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Gut microbiota signatures in food allergy children without and with malnutrition: a cross-sectional study

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Abstract

Background Altered gut microbiota (GM) profiles have been documented in children with food allergies (FA) and experiencing malnutrition. This study explored the GM composition in children with FA across varying degrees of malnutrition including those without malnutrition and those with different severity levels.

Methods Fresh faecal samples were collected from 120 children aged 1–6 years, including 40 FA children with adequate weight (FANM), 40 FA children with malnutrition (FAM), and 40 healthy controls. The hypervariable region of the 16 S rDNA gene was subsequently sequenced to assess bacterial communities.

Results Compared with healthy controls, the FANM group displayed a greater increase in the alpha diversity index. The FAM group exhibited an increase in seven genera, including *Alistipes* and *Parabacteroides*, compared to the control group, whereas nine genera were enriched in the FANM group. An analysis of clinical characteristics revealed a positive correlation between the relative abundance of the genus *Faecalibacterium* and the total IgE level. Fourteen pivotal microbial markers demonstrated substantial classification potential (AUC: 89.86%, 95% CI: 76.40–99.73% for FAM; AUC: 88.92%, 95% CI: 73.58–99.65% for FANM).

Conclusion FA children exhibit distinct GM profiles depending on the presence of malnutrition, which suggests the need for tailored microbiota-targeted therapies.

Keywords Allergy and immunology, Malnutrition, Child, Gut microbiota

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Introduction

Food allergy (FA) is an immune response that can be potentially life-threatening and is triggered by the ingestion of food protein antigens [1]. The onset of FA typically manifests during childhood and impacts 10% of infants and 5-8% of children [2]. FA poses a dual risk and encompasses potentially life-threatening reactions and significant implications for quality of life along with imposing substantial health care costs [3, 4]. Furthermore, the widespread avoidance of foods, such as chicken eggs and cow milk, which are vital nutrient sources for young children with FA, may lead to nutritional deficiencies and potential growth impairment [5, 6]. Consequently, FA often coincides with various physical manifestations, such as weight loss, in children [7]. However, the mechanisms underlying the poor nutritional status in children with FA remain inadequately understood.

Recently, the potential impact of the gut microbiota (GM) on host health has garnered significant attention. Emerging evidence suggests a link between the GM and various diseases including FA and weight status [8, 9]. Disruptions in the GM, such as decreases in microbial richness, diversity, and evenness, coupled with increases in the Firmicutes/Bacteroidetes ratio, have been observed in allergic as well as malnourished children [10–12]. Additionally, dysbiosis of the GM has been identified in individuals with FA, and the transfer of dysbiotic GM from individuals with FA to mice has exacerbated allergic symptoms [12, 13]. Furthermore, studies using rat models of FA and meta-analyses of randomized human clinical trials suggest that administering probiotics can reduce the occurrence of FA [14, 15]. However, several studies on GM transplantation indicate a possible causal link between GM and weight status [16, 17]. These findings underscore a strong association between GM, FA and nutritional status and highlight the importance of GM in regulating FA and growth in children. Nonetheless, specific alterations in GM among FA children with differing nutritional statuses have not been systematically examined.

Hence, by employing an exploratory methodology the aim of this cross-sectional study was twofold: (1) to compare GM profiles among children with FA, those with FA and poor nutritional status, and healthy control children matched for sex and age distribution, and (2) to investigate whether distinctions in GM composition are discernible among children with FA and varying severity levels of malnutrition compared with healthy control children.

Materials and methods

Study population

A total of 40 children exhibiting food allergies and average weight, alongside 40 children experiencing food allergies with malnutrition, and an additional 40 healthy control children (all matched for age and sex, aged 1–6 years), were recruited from the Zhuhai Maternal and Children Care Center for a cross-sectional study from June 2022 to October 2023. The exclusion criteria included (1) preterm birth, postmaturity, or congenital malformations; (2) gastrointestinal symptoms stemming from organic or infectious diseases, including acute and chronic gastritis, peptic ulcers, colitis, and appendicitis; (3) infectious urticaria and infectious respiratory diseases; (4) treatment with systemic antibiotics or probiotics within one month prior to recruitment; and (5) treatment with systemic immunosuppressive agents. Simultaneously, a group of age-matched healthy children with no history of allergic conditions or malnutrition were enrolled during the same study period. Informed consent was obtained from the guardian of each child. The study was conducted in compliance with the Declaration of Helsinki and approved by the Ethics Committee of the Zhuhai Maternal and Children Care Center (No.2022011011).

Diagnosis of FA and malnutrition

The diagnosis of a food allergy followed the protocol outlined by Osborne et al. [2] Children received escalating doses of food-specific allergens, including peanuts, wheat, fish, milk, egg whites, and soybeans, at 15–30-minute intervals until all doses were tolerated or a reaction occurred. A positive food challenge was defined by the presence of objective signs within two hours of the final dose including ≥ 3 concurrent, noncontact hives lasting ≥ 5 min, angioedema, vomiting, or anaphylaxis [18]. The children who did not react on the first day continued consuming the challenge food for the next seven days. Reactions within the subsequent seven days that met the stopping criteria were considered late reactions and indicative of a positive food challenge. Negative challenges were determined if the children tolerated all doses on the challenge day, and reported no late reactions. Blood samples were collected on the challenge day and the total IgE level was quantified using a latex-enhanced immunoturbidimetric method (Reebio, Ningbo, China).

The body weight (rounded to the nearest 0.05 kg) and height (rounded to the nearest 0.5 cm) of each child were measured and used to calculate body mass index (BMI; body weight/height^2 [kg/m^2]). We utilized the WHO Anthro (version 3.2.2), which is an anthropometric Z score calculator, to determine anthropometric parameters including the weight-for-age Z score (WAZ), height-for-age Z score (HAZ) and BMI-for-age Z score (BAZ). Malnutrition was defined as a standardized WAZ <-2 based on anthropometric measurements according to WHO Child Growth Standards [19]. Moderate malnutrition (-2 ≤ WAZ≤-2.9) or severe malnutrition (WAZ≤-3)

was categorized according to the recommended identification of paediatric malnutrition [20].

Clinical characteristics

Demographic and clinical profiles were comprised of age (continuous), gestational weeks (continuous), birth weight (continuous), birth height (continuous), gender (male or female), mode of delivery (caesarean or vaginal delivery), duration of breastfeeding (continuous), maternal educational level (junior high school and below, senior high school, bachelor's degree and postgraduate and above), maternal occupation (clerks, housewives, and others), and household average monthly income (≤ 8000 yuan [≤ 1095.5 USD], 8001–10000 yuan [1095.6-1369.3 USD], 10001–12000 yuan [1369.4-1643.2 USD], and ≥ 12001 yuan [≥ 1643.3 USD]) and were obtained through face-to-face interviews. Feeding issues experienced by children were assessed using the Chinese version of the Montreal Children's Hospital Feeding Scale, which previously demonstrated good validity and reliability in Chinese children [21].

Sample collection and DNA extraction

Using a sterile specimen faecal collection container (MANTACC, Shenzhen, China), faeces were collected while preventing their disposal into the toilet and to mitigate the risk of sample contamination by urine and other substances. Participants were then required to employ a collection spoon to transfer no less than 10 g of faeces into a preservation tube containing DNA-later solution and promptly froze the sample at -80 °C within 1 h. Microbial community genomic DNA was extracted from faecal samples using the TIANamp Stool DNA Kit (TIANGEN, Beijing, China) by following the manufacturer's instructions. The extracted DNA was subsequently assessed on a 1% agarose gel. Moreover, the DNA concentration and purity were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) by examining ratios of 260/280 nm and 260/230 nm, respectively.

PCR amplification and sequencing

A Miulab PR-96E PCR thermocycler (Miu Instrument Co., Hangzhou, China) was used to amplify the V3-V4 hypervariable region of the bacterial 16 S rDNA gene using the primer pairs 515 F (50-GTGCCAGCMGCCGCGGTAA-30) and 806R (50-GGACTACHVGGGTWTCTAAT-30). PCR amplification of the 16 S rDNA gene included initial denaturation at 98 °C for 1 min followed by 30 cycles of denaturation at 98 °C for 15 s, annealing at 58 °C for 15 s, and elongation at 72 °C for 15 s; a final extension occurred at 72 °C for 1 min followed by cooling to 4 °C. PCR mixtures consisted of 25 μL of PCR Master Mix, 5 μL of forward primer, 5 μL of reverse primer, 10 μL of gDNA, and 5 μL of H2O. PCR reactions were conducted in triplicate. Following amplification, the PCR product was extracted from a 2% agarose gel, purified using the Vazyme Hieff NGS® DNA Selection Beads (Vazyme, Shanghai, China) following the manufacturer's protocol, and quantified using a Qubit™3 (Thermo Fisher Scientific, Waltham, USA). Purified amplicons were then pooled equimolarly and subjected to paired-end sequencing on an Illumina NovaSeq 6000 platform (BGI, Shenzhen, China).

Processing of sequencing data

Raw paired-end sequencing data were processed using Mothur (version v1.45.3). Quality filtering was performed using sliding window-based trimming where sequences were truncated if the average quality score within a 50-bp window fell below Phred Q20 (qwindowaverage = 20, qwindowsize = 50). Primer sequences targeting the V4 region were removed from merged sequences using the trim.seqs command and allowed up to two mismatches in the primer regions (pdiffs = 2, bdiffs = 0) to ensure accurate extraction of the amplification region. Sequences between 250 and 275 bp with no ambiguities or homopolymers exceeding 8 bp were retained. Chimeric sequences were identified and removed using the UCHIME algorithm with the SILVA SSU Ref NR99 v138.1 alignment as the reference database (reference = silva.nr_v138.align). High-quality reads, which were defined by a 97% nucleotide similarity threshold, were clustered into operational taxonomic units (OTUs). The representative sequences for each OTU were classified using the classify.seqs command (cut-offs = 80) with the SILVA version 138 database. Taxonomic distributions of OTUs were summarized using these taxonomies and enabled the calculation of relative abundances of microbiota at various levels, including phylum, class, order, family, and genus.

Descriptive analyses

Data analysis was conducted using R version 4.3.0. Continuous variables were compared between groups using analysis of variance (ANOVA), whereas categorical variables were analysed using the $\chi 2$ test. For nonnormally distributed variables, group comparisons were conducted using the Kruskal-Wallis test.

Alpha and Beta diversity analyses

The R package MicrobiotaProcess was utilized to assess microbial alpha and beta diversity indices. Bacterial alpha diversity was calculated using the observed species, Chao, ACE, Shannon, Simpson and Pielou's evenness indices. The Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test were used to detect differences in alpha diversity. Principal coordinate analysis

(PCoA) using Bray-Curtis dissimilarity was performed to assess beta diversity. Permutational multivariate analysis of variance (PERMANOVA) was used to compare differences in beta diversity between groups.

Differential taxonomic compositions of GM among groups

For a more detailed examination of microbiota differences and identification of potential biomarkers among the three groups, we employed the linear discriminant analysis effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/galaxy). Initially, the Kruskal-Wallis sum-rank test was conducted to assess changes and dissimilarities among classes; this was followed by logarithmic linear discriminant analysis (LDA) to ascertain the effect size of each distinctly abundant taxon. Specifically, taxa with an LDA score exceeding 3.0 and false discovery rate (FDR)-adjusted *P* values below 0.05 were considered significant.

Associations between GM and clinical parameters

Spearman's correlation coefficients were evaluated to examine associations between GM at the genus level and clinical factors with the FDR controlled using the Benjamini-Hochberg method to account for multiple tests. A correlation was deemed significant when the absolute value of Spearman's rank correlation reached statistical significance ($P < 0.05$). The pheatmap package was utilized to depict these associations via a correlation heatmap.

Biomarker identification

A tenfold cross-validation comprised of five trials was executed to pinpoint the optimal microbial biomarkers at the genus level using a random forest model with the cut-off point determined by the mean of the minimum cross-validation error. The most discriminative biomarkers were selected based on the mean decrease accuracy, a feature importance score in the random forest model, and constituted of the optimal set with the lowest error rate. The probability of disease (POD) index was subsequently calculated as the ratio of samples predicted as cases to healthy controls among the randomly generated decision trees in the random forest model. The identified optimal set of biomarkers was then utilized to calculate the POD index for the training dataset (60%) and the testing dataset (40%). Receiver operating curve (ROC) analysis and the area under the curve (AUC) were employed to assess the strength of the constructed models. All random forest models were adjusted for confounders, which were identified as differential factors between the three groups with *P* values ≤ 0.2 in the univariate analysis, including BAZ, maternal education, maternal occupation and feeding difficulty scores.

Results

Characteristics of participants

Population demographics and disease attributes are detailed in Table 1. Within the FAM group, there was a tendency towards lower odds of mothers being clerks and higher scores for feeding difficulties (all $P < 0.05$). Additionally, a trend towards lower birth weight was noted in the FAM group, although the disparity was marginally significant ($P = 0.06$). Participants across the three groups were comparable in terms of mode of delivery, maternal education, average household monthly income, and other pertinent variables. In terms of GM composition, *Firmicutes* and *Bacteroidetes*, the two predominant phyla, presented varying ratios among groups at the phylum level (Supplementary Fig. 1).

Alpha diversity

An intragroup analysis was conducted to compare alpha diversity among the FAM, FANM, and control groups (Fig. 1). Notably, the FA group presented lower ACE, Chao1 and observed OTU values than the control group, although no statistically significant difference was detected in the FAM group compared with the control group. Upon further classification of the FAM group into two categories, 19 children were categorized as belonging to the FA with moderate malnutrition (FAMM) group, whereas 21 children were classified as belonging to the FA with severe malnutrition (FASM) group. GM richness, as indicated by the observed OTUs and the Chao1 and ACE indices, was significantly lower in the FAMM group than in both the control and FASM groups (Supplementary Fig. 2).

Beta diversity

By utilizing the Bray-Curtis distance metric at the OTU level, PCoA revealed a similar divergence between the FAM and FANM groups and the control group across the initial two principal coordinates. This discrepancy was substantiated by the PERMANOVA test results ($R^2 = 0.02$, $P = 0.33$) (Fig. 2). Furthermore, no notable differences in beta diversity were evident among the FASM, FAMM, FANM, and control groups ($R^2 = 0.03$, $P = 0.41$; Supplementary Fig. 3).

Differential taxonomic compositions of gut microbiota among groups

To delve deeper into nuanced variations in GM, LEfSe and LDA analyses were conducted to compare the FAM and FANM groups with the control cohorts and revealed significant differences in the predominant GM compositions (Fig. 3). These dominant GM were identified based on microbial taxa with average relative abundances exceeding 1% in any of the three groups. At the genus level, FAM were enriched in *Alistipes*,

Table 1 Demographic and clinical profile of participants

Characteristics	FAM, *N* = 40	FANM, *N* = 40	Control, *N* = 40	*P*[a]
Sex				0.74
Male	16 (40.00)	19 (47.50)	19 (47.50)	
Female	24 (60.00)	21 (52.50)	21 (52.50)	
Age, Mean ± SD, years	3.00 ± 1.64	2.65 ± 1.44	2.75 ± 1.49	0.58
Mode of delivery				0.64
Vaginal delivery	25 (62.50)	22 (55.00)	21 (52.50)	
Cesarean delivery	15 (37.50)	18 (45.00)	19 (47.50)	
Gestational weeks, Mean ± SD, weeks	38.70 ± 1.14	38.75 ± 1.01	38.75 ± 1.08	0.97
Birth weight, Mean ± SD, g	3.06 ± 0.36	3.25 ± 0.31	3.32 ± 0.70	0.06
Birth height, Mean ± SD, cm	49.33 ± 1.40	49.65 ± 0.92	49.98 ± 1.48	0.08
BAZ, Mean ± SD	-1.69 ± 0.86	-0.17 ± 0.79	0.07 ± 0.59	< 0.01
Duration of breastfeeding, Mean ± SD, months	9.15 ± 4.17	8.43 ± 5.19	9.10 ± 6.46	0.80
Maternal education				0.12
Junior high school and below	2 (5.00)	0 (0.00)	3 (7.50)	
Senior high school	3 (7.50)	0 (0.00)	3 (7.50)	
Bachelor degree	3 (7.50)	8 (20.00)	3 (7.50)	
Postgraduate and above	32 (80.00)	32 (80.00)	31 (77.50)	
Maternal occupation				< 0.01
Clerks	11 (27.50)	29 (72.50)	26 (65.00)	
Housewives	7 (17.50)	0 (0.00)	5 (12.50)	
Others	22 (55.00)	11 (27.50)	9 (22.50)	
Household average monthly income				0.56
≤ 8000 yuan	11 (27.50)	5 (12.50)	6 (15.00)	
8001–10,000 yuan	2 (5.00)	5 (12.50)	6 (15.00)	
10,001–12,000 yuan	6 (15.00)	6 (15.00)	6 (15.00)	
≥ 12,001 yuan	21 (52.50)	24 (60.00)	22 (55.00)	
Feeding difficulty scores, Mean ± SD	55.40 ± 5.28	52.85 ± 5.62	47.53 ± 3.57	< 0.01
Total IgE, Mean ± SD, IU/mL	123.85 ± 73.13	158.87 ± 37.27	60.00 ± 55.10	0.44

Values are no. (%) except as indicated

FANM, food allergy children without malnutrition; FAM, children with food allergy and malnutrition; BAZ, BMI-for-age Z score; SD, standard deviation

[a]Pearson's Chi-squared test; One-way ANOVA; Fisher's exact test

Parabacteroides, *Ruminococcus_torques_group*, *Lachnospiraceae_NK4A136_group*, *Incertae_Sedis_f__Ruminococcaceae*, *Subdoligranulum* and *Phascolarctobacterium*. Notably, nine genera, including *Ruminococcus_torques_group*, *Agathobacter*, *Anaerostipes*, *Lachnospira*, *Lachnospiraceae_NK4A136_group*, *Monoglobus*, *Faecalibacterium*, *Incertae_Sedis_f__Ruminococcaceae*, and *Subdoligranulum* were more prevalent in the FANM group than in the control group. Furthermore, alterations in microbiome composition among the FAMM, FASM and healthy control groups were also analysed (Supplementary Fig. 4).

Associations between gut microbiota and clinical characteristics

Correlations between the aforementioned significant taxa and clinical characteristics were examined (Fig. 4). Following FDR adjustment, *Faecalibacterium* was positively associated with the total serum IgE level and mode of delivery. Specifically, *Faecalibacterium* was more abundant in children who were delivered via caesarean section than in those who were delivered vaginally. Notably, no significant correlation between these taxa and BAZ was detected.

Identification and validation of GM markers for the FAM and FANM

To demonstrate the diagnostic potential of GM for the FAM and FANM, we developed two random forest classifier models designed to specifically distinguish the FAM and FANM samples from those of healthy controls (Figs. 5 and 6). In the training phase, 60% of samples were randomly allocated to the training dataset, which were comprised of 24 FAMs vs. 24 controls and 24 FANMs vs. 24 control samples, with the remainder reserved for the validation dataset. The cross-validation error slope stabilized when the number of OTUs exceeded fourteen in the discovery and validation phases (Figs. 5A and 6A). Thus, fourteen optimal genera were selected as the marker set for both models based on their importance (Figs. 5B and 6B). The POD value was significantly greater in the FAM group than the control group in the training and validation phases (Figs. 5C and 6C). In addition, the POD value significantly increased in the FANM group compared with the control group (Figs. 5D and 6D). The POD index achieved an AUC value of 89.86% (95% CI: 76.40–99.73%) for the FAM vs. the control group and 88.92% (95% CI: 73.58–99.65%) for the FANM vs. the control group during the validation phase.

Discussion

In our study, differences in GM composition and a microbiome signature were more effectively differentiated between FA with and without malnutrition from sex- and

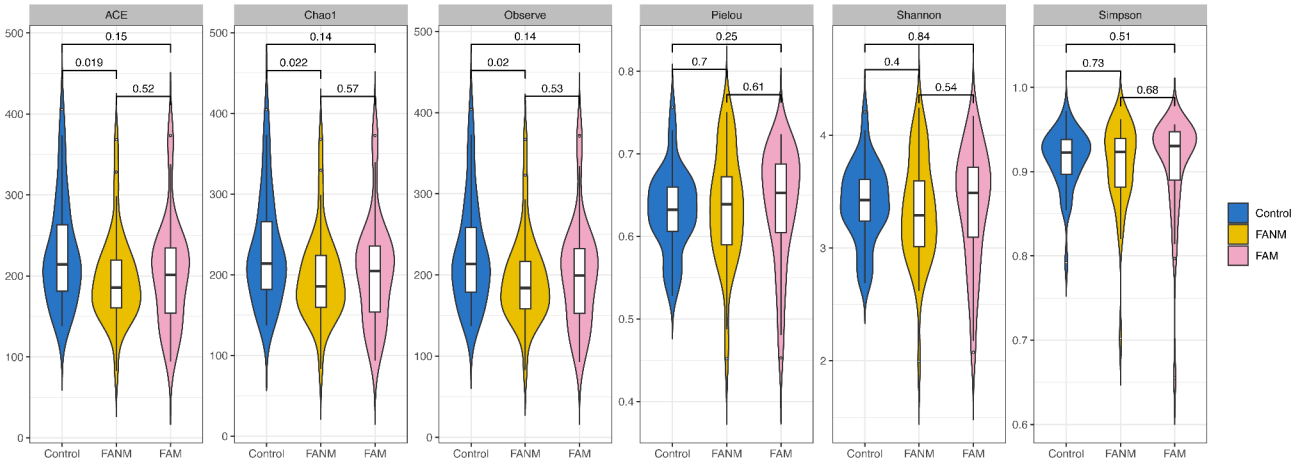

Fig. 1 The a-diversity analysis (ACE, Chao1, Observe, Pielou, Shannon and Simpson index) of the gut microbiota among the FAM, FANM, and control groups. The *P* values calculated using Kruskal–Wallis-test among three groups, and post hoc group comparisons evaluated by false discovery rate adjustment. FANM, food allergy children without malnutrition; FAM, children with food allergy and malnutrition

age-matched healthy children. Identifying unique microbiome signatures for FA with and without malnutrition were successfully determined in both the training and testing cohorts. Furthermore, correlations were noted between these microbial taxa and clinical characteristics such as total IgE levels. These findings collectively suggest that GM analysis holds promise as a noninvasive method for identifying potential markers of FAM and FANM.

Our findings reveal a significant disparity in microbial richness and evenness as assessed by alpha diversity indices, between FANM and control groups, which was inconsistent with findings of previous studies [22, 23]. Lin et al. reported comparable microbiota diversity in FA children compared with healthy controls [22]. The Canadian Healthy Infant Longitudinal Development Study, which was comprised of 166 infants up to one year old, indicated lower microbiota richness only at three months and not 12months [23]. However, they did not match children by sex or age. Additionally, our study revealed no difference in microbial richness between FAM and control groups [24, 25]. Hence, malnutrition arising from FA might impact the dysbiosis of GM in FA children. This notion was indirectly supported by significant differences observed in alpha diversity indices among FA children with moderate malnutrition in contrast with no differences in those with severe malnutrition in the present study.

In this study, the taxonomic compositions of microbiota in the FANM group were notably different from those in the control group. At the genus level, *Agathobacter*, *Anaerostipes*, *Lachnospira*, *Lachnospiraceae_NK4A136_group*, *Monoglobus*, *Faecalibacterium*, *Incertae_Sedis_f__Ruminococcaceae*, and *Subdoligranulum* emerged as predominant species in the FANM group. *Faecalibacterium*, which is recognized as a crucial commensal bacterium, primarily encompasses *Faecalibacterium prausnitzii* as its sole identified species. Research has shown that *Faecalibacterium prausnitzii* has the capacity to elicit a tolerogenic response in experimental colitis models and adult peripheral blood mononuclear cells [26, 27]. Despite being typically viewed as a bacterial biomarker for intestinal health, our findings highlight a consistent overrepresentation of *Faecalibacterium* in children with FA and adequate weight. Furthermore, various conditions, including FA and chronic spontaneous urticaria, have been linked to the absence or low levels of *Subdoligranulum* [28, 29]. Notably, *Subdoligranulum* has the potential to induce RORγt + Treg cells and mitigate the Th2 immune response in mice with FA [29]. Consequently, results regarding these two genera warrant further external validation.

A noteworthy increase in the abundance of the genera *Parabacteroides* and *Alistipes* in children diagnosed with FAM was observed in our study. The presence of the genera *Parabacteroides* or *Alistipes* in children affected by allergic diseases or malnutrition was not evident, although several studies have provided significant results [30–32]. A cross-sectional study identified *Alistipes* as one of the most significantly differentiated bacterial genera in patients with allergic rhinitis when compared with controls [30]. Similarly, a case-control study conducted in Taiwan reported substantial alterations in the abundance of *Parabacteroides* and *Alistipes* in children with food sensitization [31]. Moreover, Lv et al., in a study of 28 lean, overweight, and normal-weight male college students, reported a negative correlation between the abundance of *Parabacteroides* and *Alistipes* and BMI [32]. These studies suggest that both FA and malnutrition can influence GM composition and may result in distinct GM profiles including those of *Parabacteroides* and *Alistipes.* The specific identification of GM in FA children with malnutrition has not been reported to date. Our findings

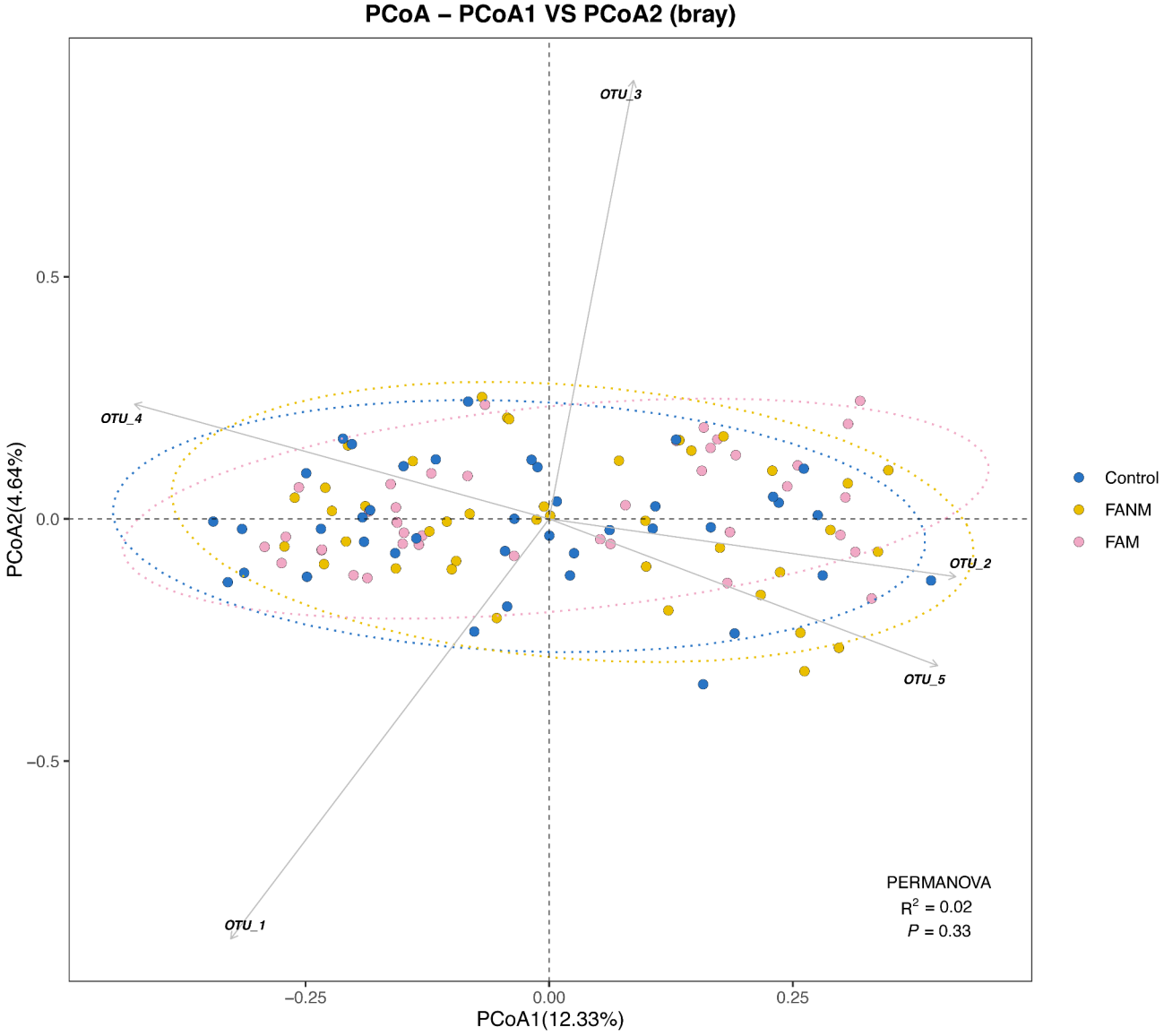

Fig. 2 Beta diversity based on the Bray-Curtis distances of the gut microbiota in the FAM, FANM, and control groups. The plot presented the first two principal coordinates (PC1, PC2) for principal coordinate analysis. FANM, food allergy children without malnutrition; FAM, children with food allergy and malnutrition

enhance the understanding of the microbial mechanisms underlying malnutrition in FA children and may present novel avenues for adjunctive therapies that target GM in clinical settings to mitigate malnutrition.

The correlation between the identified GM and clinical features in this study further underscores the potential influence of GM on immune responses. The positive correlation between the *Faecalibacterium* genus and total IgE levels contrasts with previous findings. Notably, *Faecalibacterium* has been reported as an anti-inflammatory commensal genus. Previous studies have demonstrated that both cells and cell-free supernatants from various *Faecalibacterium* species and strains can mitigate inflammation induced by chemicals in murine models [33–35]. Additionally, Qiu et al., through their research on the anti-inflammatory and immunomodulatory properties of *Faecalibacterium prausnitzii*, reported that supernatants from multiple *Faecalibacterium* species stimulated the production of IL-10 and TGF-β1 in cellular and animal models [27]. The positive relationship observed in our study between *Faecalibacterium* and total IgE levels may be mainly attributed to the FANM group, which presented the highest IgE levels among the three groups and a greater abundance of *Faecalibacterium* than the control group. Several factors associated with *Faecalibacterium*, such as physical activity, may help explain this observation [36, 37]. Specifically, an increase in the abundance of several *Faecalibacterium* species has been noted with

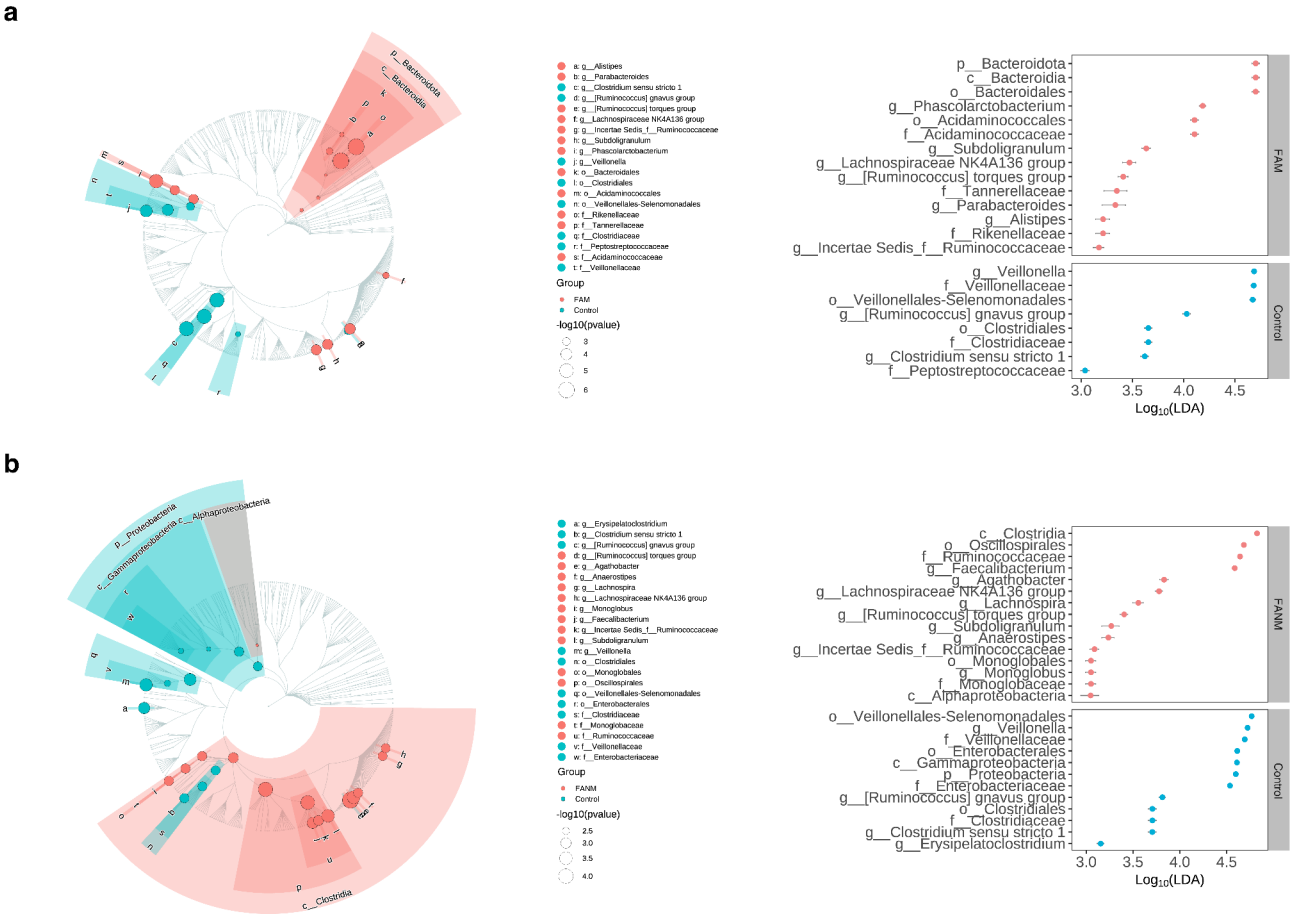

Fig. 3 The linear discriminant analysis effect size (LEfSe) identified the differentially abundant genera between groups (LDA significance threshold > 3). (**A**) FAM vs. Control; (**B**) FANM vs. Control. FANM, food allergy children without malnutrition; FAM, children with food allergy and malnutrition

increased physical activity, which is a factor that was not considered in our study. Therefore, these results should be interpreted cautiously, and future research should account for physical activity to elucidate these associations more clearly.

We pinpointed specific GM markers to differentiate between FA with and without malnutrition and sex- and age-matched healthy controls. The random forest model classifier, which utilizes fourteen optimal differentially abundant genus-level markers, displayed high accuracy in distinguishing FA with malnutrition from controls in both the training and validation phases, as well as in distinguishing FA without malnutrition from healthy controls. These findings underscore the potential of faecal microbiota-based biomarkers in predicting risks associated with FA and malnutrition. Given the recommendation of early intervention (before six months) in preventing FA by the European Academy of Allergy and Clinical Immunology, the high accuracy of our study in noninvasively detecting FA with malnutrition could contribute to increasing awareness and implementation of targeted screening strategies [38]. This may help reduce the incidence of FA and malnutrition in young children with microbiome signatures and address associated socioeconomic challenges linked to early screening.

Nonetheless, it is important to acknowledge that there were several limitations. First, our study was confined to a single centre, which potentially limits its generalizability. However, our sample size rivalled or exceeded that of comparable paediatric studies and enhanced the statistical robustness of our study. Second, dietary habits and physical activities of individuals can significantly influence the composition of their GM; unfortunately, we did not collect this information in our study [36, 39, 40]. Third, our assessment of GM relied on faecal rather than gut samples, which resulted in the potentially inadequately capture of the full spectrum of the intestinal GM. Finally, the cross-sectional nature of our study precludes causal inference. Hence, further investigations employing meticulously designed cohort studies are essential in corroborating our findings. Despite these limitations, our study is the first to reveal GM alterations in FA children with malnutrition and elucidate the association between GM and malnutrition severity in FA children.

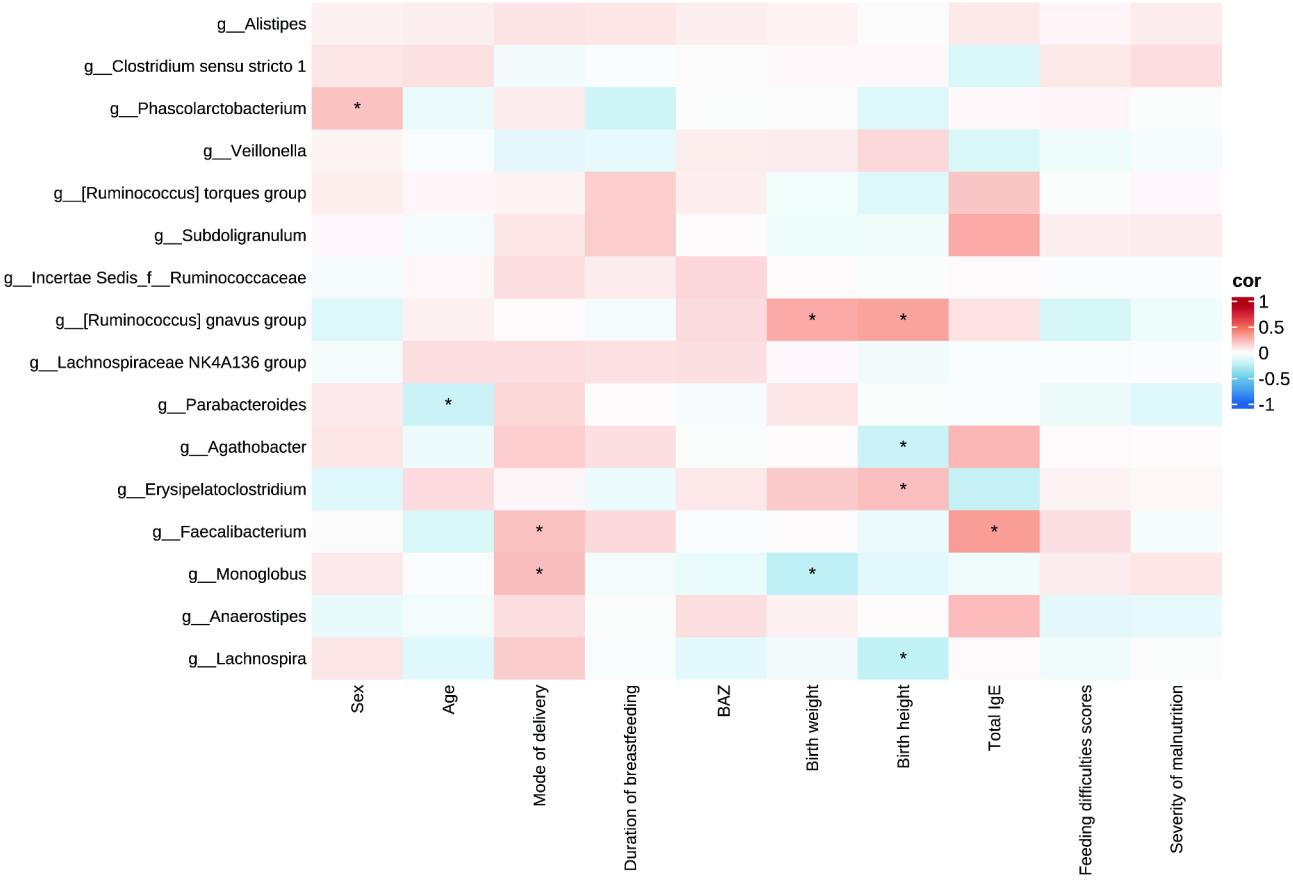

Fig. 4 Correlation of the significantly differential gut microbiota at the genus level with clinical characteristics. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$. BAZ, BMI-for-age Z score

Conclusion

FA children with and without malnutrition exhibit distinct gut microbiota profiles, which suggests the need for tailored microbiota-targeted therapies.

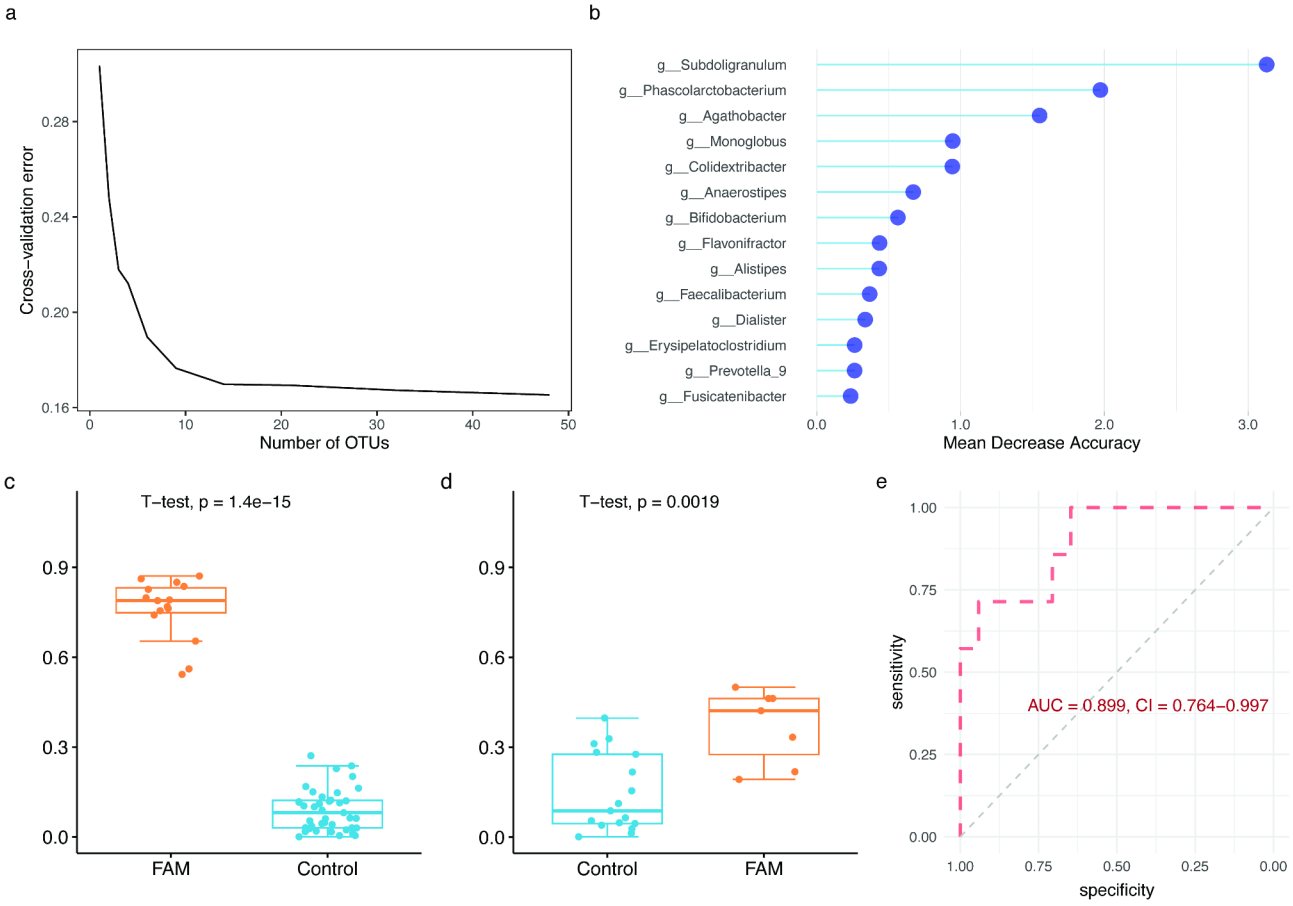

Fig. 5 Identification of microbial markers of FAM by random forest models. (**A**): A tenfold cross-validation on a random forest model between 40 FAM and 40 control in the training set; (**B**) Top 14 differentially abundant genera markers were selected as the optimal marker set based on random forest. The x-axis presents the mean decrease accuracy to each marker, which indicates the contribution to the accuracy of the model; (**C**): The POD value in FAM versus control in the validation set; (**D**): The POD value in FAM versus control in the testing phase; (**E**): The AUC value between FAM and control in the validation phase. FAM, children with food allergy and malnutrition

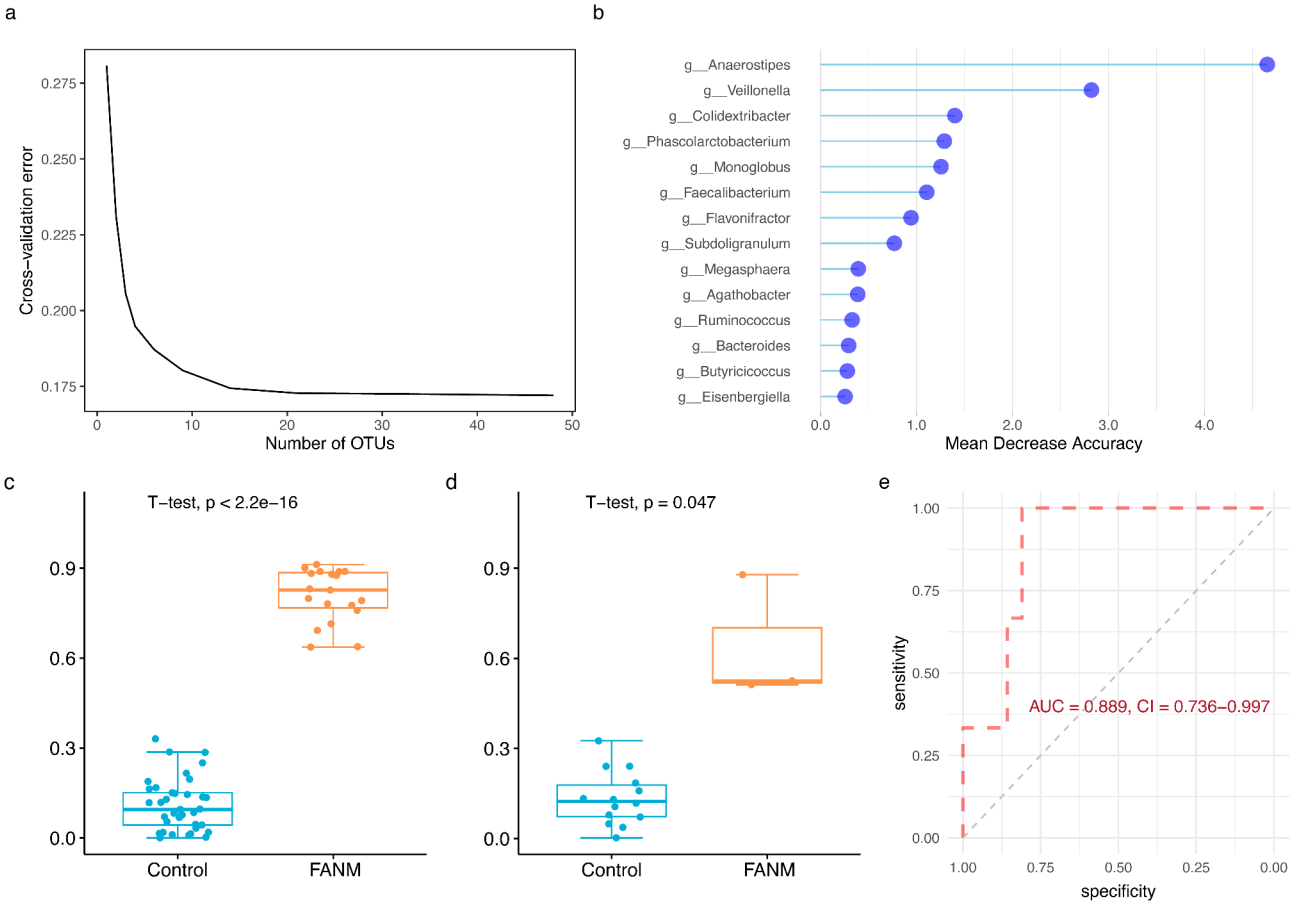

Fig. 6 Identification of microbial markers of FANM by random forest models. (**A**): A tenfold cross-validation on a random forest model between 40 FANM and 40 control in the training set; (**B**) Top 14 differentially abundant genera markers were selected as the optimal marker set based on random forest. The x-axis presents the mean decrease accuracy to each marker, which indicates the contribution to the accuracy of the model; (**C**): The POD value in FANM versus control in the validation set; (**D**): The POD value in FANM versus control in the testing phase; (**E**): The AUC value between FANM and control in the validation phase. FANM, food allergy children without malnutrition

Abbreviations

GM	Gut microbiota
FA	Food allergy
BMI	Body mass index
WAZ	Weight-for-age Z score
HAZ	Height-for-age Z score
BAZ	BMI-for-age Z score
OTUs	Operational taxonomic units
PCoA	Principal coordinates analysis
PERMANOVA	Permutational multivariate analysis of variance
FDR	False discovery rate
POD	Probability of disease
ROC	Receiver operating curve
AUC	Area under the curve
FAMM	Children with food allergy and moderate malnutrition
FASM	Children with food allergy and severe malnutrition
FANM	Food allergy children without malnutrition

Supplementary Information

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Supplementary Material 1

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Author contributions
XJZ, HZZ and HYW contributed the central idea. HYC and XJZ analyzed data and wrote the initial draft of the paper. XJZ, HYW, HZZ and YYW helped in revising the manuscript. QJX, XZQ and LC contributed to the collection of data and quality control. QZX and YHL contributed to processing, sequencing and quality control of fecal specimen. The authors read and approved the final manuscript.

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Data availability
The data sets used and/or analyzed during the current study are available from the corresponding author (Ms. Wu, email: whypq13@126.com) upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted in compliance with the Declaration of Helsinki and approved by the Ethics Committee of the Zhuhai Maternal and Children Care

Center (No.2022011011). Informed consent was obtained from the guardian of each child.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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