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Insights into the gut microbiome of vitiligo patients from India

Sudhir Kumar¹, Shruti Mahajan¹, Deeksha Kale¹, Nidhi Chourasia², Anam Khan², Dinesh Asati², Ashwin Kotnis^{2*} and Vineet K. Sharma^{1*}

Abstract

Background Vitiligo is an autoimmune disease characterized by loss of pigmentation in the skin. It affects 0.4 to 2% of the global population, but the factors that trigger autoimmunity remain elusive. Previous work on several immune-mediated dermatological disorders has illuminated the substantial roles of the gut microbiome in disease pathogenesis. Here, we examined the gut microbiome composition in a cohort of vitiligo patients and healthy controls from India, including patients with a family history of the disease.

Results Our results show significant alterations in the gut microbiome of vitiligo patients compared to healthy controls, affecting taxonomic and functional profiles as well as community structure. We observed a reduction in the abundance of several bacterial taxa commonly associated with a healthy gut microbiome and noted a decrease in the abundance of SCFA (Short Chain Fatty Acids) producing taxa in the vitiligo group. Observation of a higher abundance of genes linked to bacteria-mediated degradation of intestinal mucus suggested a potential compromise of the gut mucus barrier in vitiligo. Functional analysis also revealed a higher abundance of fatty acid and lipid metabolism-related genes in the vitiligo group. Combined analysis with data from a French cohort of vitiligo also led to the identification of common genera differentiating healthy and gut microbiome across populations.

Conclusion Our observations, together with available data, strengthen the role of gut microbiome dysbiosis in symptom exacerbation and possibly pathogenesis in vitiligo. The reported microbiome changes also showed similarities with other autoimmune disorders, suggesting common gut microbiome-mediated mechanisms in autoimmune diseases. Further investigation can lead to the exploration of dietary interventions and probiotics for the management of these conditions.

Keywords Gut Microbiome, Vitiligo, Autoimmune disease, Gut-skin axis, Dysbiosis

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Background

The members of the human gut microbiome have direct or indirect interactions with cells in the innate and adaptive immune system. Gut bacteria have been implicated to play inflammatory and protective roles in many diseases. An imbalance in the gut microbiome, also known as dysbiosis, can trigger several autoimmune disorders by modulating the immune system [1]. In recent times, the gut microbiome has emerged as an important factor in understanding the pathobiology of many autoimmune diseases like Crohn's disease (CD), inflammatory bowel disease (IBD), type 1 diabetes (T1D), etc [2, 3]. A remarkable number of studies have shown dysbiosis in various dermatological autoimmune disorders like systemic lupus erythematosus (SLE), psoriasis, and alopecia areata [4–7].

One such important autoimmune disease is vitiligo. It is a skin depigmentation disorder characterized by the loss of functional melanocytes in the affected areas, which leads to the development of non-scaly, chalky white macules with distinct margins [8]. Vitiligo is classified into two major types, Segmental Vitiligo (SV) and Non-segmental Vitiligo (NSV), based on the distribution and progression pattern of patches on the body. Vitiligo affects 0.4 to 2% of the world's population [8–10]. Although the disease is not associated with serious health implications, pigmentation has psychologically devastating effects on the patients. Investigations into genetic determinants and disease association studies have also pointed to a higher co-occurrence of vitiligo with many serious conditions like autoimmune thyroid disease, pernicious anemia, Addison's disease, and SLE, thus making vitiligo patients more vulnerable to these diseases [11, 12].

The pathogenesis of vitiligo is largely understood as an autoimmune disorder. Tyrosinase, encoded by TYR gene, is an important enzyme that catalyzes the rate-limiting steps of melanin biosynthesis, it is identified as a major autoantigen in generalized vitiligo [8]. Recent work also identified the role of CXCR3-B, a chemokine receptor variant, in the induction of apoptosis [13]. Additionally, various chemokines have been associated with vitiligo, playing roles in recruitment of cells towards melanocytes [14]. Considerable variations of cytokines in lesional and perilesional skin of vitiligo patients have been reported. Studies have shown a significant increase in the expression of cytokines IL-10 (Interleukin 10), IFN- γ (Interferon-gamma), and TNF- α (Tumor necrosis factor alpha) in vitiligo when compared to controls [15–19], indicating that cytokine generation in the epidermis may be involved in the development of vitiligo [15, 20–22]. Presence of polymorphic genes can cause variable immune responses in some subjects, which suggests that polymorphisms in cytokine genes may reflect or regulate the severity and course of different diseases [15]. IL-10

possesses anti-inflammatory properties, it is also a key immunoregulator and mediator of the inflammatory process due to its direct capacity to down-regulate the production of TNF- α , IL-1, IL-8, and IFN- γ . Additionally, IL-10 is a strong up-regulator of B-cell differentiation and production [23, 24]. The promoter region of IL-10 gene is highly polymorphic, this polymorphism is correlated with differences in transcription. Two SNPs in the promoter region of the IL-10 gene (–1082G/A and –819 C/T) have been linked to a variety of diseases including vitiligo [15].

Recently, many dermatological autoimmune diseases have been shown to have gut dysbiosis [5–7, 25]. The existence of a gut-skin axis mediated by the immune system has been proposed to explain interactions between the gut microbiome and skin-associated diseases [26]. Previous studies have explored the skin microbiome of vitiligo patients, some work on vitiligo gut microbiome has also revealed dysbiosis of gut microbiome in French and Chinese subjects [27–31]. Furthermore, a study reported that antibiotic-mediated dysbiosis in gut exacerbated depigmentation in mouse models, showing important role of gut microbiome homeostasis in symptom progression of vitiligo [32]. Similar observations have also been made in psoriasis [7, 33]. As the gut microbiome can play an important role in the regulation of the immune system, dysbiosis in the gut can be a potential factor in vitiligo pathogenesis. Therefore, studying the gut microbiome of vitiligo patients can provide significant clues to vitiligo pathogenesis and highlight involvement of gut microbiome in several related autoimmune conditions.

In this study, we sequenced and analyzed the gut microbiome from an Indian cohort of vitiligo patients with age and gender-matched healthy controls using 16S rRNA amplicon sequencing. We also included the information on family history in first-degree relatives as a proxy for the genetic risk of vitiligo. We then used predictive metagenomic profiling in this cohort and identified several microbial functions differentially abundant in the gut microbial communities of vitiligo and controls. The co-occurrence network analysis was used to compare the community structure of the gut microbiome between healthy and vitiligo groups. We also identified common genera affected in the gut microbiome of vitiligo patients across cohorts, which can act as biomarkers irrespective of the population-specific compositional variation. This study reports the first data from a non-western cohort of vitiligo patients from India. The results suggest the important role of gut microbiome in vitiligo and add to the growing evidence of gut microbiome dysbiosis in many autoimmune dermatological disorders.

Methods

Study design and subject recruitment

The study cohort comprised two main groups, the first group consisted of 10 healthy individuals and the second group had 22 vitiligo patients. To rule out the genetic propensity of the disease as a factor in affecting the gut microbiome, we included patients with and without a first-degree relative with the disease, further dividing disease groups into 12 patients with no family history and a second group of 10 patients with a family history of vitiligo. The groups were matched for age and gender.

The study participants were accrued in the Department of Dermatology at AIIMS Bhopal for a duration of two years (2018–2020). The patients were diagnosed by a dermatologist in the outpatient department on the individuals visiting the clinic with complaints of vitiligo or skin depigmentation. The diagnosis was made by visual inspection of the lesion, and other forms of depigmentation, such as melasma, were ruled out for confirmation of vitiligo. The gender, age, vitiligo onset age, vitiligo type, diet type, family history of vitiligo, and vitiligo activity state were recorded for all the recruited individuals.

Subject inclusion/exclusion criteria

Participants consenting to participate in the study and willing to provide blood or stool samples were interviewed. Participants with confirmed vitiligo lesions were recruited. One-time blood and stool collection were made from participants matching the inclusion and exclusion criteria (Table S1, Fig. S1). Individuals receiving antibiotic or steroid treatment were excluded from this study. The control group included adult participants who were apparently healthy and unrelated to others.

Measurement of T-cell populations

To obtain T-cells subset counts (CD4⁺, CD8⁺, and Regulatory T cells), 100 µL of whole blood in sheath buffer (Beckman Coulter) with antibodies targeting CD4, CD8, CD25, and CD127 (PathnSitu) was sorted and counts were obtained using flow cytometer (Beckman Coulter).

IL-10 genotyping and ELISA

Genotyping of the IL-10 (-1082 A<G) and IL-10 (819 C<T) SNPs was done using ARMS-PCR assay from genomic DNA extracted from the whole blood. An amplicon containing the SNP was generated using the sense and antisense Primer (Table S1). PCR products were run on 1% agarose gels and visualized with UV light after ethidium bromide staining in gel documentation system to assess the size of the amplified product.

The separated sera stored at -80 °C were used to perform ELISA using the ELISA kit (R&D, DuoSet ELISA, Catalog Number DY217B-05) following manufacturer protocol. Sera samples were first treated with Mouse

Anti-Human IL-10 Capture Antibody followed by incubation and treatment with Biotinylated Goat Anti-Human IL-10 detection Antibody and finally immune complexes were formed using Streptavidin HRP. The samples were washed according to the kit's protocol and then the concentration of marker was determined at 450 nm using the ELISA Plate reader (Eon Biotek Microplate Spectrophotometer, Winooski VT, USA).

Sample processing and DNA extraction

Whole blood samples were collected in EDTA vacutainers by trained personnel and transported to the laboratory for further analysis. Preferably early morning stool samples were collected from participants in sterile containers and were transported to the laboratory for storage at -80 °C. The samples were transported in cold chain to IISER Bhopal and stored at -80 °C until further processing. The collected fecal samples were processed with the QIAamp Fast DNA Stool Mini kit (Qiagen, CA, USA) following the manufacturer's instructions for metagenomic DNA extraction. The metagenomic DNA was quantified on a Qubit 2.0 fluorometer using a Qubit dsDNA High Sensitivity (HS) assay kit (Invitrogen, CA, USA). The DNA was stored in -20 °C refrigerator until further processing.

Library preparation and sequencing

Approximately 10 ng of extracted metagenomic DNA was used to amplify the V3 hypervariable region of 16S rRNA for each sample. The primers used were Nextera XT adaptor-ligated 341F-ADA-2B and 534R-ADA-2B as forward and reverse primers, respectively. The sequences are available in table (Table S1) [34]. The amplicons were checked on 2% agarose gel electrophoresis, and were used to prepare libraries following the Illumina 16S metagenomic sequencing library preparation guide. The quality of libraries was checked on TapeStation 4150 using high-sensitivity D1000 ScreenTapes. The libraries were quantified on Qubit 4.0 fluorometer using a Qubit dsDNA HS assay kit (Invitrogen, CA, USA). Final libraries were sequenced for 150 bp paired-end reads on Illumina NovaSeq 6000 platform (Illumina, CA, USA).

Read processing and data analysis

The reads were quality-filtered using the NGSQC toolkit allowing zero ambiguous bases, and high-quality reads with 70% bases above Q20 were filtered out (with parameters N 1 -l 70 -s 20) [35]. Primer sequences were trimmed using Cutadapt v2.10 [36]. After preprocessing, the paired-end reads were imported into QIIME 2 for further processing. Denoising and chimera detection were performed with DADA2, and the resulting feature table was filtered for removing low-abundant features [37, 38].

Diversity metrics, namely the number of observed features, Pielou's evenness, Faith phylogenetic diversity (Faith's PD), and Shannon entropy were used to estimate alpha diversity in samples. Beta diversity was estimated by calculating inter-sample distances using Jaccard, Bray-Curtis, and UniFrac distances. Taxonomic assignment of Amplicon Sequence Variants (ASVs) was carried out using the q2-greengenes2 plugin with the Greengenes2 database [39]. The relative abundance at the phylum, genus, and species level was calculated after taxonomic assignment. Differentially abundant features were identified using LEfSe for genus and species-level relative abundance [40].

Prediction of metagenomic functions

Prediction of metagenomic functions was carried out using PICRUSt2 version 2.5.1 (<https://github.com/picrust/picrust2>) [41]. The resulting table of functional abundance measured as KEGG ortholog abundance was further filtered to remove low abundant KOs, only the KOs present in relative abundance of more than 0.0001%, and present in at least six samples (~20% samples) were kept for further analysis. Differentially abundant features were identified using LEfSe in the same way as done for the genus and species level data. The abundance of MetaCyc pathways was also calculated to identify specific metabolic pathways.

Network construction

Microbial communities can be represented as complex networks of taxa interacting in various ways. The resulting co-occurrence networks can be used to describe changes in the community structure between healthy and disease-state microbiomes [42]. Co-occurrence network analysis was carried out separately for control and vitiligo groups using genus-level abundances, and networks statistics were compared. Pairwise correlations were calculated with sparCC with SCNIC (Sparse Cooccurrence Network Investigation for Compositional data) (<https://github.com/lozuponelab/SCNIC>) [43]. We implemented sparcc-filter and used 10 iterations for calculating the p-value. Further, the resulting networks were summarized into modules using SCNIC with a minimum R-value of 0.35.

Supervised learning classification with other vitiligo datasets

To identify potential biomarkers and explore any common patterns in the gut microbiome of vitiligo across populations, we used supervised learning to classify samples based on their health status. For this analysis, we also used publicly available data from a French cohort that studied gut microbial composition and diversity in patients with vitiligo ($n=10$) and respective healthy

controls ($n=10$) [30]. To allow comparability in the two datasets (Indian and French), we used close reference clustered genus-level feature tables. This approach was used to avoid bias due to the use of different amplicon regions in the two studies. Further, the table was filtered for genera present in more than 10% of the samples and with >0.001% relative abundance. Using this data, we trained a random forest-based supervised classifier model to classify vitiligo and healthy samples using the q2-sample-classifier plugin with 5-fold cross-validation [44]. Further, the most important genera for distinguishing between healthy and vitiligo samples were identified using feature selection.

Statistical analysis and data visualization

Alpha and beta diversity metrics were calculated in QIIME 2. The significance of differences in alpha diversity in groups was calculated using the Wilcoxon test. The significance of differences in beta diversity was calculated using PERMANOVA (permutational multivariate analysis of variance) implemented in R using the adonis2 function with 999 permutations. Differentially abundant features were identified using LEfSe analysis using default LDA cutoff of 2.0 [40]. Plots were made using R package ggplot2 [45]. Cytoscape version 3.9.1 was used for calculating network statistics [46].

Results

Study cohort description and sequencing

We collected and sequenced fecal samples for microbiome analysis from 32 individuals. Out of those, 10 were healthy and 22 were diagnosed with vitiligo consisting of family history and non-family history subgroups (Table S1). For 32 fecal samples, 74,621,554 ($2,331,923.56 \pm 788,259.31$, mean $\pm$ sd) paired-end reads were generated. After quality filtration steps, 67,723,568 ($2,116,361.5 \pm 703,127.8$, mean $\pm$ sd) high-quality reads remained, which were used for further processing (Table S1).

Immunological measurements

The frequency of alleles from IL-10 genotyping revealed variation of SNPs in Indian vitiligo patients and healthy controls. No notable differences were observed in T-cell subpopulations in the control and vitiligo groups. The levels of serum IL-10 were significantly higher in the serum of controls than in vitiligo patients (Table S2 and Fig. S2).

Amplicon data analysis

After denoising and chimera cleaning in DADA2, we detected 14,359 ASVs with a total frequency of 58,282,407. The resulting feature table was filtered to remove low abundant ASVs by removing features with a

total count of <10 across samples. The final feature table with 12,927 ASVs was used for further analysis (Table S3).

Alpha and Beta diversity

Alpha diversity for both groups was calculated using the number of observed features, Pielou's evenness, Faith PD, and Shannon entropy. Although the median observed features and Faith PD values were slightly lower for the vitiligo group, there was no significant difference ($p=0.063$ and $p=0.119$) in alpha diversity metrics between the two groups (Fig. 1[c], Table S5). We found no differences in alpha diversity between groups with and without a family history of vitiligo.

Comparison of beta diversity between groups with and without a family history of vitiligo revealed no significant differences in gut microbiome between the two groups (Bray-Curtis, $p=0.474$, $R^2=0.045$), ruling out genetic

susceptibility of disease in affecting community composition in the gut (Fig. S4). Additionally, we did not find a significant difference in microbiome composition with respect to diet and gender groups ruling out effects of these covariates (Fig. S4 and Table S5). We did not find any correlations between T-cell subpopulations counts ($CD4^+$, $CD8^+$, and Treg cells) and serum levels of IL-10 with alpha diversity metrics (Table S5).

Principal Coordinate Analysis (PCoA) showed separate clustering of the control and vitiligo groups. PERMANOVA analysis showed significant differences between vitiligo and control groups using Bray-Curtis distance (PC1 16.05%, PC2 12.99%, $p=0.005$, $R^2=0.058$) and weighted UniFrac distances (PC1 34.86%, PC2 18.52%, $p=0.004$, $R^2=0.0936$) (Fig. 1[a], Fig. S3).

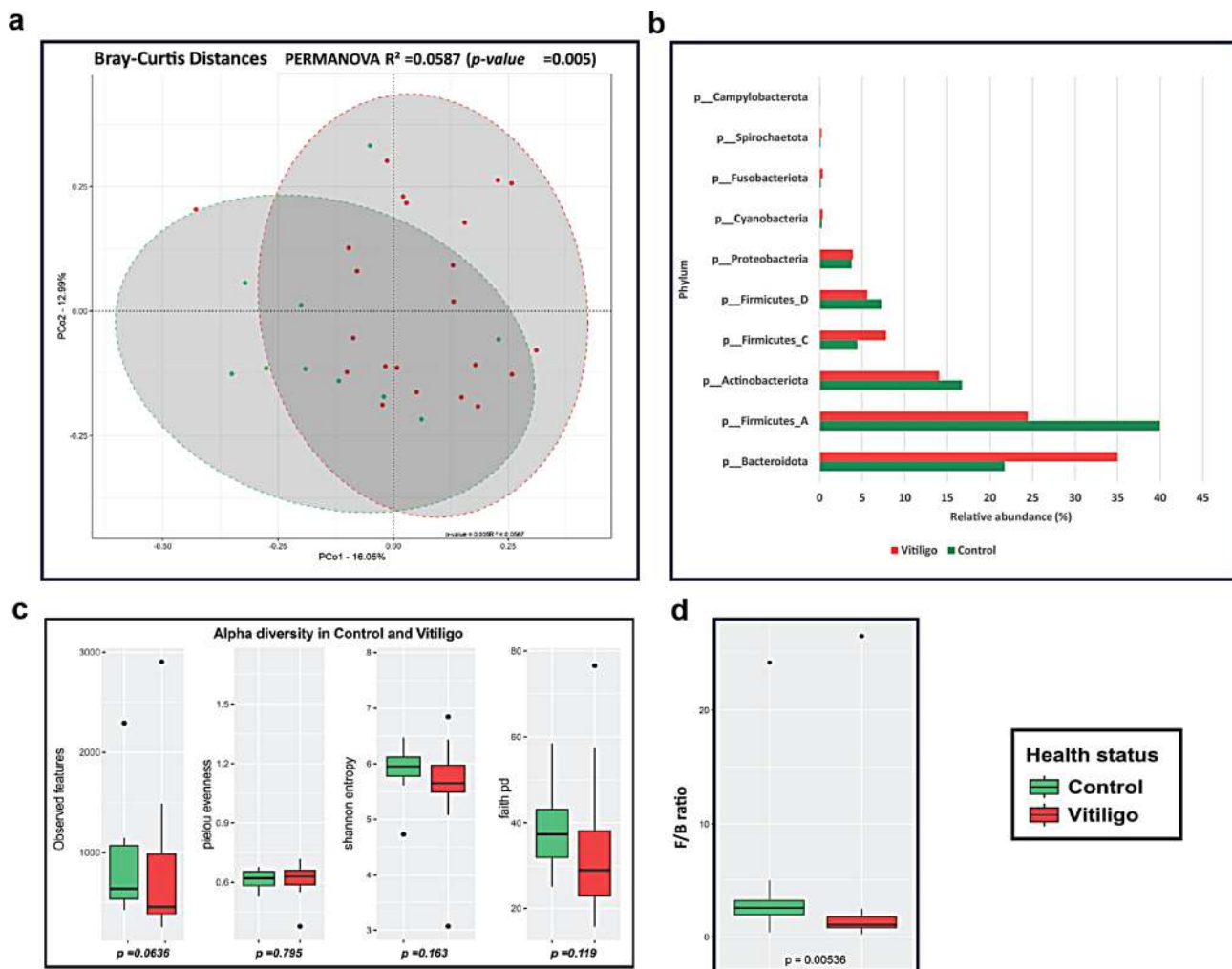


Fig. 1 Diversity of the gut microbiome in the control and vitiligo samples. **(a)** PCoA plot representing beta diversity differences between the control and vitiligo samples (PERMANOVA p -value is shown). **(b)** Average relative abundance (%) of Phyla in both groups. **(c)** Comparison of alpha-diversity metrics in sample groups (Wilcoxon rank sum test p -values are shown). **(d)** Comparison of *Firmicutes* to *Bacteroides* ratio in the control and vitiligo groups

Taxonomic composition and differentially abundant taxa

Taxonomic annotation using the Greengenes2 resulted in the identification of 19 bacterial phyla, 172 families, and 504 genera. At the phylum level, the control group had *Firmicutes_A* (39.96%) as the most abundant group, this was followed by *Bacteroidota* (21.70%), *Actinobacteria* (16.72%), *Firmicutes_D* (7.23%), and *Firmicutes_C* (4.41%). In the vitiligo group, the most abundant phylum was *Bacteroidota* (34.98%), followed by *Firmicutes_A* (24.46%), *Actinobacteria* (14.03%), *Firmicutes_C* (7.81%), and *Firmicutes_D* (5.56%) (Fig. 1[b]). The average relative abundance of phyla in both groups is given in supplementary data (Table S6).

Change in the ratio of *Bacteroides* to *Firmicutes* (F/B ratio) is often related to dysbiosis in gut microbiota in many diseases. A comparison revealed a significantly

lower F/B ratio ($p=0.005$) in the vitiligo group compared to the controls (Fig. 1[d]).

At the genus level, *Prevotella* (16.24%) was the most abundant taxa in the control group, followed by *Bifidobacterium_388775*, *Paraclostridium*, *Collinsella*, *Faecalibacterium*, *Catenibacterium*, *Gemmiger_A_73129*, *Agathobacter_164117*, *Cryptobacteroides*, and *Clostridium_T*. The vitiligo group had *Prevotella* (29.80%), followed by *Bifidobacterium_388775*, *Faecalibacterium*, *Collinsella*, *Agathobacter_164117*, *Dialister*, *Catenibacterium*, *Alloprevotella*, *Megasphaera_A_38685*, and *Paraclostridium* (Fig. 2, Table S6).

Differentially abundant genera in the control and vitiligo groups were identified using LEfSe. *Prevotella* was found to be roughly 1.8 times more abundant in the vitiligo group. Whereas *Eubacterium_B* (*Anaerovaccaceae*), *Pectobacterium* (*Enterobacteriaceae*), *Pygmaibacter* (*Ruminococcaceae*), *Nanosynbacter* (*Nanosynbacteraceae*), *Exiguobacterium_A* (*Exiguobacteriaceae*), *Romboutsia_D* (*Peptostreptococcaceae*), *Pseudomonas_E_647464* (*Pseudomonadaceae*), *Kosakonia_683478* (*Enterobacteriaceae*), *Gemella* (*Gemellaceae*), *Lachnospira* (*Lachnospiraceae*) and many others were found to be depleted in the vitiligo group and were at higher abundance in the control group (Fig. 3[a], Table S7).

We also identified differentially abundant species in vitiligo and healthy gut microbiome. At the species level, LEfSe identified *P. copri* and *Parabacteroides_B_862066 merdae* to be higher in the vitiligo group. In the control group, several taxa were found to be more abundant compared to the disease group, these include *Romboutsia_B_ilealis*, *Peptostreptococcus stomatis*, *Parafannyhessea umbonate_A*, and *Bulleidia massiliensis*, *Anaerobutyrium soehngenii* among others (Table S7, Fig. S5).

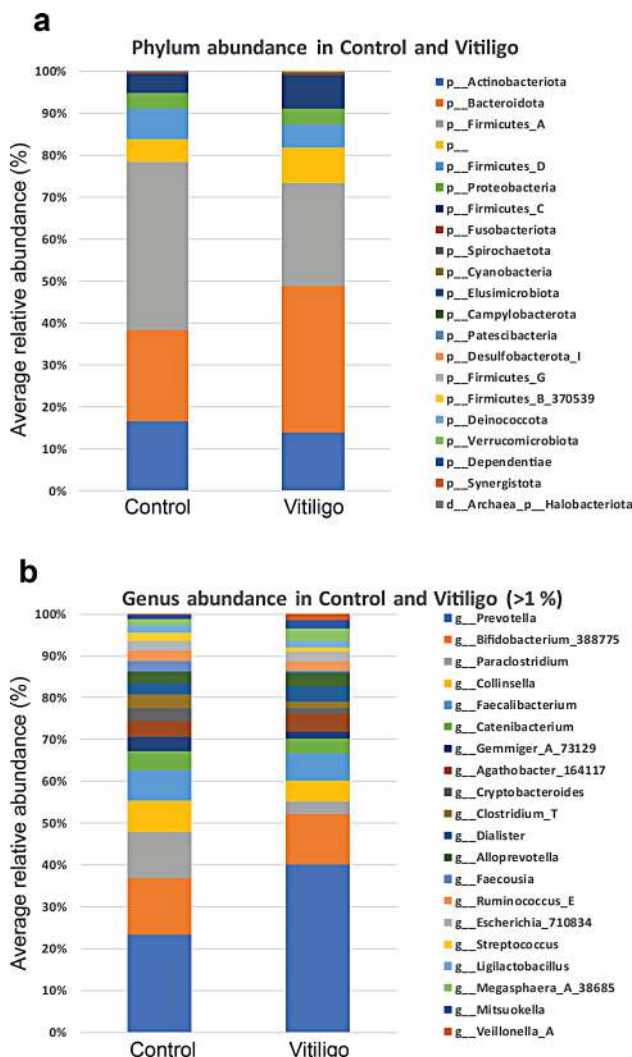


Fig. 2 (a) Composition and relative abundance (%) of microbes in the controls and vitiligo groups at the phylum level. (b) Composition and relative abundance (%) of microbes in the control and vitiligo groups at the genus level, only genera with more than 1% relative abundance are shown

Predictive metagenomic profiling using PICRUST-2

It is established that phylogenetically unrelated taxa can carry similar genes and contribute to common functions in different individuals. We looked at predicted functional profiles of vitiligo and healthy microbiomes. PCoA based on Bray-Curtis distance using the relative abundance of functions reflected similar clustering patterns and confirmed the results from ASV level clustering (Bray-Curtis PC1=60.09% and PC2=13.32%, $p=0.004$, $R^2=0.17$) (Fig. 3[b]).

LEfSe analysis identified several functions (KOs) differentially abundant in the vitiligo and control groups. LEfSe identified 39 differentially abundant KOs that were at higher abundance in the vitiligo gut microbiome. These KOs included genes associated with carbohydrate metabolism (5), glycan biosynthesis and metabolism (5), lipid metabolism (3), metabolism of amino acids (1), metabolism of cofactors and vitamins (1), biosynthesis

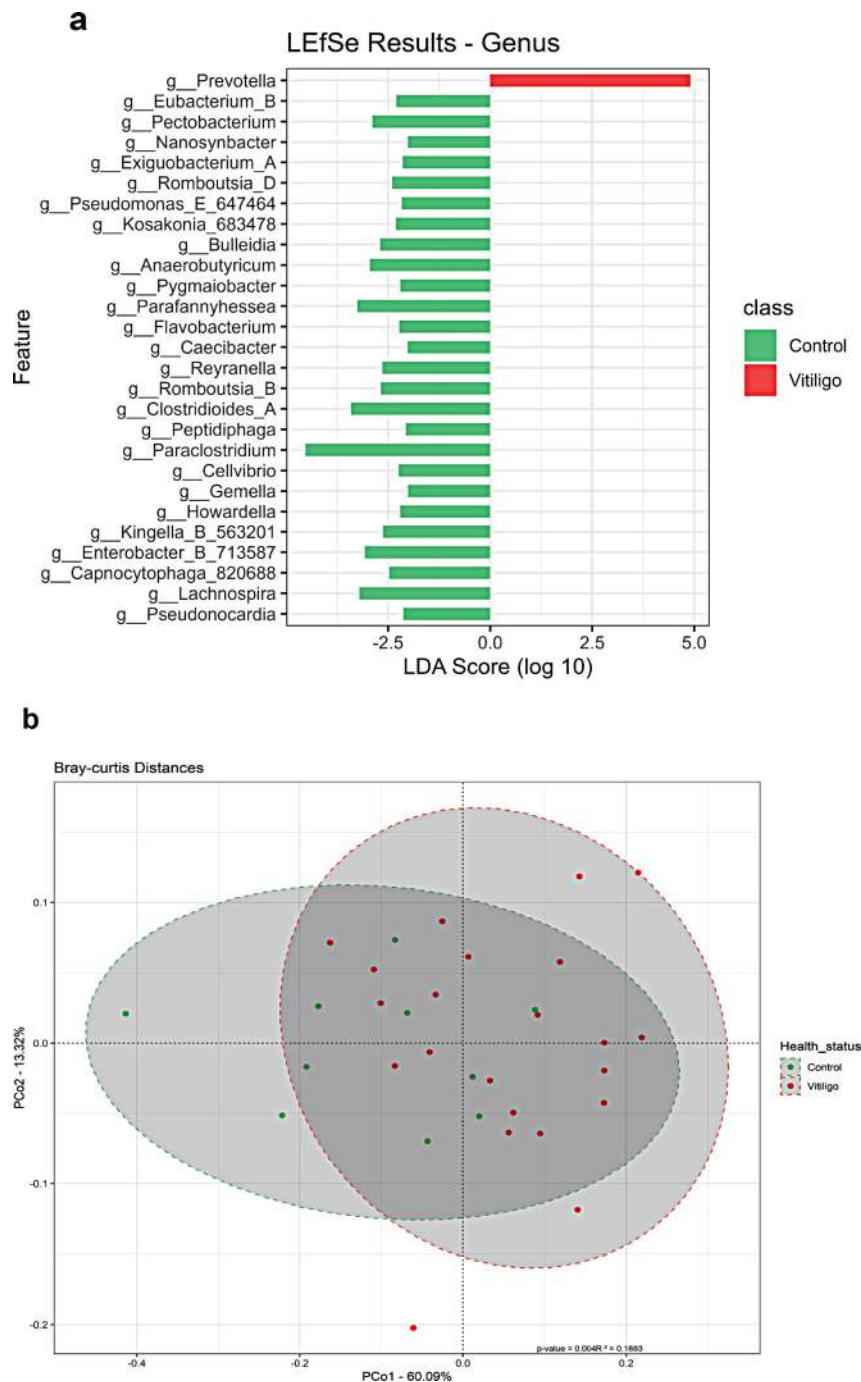


Fig. 3 (a) Differentially abundant genera identified by LEfSe analysis in the control and vitiligo groups. (b) PCoA plot based on Bray-Curtis distance using KO abundance data

of other secondary metabolites (1), antimicrobial drug resistance (3), etc., these KOs were mainly enzymes (19) and transporters (11) (Table S8, Fig. S6).

Interestingly, K12373 (HEXA_B; hexosaminidase), K01206 (FUCA; alpha-L-fucosidase), K05970 (SIAE; sialate O-acetyltransferase), and K02429 (fucP; MFS transporter, FHS family, L-fucose permease) were highly abundant in the vitiligo gut microbiome (Fig. 4 [a]). The

above-mentioned KOs have potential involvement in mucosal barrier loss by glycan degradation.

Along with these, we found K09808 (lolC_E; lipoprotein-releasing system permease protein), K12373 (HEXA_B; hexosaminidase), K07107 (ybgC; acyl-CoA thioester hydrolase), K00677 (lpxA; UDP-N-acetylglucosamine acyltransferase), K01897 (ACSL, fadD; long-chain acyl-CoA synthetase) were higher in vitiligo compared

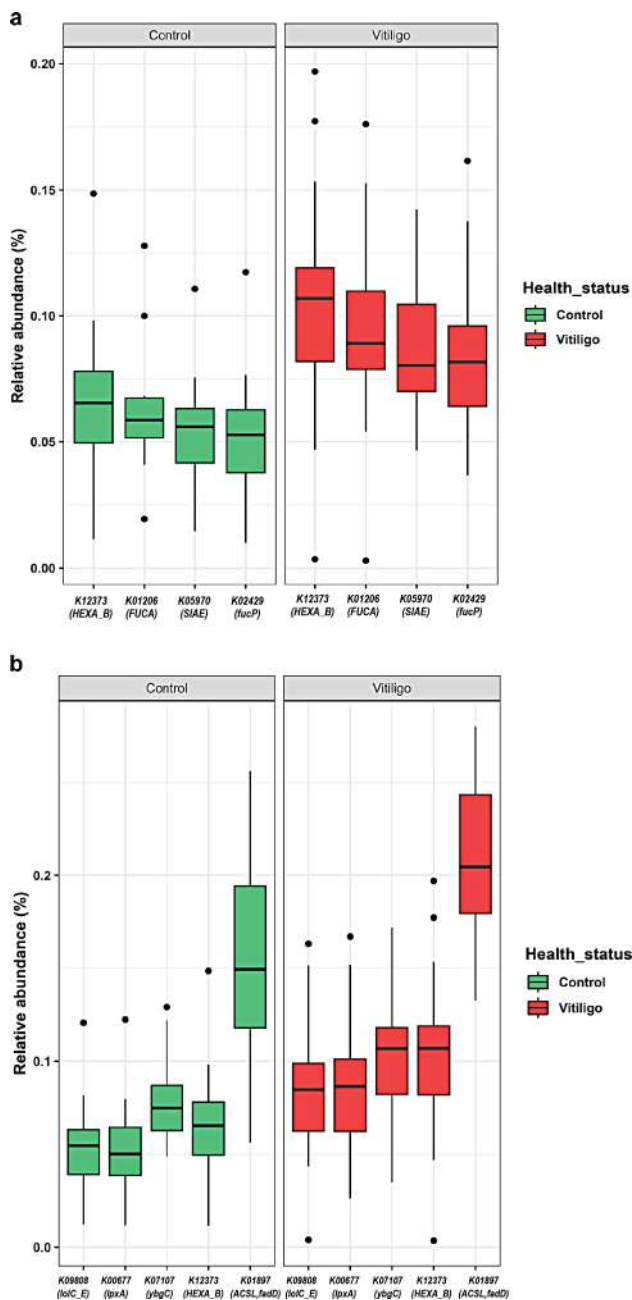


Fig. 4 (a) Relative abundance of genes related to possible role in mucus degradation identified by LEfSe analysis in the control and vitiligo groups. (b) Relative abundance of genes involved in Fatty acid and lipid metabolism functions identified by LEfSe in the control and vitiligo group

to the control group. These genes have potential roles in lipid metabolism, SCFA biosynthesis, LPS (Lipopolysaccharide) biosynthesis, and lipid transport (Fig. 4 [b], Table S8).

LEfSe analysis identified 21 KOs to be higher in the control group. These included genes of various types of transporters involved in transport of sugar, peptides, amino acid, iron, and sulfur. Many transcription factors were also higher in the control group. Additionally, genes

for the biosynthesis of amino acids, and carbohydrate metabolism (2) were also observed. The functional annotation of genes (KOs) in each group is given in the table (Table S8).

Network analysis

Based on the pairwise correlation calculated using genus-level abundance tables, two co-occurrence networks were generated for vitiligo and control group. The control network had 405 nodes and 11,330 edges, whereas in the vitiligo networks, there were 340 nodes and 3,169 edges. The control network had a higher average number of neighbors, clustering coefficient, and network density. A higher network density in healthy network suggests a more tightly connected community that is potentially robust to environmental perturbations. A high clustering coefficient in controls also indicates a more closely connected community.

The vitiligo network showed a higher number of modules, a higher network diameter, a longer characteristic path length, and a higher expected distances between two connected nodes, all suggesting relatively loose connectivity in the community. Characteristic path length which indicates the expected distance between connected nodes was higher in vitiligo. In brief, network analysis showed that topological characteristics of the vitiligo network were different from those of controls, suggesting altered community structure, which might have an influence on gut microbiome community assembly and its robustness in response to perturbations (Table S9).

Supervised learning classification with other vitiligo datasets

We used combined data from two populations, and utilized a random forest classifier to classify samples as healthy or vitiligo. The model trained on cross-cohort data from Indian and French populations could accurately predict the health status of samples with high accuracy (81.81% accuracy) using genus abundance data. *Bifidobacterium* and *Lactobacillus* genera and several *Lachnospiraceae* family members (*Bariatricus*, *Anaerobutyricum*, *Lachnospira*, *Howardella*, etc.) were among the most important genera identified in cross-cohort analysis (Fig. 5, Table S11). These genera represent the key differences between the healthy and vitiligo gut microbiome that are robust to population-specific differences in microbiome composition. Thus, it is apparent that the analysis of gut microbiome dysbiosis can provide valuable insights into the disease mechanisms.

Discussion

There has been extensive research into the role of human gut microbiota in a variety of health conditions, diets, populations, etc. However, studies from Western

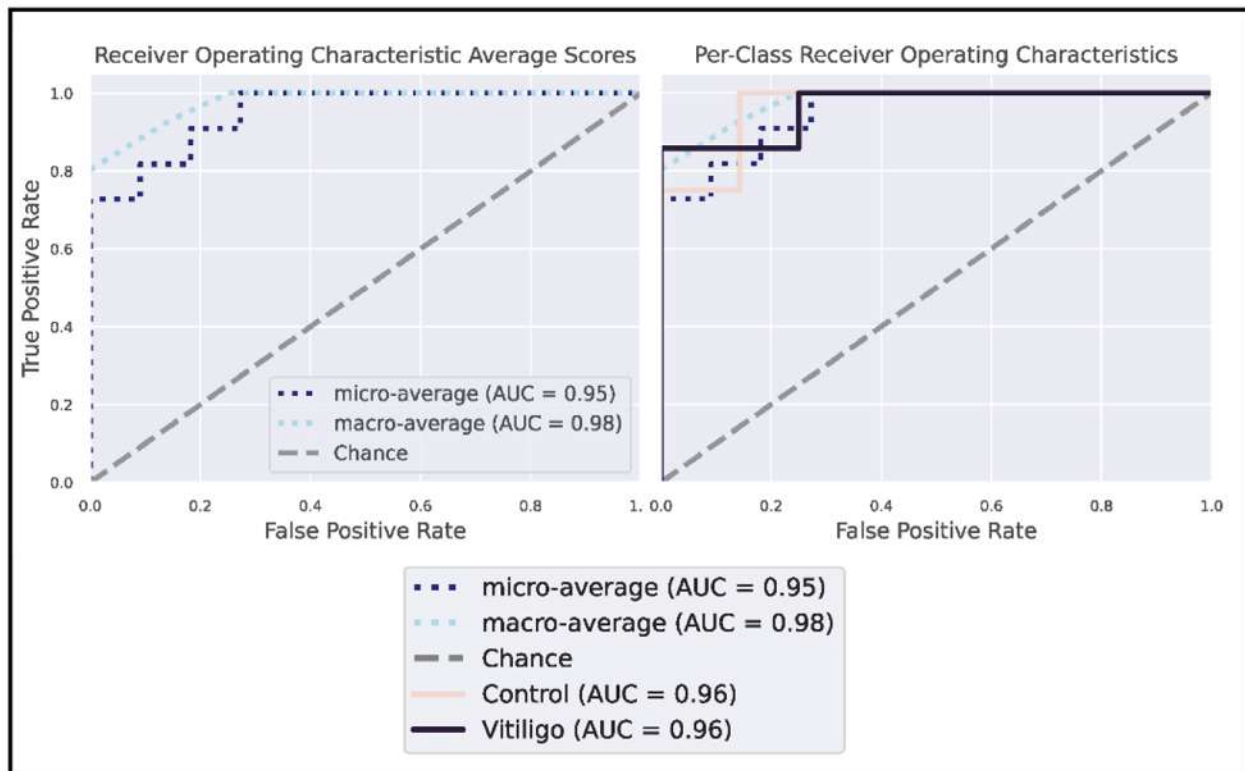
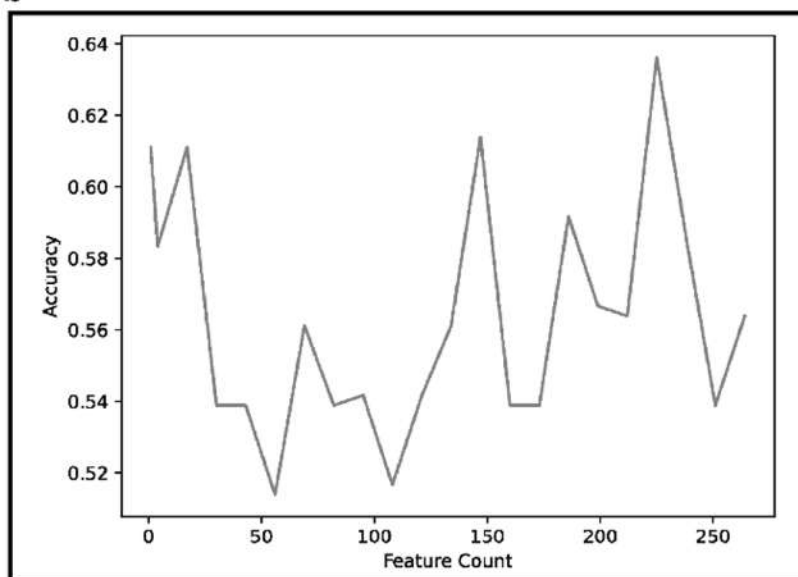
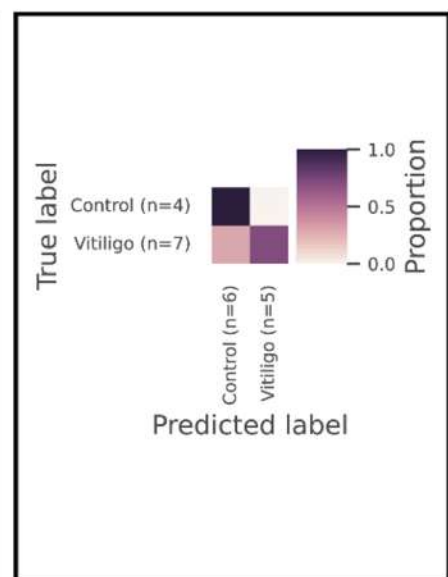
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Fig. 5 (a) Receiver operating characteristic plots for random forest classifier trained on cross-cohort data. (b) Plot of recursive feature extraction showing changes in accuracy with the number of features used. (c) Heatmap of disease predictions based on genus abundance data

populations continue to be overrepresented, with very few studies from non-Western populations such as India and Africa. This lack of representation also extends to autoimmune disease microbiome research [34, 47–51]. The interaction of the gut microbiome with the immune

system is becoming increasingly important in understanding autoimmune diseases. Widely studied diseases like IBD and Coeliac disease also show skin co-morbidities like dermatitis and psoriasis [52, 53]. There is accumulating evidence on the role of gut microbiota in

various immune-mediated dermatological diseases like psoriasis and AD, the existence of a gut-skin axis has also been proposed [7, 53]. Previously, antibiotic-driven gut dysbiosis has been shown to increase depigmentation in mice [32]. There are also case reports where fecal microbiota transplant (FMT) mediated restoration of the gut microbiome led to the reversal of symptoms in Alopecia areata, showing the importance of a healthy gut microbiome in pathogenesis [54, 55]. These observations suggest the potential role of microbiome dysbiosis in the pathogenesis of vitiligo (Fig S7). The gut microbiota may exert its influence through systemic effects of the immune system or through secreted metabolites. Studying the gut microbiome in these diseases can provide insights into their pathogenesis and possibly lead to novel therapeutic strategies.

In this study, we explored the changes in taxonomic and functional composition of gut microbiome in an Indian cohort of vitiligo patients and healthy controls. We observed a decrease in the *Firmicutes* to *Bacteroidetes* ratio (F/B ratio) in the vitiligo group. Similar shifts have been observed in IBD and autoimmune dermatological diseases like SLE and psoriasis in different populations [7, 56, 57]. Although, it must be noted that these findings remain inconsistent across studies. Our observations of F/B ratios are also contrasting with previous reports of vitiligo in French and Chinese subjects [29, 30]. Reported differences in the F/B ratio in the population may be confounded by dietary differences in Western and non-Western populations.

The observation of a higher abundance of *Prevotella* in the disease group is similar to many studies in psoriasis, it has also been known to exacerbate inflammatory phenotype in RA [33, 58, 59]. However, it should be noted that most of these studies come from western populations. As *Prevotella* is the most abundant genera with *P. copri* as the dominant species in the gut microbiome of the Indian population, the observed increase in the disease group should be looked at with caution (Table S7 and Table S10). Strain level variability in *Prevotella* needs to be explored to identify any vitiligo associated *Prevotella* strains for further insights. Also, it is tempting to speculate that the reduction in the abundance of several healthy gut commensals in Indian vitiligo patients as discussed in the following paragraph could be the driver for the further increase in abundance of *P. copri*, which is the most abundant species in a healthy Indian gut microbiome [48–50]. Thus, the observed increased abundance of *P. copri* could be a result of the depletion of other commensals and may not be associated with vitiligo. Further, an increase in this species was not noted in the analysis of the Chinese and French gut microbiome studies [29, 30, 60].

The vitiligo group showed a substantially reduced abundance of numerous microorganisms commonly linked to healthy gut microbiomes. Genera like *Lachnospira*, *Romboutsia* (*R. hominis* and *R. ilealis*), *Eubacterium*, and *Anaerobutyricum* (*A. soehngenii*) have been consistently associated with healthy gut microbiome in phenotype comparisons [61]. *Lachnospira*, *Anaerobutyricum*, and *Howardella* are members of the family Lachnospiraceae and are important butyrate-producing taxa. Butyrate production is associated with epithelial cell health and the maintenance of mucous barrier function [62–64]. Similarly *Eubacterium* members have also been shown to have healthy effects and explored as a potential probiotic [65, 66]. Their positive influence on gut microbiota can be attributed to SCFA production capabilities, which serve as important energy sources for intestinal epithelial cells [61, 67, 68]. SCFAs can also enhance regulatory capacity of Tregs which contribute to immune suppression, thus having critical role in autoimmune disease [1].

Interestingly, many taxa associated with healthy gut microbiome were also among the most important features for classification of healthy or disease in the cross-cohort dataset, these included many members of *Lachnospiraceae* (Table S11). The changes in the genus and species abundance show similarities to previous reports of other diseases, differences in these observations can also be attributed to confounders like population, age, diet, and disease severity.

With increase in availability of genome data for bacterial species, the accuracy of prediction of functional potential using PICRUSt2 is improving. The observation of several genes related to mucin degradation in LEfSe selected differentially abundant features suggests microbially mediated degradation of the gut mucosal layer. Mucin-degrading bacteria have sialidases and fucosidases to degrade terminal residues of mucin glycoproteins. Degradation of mucin by the activity of gut microbiota can result in a compromised intestinal mucosal barrier, which can lead to increased intestinal permeability leading to translocation of antigens in systemic circulation [69]. This loss of intestinal permeability in vitiligo has been confirmed by another study utilizing zonulin-based assays [70].

Previous work on serum metabolomics has shown the role of fatty acid metabolic pathways in vitiligo. For example, the serological level of alpha-linolenic acid (ALA) was upregulated and also had positive correlations with disease severity in vitiligo [29, 71]. The observation of a higher abundance of fatty acid and lipid metabolism-related functions in vitiligo in this work also suggests similar changes. The presence of free fatty acids in serum has also been confirmed in other diseases with intestinal dysbiosis [56]. It is important to explore

microbial metabolism of free fatty acids (FFA) as they can be active players in the modulation of the immune system by gut bacteria [72]. We also found an increased abundance of folate biosynthesis gene *folK* (K00950) in the vitiligo group, which has been previously reported to be high in vitiligo [73]. Interestingly, a higher abundance HEXA_B; hexosaminidase (K12373) in the vitiligo group was observed, HEXA_B has been reported to play protective roles in intestinal inflammation by modulating T-cell populations [74]. Additionally, a comparison of co-occurrence networks also revealed structural differences in the community assembly of vitiligo and control groups. Such changes can have effects on the robustness of the gut microbial community in scenarios of environmental perturbations like disease, stress, and antibiotic consumption [42].

Further, the model trained on cross-cohort data of genus abundance from Indian and French populations could accurately classify samples as healthy or vitiligo samples and identified differentiating taxa similar to the observations made by the microbiome analysis, affirming the presence of key differences between the healthy and vitiligo gut microbiome. Among the genera that showed predictive capacity for healthy or vitiligo samples, *Bifidobacterium* emerged as the most important genus, which is also a widely distributed commensal in human gut. Different strains of *Bifidobacterium* species are known to modulate the immune system by regulating suppressive regulatory T cells. The absence or reduction of this genus has been linked to autoimmune and inflammatory conditions like CD. *Bifidobacterium* is also a potent acetate producer and its metabolites can affect the community structure by modulating cross-feeding relations in the community [75]. Presence of several other SCFA producers including *Lachnospira*, *Anaerobutyricum*, and *Roseburia*, in cross cohort datasets also supported the observation of reduced SCFA producing bacteria in vitiligo [76]. Such machine learning-based approaches can help identify protective and risk factors in microbiome data that are consistent across cohorts and are less prone to confounders of lifestyle factors, population origin, and diet.

Conclusions

In conclusion, this study examined the gut microbiome data from the Indian cohort of vitiligo patients and showed alteration in taxonomic composition, functional composition, and community structure of gut microbiome in vitiligo patients. Several genera associated with healthy gut microbiome in many phenotype comparisons on the GMrepo database were depleted in the gut microbiome of the vitiligo group compared to the healthy group. These genera might be involved in beneficial activities such as production of SCFA that have positive

protective effects by maintaining the epithelial layer [61]. The findings also suggest potential functional alterations in the gut microbiome of vitiligo patients which might have roles in modulating immune system, thus affecting the pathophysiology of the disease. The inherent mechanisms can be further investigated in future studies. The results also strengthen the possible roles of the gut microbiome in the pathogenesis of vitiligo and provide clues to explore the dietary interventions and probiotics for its management by modulating the gut microbiome [26].

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-024-03529-5>.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10
Supplementary Material 11
Supplementary Material 12

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Author contributions

VKS and AK conceived the work and designed the study. NC and DA performed subject recruitment and sample collection. SM performed sample processing, library preparation, and sequencing work with the help of DK. AK performed ELISA and genotyping. SK designed the framework of computational analysis and carried out data processing, statistical analysis, and interpretation of results. SK prepared the first draft of the manuscript under the supervision of VKS. All authors have read and approved the final manuscript.

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Data availability

The datasets generated during the current study are available at NCBI under BioProject ID PRJNA1064480.

Declarations

Ethics approval

The study design was reviewed and approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Bhopal, India. Written informed consent was obtained from all the subjects prior to any study-related procedures.

Competing interests

The authors declare no competing interests.

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