

# Analysis of Gut Microbiota in Patients with Parkinson's Disease

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 162, No. 12, pp. 700-703, December, 2016  
Original article submitted April 16, 2016

Gut microbiota of patients with Parkinson's disease and healthy volunteers was analyzed by the method of high throughput 16S rRNA sequencing of bacterial genomes. In patients with Parkinson's diseases, changes in the content of 9 genera and 15 species of microorganisms were revealed: reduced content of *Dorea*, *Bacteroides*, *Prevotella*, *Faecalibacterium*, *Bacteroides massiliensis*, *Stoquefichus massiliensis*, *Bacteroides coprocola*, *Blautia glucerasea*, *Dorea longicatena*, *Bacteroides dorei*, *Bacteroides plebeus*, *Prevotella copri*, *Coprococcus eutactus*, and *Ruminococcus callidus*, and increased content of *Christensenella*, *Catabacter*, *Lactobacillus*, *Oscillospira*, *Bifidobacterium*, *Christensenella minuta*, *Catabacter hongkongensis*, *Lactobacillus mucosae*, *Ruminococcus bromii*, and *Papillibacter cinnamivorans*. This microbiological pattern of gut microflora can trigger local inflammation followed by aggregation of  $\alpha$ -synuclein and generation of Lewy bodies.

**Key Words:** gut microbiota; Parkinson's disease; 16S rRNA sequencing

Human body is now viewed in the context of its symbiotic relationships to microbiota, an ensemble of bacteria, viruses, protozoa, fungi, and archaea colonizing all body biotopes (skin, gastrointestinal tract, airways, and urogenital system) [3]. Growing evidence appears on the potential role of microbiota in the pathogenesis of human diseases, in particular nervous system disorders [14]. Microbiota is as an important part of the body. On one hand, its content is partly stipulated by human genotype and regulated by immune mechanisms [10], on the other, it depends on environmental conditions including nutrition and ecological situation in the habitat [5,7].

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Little is known about its etiology, result from the influence of environmental factors on a human with genetic predisposition for the disease [13]. Recent studies demonstrated changes in gut microbiota in patients with PD, which can serve as both biomarker of PD or its possible trigger inducing misfolding of  $\alpha$ -synuclein, a component of Lewy bodies, that launch neurodegeneration in PD [8,13].

Our aim was the search of PF-specific peculiarities of gut microbiota.

## MATERIALS AND METHODS

Microbiota of 89 patients with confirmed diagnosis of PD (main group) and 66 patients without severe somatic pathology and manifestations of parkinsonism (control group) was studied using the identical protocol of sequencing and data analysis [4]. The

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groups did not differ by the age (67 [65.00; 69.75] and 63 [61.50; 67.50], respectively,  $W=203.5$ ,  $p=0.06$ ) and body mass index (26.72 [25.40; 28.84] and 26.10 [23.82; 28.66], respectively;  $W=261$ ,  $p=0.59$ ). Physical examination of each patient was performed, medical history was analyzed, and biomaterial (fecal sample) was collected. DNA was isolated as described previously [4]. Preparation of libraries and amplicon sequencing of a marker variable area of V3-V4 bacterial genes 16S rRNA were performed on a MiSeq platform (Illumina) using a standard manufacturer protocol.

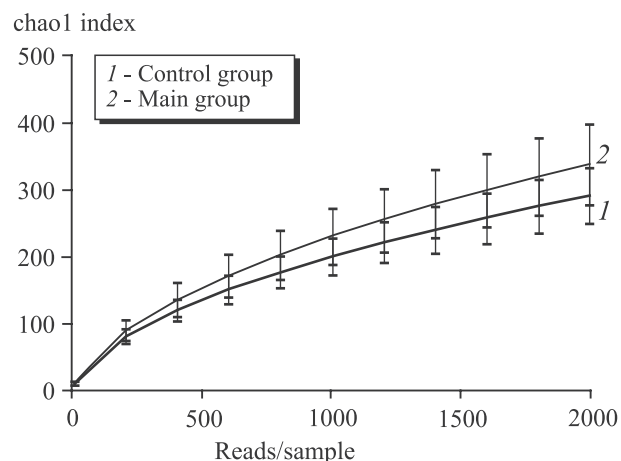
Quality filtering of the reads and their taxonomic classification were conducted using QIIME software [2] by comparing the obtained reads with HITdb database [12]. Alpha- and beta-diversities were also evaluated using QIIME software. To evaluate alpha-diversity (taxonomic diversity of microbial populations), rarefaction of the samples was performed at a depth of 2000 sequences per sample with further calculation of the chao1 index in the experimental and control groups. Beta-diversity (the degree of pair-wise similarity in the species composition among populations) was assessed with principal coordinates analysis (PCoA) on weighted Unifrac metric. The effect size  $R$  and statistical significance  $p$  were determined by ANalysis Of SIMilarity (ANOSIM), assessing statistical significance with 9999 permutations.

Statistical analysis of bacterial taxa was performed by a non-parametric White's  $t$  test (STAMP software) [11]. Differences were significant at  $p<0.05$  at 10,000 permutations. Multiple comparisons adjustment of the  $p$  values was performed using the Benjamini—Hochberg FDR-controlling procedure.

## RESULTS

Evaluation of taxonomic pattern of gut microbiota in PD patients (Fig. 1) showed that the level of alpha-diversity calculated using chao1 index was higher in the control group, which reflects reduction of gut microbiota diversity during PD ( $627.567 \pm 102.988$  and  $699.481 \pm 112.524$ , respectively;  $T=4.105$ ;  $p=0.001$ ). It is known that reduced gut microbiota diversity is associated with the development of inflammatory processes in the intestine [9]. Reduction in taxonomic diversity of gut microbiota in patients with PD can be a result of latent inflammatory process in the intestine, which is considered by several investigators as a trigger factor for  $\alpha$ -synuclein misfolding in gut neurons [8,13].

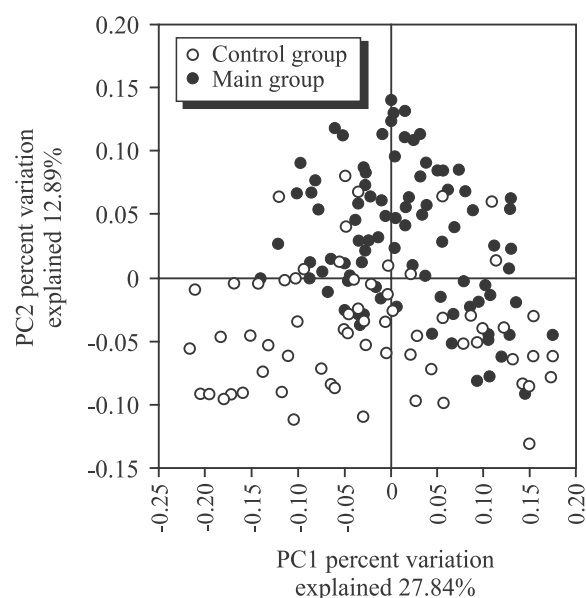
We analyzed taxonomic composition of metagenomics populations of samples of gut microbiota from patients with PD comparing to the control group using analysis of main coordinates on the weighted Unifrac metrics (Fig. 2). The distance between dots on the



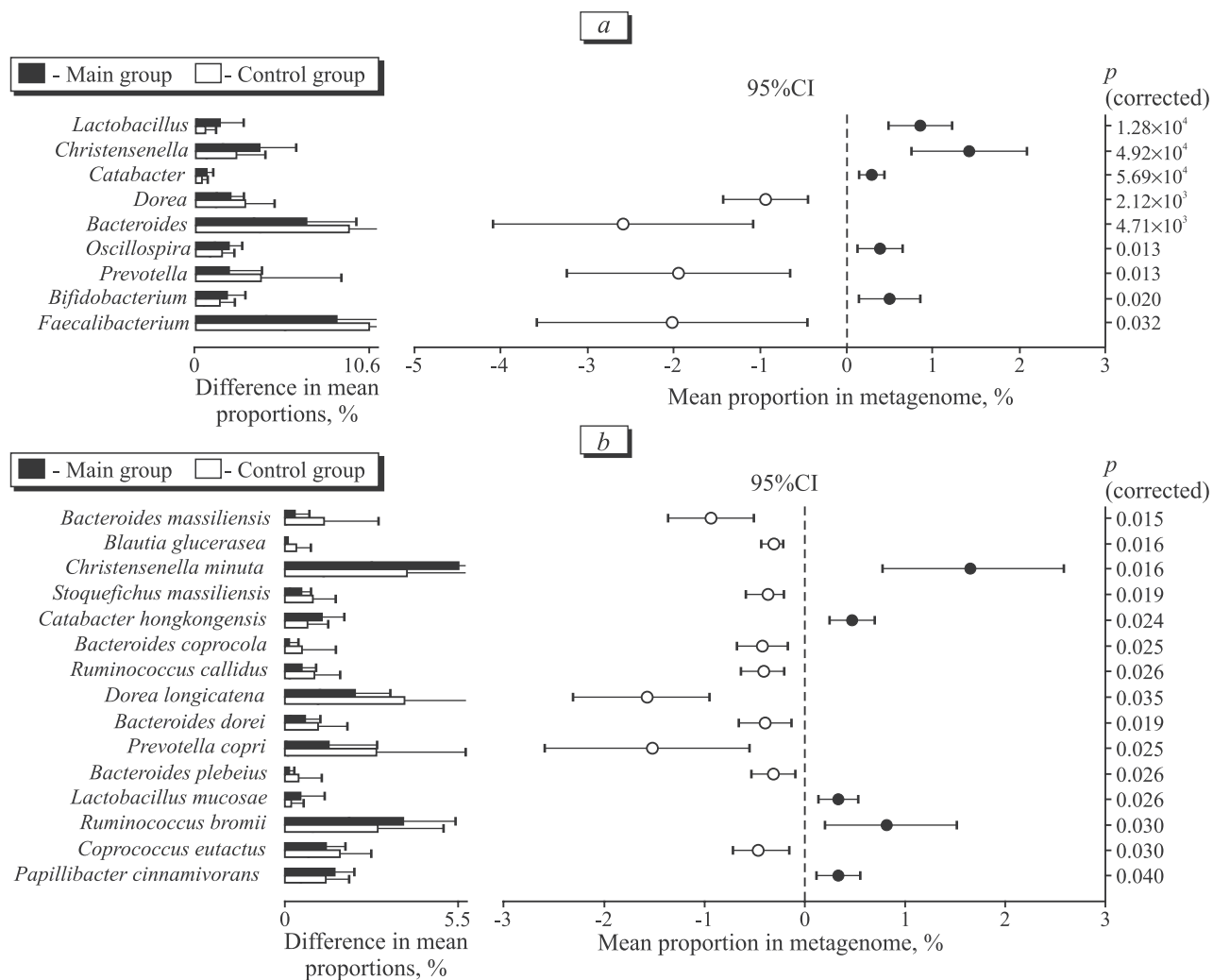
**Fig. 1.** Alpha-diversity curves of gut microbiota in the control ( $N=66$ ) and main ( $N=89$ ) groups under sample rarefaction at a depth of 2000 sequences per sample.

plot indicates the degree of similarity of taxonomic composition of the samples. Significant differences in bacterial composition ( $p=0.0001$ ;  $R=0.214$ ) were revealed after comparison of microbiota samples from patients with PD and healthy controls, which agrees with previous reported data in Finnish population [13].

Comparison of data of analysis of taxonomic composition of gut microbiota from patients with PD and control group showed statistically significant differences in the content of some microbial genera and species in these groups (Fig. 3). At genus level, gut microbiota of PD patients contained high levels of *Christensenella*, *Catabacter*, *Lactobacillus*, *Oscillospira*, and *Bifidobacterium*. The control group was characterized by higher content of *Dorea*, *Bacteroides*,



**Fig. 2.** PCoA plot of the taxonomic composition of gut microbiota in PD patients and control subjects.



**Fig. 3.** Differences in the content of bacterial taxa in PD patients and control subjects at the level of genera (a) and species (b). *p* level is corrected for multiple comparison.

*Prevotella*, and *Faecalibacterium*.

Differences were also observed at the species level. Microbiota of patients with PD was characterized by higher levels of *Christensenella minuta*, *Catabacter hongkongensis*, *Lactobacillus mucosae*, *Ruminococcus bromii*, and *Papillibacter cinnamivorans*, while gut microbiota from the control subjects contained more *Bacteroides massiliensis*, *Stoquefichus massiliensis*, *Bacteroides coprocola*, *Blautia glucerasea*, *Dorea longicatena*, *Bacteroides dorei*, *Bacteroides plebeius*, *Prevotella copri*, *Coprococcus eutactus*, and *Ruminococcus callidus*.

Twin studies showed that *Christensenellaceae* bacteria and *Methanobacteriaceae* archaea are highly heritable prokaryotic taxa [6]. Carriage of these microorganisms is associated with increased abundance of bacteria of the *Oscillospira* genera, which were also present in gut microbiota of PD patients [6]. At the same time, carriage of these microorganisms

is associated with low body mass index in humans and weight loss in gnotobiotic mice infected with these prokaryotes [6]. It is known PD is associated with reduced body weight; body weight loss in PD is associated with impaired quality of life and more severe stage of the disease in accordance to Hoehn and Yahr scale [1].

An increase in *Lactobacillus* content in the microbiota of patients with PD is also typical for Finnish population. It is shown that these bacteria can affect the secretion of  $\alpha$ -synuclein via interactions with gut neurons [14].

Thus, we showed that gut microbiota in PD patients is characterized by a decrease in taxonomic diversity and significant differences in representation of 9 genera and 15 species of microorganisms. This microbiological pattern can trigger local inflammation following by aggregation of  $\alpha$ -synuclein and formation of Lewy bodies.

Experiments were supported by the Federal Target Program “Identification of Biomarkers of Gut Microbiota for Early Preclinical Diagnostics of Parkinson’s Disease” (No. 14.604.21.0150; RFMEFI60414X0150).

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