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Decoding Gut Microbiome Dysbiosis as a Non-Invasive Diagnostic Biomarker for Alopecia Areata

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Abstract

Alopecia areata (AA) is a chronic autoimmune disease characterized by non-scarring hair loss, where pathogenesis is closely linked to the collapse of hair follicle immune privilege and dysregulated T-cell responses. Increasing evidence suggests that gut dysbiosis may contribute to systemic immune alterations relevant to autoimmune disorders, yet its role in AA remains largely unexplored. In this study, we aimed to characterize the gut microbiota composition of AA patients and evaluate its potential as a biomarker for disease discrimination. Fecal samples from patients with AA and healthy controls were analyzed by 16S rRNA sequencing and processed through QIIME2 and MicrobiomeAnalyst platforms. Diversity metrics, differential abundance, and microbial network correlations were assessed, and supervised machine learning models were developed to classify AA versus control profiles. Our results revealed distinct microbial signatures in AA, with enrichment of pro-inflammatory genera such as *Methanobrevibacter*, *Collinsella*, and *Ruminococcus gnavus*, and depletion of immunoregulatory commensals, including *Faecalibacterium* and *Eubacterium eligens group*. Network analyses showed more complex microbial interactions in AA, and Random Forest models achieved 92% accuracy in discriminating AA from controls. These findings indicate that gut dysbiosis may play a role in AA pathogenesis, providing potential diagnostic biomarkers and supporting microbiota-targeted interventions as future therapeutic strategies.

Keywords: alopecia areata; microbiota; microbiome; gut microbiota

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1. Introduction

Alopecia areata (AA) is a common, chronic autoimmune disorder characterized by non-scarring hair loss that affects approximately 2% of the global population at some point in their lifetime, with no clear predilection for sex or age [1–3]. The disease exerts a substantial psychosocial burden, often associated with impaired quality of life, anxiety, and depression [4]. From a pathogenic standpoint, AA is driven by the collapse of the hair follicle immune privilege and the activation of autoreactive $CD8^+$ $NKG2D^+$ T lymphocytes, accompanied by the release of interferon-γ (IFN-γ) and other pro-inflammatory cytokines that perpetuate follicular damage [5–7].

Recent research has focused on the interplay between immunological, genetic, and environmental factors in AA. Genome-wide association studies have implicated susceptibility loci in both innate and adaptive immunity, including HLA, CTLA4, and genes of the

JAK-STAT pathway [8,9]. Environmental factors such as psychological stress, infections, and metabolic alterations have also been proposed as disease triggers [10]. Within this multifactorial context, the gut microbiota has emerged as a key regulator of systemic immunity and a potential contributor to autoimmunity, including AA [11–13].

The gut microbiota regulates immune homeostasis through multiple mechanisms: the production of metabolites such as short-chain fatty acids (SCFAs), induction of regulatory T cells, modulation of intestinal barrier integrity, and signaling along the gut–skin axis [14–16]. Dysbiosis, defined as a disruption in the normal composition and function of the microbiota, has been associated with several autoimmune conditions, including systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease [17–20]. Despite this, the contribution of intestinal dysbiosis to AA has only been superficially investigated, with preliminary reports suggesting altered microbial profiles in affected patients [21–23].

The gut–skin axis hypothesis proposes that intestinal microbial changes can modulate cutaneous inflammation either through microbial metabolites entering systemic circulation or through the activation of mucosal immune responses with downstream peripheral effects [24–26]. In dermatological disorders such as psoriasis, atopic dermatitis, and acne, distinct intestinal microbial alterations have been documented [27–29], supporting the plausibility of a similar contribution in AA.

Beyond its mechanistic relevance, microbiome profiling represents a promising non-invasive source of biomarkers. Advances in 16S rRNA gene sequencing and multivariate bioinformatics analyses allow for the identification of disease-specific microbial signatures [30]. Furthermore, the integration of these datasets with artificial intelligence (AI)-based classification models provides opportunities for developing diagnostic and prognostic tools [31,32].

In this study, we aimed to comprehensively characterize the gut microbiota composition of patients with AA compared with healthy controls, using high-throughput sequencing and state-of-the-art bioinformatics approaches. We analyzed microbial diversity, differential abundance, and co-occurrence networks, and we evaluated the performance of supervised machine learning models for discriminating AA from control profiles. Through this approach, we sought to elucidate the role of intestinal dysbiosis in AA pathogenesis and explore its potential as a diagnostic biomarker and therapeutic target.

2. Materials and Methods

2.1. Data Acquisition

Clinical and demographic data were collected over a 24-week period in a cohort of patients with alopecia areata (AA). The study was conducted at the Centro Dermatológico Estético (Alicante, Spain) with approval from the Ethics Committee of the University Hospital Vinalopó (Elche, Spain) and was registered at ClinicalTrials.gov (identifier: NCT05599607). The control group of healthy volunteers was selected from a database owned by the Research Center Boithas s.l., located in the city of Alicante, Spain, and the subjects were chosen with age and gender similar to those of the cases.

A conceptual map showing the research workflow with each step linked to its expected outcome is shown in Figure 1.

2.2. Clinical and Demographic Characteristics

Eligible participants were men and women aged $\geq$ 18 years with a confirmed diagnosis of AA, presenting at least two signs of active disease identified through trichoscopy. The control group was healthy men and women aged $\geq$ 18.

Demographic and clinical data of the 102 healthy volunteers are included in Table 1. The mean age was 37 years, with an average BMI of 24.45 kg/m^2. Nearly half of the

population was women (51.96%). The prevalence of allergies and intolerances was low (8.2% and 0.9%, respectively). A minority of participants reported previous pathologies, mainly respiratory (4.5%), metabolic (2.7%), cardiovascular (1.8%), digestive (0.9%), skin (0.9%), and uro-gynecological (0.9%).

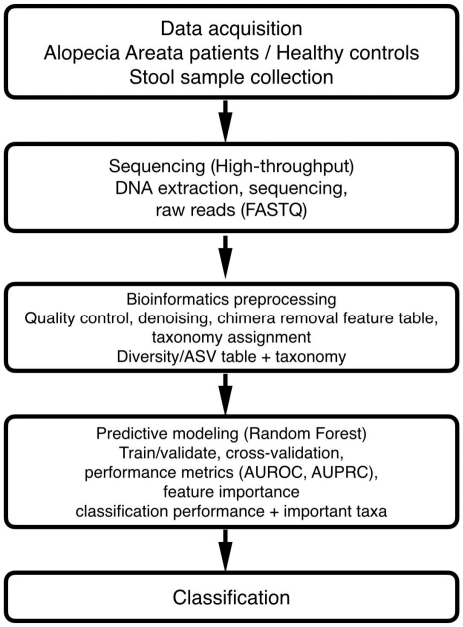


Figure 1. Study workflow.

Table 1. Demographic and clinical data of the volunteers included in the study.

Variable	Value
Gender (Women), N (%)	53 (51.96%)
Age (years), Average ± SD	37 ± 12
Weight (kg), Average ± SD	71.9 ± 12.9
Height (cm), Average ± SD	171 ± 9
BMI (kg/m^2), Average ± SD	24.45 ± 2.92
Allergies, N (%)	9 (8.18%)
Intolerances, N (%)	1 (0.91%)
Previous pathologies, N (%)	12 (10.91%)
Cardiovascular, N (%)	2 (1.82%)
Respiratory, N (%)	5 (4.54%)
Metabolic, N (%)	3 (2.73%)
Digestive, N (%)	1 (0.91%)
Skin, N (%)	1 (0.91%)
Gynecological/Uro-gynecological, N (%)	1 (0.91%)

Demographic and clinical data of the 19 alopecia areata patients included in the study are shown in Table 2. Women represented the majority of the sample (68.42%), with a mean age of 39.6 years and an average BMI of 25.9 kg/m^2. Allergies were reported by 19.2% of participants. The most frequent comorbidities were skin conditions (11.5%), followed by metabolic (3.9%), digestive (3.9%), and gynecological disorders (3.9%).

Table 2. Demographic and clinical data of the patients with alopecia areata included in the study.

Variable	Value
Gender (Women), N (%)	13 (68.42%)
Age (years), Average ± SD	39.60 ± 12.59
Weight (kg), Average ± SD	71.62 ± 18.70
Height (cm), Average ± SD	166.40 ± 7.80
BMI (kg/m^2), Average ± SD	25.9 ± 6.9
Allergies, N (%)	5 (19.20%)
Intolerances, N (%)	Not reported
Previous pathologies, N (%)	Reported
Cardiovascular, N (%)	Not reported
Respiratory, N (%)	Not reported
Metabolic, N (%)	1 (3.85%)
Digestive, N (%)	1 (3.85%)
Skin, N (%)	3 (11.50%)
Gynecological/Uro-gynecological, N (%)	1 (3.85%)

2.3. Exclusion Criteria for Healthy Volunteers

Exclusion criteria included pregnancy or breastfeeding, use of topical or systemic antifungals or antibiotics within the previous two weeks, and probiotic consumption within two months prior to enrollment. All participants provided written informed consent prior to data collection (Table 3).

Table 3. Exclusion criteria for healthy volunteers.

Exclusion Criterion
Chronic disease that, in the opinion of the investigator, contraindicates participation in the study.
Body Mass Index greater than 30.
Acute illness within the last 3 months requiring steroid treatment.
Medical treatment or nutritional supplement that, in the opinion of the investigator, could interfere with the study parameters.
Drug use or mild alcoholism (daily alcohol intake >20 g in women and >40 g in men).
Use of antibiotics and/or probiotics within the last 15 days.
Severe allergic diseases.
Inability to communicate with the investigator.
Pregnancy or breastfeeding.

2.4. Massive Genome Sequencing and Data Processing

Firstly, the intestinal bacterial composition in alopecia areata patients and volunteers was analyzed by massive genome sequencing. The NGS was carried out following the protocol provided by Illumina, which targets the amplification of the V3–V4 regions of the 16S rRNA. Taxonomic assignment was performed using the SILVA v132 database.138.2 as of 11 July 2024.

Based on 16s rRNA, inspecting a mean of over 85,000 sequences per sample (raw number of sequences), quality control of the raw data (FastQ files) was analyzed using

FastQC software v-0.11.9 [33]. The analysis of the sequencing data was performed using the QIIME2 v2023.7.0 bioinformatics software platform [34]. The DADA2 [35] denoising algorithm was used for denoising, an open-reference clustering method was used to group denoised reads into OTUs, and those were classified using a Naïve Bayes algorithm against the SILVA database [36]. The MicrobiomeAnalyst platform [37], in version 2.0, was used in order to perform the post-processing (filtering and normalization) and microbial diversity analysis. OTUs with fewer than 4 reads were removed, as well as those present in less than 10% of the samples and those accounting for less than 5% of the measured variation according to the interquartile range. Centered log-ratio (CLR) normalization was used for microbial diversity analysis.

2.5. Analysis of the Microbial Communities and Comparisons

The composition of the microbial community was evaluated through the alpha and beta diversity indices. The alpha diversity was measured using the observed richness (Chao1), while diversity was measured with the Shannon index. A *T*-test analysis was also used to determine in-depth differences in alpha diversity indices of microbial communities across different groups. On the other hand, beta diversity was measured using the Bray–Curtis distance, and the statistical comparisons were evaluated using Permutational multivariate analysis of variance (PERMANOVA), with a PCoA ordination method for results visualization. Significant differences were noted when the *p*-value was less than 0.05.

In order to check whether the different confounders (sex, age, weight, etc.) can affect the microbiota distribution, a Mantel test was performed. This test is a statistical analysis used to evaluate the association or correlation between two data matrices in terms of distances. Bray–Curtis distance was used to calculate the microbiome matrix.

In a second step, to identify those microorganisms whose abundances differed between study conditions (Alopecia Areata vs. Healthy volunteers), the DESeq2 (version 1.42.1) [38] algorithm was used, applying its default size factor normalization method to correct for differences in sequencing depth. Microorganisms were selected among those that showed statistically significant differences in abundance (FDR < 0.05) and fold changes above or below $\pm$ 1. The microbiome data were analyzed at the genus level.

2.6. Network Correlation Analysis

The SparCC (Sparse Correlations for Compositional data) algorithm was used to identify co-occurrence and correlation relationships among microorganisms present in a community, while Cytoscape (version 3.9.1) and the Cytohubba tools were used for the representation. The SparCC correlation method was applied, considering 100 permutations, and retaining features with a correlation coefficient higher than $\pm$0.5 and respective *p*-value $<$ 0.05. To assess the topological importance of each node, two centrality metrics were applied: Degree, which quantifies the total number of connections for each node, and the MCC (Maximal Clique Centrality) algorithm, which identifies nodes involved in a greater number of maximal cliques (fully connected subgroups), reflecting their structural relevance within the network.

2.7. IA Modelling

The information from microbial diversity and differential abundance analysis was used to construct a supervised machine learning model capable of classifying unknown samples as healthy or diseased (alopecia areata) based on their gut microbiota. Python (version 3.10.12) and Scikit-learn library (version 1.2.0) [39] were used to create, train, and validate the model, and several classification algorithms such as Support Vector Machine (SVC), Decision Tree, Neural Net, Nearest-Neighbors, and Random Forest were tested, and their classification capability was evaluated against each other. Eleven selected genera

from TMM normalized reads were used, and alopecia areata and healthy samples were randomly divided into two groups: train (80%, build the model) and validation (20%, test the model). The Synthetic Minority Over-sampling Technique (SMOTE) algorithm from Imbalanced-learn library [40] (version 0.14.0) was used due to the unbalanced dataset (102 healthy samples and 19 alopecia samples) to complete the same number of training healthy samples (81) with synthetic alopecia samples. This approach synthetically generates new samples from the minority class based on the distance to their k-nearest neighbors (k = 5) in feature space, resulting in a more balanced training dataset. Moreover, to avoid overfitting, a cross-validation strategy was carried out to perform the training of the models, using balanced accuracy as the precision score. A total of 10,200 models and their parameters were tested and evaluated, each one executed 5 times for the cross-validation.

3. Results

3.1. Analysis of the Microbial Communities

An average of 8.593 16S rRNA gene reads per sample were analyzed using QIIME, resulting in the identification of 1.319 operational taxonomic units (OTUs) at a 99.3% identity level. After the filtering and selection process, 314 taxonomy-assigned OTUs were acquired. An overall analysis revealed differences in the composition and abundance of the microbiome of healthy controls and patients suffering from alopecia areata (AA). The distribution of the 20 most representative genera (Figure 2A) revealed a decrease in the relative proportion of bacterial genera from the families *Oscillospiraceae* and *Ruminococcaceae*, such as *Faecalibacterium* in alopecia patients, while an increase in genera such as *Blautia* and *Dorea* was observed compared to the control condition.

The analysis of alpha diversity showed no statistically significant differences in the measure of richness using the Chao1 index (p-value of 0.2136) or in the measure of diversity using the Shannon index (p-value of 0.6923) (Figure 2B).

However, the measurement of beta diversity showed that there are significant differences in the microbial communities of healthy controls and patients with alopecia areata, with a PERMANOVA F-value of 6.544 and an FDR-corrected p-value of 0.001 using Bray–Curtis distance (Figure 2C).

These measures clearly reflect the significant role that microorganisms play in this type of pathology. The onset of alopecia alters the relationships between the microorganisms in the microbiome of patients. The objective of correlation networks is to identify potential interactions between microbes that could represent mutualistic, commensal, parasitic, or even competitive relationships. Unlike traditional linear correlation approaches, SPARCC relies on the stability of correlations across subsets of data, which helps to reduce spurious correlations caused by noise in the data. In any analysis of interactions and relationships, the position and degree of interaction of the different nodes are important characteristics in determining the relevance of that node within the study network.

At the genus level, healthy controls did not show many statistically significant correlations among different bacterial genes, with only positive correlations between *Christensenellaceae_R_7_group* and *UCG_005*, or between *Blautia* and *Anaerostipes* (Figure 3A). However, in patients with alopecia, a greater number of correlations among different bacterial genera appear. This is particularly evident in patients with alopecia, where the number of statistically significant correlations increases significantly compared to healthy controls. Here, the analysis showed that the genera *Family_XIII_AD3011_group*, *Methanobrevibacter*, *vadinBE97*, *Christensenellaceae_R_7_group*, *Eubacterium__ventriosum_group*, *Eubacterium__ruminantium_group*, and *Lachnospiraceae_UCG_004* are the main genera involved in regulating these networks that form the patients' microbiome (Figure 3B).

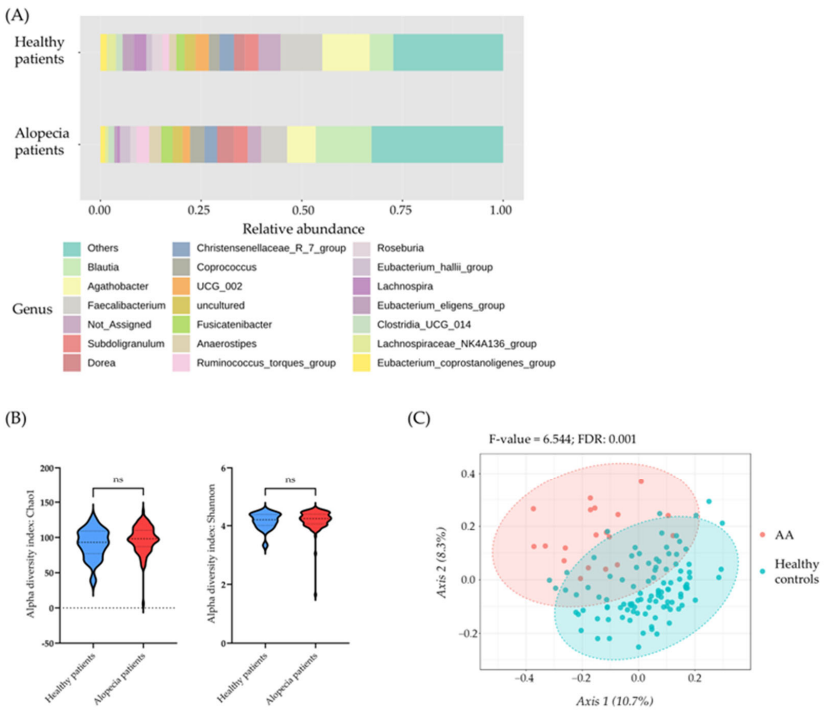


Figure 2. Microbial community profile in healthy individuals and patients with alopecia: (**A**) Bar plot showing the microbiome composition at the genus level (percentage of abundance) in healthy individuals and patients with alopecia. (**B**) Alpha diversity measured using the Chao1 index (left) and Shannon index (right) for each sample within the study groups: healthy individuals and patients with alopecia. Statistical differences were assessed using the value of each index per sample and analyzed by One-Way ANOVA, followed by Tukey's test for multiple hypothesis correction. (**C**) Representation of beta diversity for each microbial community using the Bray–Curtis index. Statistically significant differences in beta diversity among the different pathologies were assessed by pairwise comparisons using the PERMANOVA algorithm. Differences were considered statistically significant at $p < 0.05$ ($p > 0.05$ = ns).

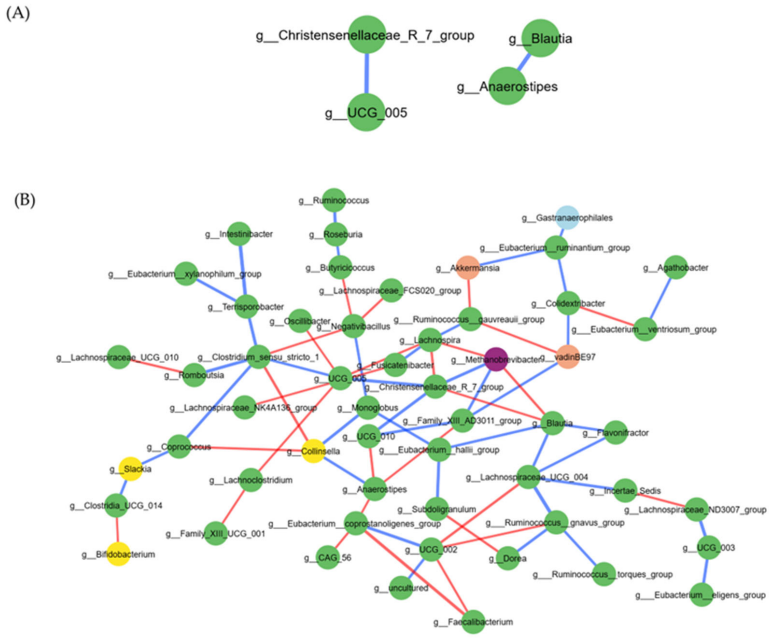


Figure 3. Correlation networks among different microbial taxa identified using the SPARCC correlation algorithm. Possible correlation relationships among different microbial taxa were defined at the genus

level: (**A**) healthy controls, (**B**) alopecia patients. The nodes were colored according to the phylum to which they belong: cyan (Cyanobacteria), yellow (Actinobacteriota), orange (Verrucomicrobiota), purple (Euryarchaeota), green (Firmicutes). Connections between the different nodes indicate the degree of existing correlation. Blue connections indicate a positive correlation, while red connections indicate a negative correlation. Additionally, the width of the connection line indicates the strength of the correlation: a thicker line represents a stronger correlation between the nodes, whether positive or negative. Correlations considered statistically significant for the study of the families were those with a p-value < 0.05 and a correlation degree of $\pm$ 0.5.

The Mantel test revealed no statistically significant correlations between the confounding factors (metadata variables) and the overall microbiome composition. In the AA group, the strongest associations were observed for age (r = 0.16, p-value = 0.07) and weight (r = 0.24, p-value = 0.08).

3.2. Identification of the Microbial Features Associated with the Alopecia Areata Gut Microbiome

The central idea of DESeq2 is to model the distributions of sequence counts (abundances) and use statistical methods to detect significant differences in these abundances between groups of samples. In this case, families and genera were selected as statistically significant if they had an FDR < 0.05 and a Fold Change value (Log_2 Fold Change) $\geq \pm 1$. The results obtained from comparing patients with alopecia to healthy controls showed a very significant increase in the abundance levels of bacterial families such as *Methanobacteriaceae*, *Coriobacteriaceae*, *Peptostreptococcaceae*, and *Eggerthellaceae*. Conversely, two bacterial families, *Gastranaerophilales* and *Victivallaceae*, had reduced presence (Figure 4A).

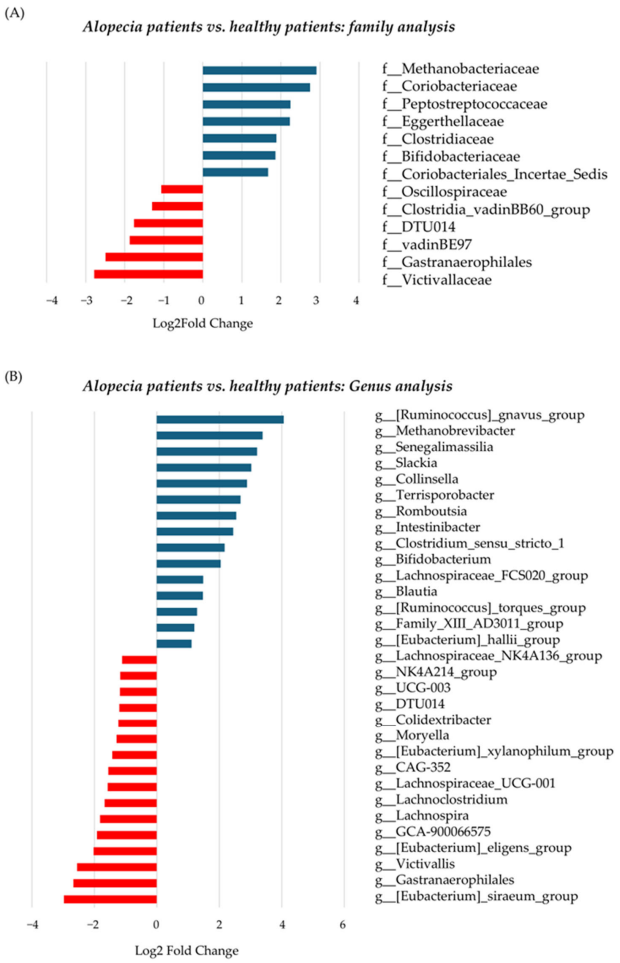


Figure 4. Differential abundance analysis of microorganisms using DESeq2 when comparing patients with alopecia and healthy individuals: (**A**) Families showing significantly different abundance levels

according to DESeq2 analysis. (**B**) Genera showing significantly different abundance levels according to DESeq2 analysis. A positive Fold Change value indicates a higher abundance in patients with alopecia compared to healthy individuals, whereas a negative Fold Change value indicates increased abundance in healthy individuals. Statistical changes were considered significant when FDR was below 0.05, and the Fold Change was greater than 1 or less than −1.

At the genus level, the results showed significant increases in different bacterial genera, among which *[Ruminococcus]_gnavus_group*, *Methanobrevibacter*, *Senegalimassilia*, *Slackia*, and *Collinsella* are noteworthy. Additionally, other bacterial genera had diminished their presence following the onset of the pathology, such as *[Eubacterium]_eligens_group*, *Victivallis*, *Gastranaerophilales*, and *[Eubacterium]_siraeum_group*, primarily (Figure 4B).

3.3. IA Model Results

Using the information from the complete microbiome analysis above, 11 microorganisms at the genus level were used as the entry for training a new supervised classification model. Samples were normalized before microorganism selection using TMM. The best classification results were obtained with a Random Forest Model, with a total number of trees (estimators) of 50, with a max depth of 5. The cross-validation training score was 98.75%, and validation results are shown in Figure 5B and Supplementary Table S1.

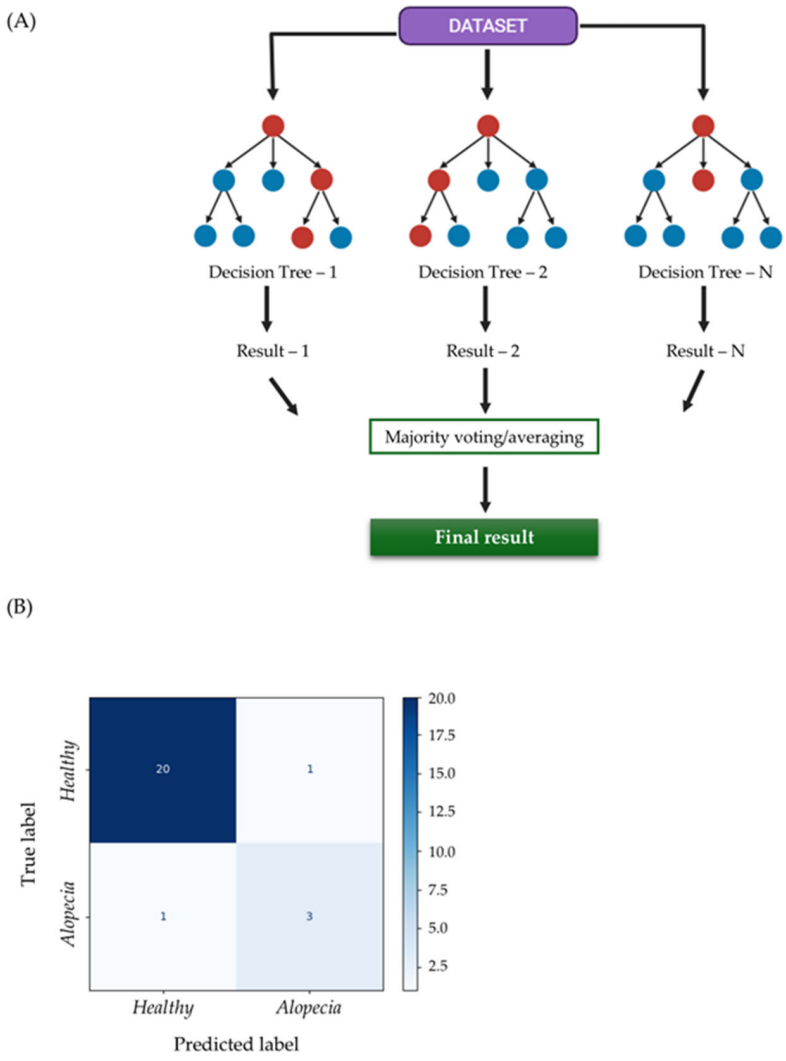


Figure 5. (**A**) Conceptual diagram of the Random Forest classification model. Each tree is trained on a random subset of data and features, and final predictions are determined by majority vote across all

trees. Blue and red dots exemplify 2 different classes of samples, (**B**) Confusion matrix illustrating the performance of the Random Forest classification model. The results are shown for both the training and validation sets, indicating the number of instances correctly and incorrectly classified for each class.

4. Discussion

The present study provides the first comprehensive characterization of gut microbiome alterations in patients with alopecia areata (AA) using high-resolution 16S rRNA sequencing coupled with bioinformatics, microbial network inference, and machine learning modeling. Our findings indicate that AA is associated with profound dysbiosis, involving not only changes in the abundance of specific bacterial taxa but also disruption of ecological interactions and predictive potential for disease classification. These results highlight the gut microbiome as a critical component of the complex immunopathogenesis of AA and support its potential utility as a non-invasive biomarker and therapeutic target.

Although no significant differences were detected in alpha diversity indices (Chao1 and Shannon), beta diversity analysis revealed a clear separation of microbial community structures between AA patients and healthy controls. This is consistent with the notion that autoimmune and inflammatory diseases often display a preserved within-sample diversity but a distinct community composition, reflecting qualitative rather than purely quantitative microbial changes [41,42]. For example, similar patterns have been reported in psoriasis [27], systemic lupus erythematosus [43], and rheumatoid arthritis [18], suggesting that the type and functional roles of microbes may be more critical than absolute diversity measures.

One of the most striking findings was the depletion of beneficial genera such as *Faecalibacterium* and *Eubacterium* groups in AA patients. *Faecalibacterium prausnitzii* is a well-recognized producer of butyrate, a short-chain fatty acid (SCFA) with anti-inflammatory effects by modulating regulatory T cells and inhibiting NF-κB signaling [44,45]. Reduced abundance of *Faecalibacterium* has been consistently observed in Crohn's disease [46], ulcerative colitis [47], and psoriasis [27], and its depletion in AA may contribute to systemic immune dysregulation and loss of tolerance. Similarly, depletion of *Eubacterium* species may reduce SCFA production and compromise epithelial barrier integrity [48].

Conversely, AA patients exhibited enrichment of *Collinsella*, *Blautia*, *Dorea*, and *Methanobrevibacter*. *Collinsella* has been implicated in promoting intestinal permeability and pro-inflammatory cytokine production in rheumatoid arthritis and type 2 diabetes [20,49]. Likewise, *Blautia* and *Dorea*, members of the Lachnospiraceae family, have been associated with metabolic inflammation and altered bile acid metabolism [50,51]. *Methanobrevibacter*, an archaeon responsible for methane production, was also markedly enriched. Previous studies have linked methanogenic archaea with delayed intestinal transit and increased production of hydrogen and methane, which may influence host immune responses [52,53]. The consistent enrichment of these taxa across autoimmune diseases suggests a shared dysbiotic signature that predisposes to chronic immune activation.

Network analysis revealed that AA patients displayed an increased number and complexity of microbial correlations compared to healthy controls. While healthy individuals showed sparse and stable networks dominated by positive associations between commensals, AA patients exhibited dense networks with multiple hub taxa, including *Methanobrevibacter*, *Christensenellaceae R-7 group*, and *Eubacterium ventriosum group*. The emergence of such complex networks may indicate ecological instability and compensatory microbial interactions in response to host inflammation [54,55]. Network-based approaches have recently been used to identify keystone taxa in autoimmune diseases, demonstrating that certain microbes exert disproportionate effects on ecosystem stability and host physiology [56]. Our findings suggest that hub taxa in AA may play critical roles in disease pathogenesis and could represent novel therapeutic targets.

DESeq2 analysis confirmed the enrichment of *Ruminococcus gnavus*, *Collinsella*, and *Methanobrevibacter* in AA patients. *Ruminococcus gnavus* is known to produce inflammatory polysaccharides that promote TNF-α secretion and have been strongly linked to Crohn's disease and systemic lupus erythematosus [57]. Its expansion in AA may contribute to activation of Th17 pathways, which are central to AA immunopathology [58]. Similarly, *Collinsella* has been shown to enhance IL-17A production and disrupt epithelial tight junctions [59]. Together, these findings suggest that microbial alterations in AA may drive disease through mechanisms involving increased intestinal permeability, altered metabolite production, and skewing of T cell responses.

Interestingly, families such as *Victivallaceae* and *Gastranaerophilales* were reduced in AA. These groups, although poorly characterized, have been associated with saccharolytic metabolism and SCFA production [59]. Their depletion further supports the hypothesis of reduced anti-inflammatory microbial functions in AA.

The application of supervised machine learning in our data demonstrated that microbial signatures can robustly distinguish AA patients from healthy controls, achieving >90% accuracy in validation. Notably, the Random Forest model highlighted *Collinsella* and *Methanobrevibacter* as the most influential taxa for classification. This aligns with their consistent enrichment in AA and their mechanistic plausibility in promoting inflammation. Previous studies have successfully applied ML to classify patients with IBD, psoriasis, and rheumatoid arthritis based on microbiome features [60–62]. Our results extend this approach to AA, suggesting that gut microbiome-based classifiers may serve as non-invasive diagnostic tools in clinical practice. In particular, our results outline the principle of the test: microbiome-derived features combined with AI/ML classifiers can deliver an objective diagnostic readout that may assist clinicians at early disease stages, and—pending validation—offer a basis for future severity stratification.

Alopecia areata is driven by a collapse of hair-follicle immune privilege with a $CD8^+$ $NKG2D^+$ T-cell-mediated attack amplified by Th1/IFN-γ and JAK–STAT signaling, alongside contributions from the Th17/IL-17/IL-23 axis within a perifollicular inflammatory milieu. In this framework, microbiome perturbations that reduce short-chain fatty acids and epithelial-barrier support, or that enrich taxa linked to pro-inflammatory metabolisms, could promote Th1/Th17 skewing and facilitate follicular inflammation. Our findings—taxonomic shifts, altered co-occurrence networks, and AI/ML features that discriminate AA—are consistent with a gut–immune pathway capable of modulating these $CD8^+$/Th17-centric mechanisms. Therefore, we interpret the observed dysbiosis as a potential immune amplifier rather than a sole cause, and we outline prospective studies to test causal links and therapeutic modulation within this immune framework [63,64]. The gut microbiome has emerged as a key regulator of systemic immunity, influencing differentiation of Tregs, Th17 cells, and NK cell activity [65]. Dysbiosis, characterized by loss of SCFA producers and expansion of pro-inflammatory taxa, can skew this balance toward autoimmunity. In murine models, transplantation of dysbiotic microbiota can trigger alopecia and exacerbate autoimmune phenotypes [66]. Although causality remains to be proven in humans, our findings support the hypothesis that gut dysbiosis may act as a trigger or amplifier of AA immune responses.

The identification of distinct microbial signatures in AA has several translational implications. First, microbial biomarkers could be integrated into multiomic panels combining genomics, transcriptomics, and metabolomics for precision diagnosis and risk stratification. Second, microbiome-targeted interventions such as dietary modulation, probiotics, prebiotics, and fecal microbiota transplantation (FMT) could be explored as adjunctive therapies. For instance, supplementation with butyrate-producing probiotics has shown promise in restoring Treg function in autoimmune conditions, while FMT has demonstrated

efficacy in recurrent Clostridioides difficile infection and is under investigation for IBD [67]. Controlled trials will be necessary to assess their efficacy and safety in AA.

5. Conclusions

In summary, this study demonstrates that alopecia areata is associated with significant gut microbiome alterations, including depletion of beneficial SCFA-producing taxa, enrichment of pro-inflammatory genera, and disruption of microbial networks. A set of eleven bacterial genera, including Blautia, Collinsella, and Bifidobacterium, was sufficient to construct a Random Forest-based classification model capable of distinguishing microbiota samples from AA patients and healthy controls with an accuracy of 92%. These microbial signatures were sufficient to classify AA patients with high accuracy using machine learning approaches, underscoring their potential as diagnostic biomarkers. This high predictive performance underscores the strong association between gut microbiome composition and the disease. Our findings highlight the relevance of the gut–immune–skin axis in AA pathogenesis and open new avenues for microbiome-based interventions. Further studies integrating multiomic approaches and interventional trials will be crucial to translate these findings into clinical applications.

Taken together, these findings support the future development of an objective, microbiome-based diagnostic aid for the early identification of alopecia areata. With external, multicenter validation, model outputs could also support clinical stratification by disease severity and inform patient management. The main achievements of this study include the following: (i) we developed the first application of predictive modeling to AA microbiome data to quantify diagnostic performance with cross-validated metrics; (ii) the identification of biologically plausible microbial features and network disruptions that discriminate AA from controls, providing testable mechanistic hypotheses.

Several limitations should be acknowledged. The relatively small sample size, particularly of AA patients, may limit generalizability and statistical power. Dietary intake and lifestyle factors were not extensively controlled, and these could influence microbiome composition. The 16S rRNA sequencing, while informative, lacks species- and strain-level resolution and does not capture functional potential, which could be addressed by metagenomics and metabolomics. The study does not have a standardization of systemic inflammatory markers of patients, and this was assessed by clinical trichoscopy. Finally, the cross-sectional design precludes causal inference. Longitudinal studies and mechanistic experiments are needed to establish whether dysbiosis precedes disease onset or results from immune dysregulation.

Despite these limitations, our study provides novel insights into the gut microbiome in AA and lays the foundation for future research exploring causal mechanisms and therapeutic interventions. These limitations will be addressed in future studies through prospective, multicenter validation; shotgun metagenomics/metabolomics for higher resolution; inclusion of standardized serum inflammatory panels; and longitudinal designs to confirm generalizability, assess causality, and define clinical utility.

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