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Gut Microbiome Shotgun Sequencing in Assessment of Microbial Community Changes Associated with *H. pylori* Eradication Therapy

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Abstract Disturbance of intestinal microbiota content and functions often results in different pathological conditions. Pharmacotherapy including antibiotics use is one of the factors leading to dysbiosis. To evaluate the influence of antibiotics use on intestinal microbiota metagenomic profiles of stool, samples of 74 patients before and after *Helicobacter pylori*—eradication therapy—were analyzed. Evaluation of taxonomic diversity changes based on Shannon index and Bray-Curtis metrics allows to range patients according to mild, moderate, and severe risk of disturbance of intestinal microbiota pathological conditions.

Keywords Intestinal microbiota · *Helicobacter pylori* · Eradication therapy · Whole genome sequencing

1 Introduction

It was shown recently that intestinal microbiota influences general human's well-being, whether directly or indirectly. Disturbance of intestinal microbiota content and functions often leads to different pathological conditions including intestinal inflammation, malabsorbtion, atherosclerosis, diabetes mellitus, rheumatoid arthritis, bronchial asthma, and other allergic diseases. Some authors even discuss the increased risk of inflammatory bowel diseases and gastrointestinal malignancies in subjects with disturbed intestinal microbiota content [1–3]. Different diets as well as stress can influence the prevalence of particular species in intestinal microbiota content. Some substances which are toxic for bacteria can dramatically change the content of microbial community. Among them, antibiotics are of particular interest as a risk of intestinal microbiota content changes which is markedly increased in subjects taking antibacterial medications. According to Maastricht IV Consensus and recommendations of Russian Gastroenterological Association, patients with *Helicobacter pylori*-associated gastrointestinal diseases should undergo eradication therapy consisting of proton pump inhibitor, amoxicillin plus clarithromycin (first line therapy) or proton pump inhibitor, bismuth subsalicylate, metronidazole, and tetracycline (second line therapy). Recommended duration of therapy is 10–14 days. Bismuth subsalicylate can be added to standard triple therapy to increase eradication rate, as well. There is a high risk of microbial species content changes and, as a consequence, their functional content disturbance after eradication therapy which can result in functional bowel disorders.

2 Material and Methods

One hundred forty-eight stool samples were taken for analysis (74 from *H. pylori*-positive patients before eradication therapy, 74—from the same patients after eradication). First line eradication therapy consisting of amoxicillin 1000 mg bid, clarithromycin 500 mg bid, and proton pump inhibitor bid in a standard dose accompanied by bismuth subsalicylate

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240 mg bid was taken by the patients for 14 days. Lactulose was added during the whole course of eradication therapy as a prebiotic. Total DNA was extracted from stool samples, and shotgun metagenomic sequencing was performed on SOLiD 5500 Wildfire platform (Life Technologies, FosterCity, CA, USA). The obtained reads were aligned and annotated using nr/nt NCBI database.

3 Results and Discussion

For evaluation of intestinal microbiota changes after *H. pylori*, eradication therapy Shannon diversity index and taxonomic distance in Bray-Curtis metrics between paired stool samples before and after therapy were used. According to the changes in these indicators, patients were divided into three groups: with mild, moderate, and severe changes in gut microbiota. Various consequences of antibiotics use can be observed depending on the individual (Fig. 1). In most cases (82 %), mild and moderate changes in microbial community content were found: changes in the diversity index varied between −1 and 1, the taxonomy distance ranged within 0 and 0.75. In case of such changes, rapid reversal of disturbances up to normal taxonomic and functional state after antibiotic use is possible which was proved by the results of analysis of the additional stool samples (2 weeks after eradication therapy, data not shown).

In case of the severe changes of gut microbiota in 18 % of patients (changes in the diversity index exceeded the limits of 1 and −1, or the taxonomic distance between samples was more than 0.75), reversal of the microbial community to the primary state is delayed for at least 2 weeks. Mild changes were associated with increasing level of Bacteroides genus (82 % of cases) and decreasing levels of Bifidobacterium (70 % of cases) and Eubacterium genus (76 % of cases) simultaneously. The group of patients with severe microbial shift revealed increased amount of Escherichia genus (83 % of cases). As for patients with moderate changes in microbial content, effects of eradication therapy were unclear and might be associated with their individual features.

For all examined patients, the following common genera in their microbial communities can be mentioned: Bacteroides, Lachnospiraceae, Faecalibacterium, Prevotella, Blautia, Coprococcus, Eubacterium, Roseburia, Parabacteroides, Alistipes, Ruminococcus, Anaerostipes, Dorea, and Bifidobacterium. These particular genera represent the normal human gut microbiota [4] and could be considered as markers for dysbiotic changes.

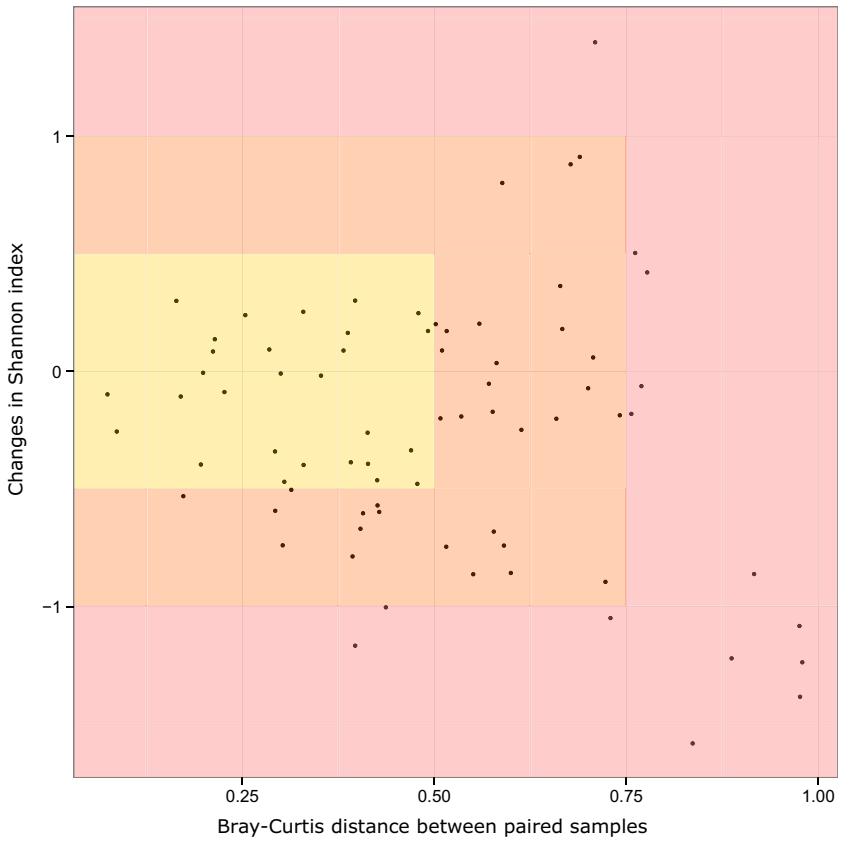

Fig. 1 Changes in the taxonomy distance (*axis X*) and Shannon index (*axis Y*). *Red color* represents severe changes in gut microbiota; *orange* represents moderate changes, and *yellow* represents mild changes

4 Conclusions

Thus, our results concerning the changes of gut microbiota after the eradication therapy could become the basis for developing the novel prognostic markers to reveal personalized risks for pathological changes of intestinal microbiota.

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