P-PM-01

Gut microbiota alteration along gastrointestinal tract in SLE (systemic lupus erythematosus)

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ABSTRACT

The balance of microbiological ecology in the intestine (homeostasis) is essential for health. Dysbiosis of gut microbiota and diversity can lead to metabolic disorders and inappropriate immune responses, hence were reported to be associated with many diseases, including systemic lupus erythematosus (SLE), a chronic autoimmune disease. Because differences of physicochemical conditions in each intestinal section (duodenum, jejunum, ileum, cecum and colon) affect the composition of gut microbiota. This study we firstly reported gut microbiota composition along each section of mouse gastrointestinal tract comparing between lupus-prone mice by genetic FcGRIIb knockout and aged-matched healthy mice, at 4 months (preclinical SLE) and 11 months (established SLE) of ages, to identify potentially beneficial and harmful bacteria. Gut microbiota were different in bacterial composition and diversity. The cecum and colon had the most significant differences between healthy and disease. In disease group, f_S24-7_unclassified, *Anaerostipes*, *Lactobacillus*, *Sutterella*, *Allobaculum*, *Adlercreutzia* and *Bifidobacterium*, as potential beneficial bacteria, decreased; whereas *Mucispirillum*, *Oscillospira*, f_Lachnospiraceae_ unclassified, *Alistipes*, and *Sporobacter*, as potential harmful bacteria, increased.

Keywords: Gastrointestinal tract, Gut microbiota, Systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that defecting immunological tolerance leads to immune responses against self-antigens. The autoreactive cells and autoantibodies cause inflammation in many organs, ranging from skin, joints, the central nervous system, kidneys, lung, heart, brain, to life threatening. SLE is predominately found in women 9 times more than men, and higher prevalence in African, Hispanic, and Asian ethnics (Kaul et al., 2016; La Paglia et al., 2017; Mu et al., 2017b). Etiology of SLE remains unknown but involves environmental and genetic factors. Although the disease more commonly results from poly-genes deficiency, a single gene that is reported associated to SLE development, including genes associated with lymphocyte activation (PTPN22, TNFSF4, IL10, SPRED2, STAT4, PXK), inflammation (TNIP1) and immune complex regulation (FCGR2A, FCGR2B, FCGR3B, ATG5, CLEC16A) (Kaul et al., 2016; Tsokos, 2011; Tsokos et al., 2016)

Recently, commensal bacteria that colonize gastrointestinal tract, or gut microbiota, have been considered as one important factor that is associated with SLE. Many studies reported that changes in gut microbiota composition play an important role in host immune responses (Kosiewicz, Zirnheld, and Alard, 2011; Rosser and Mauri, 2016). For instances, female lupus-prone mice via lymphoproliferation spontaneous mutation (MRL/lpr), the fecal gut microbiota demonstrated a decrease of Lactobacillaceae, while Lachnospiraceae and overall bacterial diversity increased (Zhang et al., 2014).

However, the genetic predisposition to SLE is various, and the colonization of bacteria along gastrointestinal (GI) tract, categorized into small intestine (duodenum, jejunum and ileum) and large intestine (cecum and colon), and feces had not been determined. The GI tract is different by a gradient of oxygen, antimicrobial peptides (e.g. bile acids) and pH (Donaldson, Lee, and Mazmanian, 2016), so the bacterial diversity is likely different. For examples, bacterial composition in GI tracts of healthy C57BL/6 mice was reported a greater proportion of Lactobacillaceae in stomach and small intestine, and anaerobes such as Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae and Ruminococcaceae in the large intestine and feces (Gu et al., 2013). We therefore compared gut microbiota and identified potentially beneficial and harmful bacteria along GI tract between lupus-prone (FcGRIIb knockout) and matched healthy mice.

MATERIALS AND METHODS

Mice and sample collections

All mice were housed and cared by Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Female C57BL/6 mice served control (wildtype, WT) and female FcGRIIb$^{-/-}$ (knockout, KO) (C57BL/6 background) mice served a test. In FcGRIIb$^{-/-}$ mice, *FCGR2B* gene was deleted causing excessively elevated immune response and development of SLE symptoms at 6 months of age. Mice, 3 replicates per group, were anesthetized and removed the intestine sections (duodenum, jejunum, ileum, cecum and colon) using sterile tweezers at 4 and 11 months of age. All samples were collected in 1.5 ml microcentrifuge tube and stored at -80 °C.

Metagenomic DNA extraction

Each sample (0.25 g) was extracted metagenomic DNA using DNeasy PowerSoil Kit (Qiagen, Inc., Hilden, Germany) according to manufacturer protocols. The NanoDrop spectrophotometer (A260 and A260/A280) were used to compute DNA concentration and quality. Agarose gel electrophoresis was also used to confirm the size and quality. The metagenomic DNA were at −20 °C.

16S rRNA gene library preparation and sequencing

The V4 region of bacterial 16S rRNA gene were amplified (2-3 independent replicates) from metagenomic DNA using universal prokaryotic primers 515F (5′ GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGAC TACHVGGGTWTCTAAT-3′). The reverse primer contained 12 nucleotide barcode sequence to indicate the sample. Thermocycling condition consists of an initial denaturation at 94°C 3 min, followed by 24-30 cycles of 94°C 40 s, 50°C 1 min and 72°C 1.30 min, and a final extension of 72°C 10 min. The size and concentration of PCR amplicon were measured using 1.75% agarose gel electrophoresis. The 381 base pairs (bp) amplicons were cut and purified by PCR Clean-Up & Gel Extraction Kit (GeneDireX, Inc., Keelung, Taiwan) and stored at -20 °C. Then, equal DNA concentration of a minimum of triplicate PCR amplicons and replicates was pooled using Qubit® 3.0 Fluorometer and Qubit® dsDNA HS Assay kit (Invitrogen, Inc., Waltham, USA) for 150-200 ng total. The DNA library and PhiX control were denatured by 0.2 N NaOH, mixed together and diluted to 7pM. After that, the prepared library was loaded onto 4 chambers of reagent cartridge, together with Read1, Read2 and index primer as sequencing primers (Illumina, Inc., 2019).

Sequencing was at Omics Sciences and Bioinformatics Center (OSBC), Faculty of Science, Chulalongkorn university using MiSeq system (Illumina, San Diego, USA) and MiSeq Reagent Kits v2 (Illumina, San Diego, USA).

Bioinformatics analyses

Sequencing data were analyzed following Mothur's Standard Operating Procedure (SOP) for Miseq (Kozich et al., 2013). Forward and reverse reads were assembled into a contig. Quality sequences were screen to remove sequences of <100 bp, ambiguous bases and chimera sequences. The quality sequences were aligned against Silva and Greengenes databases to identify operational taxonomic unit (OTUs) at phylum to genus levels. Mitochondria, chloroplast and unclassified bacteria were removed. Alpha diversity (rarefaction curves, number OTUs, Good's coverage to estimate sequencing coverage, Chao, and Shannon) and beta diversity (principal coordinate analysis (PCoA), phylogenetic tree, and Metastats) were performed to analyze intra and inter bacterial diversity correlations. Species by OTUs and by BLASTN (Altschul et al., 1990) were also determined.

RESULTS

Analysis of 16S rRNA gene sequences

Total number of reads from Miseq sequencing were 11,680,536 reads, and the number was reduced to 9,741,894 reads after quality sequence processing and removing of mitochondria, chloroplast and unclassified bacteria. This equaled to 83.40% of quality and denoted prokaryotic OTUs out of the total. The number of reads in each sample were described in Table 1. The highest and lowest numbers of reads were 13,346 (ColonKo4m2) and 373,225 (CecumKo11m1) reads, respectively.

Table 1. Number of 16S rRNA gene sequences before and after quality Screening and removed of non-prokaryotic OTUs.

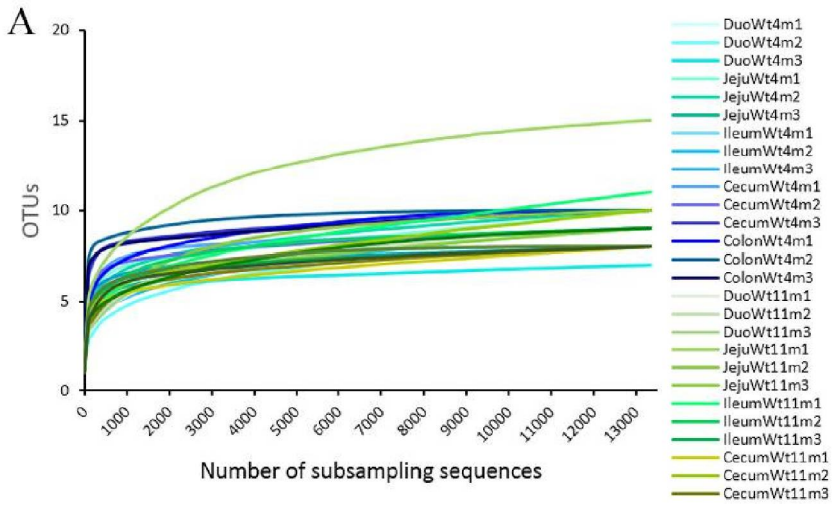
Sample ID	Quality screening		Sample ID	Quality screening	
	Before	After		Before	After
DuoWt4m1	139822	127446	DuoKo4m1	122024	107753
DuoWt4m2	138410	124961	DuoKo4m2	257073	224185
DuoWt4m3	381697	344681	DuoKo4m3	344901	288295

Table 1. Number of 16S rRNA gene sequences before and after quality Screening and removed of non-prokaryotic OTUs. (Cont.)

Sample ID	Quality screening		Sample ID	Quality screening	
	Before	After		Before	After
JejuWt4m1	308264	286065	JejuKo4m1	248567	195485
JejuWt4m2	274551	262640	JejuKo4m2	344546	299473
JejuWt4m3	118133	99568	JejuKo4m3	380378	258978
IleumWt4m1	153569	133413	IleumKo4m1	153525	145527
IleumWt4m2	126756	116417	IleumKo4m2	250586	218940
IleumWt4m3	113785	99675	IleumKo4m3	165878	141111
CecumWt4m1	403471	334737	CecumKo4m1	296903	268025
CecumWt4m2	165559	137599	CecumKo4m2	132865	121879
CecumWt4m3	343752	294418	CecumKo4m3	251818	233015
ColonWt4m1	335560	293134	ColonKo4m1	164888	159636
ColonWt4m2	179090	153374	ColonKo4m2	433951	373225
ColonWt4m3	195516	154747	ColonKo4m3	144376	129894
DuoWt11m1	326369	277280	DuoKo11m1	176954	102516
DuoWt11m2	261068	235598	DuoKo11m2	335449	288230
DuoWt11m3	308998	234413	DuoKo11m3	268564	210457
JejuWt11m1	116272	58332	JejuKo11m1	182239	161843
JejuWt11m2	290152	259018	JejuKo11m2	142213	117596
JejuWt11m3	236392	209109	JejuKo11m3	201739	165961
IleumWt11m1	112111	91530	IleumKo11m1	62425	46888
IleumWt11m2	132709	91268	IleumKo11m2	38232	33319
IleumWt11m3	84860	53361	IleumKo11m3	136276	114670
CecumWt11m1	330078	262078	CecumKo11m1	19634	13346
CecumWt11m2	87920	66271	CecumKo11m2	38846	23790
CecumWt11m3	94691	72129	CecumKo11m3	51463	30921
ColonWt11m1	210195	150082	ColonKo11m1	22363	15078
ColonWt11m2	51140	35999	ColonKo11m2	62327	41645

Alpha diversity

Because qualified bacterial sequences in each sample were not equal, we random subsampling at equal sequencing depth (13,346 reads per sample), according to the normalized the data by Mothur's SOP (Kozich et al., 2013). This sequencing depth was sufficient as their lowest rarefaction curves at phylum (Figure 1) and genus (Figure 2) levels, increase and reach plateau (relatively constant number of OTUs albeit increasing number of sequencing). Supportively, Good' s coverage indices at phylum (Table 2) and genus (Table 3) levels were all more than 99 % in all samples. Together, this indicated that this subsampling sequencing depth was sufficient and covered > 99 % of bacterial OTUs diversity. Tables 2 and 3 also listed numbers of OTUs at phylum level (880 total OTUs, and ranged from 7-17 OTUs per sample), and genus level (4,944 total OTUs, and ranged from 46-154 OTUs per sample). At phylum level, DuoKo11m2, JejuKo11m2, JejuWt11m1, and DuoKo4m1 relatively exhibited the highest numbers of OTUs, whereas DuoWt4m3, and ColonKo4m1 exhibited the lowest numbers of OTUs. At genus level, DuoKo11m2, JejuWt11m1, and JejuKo11m2 relatively exhibited the highest numbers of OTUs, whereas DuoWt4m2, JejuWt4m3, and IleumKo11m3 exhibited the lowest numbers of OTUs.



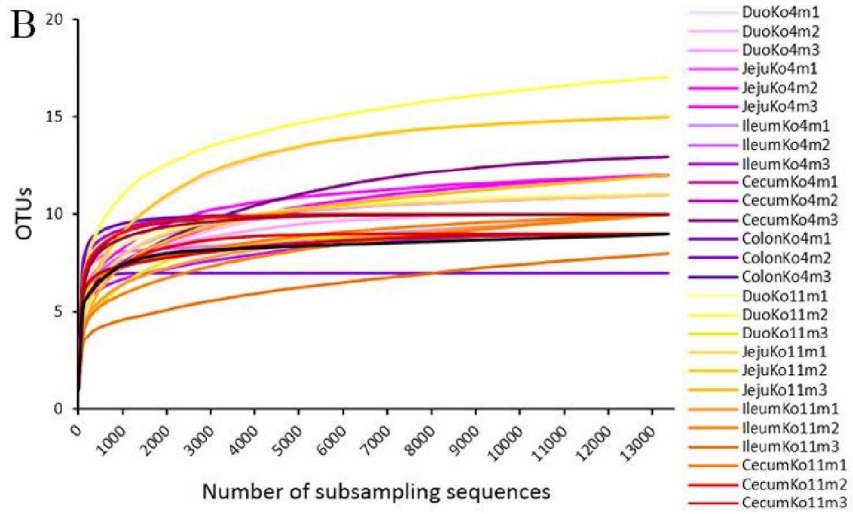


Figure 1. Rarefaction curve of 16S rRNA gene sequences at phylum level of wild type (A) and FcGRIIb knockout (B) groups.

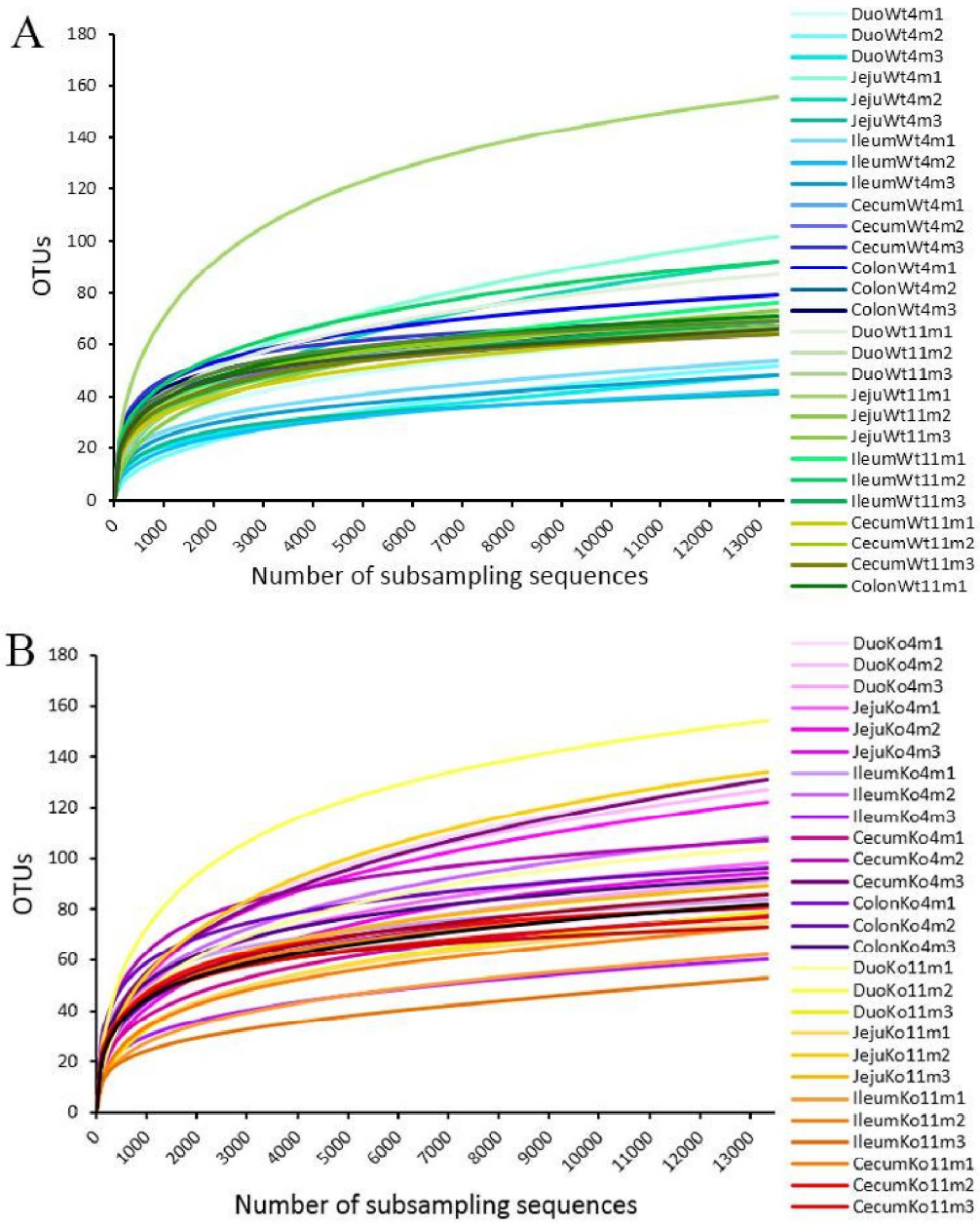


Figure 2. Rarefaction curve of 16S rRNA gene sequences at genus level of wild type (A) and FcGRIIb knockout (B) groups.

Table 2. Estimates of sample coverage and OTU number at phylum level.

Phylum level					
Wildtype group			Knockout group		
Sample ID	Coverage	OTUs	Sample ID	Coverage	OTUs
DuoWt4m1	1.000000	8	DuoKo4m1	0.999925	15
DuoWt4m2	0.999925	8	DuoKo4m2	0.999925	12
DuoWt4m3	0.999925	7	DuoKo4m3	1.000000	10
JejuWt4m1	0.999775	10	JejuKo4m1	1.000000	9
JejuWt4m2	0.999850	10	JejuKo4m2	0.999925	12
JejuWt4m3	0.999925	8	JejuKo4m3	0.999925	12
IleumWt4m1	0.999925	8	IleumKo4m1	0.999850	10
IleumWt4m2	0.999850	9	IleumKo4m2	0.999925	11
IleumWt4m3	0.999925	8	IleumKo4m3	1.000000	9
CecumWt4m1	0.999925	9	CecumKo4m1	1.000000	9
CecumWt4m2	0.999925	9	CecumKo4m2	1.000000	10
CecumWt4m3	0.999925	10	CecumKo4m3	0.999925	13
ColonWt4m1	1.000000	10	ColonKo4m1	1.000000	7
ColonWt4m2	1.000000	10	ColonKo4m2	1.000000	10
ColonWt4m3	0.999925	10	ColonKo4m3	1.000000	10
DuoWt11m1	0.999925	8	DuoKo11m1	1.000000	11
DuoWt11m2	0.999850	9	DuoKo11m2	0.999850	17
DuoWt11m3	1.000000	8	DuoKo11m3	1.000000	9
JejuWt11m1	0.999850	15	JejuKo11m1	0.999925	11
JejuWt11m2	0.999850	9	JejuKo11m2	0.999925	15
JejuWt11m3	0.999925	10	JejuKo11m3	0.999850	12
IleumWt11m1	0.999700	11	IleumKO11m1	0.999850	10
IleumWt11m2	0.999925	9	IleumKO11m2	0.999850	10
IleumWt11m3	0.999925	8	IleumKO11m3	0.999850	8
CecumWt11m1	0.999850	8	CecumKO11m1	0.999925	10
CecumWt11m2	0.999775	10	CecumKO11m2	1.000000	10
CecumWt11m3	0.999925	8	CecumKO11m3	1.000000	9
ColonWt11m1	0.999925	9	ColonKO11m1	1.000000	9
ColonWt11m2	1.000000	8	ColonKO11m2	1.000000	10
ColonWt11m3	0.999925	8	ColonKO11m3	0.999925	9

Table 3. Estimates of sample coverage and OTU number at genus level.

Genus level					
Wildtype group			Knockout group		
Sample ID	Coverage	OTUs	Sample ID	Coverage	OTUs
DuoWt4m1	0.998127	71	DuoKo4m1	0.997153	131
DuoWt4m2	0.999176	46	DuoKo4m2	0.997003	128
DuoWt4m3	0.998576	53	DuoKo4m3	0.998876	90
JejuWt4m1	0.997977	96	JejuKo4m1	0.998277	102
JejuWt4m2	0.997752	91	JejuKo4m2	0.996853	135
JejuWt4m3	0.999101	46	JejuKo4m3	0.997902	103
IleumWt4m1	0.998876	49	IleumKo4m1	0.998651	81
IleumWt4m2	0.999026	49	IleumKo4m2	0.998202	106
IleumWt4m3	0.999251	49	IleumKo4m3	0.998801	57
CecumWt4m1	0.999326	64	CecumKo4m1	0.998651	83
CecumWt4m2	0.999326	63	CecumKo4m2	0.998876	109
CecumWt4m3	0.999401	69	CecumKo4m3	0.997527	127
ColonWt4m1	0.998801	81	ColonKo4m1	0.998277	86
ColonWt4m2	0.999550	63	ColonKo4m2	0.999026	96
ColonWt4m3	0.999176	70	ColonKo4m3	0.998576	94
DuoWt11m1	0.998501	89	DuoKo11m1	0.997677	109
DuoWt11m2	0.999101	69	DuoKo11m2	0.997677	154
DuoWt11m3	0.998277	77	DuoKo11m3	0.999101	67
JejuWt11m1	0.997977	149	JejuKo11m1	0.998202	76
JejuWt11m2	0.998951	67	JejuKo11m2	0.997003	137
JejuWt11m3	0.998352	73	JejuKo11m3	0.998501	91
IleumWt11m1	0.998576	76	IleumKO11m1	0.998501	66
IleumWt11m2	0.997977	90	IleumKO11m2	0.998576	73
IleumWt11m3	0.998801	70	IleumKO11m3	0.999026	47
CecumWt11m1	0.998726	61	CecumKO11m1	0.998726	82
CecumWt11m2	0.999401	66	CecumKO11m2	0.998801	84
CecumWt11m3	0.998801	69	CecumKO11m3	0.998726	80
ColonWt11m1	0.999251	66	ColonKO11m1	0.999326	72
ColonWt11m2	0.999625	66	ColonKO11m2	0.999026	79
ColonWt11m3	0.999101	69	ColonKO11m3	0.999026	82

The alpha diversity indices (Chao and Shannon) were calculated to investigate bacterial diversity within each sample. The alpha diversity especially at wildtype young age (Wildtype_4m) different from small to large intestine sections, and highest diversity in the final intestine section, the colon.

sample groups (Figures 3 and 4). In contrast, the alpha diversity in early sections of lupus-prone mice tended to increase when compared to aged matched healthy mice (Figures 3 and 4; e.g. Shannon Knockout_4m duodenum, jejunum and ileum.

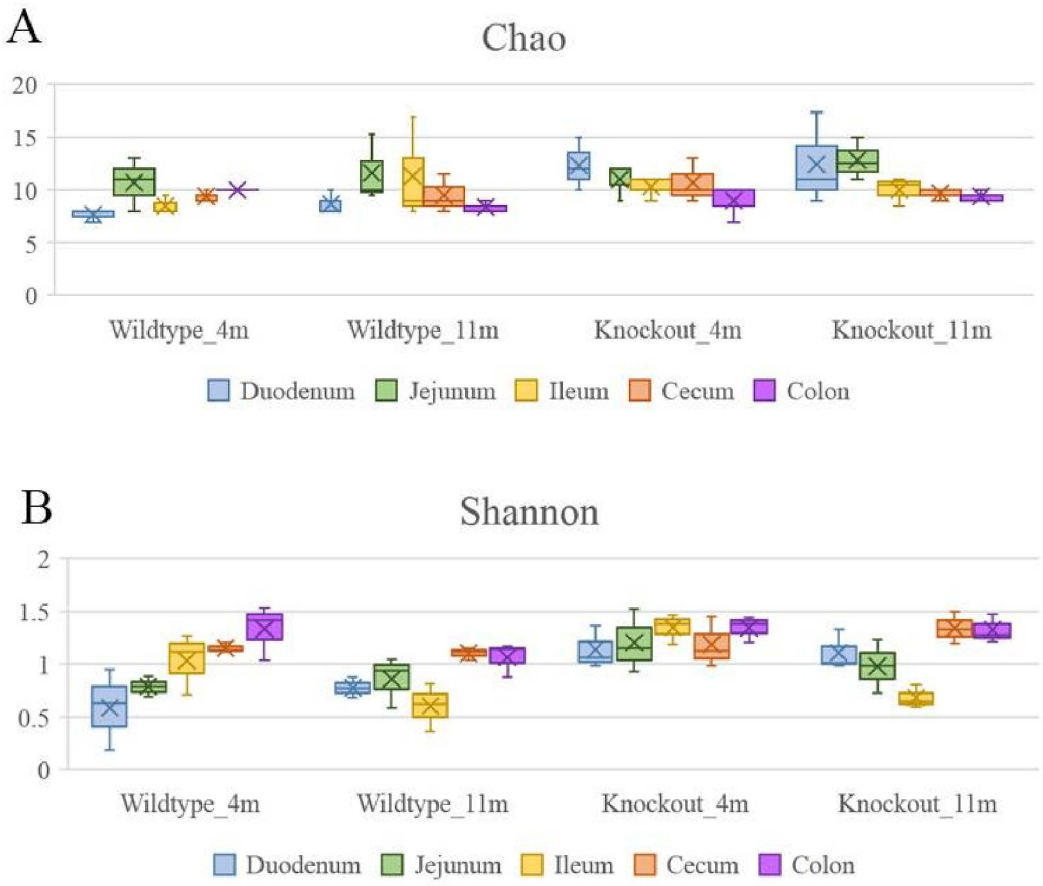


Figure 3. Alpha diversity including Chao (species richness) (A) and Shannon (species diversity) (B) indices at phylum level among healthy mice (Wildtype_4m, Wildtype_11m) and lupus-prone mice (Knockout_4m, Knockout_11m).

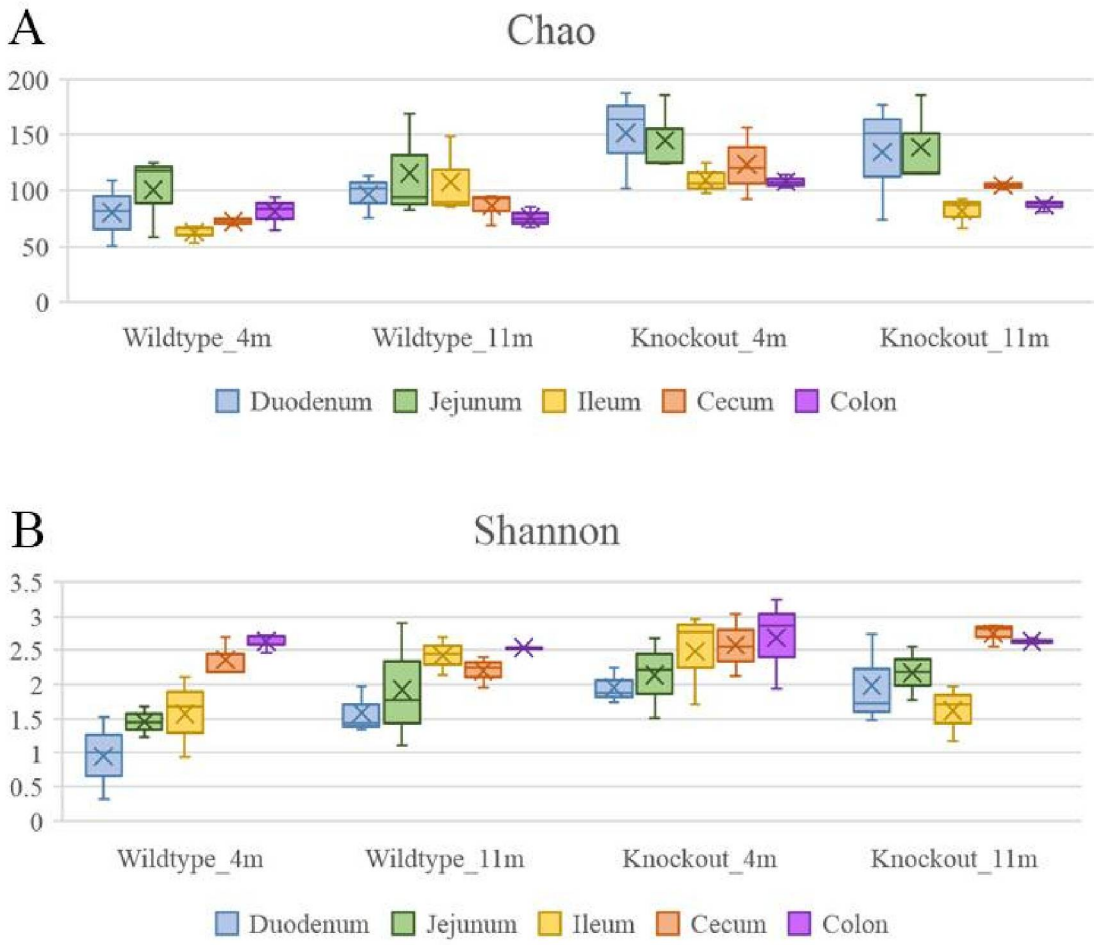


Figure 4. Alpha diversity including Chao (species richness) (A) and Shannon (species diversity) (B) indices at genus level among healthy mice (Wildtype_4m, Wildtype_11m) and lupus-prone mice (Knockout_4m, Knockout_11m).

Beta diversity and association with SLE

Beta diversity, or analyses of gut microbiota similarities and differences among sample groups representing different GI tract sections, of healthy and SLE. Cecum and colon of pre-clinical (4 months) and established SLE (11 months) mice showed obviously different from age-matched healthy mice for phylum (Figure 5) and genus (Figure 6) OTU found compositions. Moreover, gut compositions of healthy young were different from old mice, and the old healthy gut microbiota seened to be closer to pre-clinical and established SLE mice (Figure 6). Thus, gut microbiota altered by age, genetic FcGRIIb knockout, and may also disease stage (preclinical and established

SLE). The predominant bactetial phyla were Bacteroidetes (42.93%), Firmicutes (36.00%) and Proteobacteria (12.23%) (Figure 5). The predominant bactetial genera in all samples were f_S24-7_unclassified (35.01), f_Lachnospiraceae_ unclassified (7.05%) and *Lactobacillus* (6.02%) (Figure 6). Interestingly, we found some bacterial OTUs were likely increase in disease mice such as p_Verrucomicrobia p_Deferribacteres, g_Olsenella, g_adlercreutzia, g_Mucispirillium, and g_Akkermansia were likely increase, whereas f_S24-7 unclassified was decrease in disease mice compared to aged matched healthy mice.

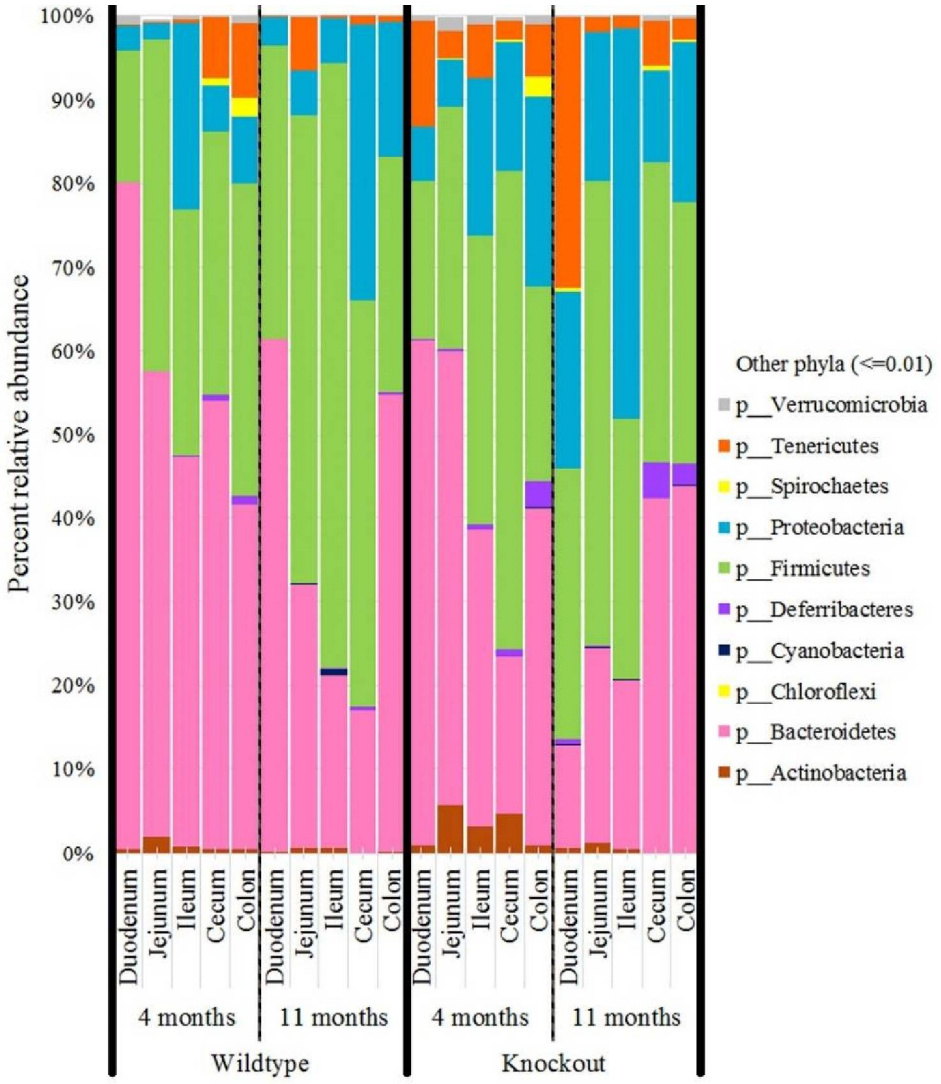


Figure 5. Difference of gut microbiota between wildtype and SLE mice in assorted intestine sections and time (4 and 11 months) (n = 3 per group), at phylum level. Data show average. Bacterial phyla below 1 percent relative abandance.

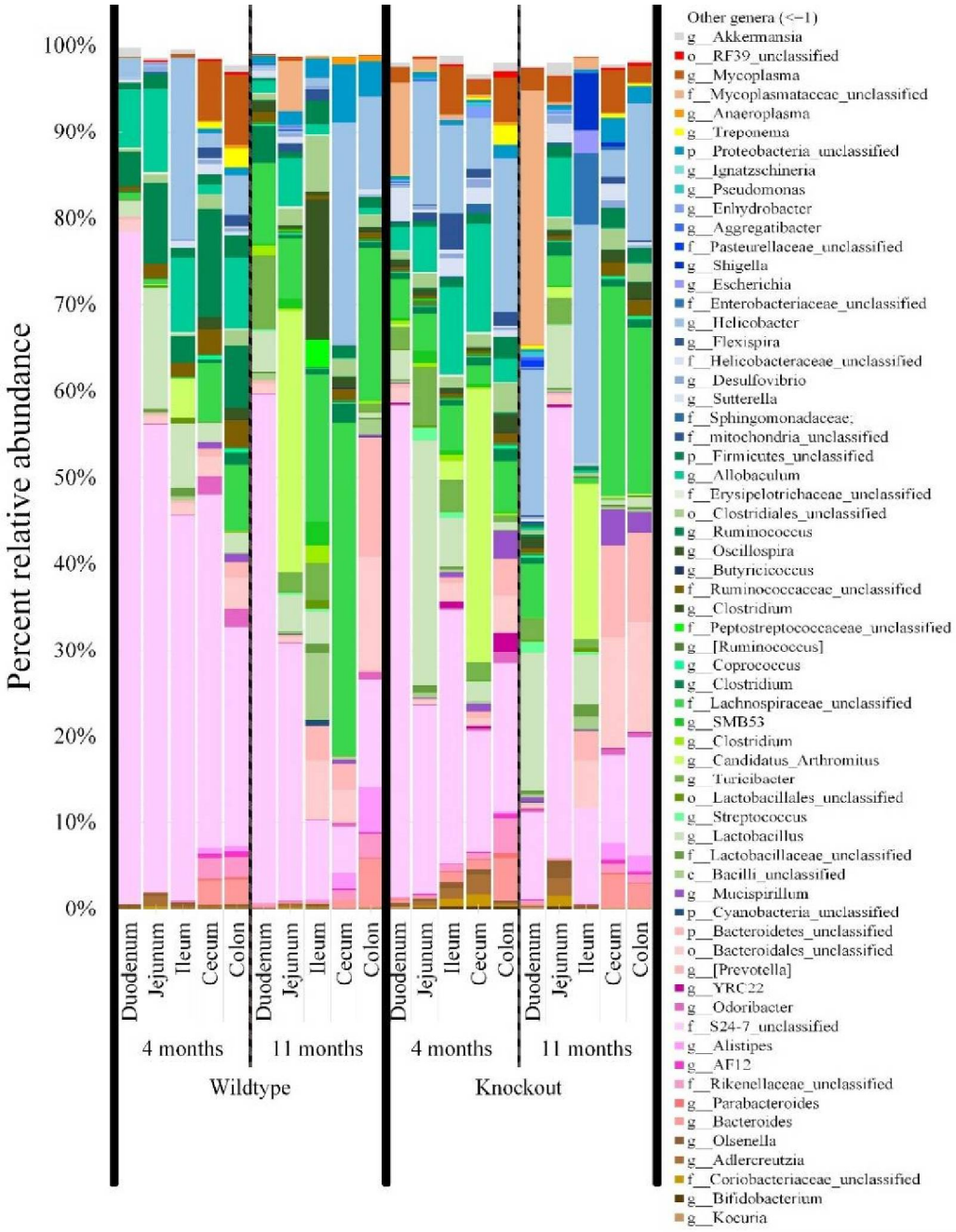


Figure 6. Difference of gut microbiota between wildtype and SLE mice in assorted intestine sections and time (4 and 11 months) (n = 3 per group), at genus level. Data show average. Bacterial phyla below 1 percent relative abandance.

Gut microbiota according to disease and healthy mice of sections along GI tract, showed significant difference between healthy and SLE disease, which might infer potential beneficial and harmful bacteria. We computed thetayc similarity distances among samples and analysed PCoA along P statistic Homava methods to compare gut microbiota structures among sample groups. Figure 7 showed the gut microbiota compositions became more different and clearly seperated as moving into the cecum and colon ($P<0.05$ or become close to 0.05).

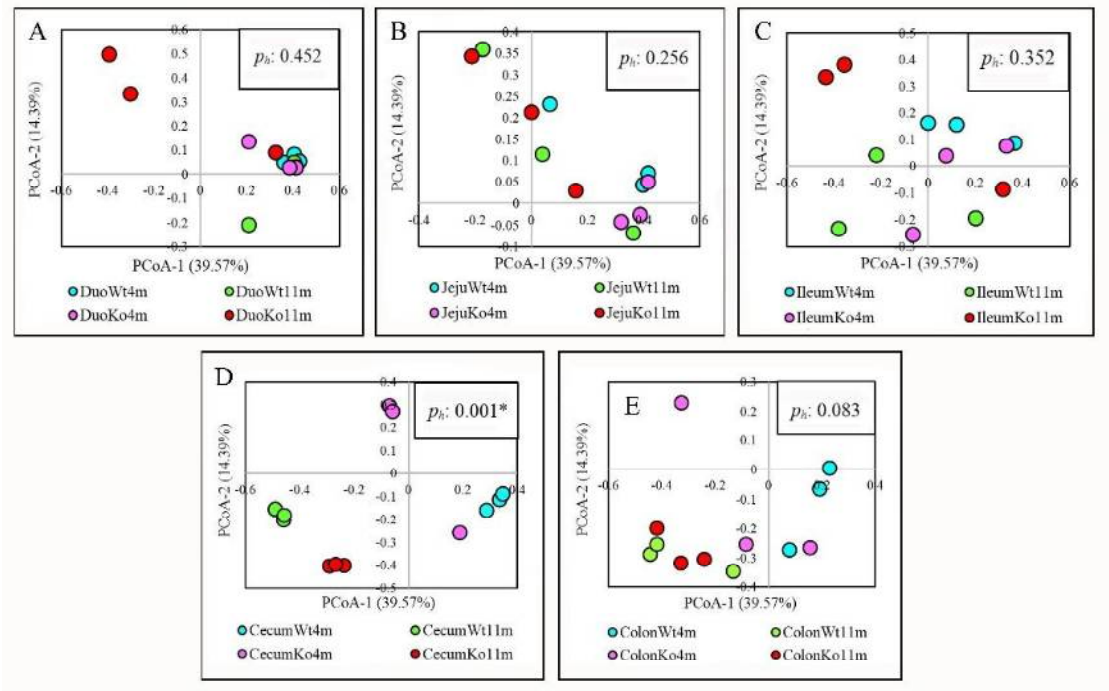


Figure 7. Principal coordinate analysis (PCoA) of gut microbiota compositions at genus level in different intestine sections including (A) duodenum, (B) jejunum, (C) ileum, (D) cecum and (E) colon. The P_h-value of less than 0.05 was considered statistically significant by Homova.

The large intestine (cecum and colon) sections were thereby region to observe the potentially beneficial and harmful bacteria associated to SLE. Noted that the Thetayc similarity distances and PCoA analyses of preclinical SLE mice were closer to healthy young mice, than between the established SLE and healthy old mices, supportively gut microbiota were altered by disease and age factors (data not shown) ; thus only the established SLE showed.

Morover, we perfromed phylogenetic clustering to investigate the relatedness of bacterial communities among different sections of intestine, and found that the small intestine sections were closer and clustered together, seperated from the large intestine sections in all samples (Figure 8). However, ileum sections were in the middle of small and large intestine causing they also were clustered with large intestine. These results supported that cecum and colon were dramatic sections to investigate the potentially beneficial and harmful bacteria.

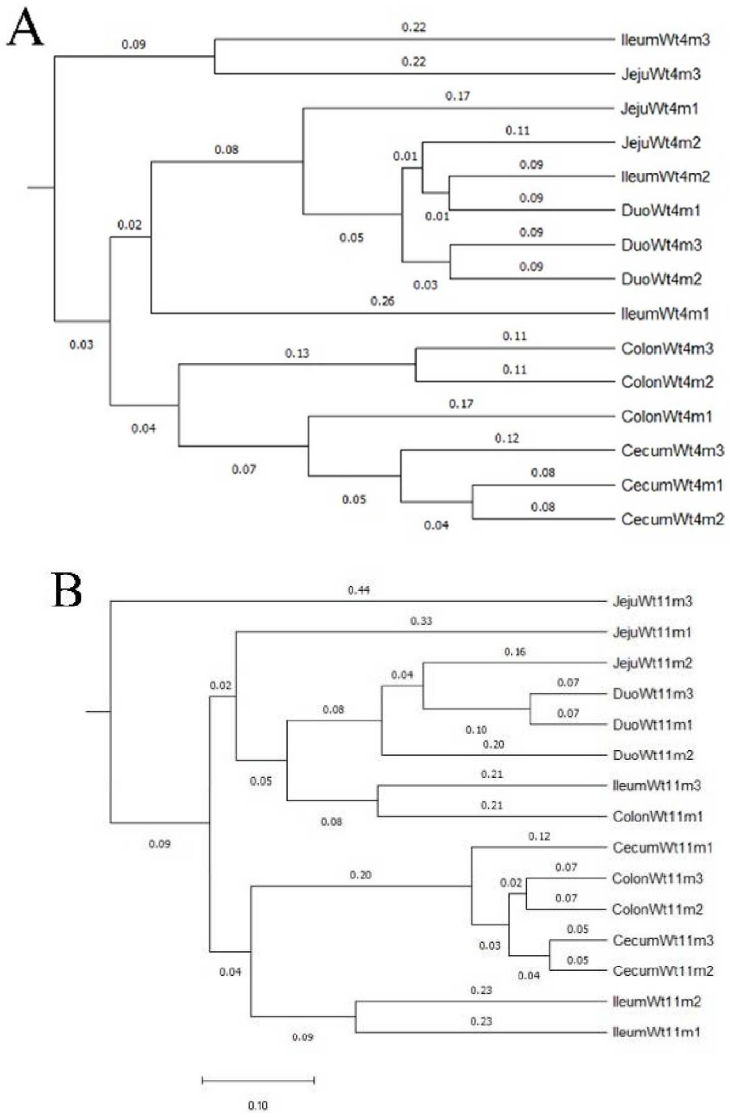


Figure 8. Phylogenetic trees (A) healthy group, (B) old group, (C) preclinical SLE and (D) established SLE mice, showing community relatedness by Braycurtis distance method. Number on bar infers unit of genetic distance.

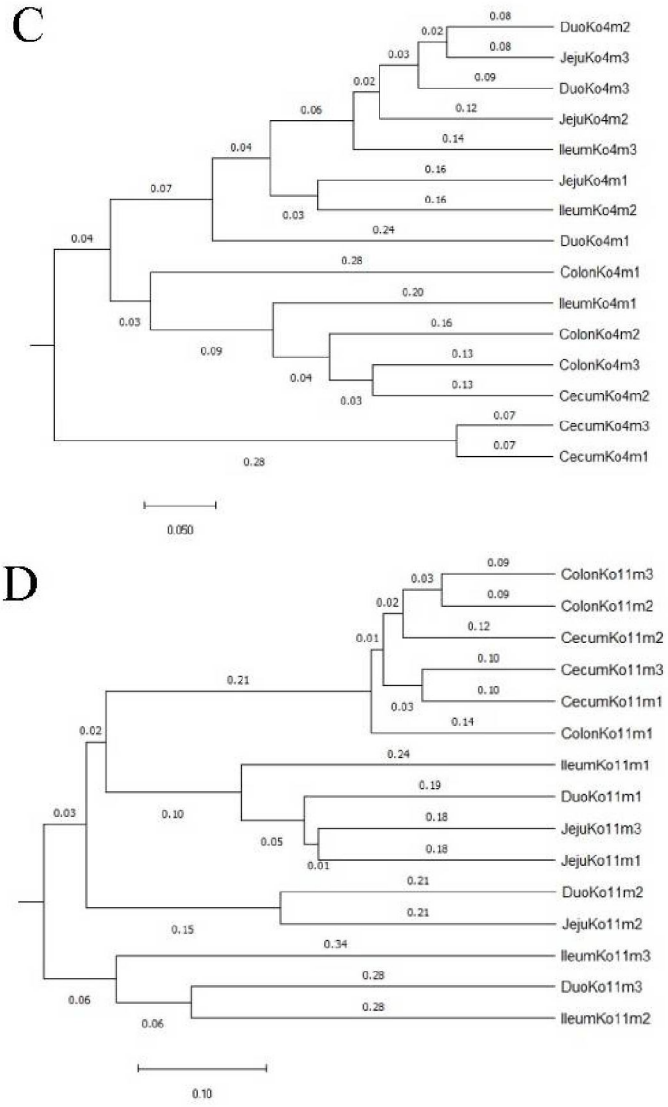


Figure 9. Phylogenetic trees (A) healthy group, (B) old group, (C) preclinical SLE and (D) established SLE mice, showing community relatedness by Braycurtis distance method. Number on bar infers unit of genetic distance.

Identification of gut genera associated with SLE establishment

To determine potential beneficial and harmful bacteria associated with SLE in cecum and colon, we used Metastats and identified bacterial genera with statistic significances ($P<0.05$). Tables 4-7 highlighted that some bacteria found only in cecum or colon and some only found in pre- and post- disease comparations or age matched healthy and post- disease comparations, as a result of genetic FcGRIIb knockout and stage of disease, given the cecum was found bacteria than the colon. The potential beneficial bacteria referred to bacterial genera which found more abundance in age matched- healthy and preclinical SLE mice, including f_S24- 7_unclassified, *Anaerostipes*, *Lactobacillus*, *Sutterella*, *Allobaculum*, *Adlercreutzia* and *Bifidobacterium*. The potential harmful bacteria referred to bacterial genera which found more abundance in established SLE mice, including *Mucispirillum*, *Oscillospira*, f_Lachnospiraceae_unclassified, *Alistipes*, and *Sporobacter*.

Table 4. Significantly relative abundances altered gut genera in cecum after SLE establishment (preclinical vs. established SLE)

Bacterial genera	CecumKo4m		CecumKo11m		p-value
	Mean	SD	Mean	SD	
f__S24-7_unclassified	0.10599	0.00103	0.07770	0.00665	0.02425
f__Lachnospiraceae_unclassified	0.00833	0.00063	0.17668	0.03466	0.02062
g__Helicobacter	0.07051	0.00325	0.02495	0.00713	0.01652
g__Lactobacillus	0.02933	0.00106	0.00373	0.00155	0.00984
o__Bacteroidales_unclassified	0.00336	0.00003	0.09205	0.00755	0.01105
p__Bacteroidetes_unclassified	0.00112	0.00009	0.07192	0.00840	0.01349
f__Mycoplasmataceae_unclassified	0.00322	0.00080	0.00015	0.00007	0.03121
g__Allobaculum	0.14761	0.02283	0.00056	0.00033	0.01530
g__Candidatus_Arthromitus	0.38672	0.02189	0.00159	0.00156	0.00739
f__Ruminococcaceae_unclassified	0.00032	0.00009	0.01160	0.00024	0.00437
p__Proteobacteria_unclassified	0.00195	0.00017	0.02064	0.00205	0.01332
o__Clostridiales_unclassified	0.00899	0.00055	0.01725	0.00111	0.01470
g__Alistipes	0.00012	0.00012	0.01587	0.00405	0.03061
g__Turicibacter	0.02602	0.00126	0.00031	0.00013	0.00558
f__Rikenellaceae_unclassified	0.00057	0.00006	0.00808	0.00033	0.00497
g__Oscillospira	0.00060	0.00009	0.00954	0.00308	0.04442
g__Clostridium	0.00035	0.00000	0.00619	0.00191	0.03923
g__Ruminococcus	0.00026	0.00014	0.00096	0.00018	0.04089
g__Sutterella	0.01838	0.00121	0.00042	0.00013	0.00923
f__Erysipelotrichaceae_unclassified	0.00362	0.00103	0.00013	0.00005	0.03459
g__Adlercreutzia	0.02763	0.00155	0.00036	0.00017	0.00801
g__Clostridium	0.00167	0.00040	0.00021	0.00012	0.03399
g__Olsenella	0.00632	0.00167	0.00012	0.00003	0.03182
f__Coriobacteriaceae_unclassified	0.01769	0.00098	0.00021	0.00010	0.00679
f__Peptostreptococcaceae_unclassified	0.00075	0.00000	0.00004	0.00004	0.00618
g__Streptococcus	0.00078	0.00009	0.00017	0.00012	0.02501
c__Clostridia_unclassified	0.00026	0.00014	0.00339	0.00060	0.01868
g__Parabacteroides	0.00012	0.00000	0.00146	0.00042	0.03594
g__Bifidobacterium	0.00362	0.00080	0.00008	0.00008	0.02259
g__Coprococcus	0.00006	0.00000	0.00192	0.00065	0.04580
o__Burkholderiales_unclassified	0.00043	0.00003	0.00008	0.00008	0.02320
g__Acinetobacter	0.00020	0.00003	0.00000	0.00000	0.01409
o__Desulfovibrionales_unclassified	0.00023	0.00006	0.00103	0.00006	0.01228
g__Dorea	0.00003	0.00003	0.00044	0.00010	0.02923
o__Bacillales_unclassified	0.00000	0.00000	0.00008	0.00002	0.02712
g__Dehalobacterium	0.00003	0.00003	0.00052	0.00017	0.04795
p__Actinobacteria_unclassified	0.00043	0.00009	0.00012	0.00007	0.04304
g__Gemella	0.00012	0.00000	0.00000	0.00000	0.00000
g__Anaerostipes	0.00080	0.00006	0.00002	0.00002	0.01044
g__SMB53	0.00471	0.00040	0.00000	0.00000	0.01166
c__Deltaproteobacteria_unclassified	0.00006	0.00000	0.00027	0.00005	0.02441
g__p-75-a5	0.00035	0.00006	0.00000	0.00000	0.01591
g__Coprobacillus	0.00014	0.00003	0.00000	0.00000	0.01928
g__Burkholderia	0.00006	0.00000	0.00000	0.00000	0.00000
g__Enterococcus	0.00006	0.00000	0.00000	0.00000	0.00000
g__Sporobacter	0.00000	0.00000	0.00006	0.00000	0.00376
g__Granulicatella	0.00006	0.00000	0.00000	0.00000	0.00000

Notes: Mean = average of relative abundances, SD = standard deviation, p_= phylum, o_ = order, c_=class, f_=family and g_=genus.

Table 5. Significantly relative abundances altered gut genera in colon after SLE establishment (preclinical vs. established SLE).

Bacterial genera	ColonKo4m		ColonKo11m		p-value
	Mean	SD	Mean	SD	
f__S24-7_unclassified	0.17819	0.03403	0.07770	0.00665	0.02492
f__Lachnospiraceae_unclassified	0.05492	0.01035	0.17668	0.03466	0.01553
o__Bacteroidales_unclassified	0.04159	0.02121	0.09205	0.00755	0.04832
c__Bacilli_unclassified	0.00056	0.00021	0.00167	0.00041	0.03933
g__Alistipes	0.00266	0.00093	0.01587	0.00405	0.01894
g__Sutterella	0.00599	0.00246	0.00042	0.00013	0.04693
f__Erysipelotrichaceae_unclassified	0.00134	0.00045	0.00013	0.00005	0.02899
g__Desulfovibrio	0.00203	0.00053	0.00687	0.00172	0.02635
c__Clostridia_unclassified	0.00109	0.00036	0.00339	0.00060	0.01631
o__Bacillales_unclassified	0.00000	0.00000	0.00008	0.00002	0.00852
f__Mogibacteriaceae_unclassified	0.00052	0.00007	0.00008	0.00002	0.00489
g__SMB53	0.00090	0.00037	0.00000	0.00000	0.03856
g__Coprobacillus	0.00023	0.00003	0.00000	0.00000	0.00355
o__HA64_unclassified	0.00033	0.00005	0.00000	0.00000	0.00411
f__Deferribacteraceae_unclassified	0.00000	0.00000	0.00013	0.00005	0.03203
Notes: Mean = average of relative abundances, SD = standard deviation, p_= phylum, o_ = order, c_=class, f_=family and g_=genus.					

Table 6. Significantly relative abundances altered gut genera in cecum between established SLE and age-matched healthy.

Bacterial genera	CecumWt11m		CecumKo11m		p-value
	Mean	SD	Mean	SD	
g__Helicobacter	0.22201	0.00036	0.02495	0.00713	0.00123
o__Bacteroidales_unclassified	0.04029	0.00025	0.09205	0.00755	0.00787
p__Bacteroidetes_unclassified	0.03282	0.00012	0.07192	0.00840	0.01124
f__Mycoplasmataceae_unclassified	0.00000	0.00000	0.00015	0.00007	0.04573
g__Mycoplasma	0.00004	0.00000	0.04132	0.01600	0.03290
p__Proteobacteria_unclassified	0.06294	0.00017	0.02064	0.00205	0.00277
g__Mucispirillum	0.00446	0.00001	0.03451	0.01267	0.03732
g__Clostridium	0.02024	0.00002	0.00619	0.00191	0.00824
g__Sutterella	0.00012	0.00000	0.00042	0.00013	0.04963
g__Desulfovibrio	0.00178	0.00000	0.00687	0.00172	0.02858
g__Flexispira	0.00000	0.00000	0.00645	0.00288	0.04358
f__Helicobacteraceae_unclassified	0.00050	0.00000	0.01499	0.00622	0.03826
g__Ruminococcus	0.00477	0.00000	0.00084	0.00030	0.01215
g__Parabacteroides	0.00046	0.00000	0.00146	0.00042	0.04478
f__Porphyromonadaceae_unclassified	0.00006	0.00000	0.00075	0.00032	0.04896
f__Deferribacteraceae_unclassified	0.00000	0.00000	0.00013	0.00005	0.02997
g__Sporobacter	0.00000	0.00000	0.00006	0.00000	0.00000
Notes: Mean = average of relative abundances, SD = standard deviation, p_= phylum, o_ = order, c_=class, f_=family and g_=genus.					

Table 7. Significantly relative abundances altered gut genera in colon between established SLE and age-matched healthy.

Bacterial genera	ColonWt11m		ColonKo11m		p-value
	Mean	SD	Mean	SD	
g__Mycoplasma	0.00006	0.00003	0.01670	0.00323	0.00442
f__Ruminococcaceae_unclassified	0.00684	0.00150	0.01224	0.00146	0.04079
p__Proteobacteria_unclassified	0.03832	0.00792	0.01450	0.00113	0.02257
g__Alistipes	0.04385	0.00436	0.01926	0.00704	0.02353
f__Rikenellaceae_unclassified	0.02070	0.00385	0.00892	0.00193	0.03501
g__Oscillospira	0.00458	0.00037	0.01111	0.00252	0.04281
f__Erysipelotrichaceae_unclassified	0.00056	0.00012	0.00012	0.00009	0.02105
g__Flexispira	0.00000	0.00000	0.00157	0.00056	0.03255
g__Clostridium	0.00197	0.00045	0.00002	0.00002	0.00644
g__Ruminococcus	0.00184	0.00041	0.00061	0.00011	0.02511
g__Streptococcus	0.00002	0.00002	0.00010	0.00002	0.02837
o__Burkholderiales_unclassified	0.00000	0.00000	0.00013	0.00005	0.03983
g__Dorea	0.00123	0.00036	0.00029	0.00013	0.04738
f__Desulfovibrionaceae_unclassified	0.00004	0.00004	0.00017	0.00003	0.03789
Notes: Mean = average of relative abundances, SD = standard deviation, p_= phylum, o_ = order, c_=class, f_=family and g_=genus.					

DISCUSSION

Previous studies reported that gut microbiota changes were associated with SLE disease that is caused by various factors such as a different genetic pathway (i. e. lymphocyte activation, inflammation, immune complex regulation) and environment (Mu, et al., 2017a; Tsokos, 2011; Tsokos et al., 2016). In this study, we investigated how gut microbiota changes in various sections of intestine during SLE development based on delete in *FCGR2B* gene. This pathway of SLE development, as well as different section of intestine had rarely been viewed elsewhere. In each intestinal section, lupus-prone mice showed altered bacterial composition and higher diversity compared to aged-match healthy mice, especially in cecum and colon, same as Zhang et al. (2014) report in MRL/Mp-Fas^{lpr} (MRL/lpr) mice, highlighting that SLE is associated with gut microbiota changes. We found that gut microbiota was altered by age (4 vs. 11 months) and genetics. Due to the most difference between healthy and SLE disease, cecum and colon represented the region to investigate the potentially beneficial and harmful bacteria associated with SLE. The potential beneficial bacteria, which were found more abundance in age matched- healthy and preclinical SLE, were f_S24-7_unclassified, *Anaerostipes*, *Lactobacillus*, *Sutterella*, *Allobaculum*, *Adlercreutzia* and *Bifidobacterium*. The potential harmful bacteria found in

Established SLE were *Mucispirillum*, *Oscillospira*, f_Lachnospiraceae_unclassified, *Alistipes*, and *Sporobacter*. Parker et al. (2018) reported that increased abundance of family S24-7 was associated with protection from an autoimmune disease including colitis. Zhixing et al. (2019) reported that *Lactobacillus*, *Sutterella*, *Allobaculum*, *Adlercreutzia* was positively associated with the bromofuranone-mediated promotion for the treatment of lupus and *Anaerostipes* were positively associated with prednisone treatment for SLE. Zhang et al. (2014) also found depletion of *Bifidobacterium* and increases of Lachnospiraceae and *Alistipes* in lupus-prone (MRL/lpr) mice compared to healthy control group. Further, scientists suggested conversion of gut microbiota dysbiosis to normal by fecal transplantation, increasing some beneficial bacteria and decreasing some harmful bacteria, and observed attenuated SLE progression (Mu, et al., 2017a; Mu, et al., 2017b).

CONCLUSION

The alteration of gut microbiota is previously suggested and supported by our data to associate with autoimmune disease. Gut microbiota composition of lupus-prone ($FcGRIIb^{-/-}$) mice had disrupted higher diversity and altered bacterial composition compared to healthy mice, particularly in the large intestine including cecum and colon of established SLE stage than the healthy young mice, followed by healthy old mice, and preclinical SLE mice, respectively. These were correlated by beta diversity analyses, PCoA, and Metastats to point out many harmful bacteria and decreasing beneficial bacteria in established SLE.

ACKNOWLEDGEMENTS

This research was supported by Microbiome Research Unit for Probiotics in Food and Cosmetics, and H.M. the King Bhumibol Adulyadej's 72nd Birthday Anniversary Scholarship (to P. Chatthanathon). The authors thanked the Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand and Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and for providing the instruments.

REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic local alignment search tool. Journal of Molecular Biology. 215: 403-410.

Donaldson, G.P., Lee, S.M. and Mazmanian, S.K. 2016. Gut biogeography of the bacterial microbiota. Nature Reviews Microbiology. 14(1): 20-32.

Gu, S., Chen, D., Zhang, J.N., Lv, X., Wang, K., Duan, L.P., Nie, Y. and Wu, X. L. 2013. Bacterial community mapping of the mouse gastrointestinal tract. PLOS ONE. 8(10): e74957.

He, Z., Kong, X., Shao, T., Zhang, Y. and Wen, C. 2019. Alterations of the gut microbiota associated with promoting efficacy of prednisone by bromofuranone in MRL/lpr mice. Frontiers in Microbiology. 10: 978.

Illumina, Inc., Miseq system denature and dilute libraries guide [Document]. 2019. [cited 2019 Sept 20]. Available from: https://support.illumina.com/ sequencing/sequencing_instruments/miseq/documentation.html.

Kaul, A., Gordon, C., Crow, M. K., Touma, Z., Urowitz, M. B., van Vollenhoven, R., Ruiz-Irastorza, G. and Hughes, G. 2016. Systemic lupus erythematosus. Nature Reviews Disease Primers. 2: 16039.

Kosiewicz, M. M., Zirnheld, A. L. and Alard, P. 2011. Gut microbiota, immunity, and disease: a complex relationship. Frontiers in Microbiology. 2: 180.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. and Schloss, P.D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology. 79(17): 5112-20.

La Paglia, G.M.C., Leone, M.C., Lepri, G., Vagelli, R., Valentini, E., Alunno, A. and Tani, C. 2017. One year in review: systemic lupus erythematosus. Clinical and Experimental Rheumatology. 35: 551-561.

Mu, Q., Tavella, V. J., Kirby, J. L., Cecere, T. E., Chung, M., Lee, J., Li, S. Ahmed, S.A., Eden, K., Allen, I.C., Reilly, C.M. and Luo, X.M. 2017a. Antibiotics ameliorate lupus- like symptoms in mice. Scientific Reports. 7(1): 13675.

Mu, Q., Zhang, H., Liao, X., Lin, K., Liu, H., Edwards, M.R., Ahmed, S.A., Yuan, R., Li, L., Cecere, T.E., Branson, D.B., Kirby, J.L., Goswami, P., Leeth, C.M., Read, K.A., Oestreich, K.J., Vieson, M.D., Reilly,

C.M., Luo, X.M. 2017b. Control of lupus nephritis by changes of gut microbiota. Microbiome. 5(1): 73.

Parker, K.D., Albeke, S.E., Gigley, J.P., Goldstein, A.M. and Ward, N.L. 2018. Microbiome composition in both wild-type and disease model mice is heavily influenced by mouse facility. Frontiers in Microbiology. 9: 1598.

Rosser, E.C. and Mauri, C. 2016. A clinical update on the significance of the gut microbiota in systemic autoimmunity. Journal of Autoimmunity. 74: 85-93.

Tsokos, G.C. 2011. Systemic lupus erythematosus. New England Journal of Medicine. 365(22): 2110-2112.

Tsokos, G.C., Lo, M.S., Costa Reis, P. and Sullivan, K.E. 2016. New insights into the immunopathogenesis of systemic lupus erythematosus. Nature Reviews Rheumatology. 12(12): 716-730.

Zhang, H., Liao, X., Sparks, J. B. and Luo, X. M. 2014. Dynamics of gut microbiota in autoimmune lupus. Applied and Environmental Microbiology. 80(24): 7551–7560.