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Fundamental and applied aspects of microbiome genetics

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Motivation and Aim: Over the past twenty years, the scientific community has actively been involved in the study of microbiota characteristics of various organisms. The microbiome is involved in paramount ecosystem processes, contributing both to host metabolism on a local scale and biogeochemical nutrient cycling globally. It was recognized that the microbial community is a key factor in determining the physiological state of the host organism. In turn, the genetic features of the host affect the microbial community, which confirms the hypothesis that the microbiome depends on the genetics of the host [1]. Cognizance of interaction mechanisms between the genome and the microbiome will make it possible to use this knowledge for the practical application both in medicine and other branches. Investigation of the interaction between the genome and the microbiome has become an overriding area of scientific interest of the Republican Centre for Microbiome Study, the Institute of Genetics and Cytology, NAS of Belarus. First of all, such a study aims to investigate the human microbiome in normal and pathological conditions, its footprint on the implementation of certain molecular genetic characteristics of the human genome, and its role in maintaining health and the pathogenesis of diseases.
Methods and Algorithms: Genetic testing of patients' biological material was performed by metagenomic analysis using the Illumina high-throughput sequencing technology. After the total DNA isolation and preparation of libraries for sequencing, V3-V4 variable regions of the 16S rRNA gene were sequenced using the MiSeq Reagent Kit v3 (600 cycles) and MiSeq Illumina (USA) according to the manufacturer's recommendations. At the initial stage, the data obtained were analyzed and visualized using the 16S Metagenomics program (Version 1.1.0) provided by Illumina as part of the BaseSpace package (https://basespace.illumina.com). Evaluation of the quality of reads and trimming of low-quality bases were carried out using FastQC and Trimmomatic programs respectively. The V3-V4 regions were analyzed using the qiime2 program. When analyzing the data, the main parameters of the patients were considered and downloaded as a file with metadata in the TVS format. The final pipeline version is available at https://github.com/IGC-bioinf/metagenome_scripts/blob/main/qiime2.sh.
Results: In recent years, a growing body of research papers has come to light indicating that the dynamics of gut microbiome modification is associated with many age-related changes and influences longevity in various species [1]. In this regard, it is of considerable interest to study the characteristics of the human intestinal microbiota, which plays an important role in the formation of healthy aging and active longevity.
A survey of 23 people (an elderly group (mean age –81 ± 0.2) was carried out. The most represented components in the studied microbiome samples included *Ruminococcaceae* and *Verrucomicrobiaceae* representatives (Table 1). In some samples, representatives of the same family (*Ruminococcaceae*, *Verrucomicrobiaceae*) consisted of more than 50 % of the detected reads. Hierarchical clustering of samples identified a cluster that included the samples with the dominant bacterium species of the *Akkermansia* genus

(from 5.89 to 52.86 %). When decreasing the dimension of the Bray-Curtis dissimilarity, a tendency for clustering the samples of people over 82 was revealed, and a high degree of divergence among the remaining samples was noted (Fig. 1). The results obtained indicate a more homogeneous composition of the microbiome in people over 80.

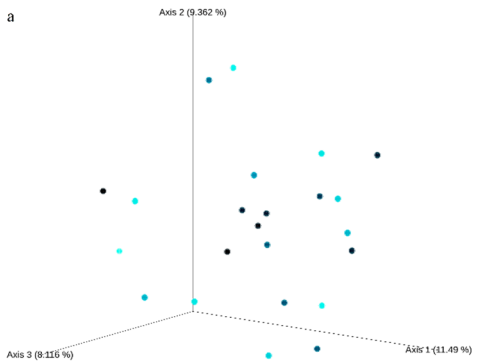

Fig. 1. Clustering of samples with colouring according to the patient's age. A darker tone of the dot indicates a higher age value

In addition to the fundamental research, metagenomic analysis in practical medicine is getting more and more widespread. Work on the identification of pathogenic and potentially pathogenic microorganisms in children with chronic inflammatory bowel diseases (CIBD) is underway at the Republican Centre for Microbiome Study. A combination of CIBD and other bacterial and viral bowel infections may significantly change a clinical pattern, characteristic of each infection separately, which complicates disease diagnosing and choosing a therapy [2]. Metagenome sequencing of DNA from colon biopsy material makes it possible to determine the totality of the microbiome community, which cannot be detected by classical methods currently used in medicine. We present a clinical case of a 2-year-old female patient with a provisional diagnosis of "Non-infectious gastroenteritis and colitis, unspecified. A risk of CIBD development". *Escherichia marmotae*. *E. marmotae* was identified using 16S rRNA sequencing of colon biopsy material, a fairly recently described pathogen phenotypically indistinguishable from *E. coli* isolated only with the advent of new metagenomic technologies [3]. The results obtained allowed us to correctly adjust a therapy regimen for the patient.

Conclusion: The Republican Centre for Microbiome Study, the Institute of Genetics and Cytology, NAS of Belarus, is a multifaceted Centre, which together with the organizations concerned of the Ministry of Health and the National Academy of Sciences allows addressing fundamental and applied problems of various branches.

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