 institutions (Figure 4), Mashhad University of Medical Sciences, Osaka University, Medical University of South Carolina, Anhui Medical University, and University of Granada had made notable contributions for more than 20 articles of each institution (Figure 4A). The collaborations among affiliations were primarily based on countries (Figure 4B). Tel Aviv University engaged in extensive and productive collaboration with various institutions, despite having fewer publications compared to Mashhad University of Medical Sciences, whose publication outputs were the most. Three Chinese institutions, Anhui Medical University, Central South University, and China Medical University, listed in the top 10 institutions in terms of publication outputs. Moreover, Central South University (15 publications) had extensive collaboration with the Medical University of South Carolina (22 publications) from the United States.

3.3 Analysis of authors and journals

Since 1991, a total of 1,675 scholars dedicated to research on this topic. Table 2 and Figure 5 presented the top 10 authors with the highest publication number and their citation counts. Luo XM, Margolles A and Shoenfeld Y were the top three authors in terms of publication outputs and had the greatest academic impact, with total citation counts of 990, 685, and 766, respectively. Pisetsky DS achieved remarkable achievements early in this field, publishing a total of 7 papers between 1991 and 1997. Shoenfeld Y published one article during this period. In the past decade, an increasing number of authors have focused on the potential role of microbiota in SLE. Among them, Luo XM had the most publication outputs and citation counts. His team primarily investigates the dysbiosis of gut microbiota and potential mechanisms in SLE, as well as the therapeutic effects of bacteria.

Academic journals serve as crucial platforms for showcasing scientific research accomplishments. We conducted an analysis using 336 papers published in 171 different journals. Table 3 presented the top 10 journals in terms of publication counts, which published a total of 114 papers, accounting for 33.93% (114/336) of the total publications. Most of these journals focused on the field of immunology. Frontiers in immunology and Lupus had the highest publication counts, 42 and 19 papers, respectively. Frontiers in Immunology (Impact Factor = 8.786) was the most cited journal. Although Annals of Rheumatic Diseases ranked eighth, it had the highest Impact factor (2021) of 28.003.

3.4 High-cited articles

We conducted a citation analysis on the 336 articles. In bibliometrics, Global Citations (GCS) refers to the total number of citations that an article has received in the WoSCC database, while Local Citations (LCS) refers to the number of citations

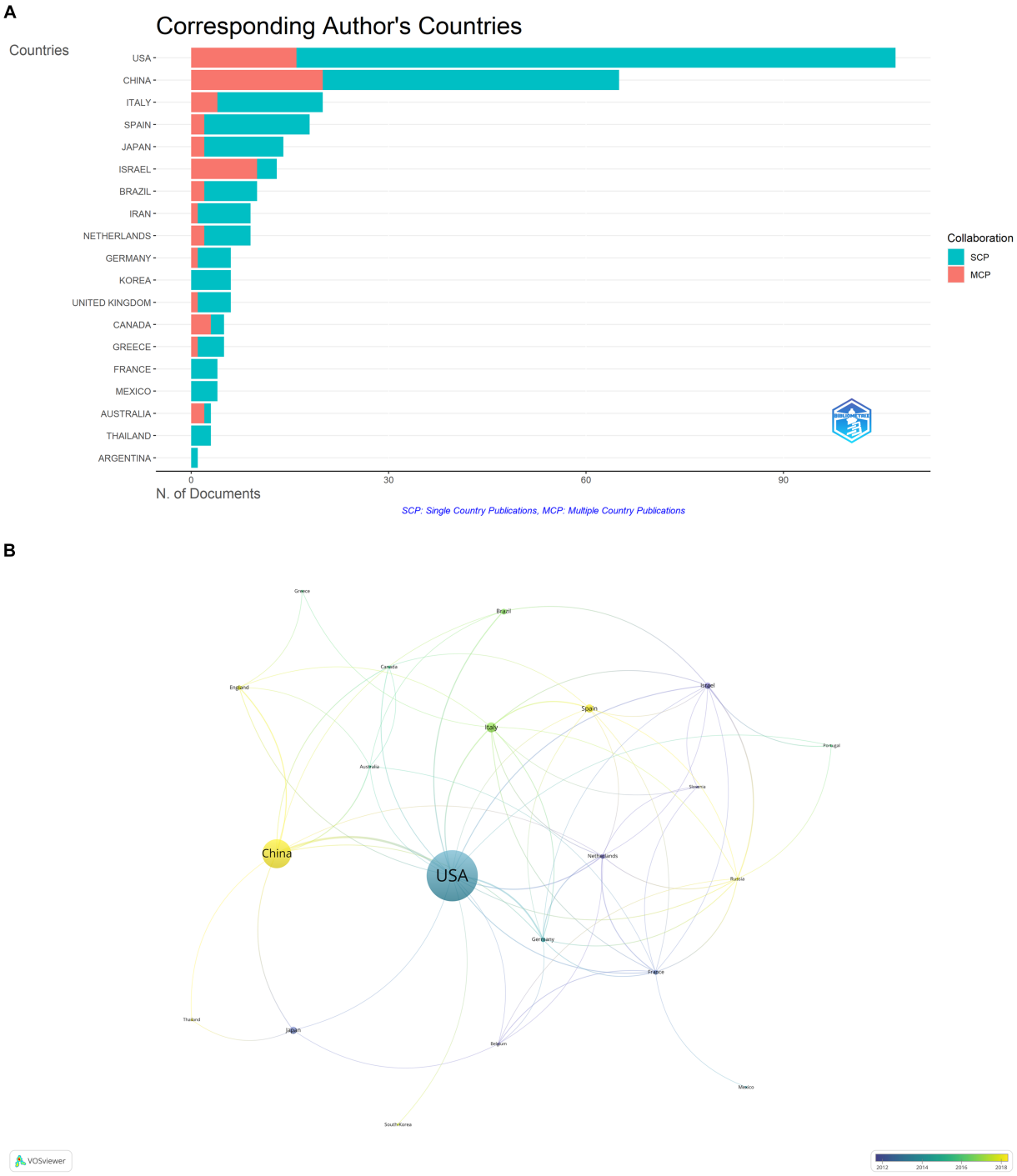

FIGURE 3
Analysis of publications on microbial research in SLE by the countries/regions. **(A)** Visualization of papers according to the country of the corresponding authors. MCP, Multiple Country Publications; SCP, Single Country Publications. **(B)** Visualization of papers according to the cooperation networks of the corresponding authors.

within our dataset, reflecting the impact in the field of microbial research in SLE. Table 4 depicted the top 10 highly cited articles within our dataset. The original research articles "10.1128/mBio.01548-14" and "10.1128/AEM.02676-14" received high global citations and local citations, indicating their significant influence in the field.

3.5 Keywords co-occurrence network

Keywords are highly concise summaries of the content of a literature. Thus, keyword co-occurrence network analysis performed using VOSviewer software can indicate the research scope and hotspots in the field, aiding to the discovery of research trends. To

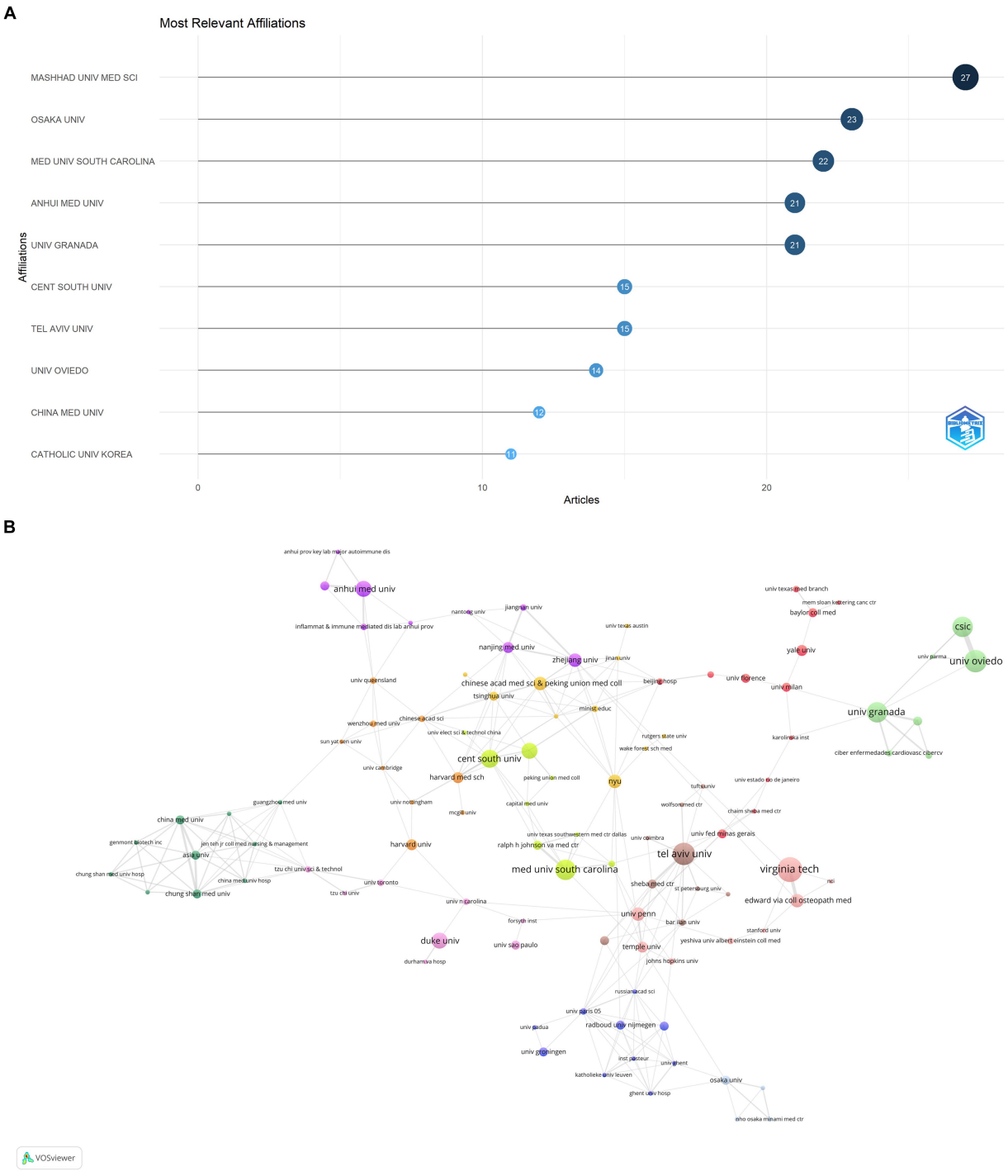

FIGURE 4
Analysis of publications on microbial research in SLE by the affiliations. **(A)** Visualization of papers according to the affiliations. **(B)** Visualization of papers according to the cooperation networks of the affiliations.

ensure readability and visual appeal of the graphs, a minimum occurrence threshold of 5 was set for the keywords in these papers. Then 101 keywords were produced and divided into six clusters represented by different colors (Figure 6). These six clusters exhibited overlapping and intersecting characteristics, indicating that researches in this field were not dispersed or isolated.

As shown in Figure 6, the purple cluster was the largest, which meant the research core in this field, encompassing keywords such as "bacterial DNA", "virus", "autoantibodies", "dendritic cells", and "autoimmunity". The second largest cluster, blue cluster contained 15 nodes, including "gut microbiota", "lupus mice", "inflammation", "diet", "fatty acids", "segmented filamentous bacteria" and "T cells". These two

TABLE 2 The top 10 authors in the microbial research of SLE.

Element	Articles	h-index	Total citations	Year of Publication_start
Luo XM (Zhang et al., 2014; Mu et al., 2015, 2017a,b, 2019, 2020; Edwards et al., 2017; Abdelhamid and Luo, 2018; Luo et al., 2018; Abdelhamid et al., 2020; Cabana-Puig et al., 2022; Christovich and Luo, 2022)	12	10	990	2014
Margolles A (Hevia et al., 2014a, 2015; Cuervo et al., 2015; Rojo et al., 2015; Sánchez et al., 2015; López et al., 2016a,b; González et al., 2017; Rodríguez-Carrio et al., 2017a; Ruiz et al., 2018)	10	8	685	2014
Shoenfeld Y (Baharav et al., 1994; Amital et al., 2008; Doria et al., 2008; Pordeus et al., 2008; Bogdanos et al., 2013; Chen et al., 2017; Ceccarelli et al., 2018; De Luca and Shoenfeld, 2019; Nogueira and Shoenfeld, 2019; Shoenfeld et al., 2019)	10	8	766	1994
Suarez A (Hevia et al., 2014a,b; Cuervo et al., 2015; Rojo et al., 2015; López et al., 2016a,b; González et al., 2017; Rodríguez-Carrio et al., 2017a; Ruiz et al., 2018)	9	8	762	2014
Sanchez B (Cuervo et al., 2015; Hevia et al., 2015; Rojo et al., 2015; Sánchez et al., 2015; López et al., 2016a,b; González et al., 2017; Rodríguez-Carrio et al., 2017a; Ruiz et al., 2018)	9	8	711	2014
Lopez P (Hevia et al., 2014a,b; Cuervo et al., 2015; Rojo et al., 2015; López et al., 2016a,b; González et al., 2017; Rodríguez-Carrio et al., 2017a; Ruiz et al., 2018)	9	8	468	2014
Mu QH (Mu et al., 2015, 2017a,b, 2019, 2020; Edwards et al., 2017; Gutiérrez-Díaz et al., 2018; Luo et al., 2018)	8	8	790	2015
Pisetsky DS (Gilkeson et al., 1991; Robertson et al., 1992; Robertson and Pisetsky, 1992; Bunyard and Pisetsky, 1994; Gilkeson et al., 1995; Wu et al., 1997; Hamilton et al., 2006; Mu et al., 2017c)	8	7	599	1991
Gonzalez S (Pisetsky, 1997; Hevia et al., 2014a; Cuervo et al., 2015; Rojo et al., 2015; Sánchez et al., 2015; González et al., 2017; Rodríguez-Carrio et al., 2017a,b; de la Visitación et al., 2019, 2020, 2021a,b)	7	6	197	2014
De La VISITACION N (Rodríguez-Carrio et al., 2017b; de la Visitación et al., 2019, 2020, 2021a,b,c)	6	6	662	2019

WOS h-index: The articles of authors and journals published are sorted in descending order based on the number of citations. Each of these articles has been cited at least H times, while the citation counts of other articles do not exceed H. This delineates the h-index of the authors and the journals.

clusters primarily explored the relationship between microbiota and SLE.

The green cluster reflected researchers' exploration of the pathogenetic effect of microbial dysbiosis in other autoimmune diseases like "rheumatoid arthritis," "inflammatory bowel disease," "antiphospholipid syndrome" and "ankylosing spondylitis."

The yellow, orange, and pink clusters displayed researches on gut microbiota in SLE disease progression. They mainly focused on two aspects: firstly, the regulation of gut microbiota, including terms "dysbiosis," "*lactobacillus*," "dietary supplements," "fecal microbiota transplantation" and "metabolites"; secondly, immunological pathogenesis involving terms such as "leaky gut," "toll-like receptors," "regulatory T cells," "dendritic cells," "B cells" and "innate immunity."

The red cluster mainly encompassed keywords related to oral microbial alterations and relevant experimental researches in SLE. The key terms were "oral microbiota," "periodontitis" and "periodontal disease". Additionally, "classification system," "disease activity" and "metabolomics" were also frequent keywords in this cluster, indicating that microbiota may induce oral disease of SLE patients through metabolites.

3.6 Hotspots and topic migration

The density visualization of the co-occurrence network of key terms is depicted in Figure 7A. The research content of these articles revolves around "SLE," "autoimmunity" and "gut microbiota," highlighting the pivotal role of immune and inflammatory responses in SLE. T cell-mediated cellular immunity stands out as a prominent area of research. Mechanistically, particular attention is directed toward intestinal permeability, the production of short-chain fatty acids by gut microbiota, and the impact of gut microbiota on T cells.

In the timeline graph of keyword co-occurrence (Figure 7B), each node is color-coded based on the average temporal multiplier of the corresponding keyword. It can be observed that high-frequency keywords exhibit an average onset time post-2015, indicating the emergence of a novel field. As demonstrated in Figure 7B, the recent research emphasis predominantly centers on the mechanistic exploration of gut microbiota involvement in SLE and the investigation of applications such as fecal microbiota transplantation.

We conducted a "trend topic" analysis employing bibliometric software packages for comprehending the historical dynamics of research hotspots in this domain. As depicted in Figure 7C, during the initial phase, the scholarly output in this field was significantly limited. Preceding 2013, there existed inadequate continuity among high-frequency keywords. In 2014, 2015, and 2016, prominent keywords encompassed "B cells," "dendritic cells," "infection," "viruses" and "regulatory T cells." This suggests that the pathogenetic research of microorganisms primarily focused on the functions of immune cells in SLE. In 2017 and 2018, high-frequency keywords included "rheumatoid arthritis" and "inflammatory bowel disease" which are also autoimmune diseases related to immune function and

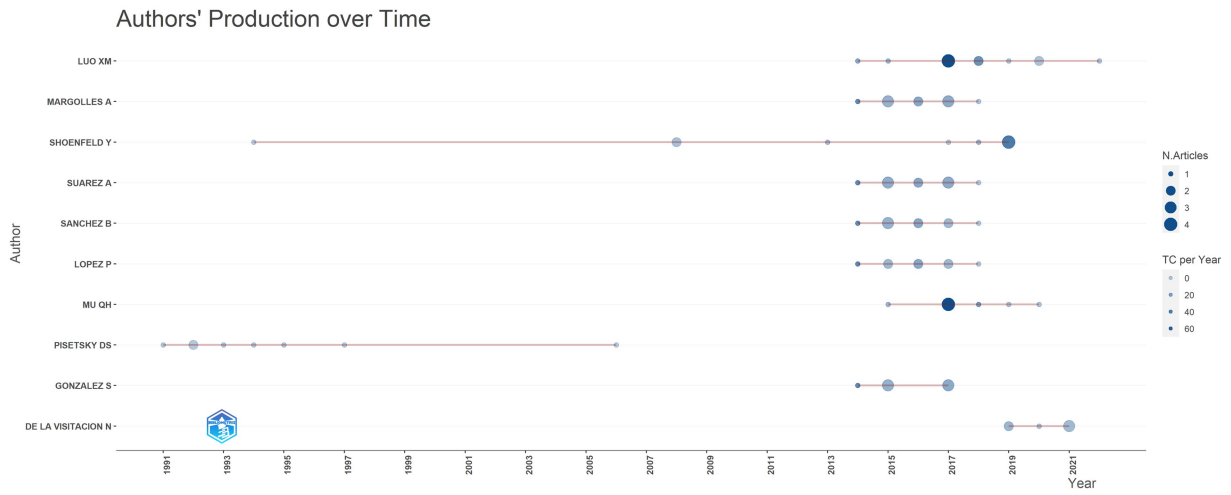

FIGURE 5
Analysis of publications on microbial research in SLE by the authors.

TABLE 3 The top 10 journals in the microbial research of SLE.

	Journal	Articles	h-index	Impact factor-2021	Total citations	Start year
1	Frontiers in Immunology	42	20	8.786	1,162	2015
2	Lupus	19	11	2.858	384	1994
3	Journal of Autoimmunity	10	7	14.551	389	2008
4	Autoimmunity Reviews	8	6	17.39	195	2006
5	International Journal of Molecular Sciences	7	6	6.208	384	1991
6	Clinical and Experimental Immunology	6	6	5.732	180	2014
7	Current Opinion in Rheumatology	6	12	5.006	358	2008
8	Frontiers in Microbiology	6	12	6.064	381	2016
9	Annals of Rheumatic Diseases	5	12	28.003	108	2019
10	Clinical Immunology	5	12	10.19	118	2019

WOS h-index: The articles of authors and journals published are sorted in descending order based on the number of citations. Each of these articles has been cited at least H times, while the citation counts of other articles do not exceed H. This delineates the h-index of the authors and the journals.

microorganisms. These diseases share common mechanisms and pathways, providing new insights for the microbial study in SLE. Since 2019, frequent keywords such as "gut microbiota," "mechanisms," "T cells," "dysbiosis" and "metabolites" have appeared, indicating that the recent research mainly focused on the mechanisms and applications of gut microbiota in SLE, which is consistent with the results shown in Figure 7B.

4 Discussion

4.1 Overview of development in the field of microbiota in SLE

In this study, we retrieved 218 research articles and 118 review articles about the microbial study in SLE from 1991 to 2022 in the WOSCC database. The annual publications were steady from 1991 to 2014. From 2015 significant attention was paid to this field. In addition to the development of microbial sequencing, such as 16S rRNA gene sequencing and metagenomic sequencing, this may be attributed to increasing interest in bacteria-induced immune response in autoimmune disease. The greatest contributors were the United States and China. Two institutions from the two countries, the Medical University of South Carolina and Central South University had intensive collaboration. Luo XM, Margolles A, and Shoenfeld Y had the greatest impact in this field. Frontiers In Immunology had the most publication numbers. Notably, the impact factors of the top 10 journals were higher than 5, indicating the high quality of articles. "10.1128/mBio.01548-14" and "10.1128/AEM.02676-14" had the highest LCS. The highly cited articles used sequencing technology to reveal microbial dysbiosis mainly in the gut as well as the pathogenesis of SLE.

TABLE 4 The top 10 high-cited articles in the microbial research of SLE.

No.	Document	DOI	Title	Component	Year	LCS	GCS
1	HEVIA A, 2014, MBIO	10.1128/mBio.01548-14	Intestinal Dysbiosis Associated with Systemic Lupus Erythematosus	Research article	2014	120	350
2	ZHANG HS, 2014, APPL ENVIRON MICROB	10.1128/AEM.02676-14	Dynamics of Gut Microbiota in Autoimmune Lupus	Research article	2014	81	173
3	LUO XM, 2018, APPL ENVIRON MICROB	10.1128/AEM.02288-17	Gut Microbiota in Human Systemic Lupus Erythematosus and a Mouse Model of Lupus	Research article	2018	65	149
4	MU QH, 2017, MICROBIOME	10.1186/s40168-017-0300-8	Control of lupus nephritis by changes of gut microbiota	Research article	2017	61	160
5	HE ZX, 2016, GUT PATHOG	10.1186/s13099-016-0146-9	Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus	Research article	2016	59	136
6	AZZOUZ D, 2019, ANN RHEUM DIS	10.1136/annrheumdis-2018-214856	Lupus nephritis is linked to disease-activity associated expansions and immunity to a gut commensal	Research article	2019	53	164
7	ZEGARRA-RUIZ DF, 2019, CELL HOST MICROBE	10.1016/j.chom.2018.11.009	A Diet-Sensitive Commensal *Lactobacillus* Strain Mediates TLR7-Dependent Systemic Autoimmunity	Research article	2019	45	141
8	LOPEZ P, 2016, SCI REP-UK	10.1038/srep24072	Th17 responses and natural IgM antibodies are related to gut microbiota composition in systemic lupus erythematosus patients	Research article	2016	44	129
9	JOHNSON BM, 2015, CLIN EXP IMMUNOL	10.1111/cei.12609	Impact of dietary deviation on disease progression and gut microbiome composition in lupus-prone SNF1 mice	Research article	2015	43	92
10	MU QH, 2017, SCI REP-UK	10.1038/s41598-017-14223-0	Antibiotics ameliorate lupus-like symptoms in mice	Research article	2017	35	67

GCS represents the total citations of an article in WOSCC, while LCS represents the number of citations in our collections.

4.2 Structure, current status, and direction of microbial research in SLE

In about 20 years, the research on microbiota in SLE mainly focused on three aspects. The first was searching for evidence of microbial dysbiosis to SLE. The second was mechanism studies, involving "molecular simulation," "Toll-like receptors," "T cells" and "fatty acids." The third was translational studies using probiotic supplements, dietary interventions and targeted microbiota-based therapeutics.

4.2.1 Microbiota dysbiosis in SLE

The activity and composition of the microbiota are influenced by genetic background, age, diet, and the overall health status of the host. In turn, the composition and activity of the microbiota also affect the host metabolism and the development of diseases (Manos, 2022). The composition and the dynamic patterns of microbiome dysbiosis can serve as early indicators of SLE onset (Rahbar Saadat et al., 2019). The researches mainly focused on gut microbiota. Additionally, the dysbiosis in oral, skin and plasma also received increasing interest.

4.2.1.1 Gut microbiota

In "10.1128/mBio.01548-14," 16S sequencing and metagenomic sequencing were performed on fecal samples from SLE patients and healthy individuals, revealing a significant decrease in the Firmicutes/Bacteroidetes ratio and overexpression of polysaccharide degradation pathways in the microbiota of SLE patients. "10.1128/AEM.02676-14" analyzed differences in gut microbiota in a lupus-prone mouse model and found a significant decrease in *Lactobacillaceae* and significant enrichment of *Clostridiaceae* family XIII and *Streptococcaceae* in lupus-prone mice, with gender differences observed in the gut microbiota changes.

The gut microbiota plays a role in the development and regulation of the immune system, potentially influencing autoimmunity by modulating the balance between tolerance and inflammatory microorganisms (Dominguez-Bello et al., 2010; Lee and Mazmanian, 2010). Currently, various mouse models have been applied to understand gut microbiota dysbiosis in SLE. Apperloo-Renkema et al. (1995) were the first to find that the antimicrobial colonization quality of the gut microbiota reduced in active SLE patients, which may lead to increased translocation of foreign bacteria, then facilitate the production of anti-double-stranded DNA (anti-dsDNA) autoantibodies. Compared to healthy individuals, non-active SLE patients have a lower abundance of the *phylum Firmicutes*, as well as a lower *Firmicutes/Bacteroidetes* (F/B) ratio (Hevia et al., 2014a). Additionally, SLE patients show a decrease in *Ruminococcaceae* and *Lachnospiraceae*, and a significant increase in *Prevotellaceae, Bacteroidaceae*, and intestinal *Streptococcus* (Hevia et al., 2014a). Furthermore, *Streptococcus pneumoniae* and *Streptococcus intermedius* (both belonging to the normal microbiota in the oral cavity and gastrointestinal tract) are increased in SLE patients, indicating a potential association between the microbiota-mediated oral-gut axis

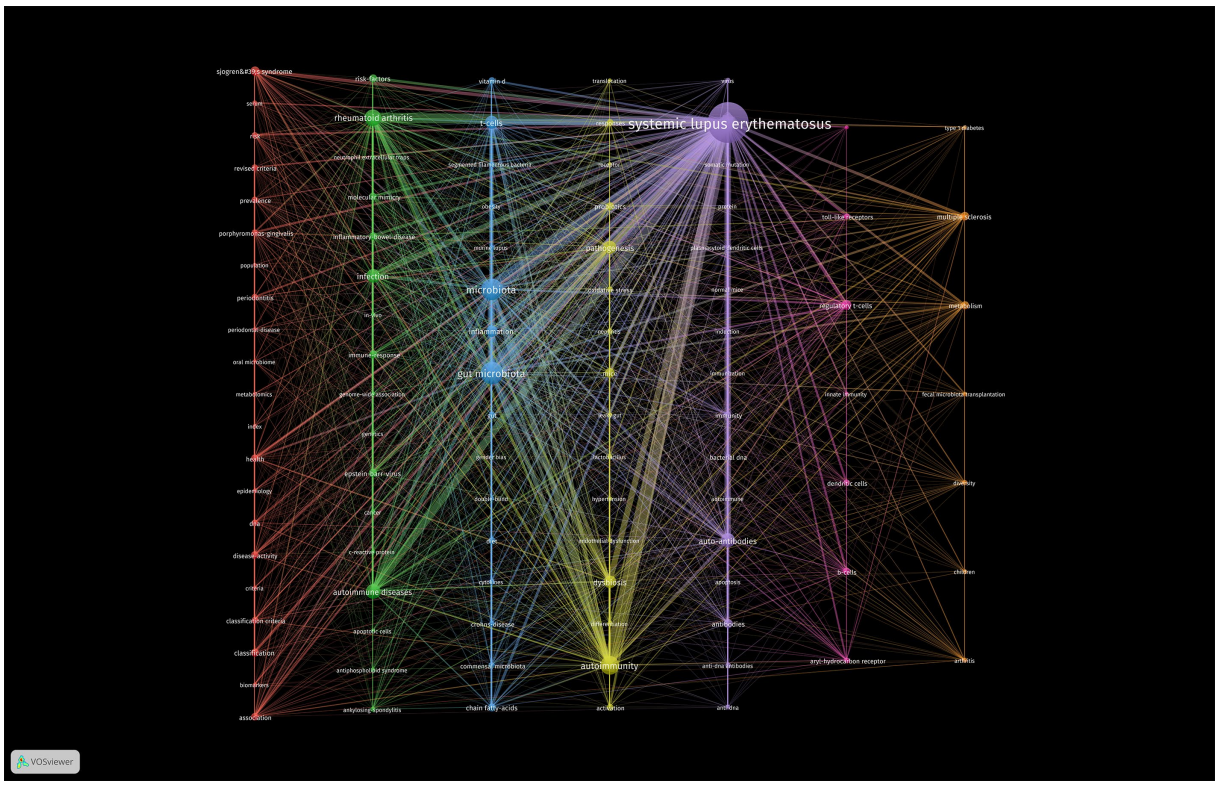

FIGURE 6
Cluster visualization of the co-occurrence network according to the authors' keywords. The minimum number of occurrences of the author's keywords was set to 5. Finally, 101 keywords were included in the co-occurrence network. These keywords were divided into seven different clusters.

and the pathological changes in SLE (Tomofuji et al., 2021). Zhang et al. (2014) discovered significant reductions in *Lactobacilli* and increased abundance of *Lachnospiraceae* in the gut microbiota of MRL/lpr mice. Furthermore, the abundance of *Lachnospiraceae* was closely associated with the disease progression, while the colonization of *Lactobacilli* in the gut showed a negative correlation with lupus activity. These findings suggest that the gut microbiota is involved to some extent in the development of SLE.

4.2.1.2 Oral microbiota

Keywords cluster showed oral microbiota was a hotspot in SLE. When the disease affects the oral mucosa, patients often experience symptoms such as dry mouth, insufficient saliva production, oral ulcers, and periodontal disease. The oral microbiome forms a highly interconnected microbial community network and can distribute throughout the human body (Avila et al., 2009). Guo et al. (2023) discovered that an increased abundance of *Prevotella* and *Alloprevotella* of the *Prevotellaceae* family, *Leptotrichia* of the *Fusobacteriaceae* family, *Veillonella* and *Megasphaera* of the *Veillonellaceae* family in the oral microbiome of SLE patients. Additionally, the abundance of 15 bacteria, including members of the *Micrococcaceae, Bacillaceae,* and *Abiotrophia,* were decreased. Furthermore, with increasing disease activity in SLE, the abundance of *Abiotrophia* and *Lactobacillus* increases, while the abundance of *Phyllobacterium* and Micrococcaceae decreases. Even the dysregulation of subgingival microbiota in SLE patients was linked to periodontal conditions and can result in a 1.76-fold higher risk of developing periodontitis (Rutter-Locher et al., 2017). SLE patients with concurrent periodontitis exhibit increased abundance of *Prevotella nigrescens*, *Prevotella oulorum, Prevotella oris, Selenomonas noxia, Lachnospiraceae,* and *Leptotrichia* in both inflamed and healthy sites (Corrêa et al., 2017). The presence of pathogenic bacteria is positively correlated with the systemic inflammatory levels in SLE patients, and patients with concurrent periodontitis show elevated IL-6, IL-17, and IL-33 (Marques et al., 2016). It is worth noting that SLE patients exhibit an enrichment of certain microorganisms in the intestinal tract, including *A. massiliensis, S. satelles,* and *A. rimae*, which are closely associated with oral inflammation. This suggests that the microbiota enriched in the intestinal tract of SLE patients may originate from the oral cavity (Chen et al., 2021). Thus alterations in the oral microbiota may lead to the dysbiosis of gut bacteria and cause inflammation in SLE.

4.2.1.3 Skin microbiota

Around 80% of SLE patients experienced cutaneous manifestations and up to 25% of patients presented with skin involvement as the initial symptom (Yell et al., 1996). SLE patients exhibit dysbiosis of the skin microbiota, characterized by an increased proportion of *Staphylococcus* and *Corynebacterium* in the lesional skin and a decreased abundance of *Cutibacterium acnes* (Zhou et al., 2021). Compared to healthy skin, SLE patients show significant reductions in the abundance of *Prevotella, Rothia* and *Klebsiella* in the lesional skin. Furthermore, there is a significant decrease in the abundance of *Acidobacteria*, *Gemellaceae* and *Corynebacterium* in both the affected and unaffected skin of SLE patients (Huang et al., 2020). In addition, the microbial community diversity of SLE lesions is associated with clinical features of the patients, including gender, renal involvement,

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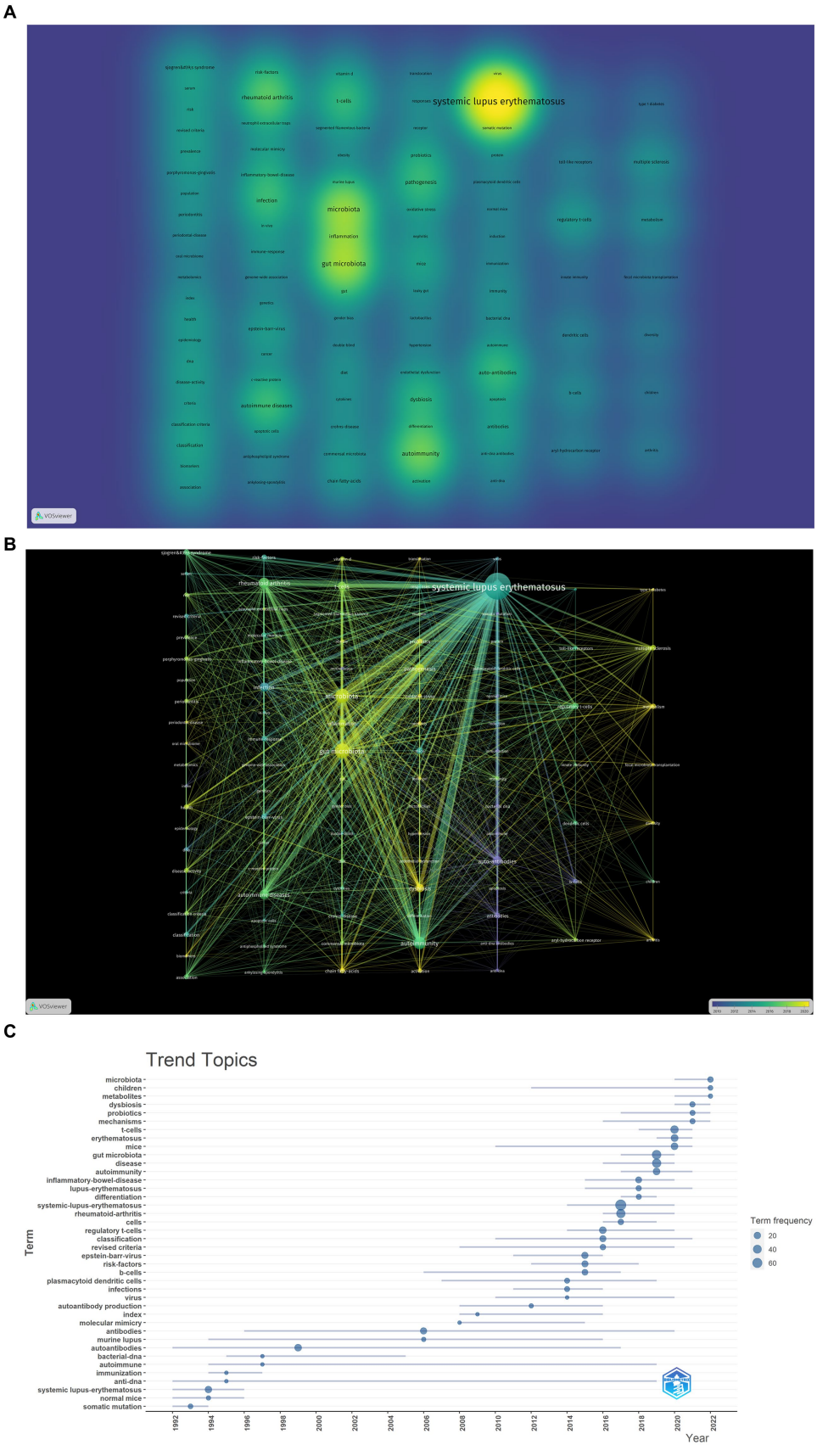

FIGURE 7
Analysis of hotspot and topic migration on microbial research in SLE. **(A)** The density visualization of keywords co-occurrence network (the figure represented the numbers of keyword occurrences). **(B)** The timestamp visualization of keywords co-occurrence network. The co-occurrence network was based on time characteristics, and each node was color-coded with a different color based on the average time multiple of the keywords. **(C)** The historical migration of research hotspots.

low serum complement levels, and myositis. Disruption of the skin microbiome may have the potential for SLE diagnosis, with a combination of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus hominis* showing high accuracy and serving as microbial biomarkers for SLE diagnosis (Huang et al., 2020).

4.2.1.4 Plasma microbiota

The dysfunction of the skin, mucosa or gut barrier will increase permeability and allow microbe and/or their products to translocate into the system, causing excessive and persistent immune stimulation. Lipopolysaccharide (LPS) is a representative marker of microbial translocation (Marchetti et al., 2013). SLE patients and their first-degree relatives exhibit elevated plasma levels of LPS, which is positively correlated with anti-dsDNA antibodies, indicating a higher degree of microbial translocation in both patients and relatives (Ogunrinde et al., 2019). Compared to healthy controls, both SLE patients and their first-degree relatives show a decrease in the abundance of *Paenibacillus* in the blood. Furthermore, the enrichment of *Desulfoconvexum, Desulfofrigus, Desulfovibrio, Draconibacterium, Planococcus*, and *Psychrilyobacter* in SLE patients is directly associated with increased plasma autoantibodies levels (Schett et al., 2002; Reveille, 2004). *Planococcus* is increased in the plasma of SLE patients and can stimulate peripheral blood mononuclear cells (PBMCs) to produce pro-inflammatory cytokines (Luo et al., 2020).

4.2.2 Potential mechanisms of microbiota in SLE

The relationship between microbial dysbiosis and SLE remains unclear. Although genetic susceptibility is an important factor contributing to dysbiosis of the microbiota in lupus-prone mice and the progression of autoimmune diseases, environmental factors appear to be more crucial than host genetics (Ulff-Møller et al., 2018). Microbial dysbiosis can lead to the development of SLE through various mechanisms.

4.2.2.1 Microorganisms interact with the host immune system

The microbiota plays a vital role in shaping and maturing the host's immune system, while the immune system reciprocally maintains crucial aspects of host-microbe symbiosis. The maturation and stability of microbiota, especially the gut microbiota, occur in parallel with the development of the immune system (Arpaia et al., 2013). Even after development ceases, the microbiota continues to interact with the host immune system. The host maintains a host-microbe symbiotic equilibrium by limiting tissue inflammation and microbial translocation by reducing microbial contact with epithelial cell surfaces. The intestinal mucosal epithelial barrier serves as a defense against the invasion of foreign toxins. Disruption of the gut microbiota community homeostasis can result in alterations in paracellular transport, cell apoptosis, or increased intercellular permeability, ultimately leading to "intestinal permeability" or "leaky gut" (Christovich and Luo, 2022). Additionally, certain bacteria and their metabolic byproducts can induce T cell differentiation and proliferation of T helper (Th17) cells. The RORγt+ T regulatory cells (Treg) cell subset is stimulated by short-chain fatty acids (SCFAs) derived from microbiota, specifically *Clostridia* (Arpaia et al., 2013). The colonization of *segmented filamentous bacteria* (SFB) can promote TH17 cell expansion (Ivanov et al., 2009). RORγt+ Treg cells and Th17 cells are crucial for controlling immune responses to gut microbiota, maintaining the gut barrier, reducing inflammation and lessening certain immune reactions mediated by Th2 cells (Yang et al., 2016; Pandiyan et al., 2019). In SLE patients, fecal samples demonstrate a significant increase in calprotectin (a biomarker of impaired intestinal barrier function), while serum levels of soluble CD14, α1-acid glycoprotein, and LPS are elevated, indicating compromised intestinal barrier function and gut bacterial translocation (Azzouz et al., 2019). Microbial dysbiosis can result in an imbalance of Treg/Th17 responses in patients with SLE, instigating immune reactions and facilitating the production of autoantibodies (Rother and van der, 2015; Pandiyan et al., 2019). Moreover, diminished levels of Treg cells can give rise to the generation of bacterial antigens, eliciting Th17-Th1 effector responses against beneficial bacteria, and promoting the development of inflammatory symbiotic reactions and autoimmune responses (Ivanov et al., 2008).

4.2.2.2 Epitope spreading and molecular mimicry

Autoantibodies are expanded induced by bacteria through epitope spreading and molecular mimicry. Anti-Ro60 antibodies are the most common and earliest preclinical antinuclear antibodies in SLE. Commensal Ro60 homologs can trigger autoimmune responses through cross-reactivity in the human body (Greiling et al., 2018). Immunization with Ro60 protein can also lead to the production of anti-Ro52, anti-La, anti-Sm, and anti-U1-RNP antibodies through intermolecular epitope spreading in mice (Deshmukh et al., 1999). Additionally, *Escherichia coli* can induce the production of RNA and dsDNA antibodies and promote the immune response against β2-glycoprotein I (Manfredo Vieira et al., 2018; Salem et al., 2019). *Fretibacterium, Lachnospiraceae, Prevotella,* and *Selenomonas* can also activate the autoimmune response through cross-reactivity with self-antibodies and contribute to the development of SLE (Corrêa et al., 2017; Mu et al., 2017a). Zhang and Reichlin (2008) discovered that antigens from *Burkholderia* can bind to dsDNA antibodies in the serum of SLE patients. Some peptides from *Odoribacter splanchnicus* and *Akkermansia muciniphila* share high similarity with Sm antigen and Fas antigen epitopes (Chen et al., 2021). The amino acid residues 35–58 of Epstein–Barr virus nuclear antigen-1 (EBVNA-1) have similar cross-reactive epitopes with Sm and Ro, leading to SLE-like diseases (Lossius et al., 2012).

4.2.2.3 Microbial biofilms and metabolites

Bacterial biofilms primarily composed of starch-binding amyloid proteins, accumulation of amyloid protein/DNA complexes can trigger intracellular DNA sensors, TLR9, stimulating immune cascade reactions that lead to the transcription of type I IFN and the production of anti-nuclear antibodies (di Domizio et al., 2012). Curli fibers in *S. typhimurium* can also bind to bacterial DNA, activate dendritic cells to secrete pathogenic type I IFN, stimulate the proliferation of activated T cells, B cells, and inflammatory monocytes (Gallo et al., 2015).

Microbial metabolites are involved in a wide range of physiological processes in the human body, can act as signaling molecules and substrates for metabolic reactions, modulating host immune function (Nicholson et al., 2012; Wang et al., 2019). Substances such as SCFAs- butyrate (Kaisar et al., 2017; Gonçalves et al., 2018), tryptophan (Mohammadi et al., 2018; Choi et al., 2020), polyamines (Sánchez-Jiménez et al., 2019) and lactic acid

(Mu et al., 2017a; Lee et al., 2018) can enhance or regulate host immune tolerance through diverse pathways, such as reducing pro-inflammatory cytokines, maintaining immune tolerance, preserving mucosal barrier integrity, and modulating intestinal immune cells. The reduction of *Firmicutes* and augmentation of *Bacteroidetes* in SLE patients result in decreased SCFAs production, thereby exacerbating inflammatory responses (Katz-Agranov and Zandman-Goddard, 2021). Resistant starch can promote the digestion of fiber by the gut microbiota into SCFAs, which has been shown to reduce the abundance of *Lactobacillus reuteri*, alleviate lupus-like symptoms, downregulate the type I interferon (IFN) pathway, and decrease lupus-related mortality (Zegarra-Ruiz et al., 2019). Elevated trimethylamine N-oxide (TMAO), derived from gut microbiota choline metabolism, may serve as a risk factor for concurrent atherosclerosis in SLE mice (González-Correa et al., 2021). Therefore, it is evident that the homeostasis of human microbiota and its metabolic products are crucial for normal physiological activities of the body.

4.2.3 Application potential of microbiota in SLE

Research on gut microbiota intervention therapy for SLE is still in its early stages, yet lessons can be gleaned from other dysbiosis-related disorders, enabling the prediction of future therapeutic approaches for SLE.

4.2.3.1 Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) involves the transplantation of gut microbiota from a healthy donor to a patient's gastrointestinal tract, either through the upper or lower digestive pathways, to restore microbial balance and improve various gastrointestinal dysfunctions. FMT has been proven to be an effective, safe, and cost-efficient method for treating recurrent Clostridioides difficile infection, with a clinical success rate exceeding 90% (Lee et al., 2016). Experimental evidence suggests that transplantation of gut microbiota from SLE-prone mice to germ-free C57BL/6 mice results in changes in the distribution of immune cells in the recipients and upregulation of lupus-susceptible genes (Ma et al., 2019). The decreased abundance of gut microbial genera, such as Ruminococcus and Alistipes, in the gut microbiota of prednisone-treated MRL/lpr mice indicates that FMT may mediate the effects of hormones such as prednisone (Wang et al., 2021). In SLE patients, FMT therapy leads to a reduction in pro-inflammatory microbial groups (*Porphyromonas, Pseudomonas genera and Alphaproteobacteria class, Prevotella and Veillonella genera,* and *the Burkholderiales order*) and an increase in SCFA-producing bacterial taxa (*phylum Firmicutes, including Eubacterium hallii group, Dorea, Marvinbryantia,* and *Papillibacter*) (Huang et al., 2022). This suggests that FMT contributes to the alleviation and improvement of systemic inflammation and clinical symptoms in SLE patients.

4.2.3.2 Probiotics, prebiotics, and synbiotics

Probiotics, prebiotics, and synbiotics can maintain microbial balance by influencing immune homeostasis, promoting the production of various nutrients, degrading toxic compounds, and generating antimicrobial compounds (Li et al., 2020). In lupus patients, probiotics and prebiotics can induce the differentiation of Treg cells, improve the imbalance of Th17/Th1, and reduce the production of autoantibodies, thereby alleviating the severity of the disease (Zhang et al., 2021). A mixture composed of five strains of lactobacilli (*Lactobacillus oris, Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus johnsonii,* and *Lactobacillus gasseri*) can repair intestinal permeability, decrease the production of intestinal IL-6, and increase the generation of IL-10, promoting the formation of an anti-inflammatory environment (Mu et al., 2017a). Additionally, the probiotic *Lactobacillus fermentum* CECT5716 (LC40) (Toral et al., 2019) and/or the *bifidobacterium Bifidobacterium breve* CECT7263 can prevent TLR9-induced hypertension and endothelial dysfunction in a mouse model of erythematous pustulosis (de la Visitación et al., 2021a). *Bifidobacteria* can prevent excessive activation of CD4+ T cells, thus maintaining the balance of Treg, Th17, and Th1 cells in SLE patients (López et al., 2016a). SLE patients exhibit increased serum levels of high-sensitivity C-reactive protein (CRP), which is associated with an increased risk of cardiovascular disease (Barnes et al., 2005; Rezaieyazdi et al., 2011). Widhani et al. (2022) found that synbiotics effectively reduce the elevated levels of high-sensitivity CRP in SLE patients, increase the *Firmicutes/Bacteroidetes* (F/B) ratio and butyrate metabolism, and decrease nucleotide sugar and amino sugar metabolism, thereby reducing disease activity. *Lactic acid bacteria*, as probiotics, can modulate immune and anti-inflammatory responses by reducing interleukin-6 (IL-6) and enhancing IL-10 levels. Supplementation of *Lactic acid bacteria* can also decrease proteinuria and levels of autoantibodies, improve renal pathology scores in MRL/lpr mice, reduce inflammatory cytokines, increase anti-inflammatory cytokine levels, and enhance the population of Tregs (Mu et al., 2017a).

4.2.3.3 Dietary intervention

The influence of diet on autoimmune diseases should not be underestimated. Consumption of a high-salt diet has the potential to activate dendritic cells (DCs) and stimulate the production of pathogenic Th17 cells via the p38/MAPK-STAT1 signaling pathway. This activation can result in dysbiosis of the gut microbiota, further exacerbating immune system dysregulation and worsening SLE (Kleinewietfeld et al., 2013; Zhang et al., 2021). Dietary modifications can impact the composition of the gut microbiota. Incorporating a moderate intake of protein, along with supplementation of vitamins, minerals, polyunsaturated fatty acids (PUFAs), and plant estrogens, can aid in improving patients' immune function, regulating systemic inflammation, and reducing disease activity. These dietary interventions have the potential to slow down the progression of SLE (Vieira et al., 2014; Putri et al., 2022).

4.2.3.4 Symbiotic microbial consortia

Symbiotic microbial consortia aim to assemble bacterial strains with favorable characteristics into beneficial microbiome components for the host organism. Tvede and Rask-Madsen cultivated a consortium comprising 10 strains of facultative and obligate anaerobic bacteria. They administered colonic therapy to five individuals with recurrent infections of the challenging pathogen *Clostridioides difficile* (*C. difficile*). None of the five patients experienced recurrence, indicating a certain efficacy of the consortium (Tvede and Rask-Madsen, 1990). A six-strain symbiotic bacterial consortium successfully eradicated *C. difficile* from the intestines of infected mice (Lawley et al., 2012). Further experiments revealed that a four-strain consortium, featuring *Clostridium scindens*, which possesses the unique capacity to convert primary bile acids into secondary bile acids known to inhibit *C. difficile* growth, significantly bolstered resistance to *C. difficile* colitis in mice (Buffie et al., 2015).

Research on symbiotic microbial consortia in SLE is limited and primarily focused on animal models. While these consortia (*Lactobacillus oris, Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus johnsonii, and Lactobacillus gasseri*) can control the progression of lupus nephritis, the primary actors in this process are *L. reuteri* and an uncultured *Lactobacillus* sp. (Mu et al., 2017a). The application of microbial consortia lies in uncovering correlations between different microbial community compositions or microbial-encoded metabolic pathways and experimental or clinical phenotypes. Given the vast diversity of commensal microbial species in the human body and the multitude of potential combinations of symbiotic species, such research poses significant challenges. The development of machine learning and artificial intelligence platforms undoubtedly will propel advancements in this field.

4.2.3.5 Engineered symbiotic bacteria

Currently, the capacity to engineer bacterial strains residing in the gastrointestinal tract also holds potential practical value. Advancements in genetic engineering techniques have facilitated significant upregulation of recombinant protein expression in *Bacteroidales* strains, achieving up to a 9,000-fold increase using the 16S ribosomal RNA gene promoter and the TetR repressor (Lim et al., 2017). Expansion of genetically manipulable *Bacteroidales* strains has been achieved through gain-of-function vector construction, enabling allelic replacement and utilization of specific polysaccharides (García-Bayona and Comstock, 2019). *Bacteroides thetaiotaomicron* has been successfully utilized for heterologous expression of tryptophan decarboxylase from *R. gnavus*, leading to enhanced tryptamine production in the lower intestinal tract (Bhattarai et al., 2018). Subsequent investigations have shown that colonization of mice with this recombinant *B. thetaiotaomicron* strain augments mucus release from goblet cells and enhances resistance to dextran sulfate sodium-induced colitis (Bhattarai et al., 2020). Research on engineered symbiotic microorganisms remains at the laboratory stage due to factors such as technological limitations and safety considerations.

The pathogenesis and organ manifestations of SLE are intricately linked to dysbiosis of the microbiota, underscoring the prospective utilization of microbiota-based interventions in the management of SLE. Contemporary investigations propose that alterations in the gut microbiota have the potential to modulate the development of SLE; however, the precise underlying mechanisms remain elusive. Consequently, further research endeavors are imperative to elucidate the intricate interplay between the microbiota and SLE. Moreover, treatment strategies should adopt a comprehensive approach, considering both genetic and environmental factors.

5 Conclusion

Over the previous decade, a remarkable upsurge in scholarly publications has occurred, attracting researchers and institutions from diverse countries/regions. Notably, China and the United States have emerged as the most active contributors in this domain. Recent research endeavors have predominantly focused on exploring the phenomenon of microbiota dysbiosis, specifically pertaining to gut microbiota dysbiosis, alongside elucidating the intricate mechanisms and applications associated with SLE, thereby establishing it as a prominent area of investigation. Future research in this field can be oriented toward several pivotal directions. Firstly, additional studies are warranted to ascertain the relationship between the microbiota and SLE, as well as to unravel the precise underlying mechanisms. Secondly, prospective research initiatives are essential to monitor the dynamic fluctuations of the microbiota and identify distinct patterns of microbiota alterations that correlate with the development of SLE. Lastly, through investigating the intricate interactions between the microbiota and SLE, potential therapeutic targets can be discerned, paving the way for the development of personalized treatment strategies targeting the microbiome.

Data availability statement

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

Author contributions

MZ: Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. XW: Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing. RL: Data curation, Formal analysis, Methodology, Writing – review & editing. KX: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from the National Natural Science Foundation of China (82202005 and 81871292), Four "batches" Innovation Project of Invigorating Medical through Science and Technology of Shanxi Province (2023XM002), Fundamental Research Program of Shanxi Province (202303021211218), the applied basic research project of Shanxi Science and Technology Department (201901D211511) and the Research Project of Shanxi Provincial Health Commission (2019006), and Shanxi Province Postgraduate Scientific Research and Innovation Project (2023KY383).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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