Fecal Microbiota Changes in NZB/W F1 Mice After Induction of Lupus Disease

Yen-Fu Chen
Linkou Chang Gung Memorial Hospital
Ao-Ho Hsieh
Linkou Chang Gung Memorial Hospital
Lian-Chin Wang
Linkou Chang Gung Memorial Hospital
Yun-Ju Huang
Linkou Chang Gung Memorial Hospital
Yun-Chen Tsai
Linkou Chang Gung Memorial Hospital
Wen-Yi Tseng
Keelung Chang Gung Memorial Hospital
Yu-Lun Kuo
Biotools Co., Ltd, New Taipei City
Shue-Fen Luo
Linkou Chang Gung Memorial Hospital
Kuang-Hui Yu
Linkou Chang Gung Memorial Hospital
Chang-Fu Kuo (zandis@gmail.com)
Linkou Chang Gung Memorial Hospital

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Abstract

Background: Gut microbiota is thought to regulate immune homeostasis. The association between gut microbiota and the development of systemic lupus erythematosus remains unclear. This study aims to investigate the differential alteration of gut microbiota after the induction of lupus in a murine model with viral peptide of human cytomegalovirus (HCMV), which is an established murine model of induced lupus.

Results: Specifically, we injected HCMVpp65 peptide in peritoneal space in NZB/W F1 mice to induce lupus. Three arms of animal treatment were prepared: intraperitoneal injection of HCMVpp65 peptide, intraperitoneal injection of adjuvant alone, and none injected. Feces of the study animals were collected before and every two weeks after the lupus induction for 16S rRNA sequencing. At 24 weeks of age, pathological investigation of renal tissue from sacrificed mice was conducted. Statistical analysis for dynamics and alteration of the fecal microbiota and lupus-like-activity-related functional prediction of bacterial communities was performed. HCMVpp65 peptide immunization induced lupus-like activities, with a higher level of anti-dsDNA antibodies, creatinine and proteinuria, and severe glomerular damage, compared to the mice treated with none or adjuvant only. A higher Simpson diversity value was measured in mice with injection of HCMVpp65 peptide than those with injection of adjuvant or none, but there have no difference in the species richness estimates, ACE and Chao1. Statistical Analysis of Metagenomic Profiles (STAMP) showed a higher relative abundance of the family (*Saccharimonadaceae*, *Marinifiaceae*, and *Desulfovibrionaceae*) *and genera* (*Candidatus Saccharimonas, Roseburia, Odoribacter, and Desulfovibrio*) that were found in HCMVpp65 peptide treated mice, compared to mice injected with adjuvant or none. The predicted metagenomic taxonomic profile showed statistically significant enrichment of flagellar assembly, bacterial motility, and chemotaxis.

Conclusion: A significant correlation between increased related genera abundance (*Candidatus Saccharimonas, Roseburia, Odoribacter, and Desulfovibrio*) and HCMVpp65 peptide immunization induced lupus-like activities was observed. This study confirmed significant changes in gut microbiota after the onset of lupus in a murine animal model.

Background

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases characterized by persistent chronic inflammation and various autoantibody productions, particularly anti-dsDNA antibody. It also results in dysregulation of cytokines that lead to severe and irreversible tissue injury [1, 2]. Although SLE etiology remains uncertain, genetic, environmental, hormonal, and epigenetic factors are associated with SLE development [3–5]. The mammalian gut is colonized by trillions of microorganisms that shape microbial diversity in the human intestine, are collectively known as the microbiota [6]. The reciprocal interplay between intestinal microbiota and the host immune system maintains tissue homeostasis [7–9]. Therefore, autoimmune diseases such as systemic lupus erythematosus (SLE) may be associated with changes in gut microbiota.

A recent cross-sectional study confirmed a decreased species richness diversity and a reduction in taxonomic complexity in feces of lupus nephritis patients compared with controls [10]. Other human evidence also links the gut microbiota changes to the presence of serum antinuclear antibody and changes of inflammatory cytokines associated with SLE progression [11, 12]. In animal studies, the alteration of microbial community structure and the greater bacterial diversity in the SLE have been reported [13, 14]. The increase in the relative family abundance of *Lachnospiraceae* and *Rikenellaceae* was reported to be associated with the severity of murine lupus activity, suggesting that gut microbiota significantly influences the host immune system and could effectively impact the development of SLE [15, 16]. Current data demonstrate that the pattern changes in the intestinal microorganisms or the presence of specific bacteria genus in the gut are associated with immune responses related to lupus.

Human cytomegalovirus (HCMV), a virus linked to the development of SLE in humans, has been shown to accelerate lupus-like disease in murine models [17, 18]. In the current study, we used HCMVpp65 peptide to immunize NZB/W F1 mice and to induce lupus. The objective of this study was to investigate the dynamics of fecal microbiota associated with the lupus-like activity in HCMVpp65$_{422\text{-}439}$-immunized mice compared to mice treated with none or adjuvant only.

Methods

Synthetic peptides

The purity of synthetic HCMVpp65$_{422\text{-}439}$ peptides $_{(GGGAMAGASTSAGRKRKS)}$ was > 99%, as per the manufacturers' guarantee (GenScript Biotech Corp). The HCMVpp65 peptide was prepared and stored according to the manufacturer’s recommendations.

NZB/W F1 mice and induction of lupus-like activity

The laboratory animal experiment center was approved by the Institutional Review Board of the Chang Gung Medical Foundation (approval number #2016062804 and #2018121402). The study was carried out in compliance with the ARRIVE guidelines [19]. NZB/W F1 female mice (3–5 weeks-old) were purchased from Jackson laboratory Co., Ltd., and housed in specific-pathogen-free (SPF) conditions in the animal center at Chang Gung Memorial Hospital. After 30 days of adaptive feeding and co-housing, the NZB/W F1 mice were separated into three groups: control group (n = 3), adjuvant group (injected with adjuvant only, n = 5) and lupus group (induced by HCMVpp65$_{422\text{-}439}$, n = 7). All mice were housed in an SPF room under a regular 12-hour light/ 12-hour dark cycle and stable air humidity. The immunization schedule was performed as previously described [20]. Briefly, immunization was administered from 12 to 14 weeks of age. On day 1, the NZB/W F1 mice received an intraperitoneal injection with 100-μg HCMVpp65$_{422\text{-}439}$ emulsified with complete Freund’s adjuvant (CFA, Sigma-Aldrich, Catalog Number: F5881). Boosting of mice was performed with HCMVpp65$_{422\text{-}439}$ in incomplete Freund’s adjuvant (IFA, Sigma-Aldrich, Catalog Number: F5506) on days 14, 28, and 42.

Serum, urine, and stool collection

Stool, urine, and blood of mice were harvested once every two weeks. For blood collection, mice were bled from the retro-orbital vein sinus, and plasma was collected from the blood by centrifugation for 10 minutes at 4°C at 13,000 rpm and stored at -80°C. The fresh stool and urine samples were collected from mice and preserved in micro-tubes that were immediately stored in liquid nitrogen.

Hematoxylin and Eosin (H&E) staining

H&E staining was conducted according to the Cold Spring Harbor protocols with small modification [21]. The frozen sections of the kidney were immersed in 100% ethanol for 30 seconds after they were sectioned and then rinsed ten times in double-distilled H_2O. Slides in the rack were put into a container filled with hematoxylin for 5 minutes, and the rack was then dipped five times into a jar containing 0.1% HCl. After dipping into tap water for 5 seconds, the rack was dipped into a jar containing 0.1% NH_4OH five times and then dipped into tap water five times. The cytoplasm stain with eosin and tissue dehydration as follows: eosin for 3 minutes, 100% ethanol with 0.1% acetic acid for five dips, 100% ethanol I for five dips, 100% ethanol II for five dips, acetone I for five dips, acetone II for five dips, xylene I for five dips and xylene II for five dips. After dehydration, the slides were mounted, and a cover glass was put on the slide for further investigation by microscopy (Olympus IX73/DP72, cellSens standard software).

Evaluation for lupus-like activity in NZB/W F1 mice

The examination for lupus-like activity was performed by serum indexes and kidney pathology using the ELISA test and hematoxylin-eosin (HE) staining [21]. The serum level of creatinine was measured by using standard ELISA test kits (MyBioSource, catalog number: MBS751125 and MBS763433, Southern California, San Diego, USA). The serum titer of anti-dsDNA autoantibodies was evaluated by anti-dsDNA ELISA Kit (Inova Diagnostics, catalog number: 708510, Southern California, San Diego, USA) according to the manufacturers' instructions. Proteinuria levels were examined by using the proteinuria strip (Medi-Test Combi 10 VET strip, MACHEREY-NAGEL, Germany).

Microbial DNA Extraction and 16S rRNA Gene Sequencing

Microbial DNA was extracted from fecal pellets of mice using the DNA Stool Mini Kit (Qiagen) according to the manufacturer's instructions. The quality and quantity of DNA extracts were determined by agarose gel electrophoresis (0.8% w/v agarose) and NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) followed by 16S rRNA sequencing.

Data Analysis and Bioinformatics of microbial diversity

Data analysis was carried out using the modified protocol, according to Chi-Cheng Huang [22]. Amplicon sequencing was performed by using 300-bp paired-end raw reads and assembled using FLASH v.1.2.7 [23]. De-multiplexing was carried out based on barcode identification. For quality control, Q < 20 reads were discarded from the pipeline of QIIME (v1.9.1)[24]. If three consecutive bases were < Q20, the read was classified as truncated. The read was preserved in the data set if it had more than 75% of the original length using split_libraries_fastq.py script in QIIME [25]. Using UCHIME algorithms, sequences were chimera-checked to acquire the effective tags and filtered from the data set by using the UPARSE function

in the peptide of USEARCH (v.7)[26–29]. The information of operational taxonomic unit (OUT) abundance was normalized to the variation and rarefied to the minimum sequence depth using the QIIME script (single_rarefaction.py). Subsequently, analysis for alpha and beta diversities was performed using the normalized data. Alpha diversity indicated the species complexity within individual samples based on seven different criteria outputs from the QIIME pipeline, including observed-OTUs, Shannon, Simpson, Chao1, and ACE [30]. Observed-OTUs reflect the number of different species identified in the microbial community. The Chao1 and ACE indices used to evaluate community richness and the relative abundance and evenness accounting for diversity were assessed by the Shannon and Simpson indices. Beta diversity analysis was conducted to evaluate the differences across samples in terms of species complexity. Principal Coordinate Analysis (PCoA) was performed using the distance matrix to acquire principal coordinates for visualization of sophisticated and multi-dimensional data [31]. For statistical analysis, the significance of all microbial species within groups at various levels of taxonomy was detected by using the differential abundance analysis with a zero-inflated Gaussian (ZIG) log-normal model executed in the “fitFeatureModel” function of the Bioconductor metagenomeSeq package [32]. Furthermore, Welch’s t-test was carried out using the Statistical Analysis of Metagenomic Profiles (STAMP) software (v2.1.3)[33]. Anosim and MRPP analyses were used to determine whether the community structures significantly differed among and within groups. Significant biomarkers were evaluated by the linear discriminant analysis (LDA) of effect size (LEfSe) analysis with non-parametric factorial Kruskal-Wallis (KW) sum-rank test and LDA to identify the differential abundance of taxa between two groups. For functional analysis, functional abundances from 16S rRNA sequencing data were analyzed for the prediction of functional genes with PICRUSt (v1.1.1)[34]. Fisher’s two-tailed unpaired and paired student's test with graphs depicting the mean ± 3 standard error of the mean (SEM) were used for comparisons between pairs of groups. All tests of statistical hypothesis were done on the 2-sided 5% level of significance. A 5% level of significance for P values was used for all analyses (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). Data were analyzed by using the SAS statistical software (Version 9.4, SAS Institute)

Results

HCMVpp65$_{422\text{-}439}$immunization induces lupus-like activity in NZB/W F1 mice

Studies indicate that HCMVpp65$_{422\text{-}439}$ immunization induces anti-dsDNA autoantibodies and initiates the glomerulonephritis in non-autoimmune prone mice [35]. To investigate the differential alteration of fecal microbiota associated viral peptide-induced lupus-like activities, we conducted HCMVpp65$_{422\text{-}439}$ immunization using NZB/W F1 mice at 12 weeks of age, and their lupus-like activities were evaluated (Fig. 1). Compared with NZB/W F1 mice treated with none (control group) or adjuvant only (adjuvant group), the levels of serum anti-dsDNA antibody, creatinine, and proteinuria were increased in HCMVpp65$_{422\text{-}439}$-immunized mice (referred as lupus group) at 12 weeks post-immunization (24 weeks of mice age, Fig. 1a-c). Also, we observed enlarged spleens and more severe renal damage in the lupus group (Fig. 1d-f). These findings suggest that HCMVpp65$_{422\text{-}439}$ immunization induced lupus successfully.

Dynamics of the microbial composition in NZB/W F1 mice

To investigate the change in the microbial community in NZB/W F1 mice, we analyzed fecal samples collected from mice before immunization (Pre-disease, 12 weeks of mice age) and at the diseased time point (lupus, adjuvant, and control groups, 24 weeks of mice age). The measurement of the alpha and beta diversity was performed to evaluate the differential alteration of microbial composition between these four groups. As shown in Fig. 2a-b, the lupus group had significantly higher Shannon's (P = 0.033) and Simpson's (P = 0.008) diversity index values (extent of microbial diversity), but there was no difference in ACE and Chao1 indexes (extent of microbial richness) compared to the control group. A higher Simpson's index value was found in the lupus group in comparison with the adjuvant group. The microbial diversity and richness had no significant difference between the adjuvant and control groups (Fig. 2a-d). For beta diversity analysis, the pattern variations in the microbial community structure between lupus, adjuvant, and control groups are listed in Table 1. The three-dimensional PCoA plot exhibited a separation of the community composition between the four groups (Fig. 2e). PC1, PC2, and PC3 showed 9.93% and 17% and 25.66% of the total variance in microbial species, respectively (Fig. 2E). Figure 2F-G shows the relative abundance of the top ten phyla and genera levels in the bacterial community of three groups (lupus, adjuvant, and control). At the phylum level, *Bacteroidetes* (59.0%) was determined to be the most abundant in the control group, followed by *Firmicutes* (39.5%), *Tenericutes* (1.0%), and *Patescibacteria* (0.3%) (Fig. 2f). In comparison with adjuvant group, the lupus group had an increase in *Firmicutes* (53.5% vs 45.7%), *Patescibacteria* (3.1% vs 1.7%), and *Proteobacteria* (0.6% vs 0.007%), as well as a decrease in *Bacteroidetes* (41.8% vs 52%) and *Tenericutes* (0.7% vs 0.9%). At the genus level, the genera (top ten) in the relative abundance of *Lachnospiraceae NK4A136 group* (*f_ Lachnospiraceae*), *Oscillibacter* (*f_Oscillospiraceae*), *Ruminococcaceae UCG 014* (*f_ Ruminococcaceae*), *Intestinimonas, Candidatus Saccharimonas* (*f_ Saccharimonadaceae*), *Ruminiclostridium*, *Ruminiclostridium 9* (*f_ Ruminococcaceae*) and *Anaeroplasma* (*f_Anaeroplasmataceae*) were observed in the four groups (Fig. 2g). The total percentage of top ten genera was 22.6%, 22.4%, 25.5%, and 31.0% in pre-disease, control, adjuvant, and lupus groups, respectively.

Table 1
Significant test of microbial community structure between two groups

	MRPP		Adonis		Anosim	
Groups	E-Δ	P	R^2	P	R	P
Adjuvant vs. Control	0.36	1.8E-2	0.21	6.3E-2	0.11	3.0E-1
Adjuvant vs. Lupus	0.45	2.0E-3	0.30	9.0E-4	0.66	3.0E-3
Control vs. Lupus	0.45	1.3E-2	0.35	1.4E-2	0.58	1.9E-2

Three different tests were performed with MRPP, Adonis and Anosim based on Bray-Curtis dissimilarity. E-Δ: Expected-delta. P: *P*-value.

Altered microbial families and genera associated with HCMVpp65$_{422-439}$immunization

The STAMP software was used for the comparison of lupus, adjuvant, and control groups to determine the significant alterations of microbiota in the lupus group. The significantly altered family and genera with changes in relative abundance are shown in Fig. 3 and Fig. 4, respectively. In comparison to the control group at the family level, the increased relative abundance of *Saccharimonadaceae* was observed in the adjuvant group (Fig. 3a-b). Meanwhile, the change in abundance of *Saccharimonadaceae*, *Marinifiaceae*, *Rikenellaceae, Desulfovibrionaceae*, and *Eggerthellaceae* was observed in the lupus group (Fig. 3c). The lupus group had a higher relative abundance of the family *Saccharimonadaceae (3.1%)*, *Marinifiaceae (2.0%)*, and *Desulfovibrionaceae (0.7%)* than the adjuvant and control group did (Fig. 3d). At the genus level, the increase in *Candidatus Saccharimonas, Turicibacter, Ruminiclostridium 5, and Ruminococcaceae UCG-009* were observed in the adjuvant group (Fig. 4a). Also, the change in abundance of *Lachnospiraceae_NK4A136_group, Candidatus Saccharimonas*, *Odoribacter, Roseburia*, *Ruminiclostridium_5*, *Alistipes, Desulfovibrio, Ruminococcaceae_UCG_005, Ruminococcaceae_UCG_010* or *Enterorhabdus* were found in the lupus group in comparison with the control group (Fig. 4b-c). Notably, *Candidatus Saccharimonas (3.1%), Roseburia (2.3%), Odoribacter (1.9%), and Desulfovibrio (0.7%)* had higher abundance in the lupus group compared to the other two groups (Fig. 4d). Linear discriminant analysis (LDA) Effect Size (LEfSe, LDA > 3) showed the differential alteration between lupus versus adjuvant and lupus versus control groups in Supplemental Fig. 1.

Functional prediction of microbial communities associated with lupus-like activities in HCMVpp65$_{422-439}$immunization

We predicted the functional potential of microbial communities using phylogenetic reconstruction of unobserved states (PICRUSt) to gain an insight into the potential functional role of fecal microbiota in the lupus group[34]. Several pathways relevant to cell motility were differentially expressed between the two groups (Fig. 5). The genetic markers with significant discriminative power in the cellular processes, including lysosome, flagellar assembly, cytoskeleton proteins, bacterial motility proteins, and bacterial chemotaxis, were detected. Spearman's rank correlation method was conducted to infer the association of fecal microbial genera and lupus-like activity. Statistically significant positive correlations between microbial genera and lupus-like activity were identified (Fig. 6). Noticeably, all four lupus-like activities correlate positively with *Odoribacter*, *Desulfovibrio, and Roseburia*. *Candidatus Saccharimonas showed* a statistically significant positive correlation with creatinine, anti-dsDNA IgG titer and severity of glomerulonephritis (Fig. 6).

Discussion

Accumulating evidence suggests that alterations in microbiota composition are related to the development of SLE [36]. In the current study, we report that the fecal microbial alteration is associated with lupus-like activity in NZB/W F1 mice. The HCMVpp65$_{422-439}$ immunization accelerated lupus progression and exacerbated glomerulonephritis. Among these animals, a significantly altered microbiota composition was observed, and had a differential bacterial community, compared to the adjuvant or control group. The abundance of significantly altered microbial families (*Saccharimonadaceae,*

Marinifilaceae, and Desulfovibrionaceae) and genera (*Candidatus Saccharimonas, Roseburia, Odoribacter, and Desulfovibrio)* in lupus group were suggested to be involved in the HCMVpp65 peptide induced lupus-like activity in NZB/W F1 mice.

Previous studies reported altered microbiota composition in lupus as well. A significant relative increase in the family *Clostridiaceae, Lachnospiraceae, Ruminococcaceae, Desulfovibrionaceae, and Rikenellaceae*, and a decrease in *Akkermansia muciniphila* were described [37]. Moreover, the same group has been reported the increased levels of *Lachnospiraceae, Ruminococcaceae*, and *Rikenellaceae*, as well as decreased levels of *Lactobacillaceae* in MRL/lpr mice at the disease stage [16]. A higher relative abundance of the *Rikenellaceae* family in SNF1 lupus mice is believed to be associated with more severe lupus symptoms [15]. The *Lactobacillaceae* and *Akkermansiaceae* families are reported to have an anti-inflammatory effect and play a crucial role in microbiota remodeling [38–40]. In the current study, the family *Saccharimonadaceae, Marinifilaceae, Rikenellaceae*, and *Desulfovibrionaceae* were more abundant in the lupus group in comparison to the control group. The significant decrease in *Akkermansiaceae* family was found in the three groups at 12 weeks post-immunization, but the decreased level of *Lactobacillaceae* did not reach the statistical significance (Supplemental Fig. 2a). Of note, the increase in *Saccharimonadaceae* and *Marinifilaceae* family has been reported to positively correlate with levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [41]. The enrichment in the relative abundance of the *Desulfovibrionaceae* family associated with Th17 expression was found in patients with multiple sclerosis [42]. The higher phylogenetic diversity and a significant change in bacterial community structure were found in the lupus group that is prone to inflammation and potentially related to the increased risk of inflammatory disease.

At the genus level, we observed an increased relative abundance of *Candidatus Saccharimonas, Roseburia, Odoribacter, and Desulfovibrio* genera in the lupus group. These microbial genera were found to link to host immune regulation or autoimmune diseases such as proinflammatry cytokine IL-17. The increased relative abundance of *Candidatus Saccharimonas* and *Roseburia* in colitis has been reported. They were found to associate with the reduction of IL-17 expression [43, 44]. *Roseburia* may promote the regulatory T cell differentiation and reduce the expression of IL-17 in inflammatory bowel disease [43]. In contrast, the abundance of *Odoribacter and Desulfovibrio* genera are found in several autoimmune diseases, including rheumatoid arthritis, ankylosing spondylitis, and inflammatory bowel disease, possibly due to the increased expression of IL-17 and Th17 cells [45–47]. The parallel changes in the abundance of microbial genera in the lupus group were suggested to mediate a synergistic or antagonistic interaction in response to the induction of lupus in NZB/W F1 mice.

In the present study, we found that the average Firmicutes and Bacteroidetes (F/B) ratio increased in the lupus group (1.72 ± 0.49) compared with control (0.72 ± 0.22) and adjuvant (0.89 ± 0.23) groups (Supplemental Fig. 2b). Our results showed significant bacterial diversity and an increasing trend of F/B ratio in NZB/W F1 mice after the induction of lupus. Both Firmicutes and Bacteroidetes are dominant microbial phyla in the human gut and have critical roles in modulating inflammation and immune status in the host [48, 49]. The functional roles of Bacteroidetes that mediate the intestinal mucosal barrier

function and reduce inflammation have been described [50, 51]. Firmicutes can lead to the increased production of lipopolysaccharides (LPS) that enter the bloodstreams and result in chronic inflammation [52, 53]. The elevated F/B ratio in an abundance of fecal microbiota is suggested to promote a pro-inflammatory environment and exhibits characteristics of multiple sclerosis [42, 54]. Recently, a research work to study the difference in gut microbiota between SLE patients and non-SLE controls reported that the value of F/B ratio was no significant difference between the two groups [37]. By contrast, Hevia et al. showed an opposite finding that the remissive SLE patients had significantly lower F/B ratios than did healthy individuals [55]. The F/B ratio has been examined extensively for gut microbiota in human or animal studies, but the correlation between the F/B ratio and diseases seems far from conclusive.

There are some limitations to this study. Long term co-housing of mice may result in hybrid-microbiota animals or cage effects. Taking advantage of the coprophagy of mice, co-housing gnotobiotic animals shortly before mice received immunization can reduce the bias in gut microbiota analysis [56]. However, we were unable to eliminate the cage effect, even though co-housing of mice before induction of lupus in mice. Moreover, the small population of mice and disease severity of mice after induction are two possible factors causing bias in this study. However, our results are not merely consistent with previously reported alterations in microbiota composition in spontaneous murine lupus model, but also observed significantly higher species richness and microbial community. These findings suggest that the orchestration of specific bacterial compositions may play a part in the development of autoimmune diseases.

In our study, we attempted to investigate the interplay between the changes in the fecal microbiota and lupus-like activity in NZB/W F1 mice. The lupus group has higher microbial diversity and an increase in the family (*Rikenellaceae Desulfovibrionaceae. Saccharimonadaceae* and *Marinifilaceae*) and genera (*Candidatus Saccharimonas, Roseburia, Odoribacter, and Desulfovibrio)*. The current study showed that the fecal microbiota composition of the viral peptide induced lupus-like activity in NZB/W F1 mice markedly differed from their controls, which may be of value for future microbiota-targeted work in the similar disease induction scheme.

Declarations

Ethics approval and consent to participate

The laboratory animal experiment was approved by the Institutional Review Board of the Chang Gung Medical Foundation (approval number #2016062804 and #2018121402). All experiments were performed with full compliance with the Guide of the Care and the Use of Laboratory Animals in Taiwan. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All sequences used in this study are available in the NCBI Sequence Read Archive. https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA693398 (Reference number PRJNA693398)

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AHH, LCW, WYT and CFK designed the project. AHH, LCW, YFC, YJH, and YCT performed the experiments, acquired the data and interpreted the results. AHH, YLK, KHY, CFK and SFL participated in the interpretation of the results and drafted and revised the manuscript. All authors reviewed the manuscript, approved the final version to be published and agreed to be accountable for all aspects of the work.

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Authors' information

Affiliations

Division of Rheumatology, Allergy and Immunology, Chang Gung Memorial Hospital, Taoyuan, Taiwan

Yen-Fu Chen, Ao-Ho Hsieh, Lian-Chin Wang, Yun-Ju Huang, Yun-Chen Tsai, Shue-Fen Luo, Kuang-Hui Yu, Chang-Fu Kuo

Division of Rheumatology, Allergy and Immunology, Chang Gung Memorial Hospital, Keelung, Taiwan
Wen-Yi Tseng

Biotools Co., Ltd, New Taipei City 221, Taiwan

Yu-Lun Kuo

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Figures

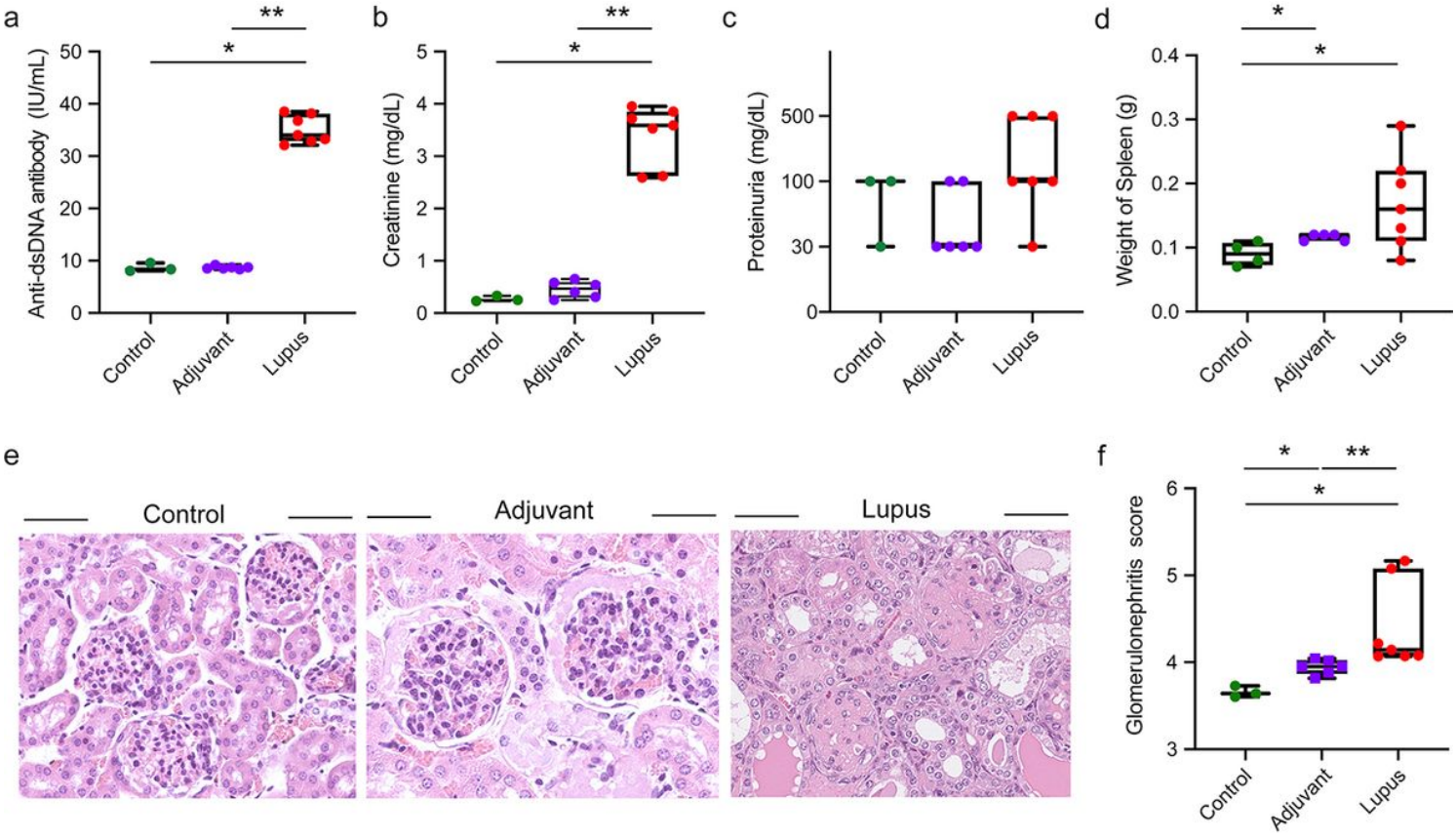

Figure 1

Induction of lupus-like activities in NZB/W F1 mice. Mice were Intraperitoneal injection with HCMVpp65422-439 peptide or adjuvant only. The levels of (a) anti-dsDNA antibody (b) serum creatinine, and (c) proteinuria were examined with blood or urine collected from control, adjuvant, and lupus groups at 12 weeks post-immunization. (d) The representative photograph and diagram show the size of the spleens collected from three groups. Data are shown as the mean ± SEM. (e) Hematoxylin and eosin staining of the glomerular from control, adjuvant, and lupus groups. (f) The glomerulonephritis score of renal lesions from three groups. Data are presented as the mean ± SEM of three independent experiments. *: P-value < 0.05 and **: P-value < 0.01. P-value of < 0.05 was considered significant.

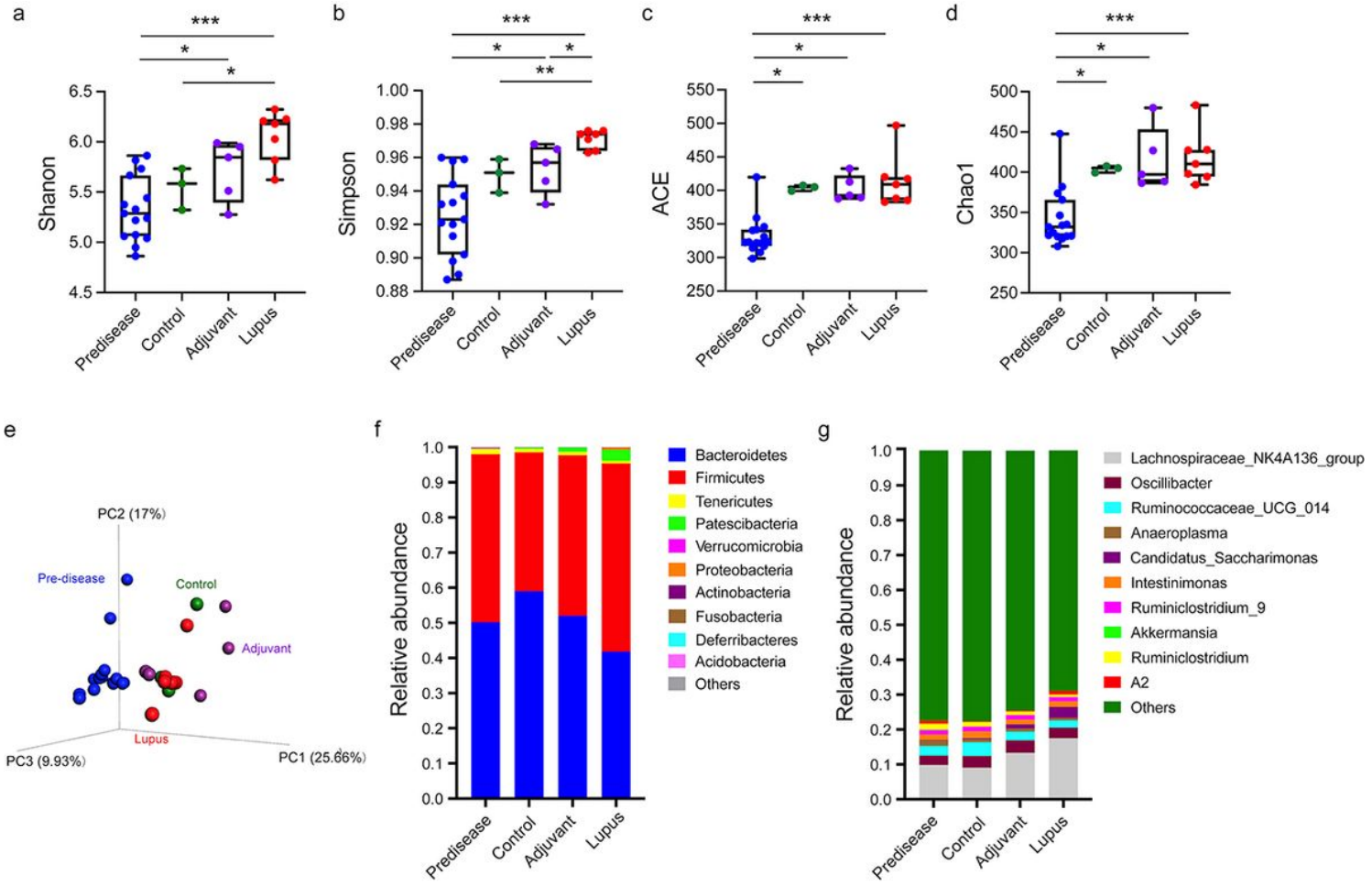

Figure 2

Comparison of microbial community structure between control, adjuvant, and lupus groups. Alpha diversity indexes, (a) Shannon; (b) Simpson; (c) ACE; (d) Chao1, were analyzed using the Kruskal-Wallis test. The statistical analysis was performed using ANOVA and Student's t-test. Beta diversity of (e) principal coordinates analysis (PCoA) was evaluated by unweighted UniFrac and Bray-Curtis distances. The relative distribution of the top ten (f) phyla and (f) genera present in three groups are shown. Data are presented as the mean ± SEM. *: P-value < 0.05, **: P-value < 0.01, and ***: P-value < 0.001. P-value of < 0.05 was considered significant.

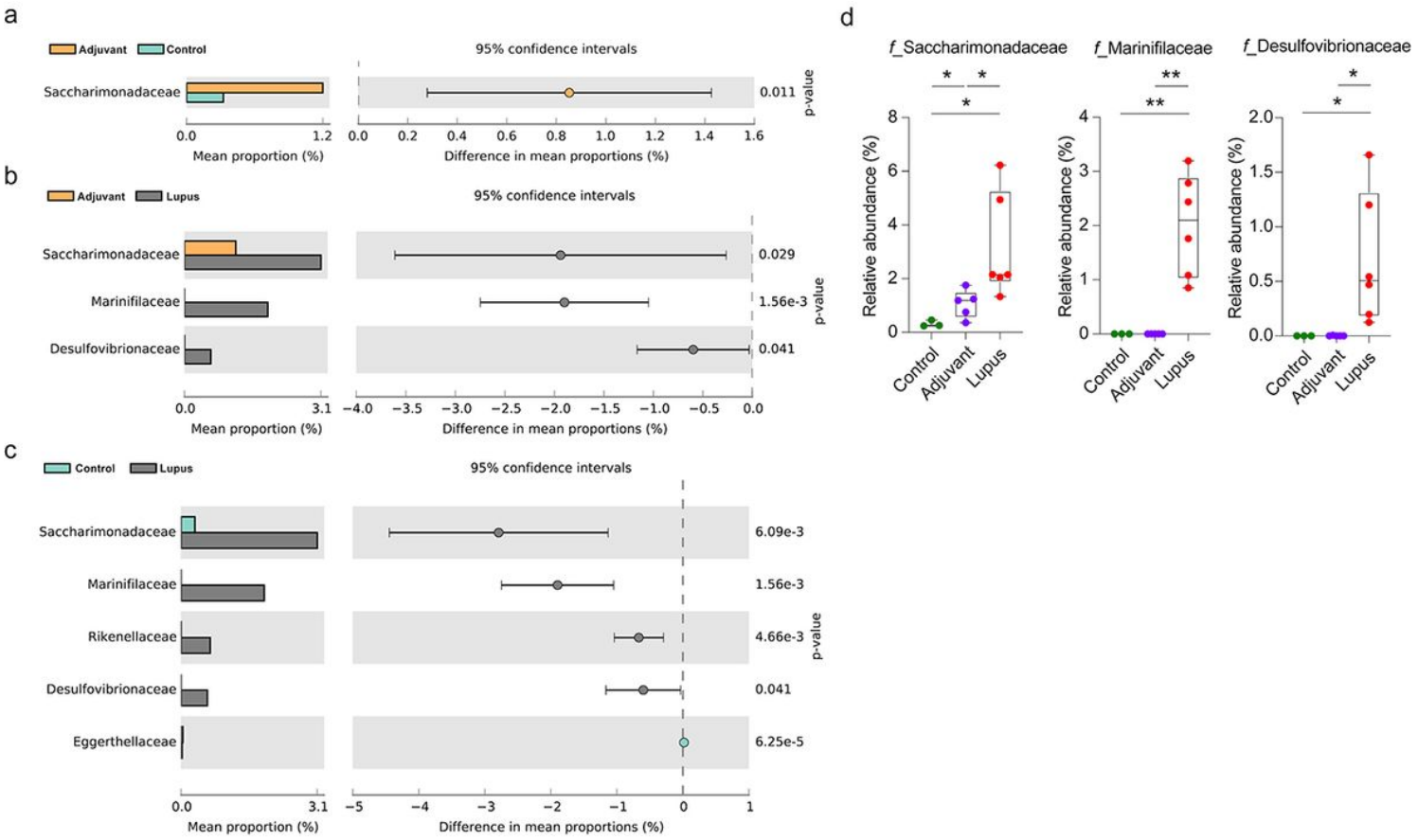

Figure 3

Comparison of relative abundance of family groups in the fecal microbiota of control, adjuvant, and lupus groups. The differential abundance analysis of family groups in (a) adjuvant vs. control, (b) adjuvant vs. lupus, and (c) control vs. lupus was performed by the STAMP software using Welch's t-test. (d) The significantly higher relative abundance of the family in lupus compared to the adjuvant and control groups. *: P-value < 0.05 and **: P-value < 0.01. P-value of < 0.05 was considered significant.

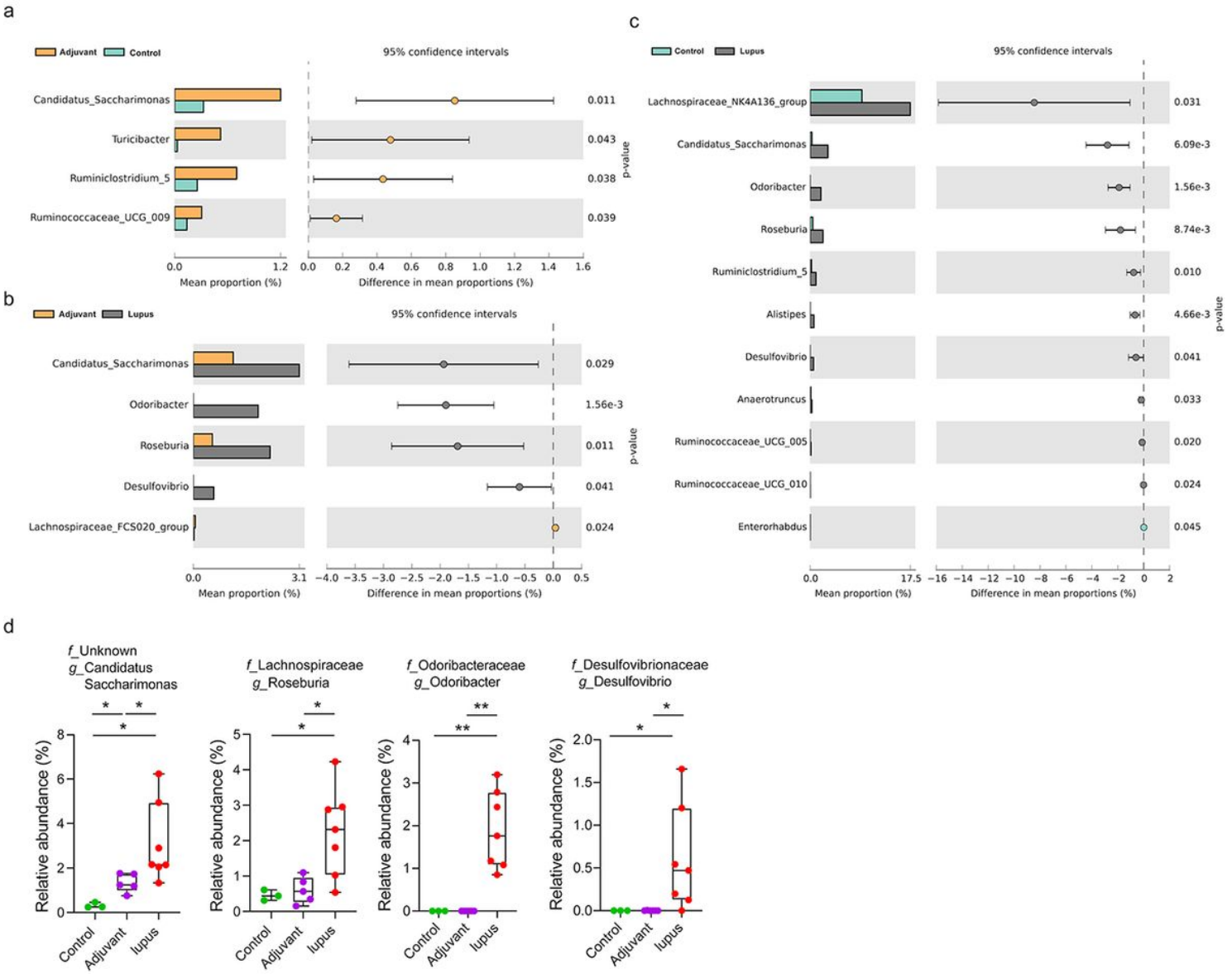

Figure 4

Comparison of relative abundance of genera in the fecal microbiota of control, adjuvant, and lupus groups. The differential abundance analysis of genera in (a) adjuvant vs. control, (b) adjuvant vs. lupus, and (c) control vs. lupus was performed by the STAMP software using Welch's t-test. (d) The significantly higher relative abundance of genera in lupus compared to the adjuvant and control groups. *: P-value < 0.05 and **: P-value < 0.01. P-value of < 0.05 was considered significant.

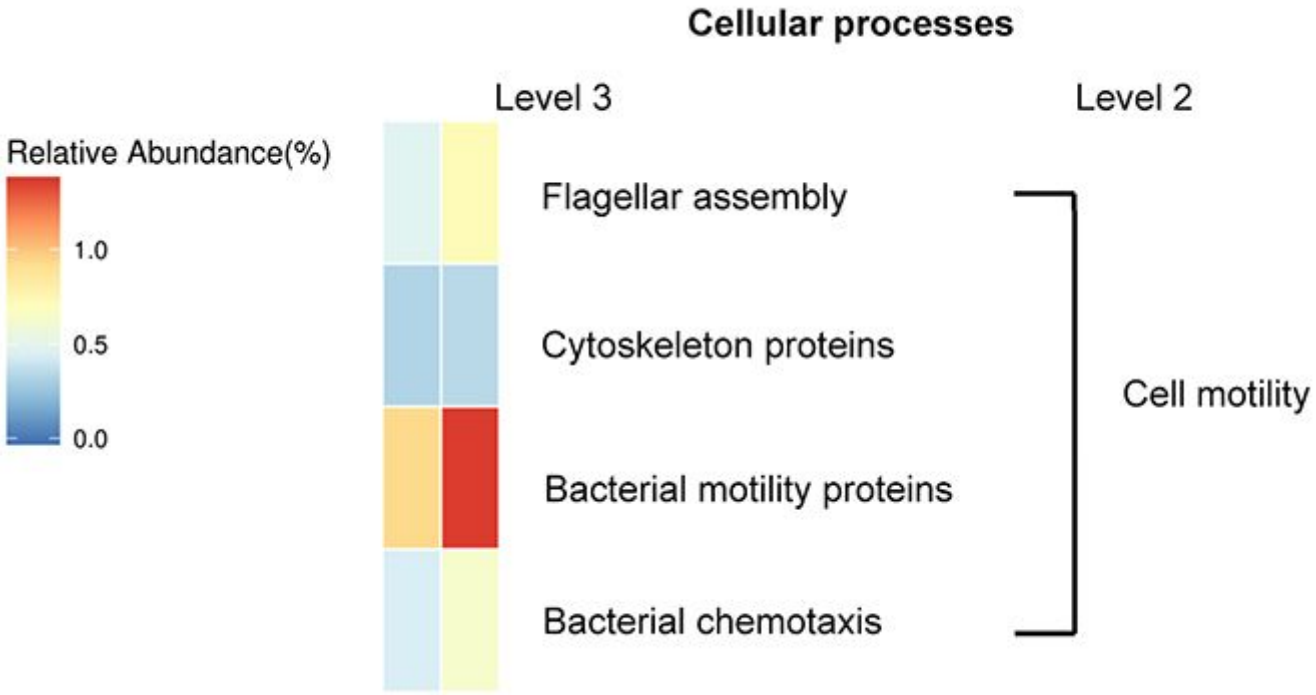

Figure 5

Metagenomic taxonomic profile to predict the functional profile of microbial communities in the lupus group by phylogenetic reconstruction of unobserved states (PICRUSt).

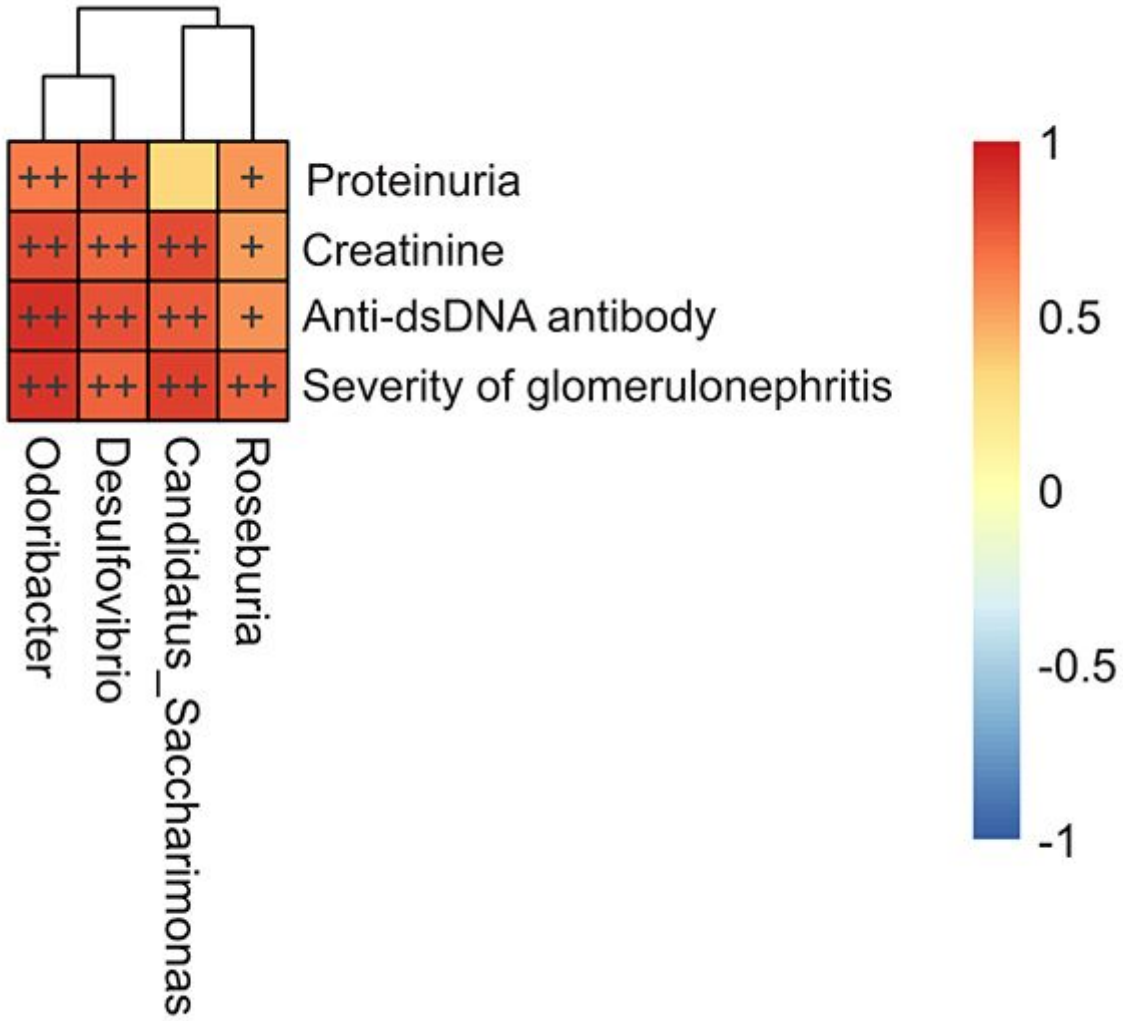

Figure 6

Spearman's rank correlation between the relative genera abundance and the lupus-like activities in the lupus group. These results are shown as a red-blue heat-map diagram for each correlation test, corresponding to the Spearman rho value: +: P-value < 0.05 and ++: P-value < 0.01.

Supplementary Files

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