Clinical Rheumatology (2019) 38:1437–1445
https://doi.org/10.1007/s10067-018-04419-8

ORIGINAL ARTICLE

Relative abundance of *Megamonas hypermegale* and *Butyrivibrio* species decreased in the intestine and its possible association with the T cell aberration by metabolite alteration in patients with Behcet's disease (210 characters)

Jun Shimizu[1] · Takao Kubota[1] · Erika Takada[1] · Kenji Takai[1] · Naruyoshi Fujiwara[1] · Nagisa Arimitsu[1] · Yuji Ueda[1] · Sueshige Wakisaka[1] · Tomoko Suzuki[1] · Noboru Suzuki[1]

Received: 11 July 2018 / Revised: 22 December 2018 / Accepted: 27 December 2018 / Published online: 9 January 2019

Abstract

Objectives We have previously demonstrated that the phylum *Actinobacteria*, the family *Lactobacillaceae*, and the genus *Bifidobacterium* increased in relative abundance of gut microbiota in patients with Behcet's disease (BD). The phylum *Firmicutes* and the class *Clostridia* were predominant in the feces of normal individuals. The class *Clostridia* includes short-chain fatty acid–producing bacteria, important for the balance between regulatory T cells and helper T type 17 (Th17) cells. It is possible that the bacterial compositional alteration causes low intestinal short-chain fatty acid concentrations, leading to skewed immune functions in patients with BD.

Methods To test the hypothesis, we examined species composition and gene functions from the 16S rRNA data by utilizing PICRUSt software.

Results We have shown that relative abundance of *Eggerthella lenta*, *Acidaminococcus* species, *Lactobacillus mucosae*, *Bifidobacterium bifidum*, *Lactobacillus iners*, *Streptococcus* species, and *Lactobacillus salivarius* increased significantly in patients with BD. Relative abundance of *Megamonas hypermegale*, *Butyrivibrio* species, *Streptococcus infantis*, and *Filifactor* species increased significantly in normal individuals compared with BD patients. In the functional annotation analysis by PICRUSt, we found prevalent gene functions of the pentose phosphate pathway and the inosine monophosphate biosynthesis in patients with BD. The data suggested that BD gut microbes altered nucleic acid and fatty acid synthesis.

Conclusions These compositional and functional alterations of gut microbes may accompany unfavorable molecular exchanges between intestinal immunocompetent cells and gut microbes, and these interactions may have an association with the immune aberration in patients with BD.

Keywords Behcet's disease · *Butyrivibrio* species · Inosine monophosphate biosynthesis · *Megamonas hypermegale* · Pentose phosphate pathway · Th17 cells

Introduction

Behcet's disease (BD) is a systemic inflammatory disease, characterized by recurrent attacks of oral aphthosis, genital ulcers, skin lesions, and uveitis [1]. We have demonstrated that helper T type 17 (Th17) cells in peripheral blood increased significantly in BD patients [2, 3]. Th17 cells, concomitantly with neutrophils, infiltrated into skin lesions [3, 4]. Naïve T cells in patients with BD produced higher levels of interleukin (IL)17 in the presence of inflammatory cytokines,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10067-018-04419-8) contains supplementary material, which is available to authorized users.

✉ Noboru Suzuki
n3suzuki@marianna-u.ac.jp

[1] Department of Immunology and Medicine, St. Marianna University School of Medicine, Sugao 2-16-1, Miyamae-ku, Kawasaki 216-8511, Japan

tumor necrosis factor (TNF)α, IL1β, and IL23, than those of normal individuals [2].

Th17 cells [5] and regulatory T (Treg) cells [6] are shown to differentiate in mucosa associated lymphoid tissue and have a relationship with several specified gut microbes. Thus, it is possible that mucosal lymphoid tissue activation and imbalances in the composition of the gut microbiota, termed as dysbiosis, occur in BD patients. Accordingly, we conducted their metagenomic analysis.

We found that the family *Lactobacillaceae* and the genus *Bifidobacterium* increased in relative abundance of gut microbes in patients with BD [7]. Relative abundance of the order *Clostridia* decreased in BD patients. The order *Clostridia* includes several bacteria which produce short-chain fatty acids [8]. Short-chain fatty acids in the intestine act as one of activators of Treg cells [8]. Recent studies suggested that bacterial compositional and metabolic alterations played a role in the immunological abnormalities shown in autoimmune diseases through the inappropriate balance between Th17 cells and Treg cells [9]. Actually, a study reported low concentrations of butyrate, a short-chain fatty acid, in the feces of BD patients [10] with the characteristic gut microbe compositional alterations [10, 11], which were similar to our data in part. We observed that secretory IgA concentrations of the feces of BD patients increased significantly and considered that the altered microbe composition may accompany the immune aberration of the intestine in patients with BD [7].

Several microbial species were observed repeatedly in the intestine of human autoimmune diseases. For example, the species *Eggerthella lenta* was predominant in the gut microbiota of patients with immune disorders [12, 13].

Here, we obtained species information from a 16S rRNA metagenomic data of BD patients from analysis by PICRUSt software. Then, we evaluated bacterial gene functions of the gut microbes of BD patients and compared the data with those of normal individuals.

Materials and methods

16S rRNA metagenomic data

We analyzed the feces of 13 BD patients and 27 normal individuals to obtain 16S rRNA metagenomic data. Demographical and clinical data of BD patients and normal individuals were summarized in Table 1 [14].

This study was approved by the institutional review boards of St. Marianna University School of Medicine and was registered with the University Hospital Medical Information Network-Clinical Trials Registry (UMIN000018937). We conducted this research according to the principles expressed in the Declaration of Helsinki. We obtained written informed consent from each individual prior to enrolment in the study. A copy of the written consent is available for review upon request.

Table 1 Demographical and clinical characteristics of patients with Behcet's disease (BD) and normal individuals (NI) at the time of sample collection

Characteristics	Behcet's disease (BD, $n = 13$)	Normal individuals (NI, $n = 27$)
Age[a]	49.2 ± 4.7	52.8 ± 2.8
Men:women	5:8	12:15
Disease duration[a]	9.6 ± 1.4	NA[c]
Disease activity parameters		
BDAI[a, b]	6.6 ± 0.68	NA[c]
CRP[a]	0.34 ± 0.15	NA[c]
Oral aphthosis, %	100	0
Skin involvement, %	85	0
Genital ulcers, %	46	0
Uveitis, %	31	0
Gastrointestinal system involvement, %	15	0
Central nervous system involvement, %	15	0
Arthritis, %	8	0
Medication		
Colchicine, %	85	0
Steroid, %	38	0
Cyclosporine, %	15	0
Azathioprine, %	8	0
Methotrexate, %	0	0
Biologic agent, %	0	0

[a] Mean ± standard error

[b] Behcet's disease activity index [14]

[c] Not applicable

The study methods are described briefly as follows. We extracted genomic DNA from fecal samples by treating them with achromopeptidase (Wako Pure Chemical Industries, Tokyo, Japan) [15]. We amplified the V1–V2 16S rRNA gene region by primers according to the procedure reported previously [16]. We purified (AMPure XP magnetic purification beads, Beckman Coulter, Tokyo, Japan), quantified (Agilent 2100 Bioanalyzer, Agilent Technologies Japan, Tokyo, Japan), and sequenced the amplicon libraries (Ion Torrent PGM, Life Technologies Japan, Tokyo, Japan).

Sequence analysis

We filtered the output file using QIIME software (version 1.9.1) with the default settings. We obtained an operational taxonomic unit (OTU) table and estimated microbial alpha and beta diversity using QIIME software.

At the same time, we obtained another OTU table by QIIME software with a Greengenes-formatted database. We

modified and predicted the file by using PICRUSt software and obtained a taxonomy file and bacterial gene function files. We have uploaded these files to the Galaxy (http://huttenhower.sph.harvard.edu/galaxy) [17].

Statistical analysis

Uploaded files were analyzed statistically with LEfSe software [18]. LEfSe (linear discriminant analysis (LDA) effect size) was an algorithm to find significant differences in genomic features (genes, pathways, or taxa) between patients and normal individuals. The software used the nonparametric factorial Kruskal-Wallis sum-rank test ($P < 0.05$) and, subsequently, a set of pairwise tests utilizing the Wilcoxon rank-sum test. Then, LEfSe software used linear discriminant analysis to estimate the effect size of each differentially abundant feature. Those features that showed a higher log LDA score than 2.0 were chosen for subsequent plotting of output charts. Consequently, LEfSe indicated those features that better discriminate between BD patients and normal individuals with the log LDA scores.

We compared demographical and clinical data of patients and normal individuals with Wilcoxon rank-sum test or Fisher's exact test. We compared the OTU numbers and alpha diversity index scores of the QIIME's OTU table by using Wilcoxon rank-sum test. A P value less than 0.05 was considered significant.

Results

Reduction of *Megamonas hypermegale and Butyrivibrio* species in patients with BD

We estimated OTU numbers and alpha diversity (Chao 1 and Shannon indexes) using QIIME software. Alpha diversity was defined as the diversity within a community. We did not find any significant differences in the OTU numbers and the index scores between BD patients and normal individuals (Fig. 1a).

We next estimated beta diversity between BD patients and normal individuals. Beta diversity was defined as the diversity between communities. We obtained a figure of principal coordinates analysis (PCoA) plots using a linear conversion formula (Fig. 1b).

We evaluated the distance between the distribution of BD patients and that of normal individuals using a two-sided Student's two-sample t test and Monte Carlo permutations of QIIME software. We obtained significant P values of the beta diversity between BD patients and normal individuals in both weighted (quantitative) and unweighted (qualitative) UniFrac PCoA even after the Bonferroni correction (both had $P = 0.01$).

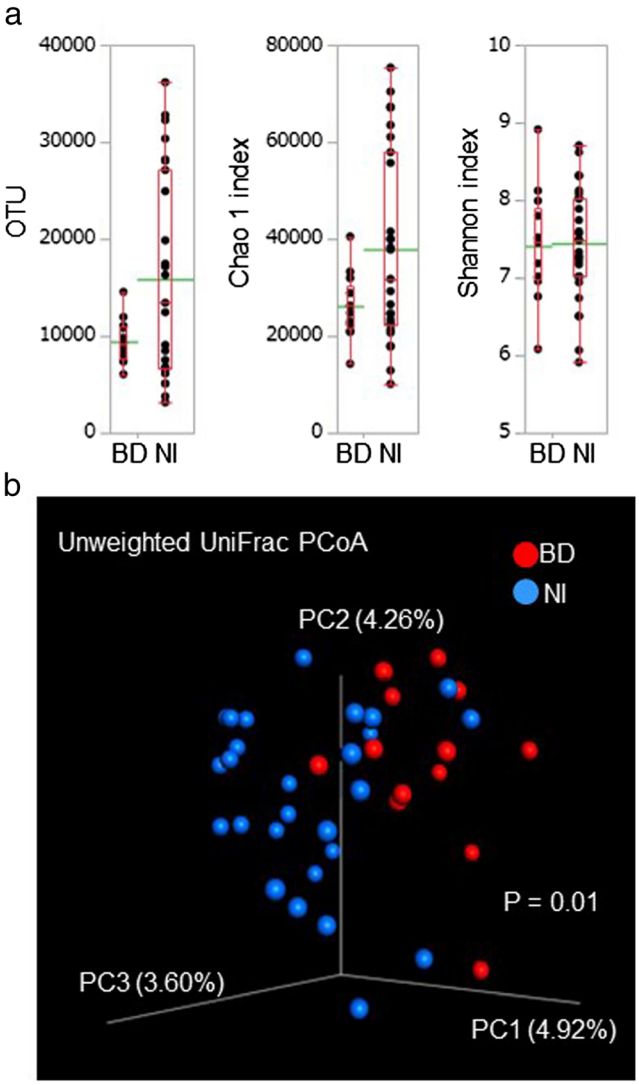

Fig. 1 Comparison of bacterial diversity between BD patients and normal individuals. **a** We counted OTU number (annotated species number) and estimated alpha diversity score (Chao 1 and Shannon indexes) of each sample. We compared the values between BD patients (BD) and normal individuals (NI). We did not find significant differences in the parameters between BD patients and normal individuals. These biological parameters of BD patients and normal individuals were displayed with dot plots. A box-plot and a mean level (green line) of each group of BD patients and normal individuals were indicated. **b** We estimated beta diversity between BD patients and normal individuals. We visualized the PCoA plots in a three dimensional structure where three axes and each contribution ratio were depicted. We evaluated the distance between the distribution of BD patients and that of normal individuals using a two-sided Student's two-sample t test and Monte Carlo permutations. We obtained a significant P value of the beta diversity between BD patients and normal individuals in both weighted and unweighted UniFrac PCoA. A panel of unweighted UniFrac PCoA plots was shown

We analyzed the metagenomic data of bacterial taxa using LEfSe analytic method to detect major taxon differences between BD patients and normal individuals. We found that there were significant differences in relative abundance of 11 bacterial species between BD patients and normal individuals (Table 2, Supplemental Figs. 1 and 2).

Relative abundance of *Eggerthella lenta*, *Acidaminococcus* species, *Lactobacillus mucosae*, *Bifidobacterium bifidum*, *Lactobacillus iners*, *Streptococcus* species, and *Lactobacillus salivarius* increased significantly in patients with BD. Relative abundance of *Megamonas hypermegale*, *Butyrivibrio* species, *Streptococcus infantis*, and *Filifactor* species increased significantly in normal individuals compared with BD patients.

The 17 most abundant KEGG pathways in BD gut microbiota

We analyzed the metagenomic data of bacterial taxa to detect gene functional differences between BD patients and normal individuals using PICRUSt software (Fig. 2 and Table 3). Functional assignment was performed according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping database, which included molecular interaction/reaction network diagrams for biological interpretation of higher level systemic functions.

LEfSe plots described the 17 most abundant KEGG pathways (lipoic acid metabolism, naphthalene degradation, glycolysis/gluconeogenesis, meiosis-yeast, nicotinate and nicotinamide metabolism, fatty acid metabolism, tyrosine metabolism, homologous recombination, nucleotide excision repair, phosphonate and phosphinate metabolism, retinol metabolism, biosynthesis of unsaturated fatty acids, cysteine and methionine metabolism, inositol phosphate metabolism, purine metabolism, pyrimidine metabolism, and peroxisome proliferator-activated receptor (PPAR) signaling pathway) in BD patients. LEfSe plots described the 8 most abundant KEGG pathways (bacterial chemotaxis, biotin metabolism, porphyrin and chlorophyll metabolism, riboflavin metabolism, sulfur relay system, beta-alanine metabolism, histidine metabolism, and plant-pathogen interaction) in normal individuals. It was thus suggested that the 8 most abundant KEGG pathways were reduced in patients with BD.

Abundant KEGG modules for pentose phosphate pathway and inosine monophosphate biosynthesis in patients with BD

We next assigned predicted gene functions of the metagenomic data according to the KEGG module database to identify prevalent modules in BD patients and normal individuals (Fig. 3, Tables 4 and 5). A KEGG module was a collection of manually defined functional units and was utilized for the annotation and biological interpretation of sequenced genomes.

Gut microbiota of BD patients demonstrated increased relative abundance of KEGG modules for inosine monophosphate biosynthesis, lysine biosynthesis, methionine biosynthesis, pentose phosphate pathway (pentose phosphate cycle), pentose phosphate pathway-oxidative phase, pyrimidine deoxyribonuleotide biosynthesis, putative spermidine/putrescine transport system, glutamate transport system, methionine degradation, and cellobiose transport system.

Gut microbiota of normal individuals demonstrated increased relative abundance of KEGG modules for riboflavin biosynthesis, F-type ATPase, ATP synthase, pantothenate biosynthesis, gluconeogenesis, and shikimate pathway, suggesting that the 6 KEGG modules (riboflavin biosynthesis, F-type ATPase, ATP synthase, pantothenate biosynthesis, gluconeogenesis, and shikimate pathway) were defective in patients with BD.

Discussion

Recently, it was reported that alterations in metabolic pathways were crucial for T cell activation and differentiation [19]. In the quiescent state, they generated adenosine triphosphate (ATP) through oxidative phosphorylation and fatty acid oxidation in the mitochondria. Upregulation of aerobic glycolysis and pentose phosphate pathway and subsequent increase of lipid/nucleic acid synthesis were important for the T cell activation [19].

Memory T cells utilized oxidative phosphorylation and fatty acid oxidation. In patients with rheumatoid arthritis, T cells

Table 2 PICRUSt/LEfSe showed significant differences in relative abundance of 11 bacterial species between BD patients and normal individuals

Abundant species in BD patients		Abundant species in normal individuals	
Names	LDA scores	Names	LDA scores
Eggerthella lenta	4.7	*Megamonas hypermegale*	4.7
Acidaminococcus species	4.5	*Butyrivibrio* species	3.8
Lactobacillus mucosae	4.3	*Streptococcus infantis*	3.4
Bifidobacterium bifidum	4.3	*Filifactor* species	3.0
Lactobacillus iners	4.1		
Streptococcus species	3.4		
Lactobacillus salivarius	2.8		

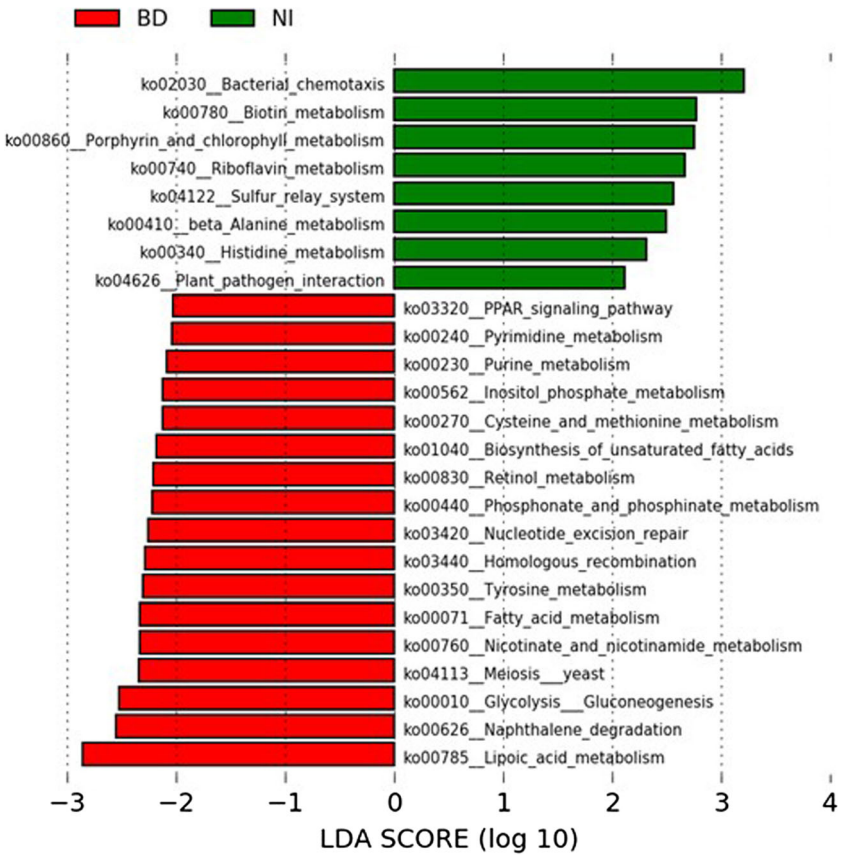

Fig. 2 Abundant KEGG pathways in BD patients and normal individuals. We assigned predictive gene functions of the metagenomic data according to KEGG pathway mapping database to identify predominant pathways in BD patients (BD) and normal individuals (NI). LEfSe plots showed the 17 most abundant KEGG pathways in BD patients and the 8 most abundant KEGG pathways in normal individuals (Table 3)

Table 3 Abundant KEGG pathways in BD patients and normal individuals

Abundant KEGG pathways in BD patients		Abundant KEGG pathways in normal individuals	
KEGG pathway identifiers	KEGG pathway names	KEGG pathway identifiers	KEGG pathway names
ko00785	Lipoic acid metabolism	ko02030	Bacterial chemotaxis
ko00626	Naphthalene degradation	ko00780	Biotin metabolism
ko00010	Glycolysis/gluconeogenesis	ko00860	Porphyrin and chlorophyll metabolism
ko04113	Meiosis-yeast	ko00740	Riboflavin metabolism
ko00760	Nicotinate and nicotinamide metabolism	ko04122	Sulfur relay system
ko00071	Fatty acid metabolism	ko00410	Beta-alanine metabolism
ko00350	Tyrosine metabolism	ko00340	Histidine metabolism
ko03440	Homologous recombination	ko04626	Plant-pathogen interaction
ko03420	Nucleotide excision repair		
ko00440	Phosphonate and phosphinate metabolism		
ko00830	Retinol metabolism		
ko01040	Biosynthesis of unsaturated fatty acids		
ko00270	Cysteine and methionine metabolism		
ko00562	Inositol phosphate metabolism		
ko00230	Purine metabolism		
ko00240	Pyrimidine metabolism		
ko03320	PPAR signaling pathway		

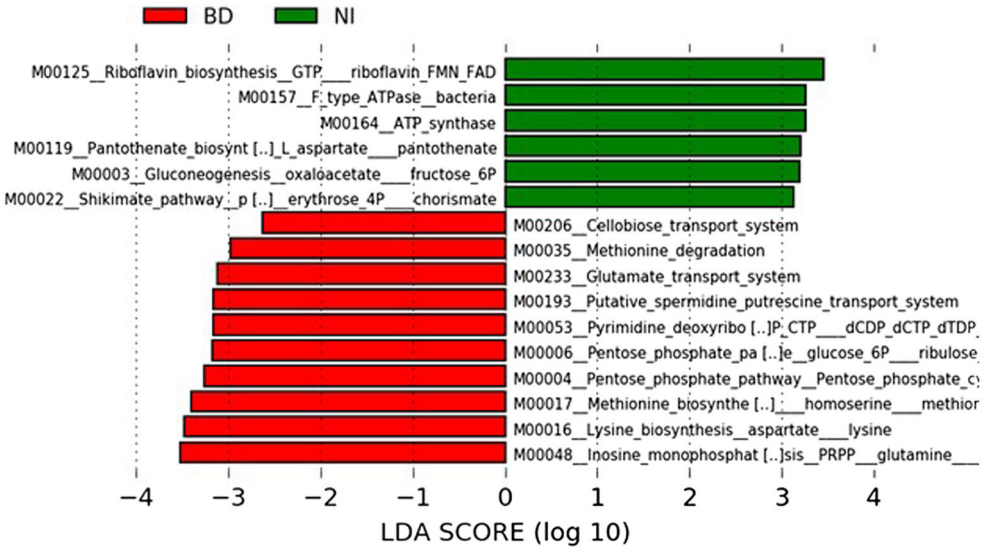

Fig. 3 Abundant KEGG modules in BD patients and normal individuals. We assigned predicted gene functions according to the KEGG module database to identify prevalent modules in BD patients (BD) and normal individuals (NI). LEfSe plots described the 10 most abundant KEGG modules in BD patients and the 6 most abundant KEGG modules in normal individuals (Tables 4 and 5)

failed to enhance glycolysis in the activation and metabolized the glucose further towards pentose phosphate pathway [20] whose enhancement was suggested to associate with Th17 cell differentiation [21].

We found that gut microbiota of BD patients demonstrated increased relative abundance of *Eggerthella lenta*, *Acidaminococcus* species, *Lactobacillus mucosae*, *Bifidobacterium bifidum*, *Lactobacillus iners*, *Streptococcus* species, and *Lactobacillus salivarius* compared with normal individuals (Table 2, Supplemental Figs. 1 and 2). Gut microbiota of normal individuals demonstrated increased relative abundance of *Megamonas hypermegale*, *Butyrivibrio* species, *Streptococcus infantis*, and *Filifactor* species (Table 2, Supplemental Figs. 1 and 2).

Accumulating evidence suggested that a list of rheumatic diseases seemed to have a relationship with gut microbiota perturbation [7, 10–12, 22–25]. Relative abundance of certain bacterial species, such as *Eggerthella lenta*, *Megamonas hypermegale*, and *Prevotella copri*, were often indicated as disease-associated bacteria in several immunological disorders [12, 13, 23, 25]. The data suggested that these species seemed to have an association with pathophysiological processes in the diseases [26, 27].

In patients with BD, bacterial compositional alteration of saliva was reported using an array-based [28], mass spectrometric-based [28], and 16S rRNA–based [29] analyses. At the species level, relative abundance of *Bifidobacterium dentium*, *Prevotella histicola*, and *Haemophilus parainfluenzae* increased significantly in BD patients. The studies demonstrated that relative abundance of several *Prevotella* and *Veillonella* species decreased significantly in the saliva of BD patients. The authors discussed the relationships between abundant bacterial

Table 4 Abundant KEGG modules in BD patients

KEGG module identifiers	KEGG module names
M00048	Inosine monophosphate biosynthesis, 5-phosphoribosyl 1-diphosphate (PRPP) + glutamine → inosine monophosphate (IMP)
M00016	Lysine biosynthesis, succinyl-diaminopimelate (DAP) pathway, aspartate → lysine
M00017	Methionine biosynthesis, apartate → homoserine → methionine
M00004	Pentose phosphate pathway (pentose phosphate cycle)
M00006	Pentose phosphate pathway, oxidative phase, glucose 6P → ribulose 5P
M00053	Pyrimidine deoxyribonuleotide biosynthesis, cytidine diphosphate/cytidine triphosphate (CDP/CTP) → 2′-deoxycytidine diphosphate/deoxycytidine triphosphate (dCDP/dCTP), deoxythymidine 5′-diphosphate/deoxythymidine triphosphate (dTDP/dTTP)
M00193	Putative spermidine/putrescine transport system
M00233	Glutamate transport system
M00035	Methionine degradation
M00206	Cellobiose transport system

Table 5 Abundant KEGG modules shown in normal individuals

KEGG module identifiers	KEGG module names
M00125	Riboflavin biosynthesis, GTP → riboflavin/flavin mononucleotide (FMN)/flavin adenine dinucleotide (FAD)
M00157	F-type ATPase, prokaryotes and chloroplasts
M00164	ATP synthase
M00119	Pantothenate biosynthesis, valine/L-aspartate → pantothenate
M00003	Gluconeogenesis, oxaloacetate → fructose-6P
M00022	Shikimate pathway, phosphoenolpyruvate + erythrose-4P → chorismate

taxa in both patients and normal individuals and severity of mucosal inflammation.

Rag2-deficient mice with depletion of T-bet gene developed ulcerative colitis [30] and their gut bacterial composition changed significantly [18]. The colitis severity correlated with relative abundance of specific species [31]. The bacteria did not induce the colitis by themselves, rather, they needed commensal bacteria to provoke the colitis. The data suggested that unbalanced microbial community, but not a specific single bacterium, contributed to the immune aberration of the disease.

It has been shown that middle- and long-chain fatty acids (LCFAs) supported Th1 and Th17 cell differentiation, whereas short-chain fatty acids (SCFAs), such as propionate and butyrate, led to increased Treg cell differentiation [32]. Indeed, dietary intake of a medium-chain fatty acid reversed the Treg/Th17 cell ratio and aggravated the encephalomyelitis [32]. The short-chain fatty acids delivered orally were suggested to improve Treg/Th17 cell unbalance [32, 33].

We found that *Megamonas* and *Butyrivibrio* species were less abundant in BD patients (Table 2, Supplemental Figs. 1 and 2). The two species were suggested to produce short-chain fatty acids, namely butyrate and propionate, in human intestine [34, 35]. Especially, researchers found that *Butyrivibrio* species, some of which located in *Clostridium* cluster XIVa [36], were mucin-adhering microbes and produced butyrate close to the epithelium [37]. We speculated that reduction of the species appeared to bring about the short-chain fatty acid depletion in the intestine, possibly leading to the skewed T cell differentiation.

An increase of the short-chain fatty acid production by gut microbes, or alternatively, oral delivery of short-chain fatty acids, may become one of the candidates to ameliorate the skewed T cell differentiation in patients with BD.

As a next step, it may be important to determine whether the metabolic aberrations which affect Th17 cell/Treg cell differentiation exist in the intestine of BD patients, and if so, whether the metabolic processes relate to the gut bacterial gene functions.

We observed significantly reduced relative abundance of KEGG pathways and modules for several vitamin B syntheses in BD patients compared with normal individuals (Figs. 2, 3, Tables 3, 4, and 5). Indeed, vitamin B levels looked important for T cell differentiation. Riboflavin metabolites were shown to promote the differentiation of mucosal-associated invariant T cells, a major subgroup of intestinal T cells and the loss of which led to gut integrity defects in an autoimmune disease [38]. Riboflavin is essential for extracellular electron shuttle which is suggested to be important to produce short-chain fatty acids in several gut microbes [39]. Conditional knockout of multivitamin transporter gene was resulted in decreased biotin and pantothenate uptake and caused persistent inflammation of intestinal tract, especially in the cecum [40].

It may be hard to ascribe single metabolic defect to the immune aberration observed in patients with BD. Rather, it is possible that combined and/or summation of bacterial compositional alterations and host predisposition affect the subsequent differentiation of immune cells. Elucidation of such interactions may be important for understanding the immune aberration of BD.

In conclusion, we found that relative abundance of *Megamonas hypermegale* and *Butyrivibrio* species significantly decreased in the gut microbiota of BD patients compared with normal individuals. It seems that altered gene functions of lipid/nucleic acid metabolism have a relationship with the immune aberration in patients with BD.

Acknowledgements The authors are grateful to Dr. Masanori A. Murayama (Department of Immunology and Medicine, St. Marianna University School of Medicine) for his help in the review process.

Funding This study was supported in part by Grants-in-Aid for Scientific Research (Grant number: 17K10009) of Japan Society for the Promotion of Science.

Compliance with ethical standards

This study was approved by the institutional review boards of St. Marianna University School of Medicine and was registered with the University Hospital Medical Information Network-Clinical Trials Registry (UMIN000018937). We conducted this research according to the principles expressed in the Declaration of Helsinki. We obtained written informed consent from each individual prior to enrolment in the study. A copy of the written consent is available for review upon request.

Conflict of interest The authors have no conflict of interest.

References

1. Sakane T, Takeno M, Suzuki N, Inaba G (1999) Behçet's disease. N Engl J Med 341:1284–1291
2. Shimizu J, Takai K, Takada E, Fujiwara N, Arimitsu N, Ueda Y, Wakisaka S, Suzuki T, Suzuki N (2016) Possible association of proinflammatory cytokines including IL1β and TNFα with enhanced Th17 cell differentiation in patients with Behcet's disease. Clin Rheumatol 35:1857–1863. https://doi.org/10.1007/s10067-015-2966-2
3. Shimizu J, Takai K, Fujiwara N, Arimitsu N, Ueda Y, Wakisaka S, Yoshikawa H, Kaneko F, Suzuki T, Suzuki N (2012) Excessive CD4+ T cells co-expressing interleukin-17 and interferon-γ in patients with Behçet's disease. Clin Exp Immunol 168:68–74. https://doi.org/10.1111/j.1365-2249.2011.04543.x
4. Shimizu J, Izumi T, Arimitsu N, Fujiwara N, Ueda Y, Wakisaka S, Yoshikawa H, Kaneko F, Suzuki T, Takai K, Suzuki N (2012) Skewed TGFβ/Smad signalling pathway in T cells in patients with Behçet's disease. Clin Exp Rheumatol 30:S35–S39
5. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139:485–498. https://doi.org/10.1016/j.cell.2009.09.033
6. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K (2013) Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500:232–236. https://doi.org/10.1038/nature12331
7. Shimizu J, Kubota T, Takada E, Takai K, Fujiwara N, Arimitsu N, Ueda Y, Wakisaka S, Suzuki T, Suzuki N (2016) Bifidobacteria abundance-featured gut microbiota compositional change in patients with Behcet's disease. PLoS One 11:e0153746. https://doi.org/10.1371/journal.pone.0153746
8. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341:569–573. https://doi.org/10.1126/science.1241165
9. Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. https://doi.org/10.1038/nri2515
10. Consolandi C, Turroni S, Emmi G, Severgnini M, Fiori J, Peano C, Biagi E, Grassi A, Rampelli S, Silvestri E, Centanni M, Cianchi F, Gotti R, Emmi L, Brigidi P, Bizzaro N, De Bellis G, Prisco D, Candela M, D'Elios MM (2015) Behçet's syndrome patients exhibit specific microbiome signature. Autoimmun Rev 14:269–276. https://doi.org/10.1016/j.autrev.2014.11.009
11. Ye Z, Zhang N, Wu C, Zhang X, Wang Q, Huang X, Du L, Cao Q, Tang J, Zhou C, Hou S, He Y, Xu Q, Xiong X, Kijlstra A, Qin N, Yang P (2018) A metagenomic study of the gut microbiome in Behcet's disease. Microbiome 6:135. https://doi.org/10.1186/s40168-018-0520-6
12. Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, Wu X, Li J, Tang L, Li Y, Lan Z, Chen B, Li Y, Zhong H, Xie H, Jie Z, Chen W, Tang S, Xu X, Wang X, Cai X, Liu S, Xia Y, Li J, Qiao X, Al-Aama JY, Chen H, Wang L, Wu QJ, Zhang F, Zheng W, Li Y, Zhang M, Luo G, Xue W, Xiao L, Li J, Chen W, Xu X, Yin Y, Yang H, Wang J, Kristiansen K, Liu L, Li T, Huang Q, Li Y, Wang J (2015) The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 21:895–905. https://doi.org/10.1038/nm.3914
13. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim SW, Morita H, Hattori M, Yamamura T (2015) Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to Clostridia XIVa and IV clusters. PLoS One 10:e0137429. https://doi.org/10.1371/journal.pone.0137429
14. Bhakta BB, Brennan P, James TE, Chamberlain MA, Noble BA, Silman AJ (1999) Behçet's disease: evaluation of a new instrument to measure clinical activity. Rheumatology (Oxford) 38:728–733
15. Morita H, Kuwahara T, Ohshima K, Sasamoto H, Itoh K, Hattori M, Hayashi T, Takami H (2007) An improved DNA isolation method for metagenomic analysis of the microbial flora of the human intestine. Microbes Environ 22:214–222
16. Kim SW, Suda W, Kim S, Oshima K, Fukuda S, Ohno H, Morita H, Hattori M (2013) Robustness of gut microbiota of healthy adults in response to probiotic intervention revealed by high-throughput pyrosequencing. DNA Res 20:241–253. https://doi.org/10.1093/dnares/dst006
17. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821. https://doi.org/10.1038/nbt.2676
18. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60. https://doi.org/10.1186/gb-2011-12-6-r60
19. Pearce EL, Poffenberger MC, Chang CH, Jones RG (2013) Fueling immunity: insights into metabolism and lymphocyte function. Science 342:1242454. https://doi.org/10.1126/science.1242454
20. Yang Z, Fujii H, Mohan SV, Goronzy JJ, Weyand CM (2013) Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. J Exp Med 210:2119–2134. https://doi.org/10.1084/jem.20130252
21. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, Winter PS, Liu X, Priyadharshini B, Slawinska ME, Haeberli L, Huck C, Turka LA, Wood KC, Hale LP, Smith PA, Schneider MA, MacIver NJ, Locasale JW, Newgard CB, Shinohara ML, Rathmell JC (2015) Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. J Clin Invest 125:194–207. https://doi.org/10.1172/JCI76012
22. Hevia A, Milani C, López P, Cuervo A, Arboleya S, Duranti S, Turroni F, González S, Suárez A, Gueimonde M, Ventura M, Sánchez B, Margolles A (2014) Intestinal dysbiosis associated with systemic lupus erythematosus. MBio 5:e01548–e01514. https://doi.org/10.1128/mBio.01548-14
23. Wen C, Zheng Z, Shao T, Liu L, Xie Z, Le Chatelier E, He Z, Zhong W, Fan Y, Zhang L, Li H, Wu C, Hu C, Xu Q, Zhou J, Cai S, Wang D, Huang Y, Breban M, Qin N, Ehrlich SD (2017) Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. Genome Biol 18:142. https://doi.org/10.1186/s13059-017-1271-6
24. Scher JU, Ubeda C, Artacho A, Attur M, Isaac S, Reddy SM, Marmon S, Neimann A, Brusca S, Patel T, Manasson J, Pamer EG, Littman DR, Abramson SB (2015) Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. Arthritis Rheumatol 67:128–139. https://doi.org/10.1002/art.38892
25. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, Rostron T, Cerundolo V, Pamer EG, Abramson SB, Huttenhower C, Littman DR (2013) Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife 2:e01202. https://doi.org/10.7554/eLife.01202
26. Pianta A, Arvikar S, Strle K, Drouin EE, Wang Q, Costello CE, Steere AC (2017) Evidence of the immune relevance of Prevotella copri, a gut microbe, in patients with rheumatoid arthritis. Arthritis Rheumatol 69:964–975. https://doi.org/10.1002/art.40003

27. Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL, Crabtree-Hartman E, Sand IK, Gacias M, Zhu Y, Casaccia P, Cree BAC, Knight R, Mazmanian SK, Baranzini SE (2017) Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc Natl Acad Sci U S A 114:10713–10718. https://doi.org/10.1073/pnas.1711235114
28. Seoudi N, Bergmeier LA, Drobniewski F, Paster B, Fortune F (2015) The oral mucosal and salivary microbial community of Behçet's syndrome and recurrent aphthous stomatitis. J Oral Microbiol 7:27150. https://doi.org/10.3402/jom.v7.27150
29. Coit P, Mumcu G, Ture-Ozdemir F, Unal AU, Alpar U, Bostanci N, Ergun T, Direskeneli H, Sawalha AH (2016) Sequencing of 16S rRNA reveals a distinct salivary microbiome signature in Behçet's disease. Clin Immunol 169:28–35. https://doi.org/10.1016/j.clim.2016.06.002
30. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, Glickman JN, Glimcher LH (2007) Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell 131:33–45
31. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH (2010) Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe 8:292–300. https://doi.org/10.1016/j.chom.2010.08.004
32. Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A, Hammer A, Lee DH, May C, Wilck N, Balogh A, Ostermann AI, Schebb NH, Akkad DA, Grohme DA, Kleinewietfeld M, Kempa S, Thöne J, Demir S, Müller DN, Gold R, Linker RA (2015) Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. Immunity 43:817–829. https://doi.org/10.1016/j.immuni.2015.09.007
33. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504:446–450. https://doi.org/10.1038/nature12721
34. De Paepe K, Kerckhof FM, Verspreet J, Courtin CM, Van de Wiele T (2017) Inter-individual differences determine the outcome of wheat bran colonization by the human gut microbiome. Environ Microbiol 19:3251–3267. https://doi.org/10.1111/1462-2920.13819
35. Duncan SH, Russell WR, Quartieri A, Rossi M, Parkhill J, Walker AW, Flint HJ (2016) Wheat bran promotes enrichment within the human colonic microbiota of butyrate-producing bacteria that release ferulic acid. Environ Microbiol 18:2214–2225. https://doi.org/10.1111/1462-2920.13158
36. Paillard D, McKain N, Chaudhary LC, Walker ND, Pizette F, Koppova I, McEwan NR, Kopecný J, Vercoe PE, Louis P, Wallace RJ (2007) Relation between phylogenetic position, lipid metabolism and butyrate production by different Butyrivibrio-like bacteria from the rumen. Antonie Van Leeuwenhoek 91:417–422
37. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, De Weirdt R, Kerckhof FM, Van de Wiele T (2013) Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. ISME J 7:949–961. https://doi.org/10.1038/ismej.2012.158
38. Rouxel O, Da Silva J, Beaudoin L, Nel I, Tard C, Cagninacci L, Kiaf B, Oshima M, Diedisheim M, Salou M, Corbett A, Rossjohn J, McCluskey J, Scharfmann R, Battaglia M, Polak M, Lantz O, Beltrand J, Lehuen A (2017) Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. Nat Immunol 18:1321–1331. https://doi.org/10.1038/ni.3854
39. Khan MT, Duncan SH, Stams AJ, van Dijl JM, Flint HJ, Harmsen HJ (2012) The gut anaerobe Faecalibacterium prausnitzii uses an extracellular electron shuttle to grow at oxic-anoxic interphases. ISME J 6:1578–1585. https://doi.org/10.1038/ismej.2012.5
40. Ghosal A, Lambrecht N, Subramanya SB, Kapadia R, Said HM (2013) Conditional knockout of the Slc5a6 gene in mouse intestine impairs biotin absorption. Am J Physiol Gastrointest Liver Physiol 304:G64–G71. https://doi.org/10.1152/ajpgi.00379.2012