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METAGENOMIC ANALYSIS OF GUT MICROBIAL COMMUNITIES IN KAZAKHSTAN INDIVIDUALS

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Abstract

Introduction. A human metagenome is 100 times larger than its own genome and determines many physiological processes in our body. The metagenome has specific characteristics for each population, which determines the markers of diseases, the course and ways of preventing and treating pathologies.

Materials and methods. The studies were carried out according to the procedures of IHMC (International Human Microbiome Consortium) standards.

Results. These studies of human metagenome are the first among the Central Asian population. Comparison of Kazakh samples of the gut microbiome with samples of other populations demonstrated the main differences and similarities and found that the microbiome depends on nutrition, climatic and geographical features, lifestyle, social factors and age.

We compared the distal gut microbiota of 149 Kazakhstan individuals aged 25 - 65 years. Our studies have shown that microbiomes are different depending on climatic and geographical features, lifestyle, social factors and age. mOTU analysis showed that a microbiome core of our population form by the genera *Faecalibacterium*, *Bacteroides, Dorea, Collinsella, Oscillibacter, Ruminococcus, Subdoligranulum, Coprococcus, Escherichia, Eberichia, Eberichia Roseburia, Parabacteroides* and *Prevotella*. The microbiome core does not change throughout life, and their ratio determines the human enterotype, that determine the risks of developing microbiome-associated diseases, especially the metabolism of drug substances and dietary features to maintain health. The Kazakh samples mostly belong to Enterotype 3. As well as at the mOTU level we found significant (Spearman FDR <0.05) associations to many categories of nutrients, which were studied using FFQ questionnaire. Due to study, the functionality of bacterial genes using the KEGG database were defined the 44 KEGG pathways with significant differences depending on clinical and laboratory characteristics, as well as an anamnesis.

Conclusion The main characteristics of the gut metagenome of Kazakhstan individuals were determined.

Keywords: *metagenome, enterotype, molecular operating taxonomic units (mOTU), biodiversity, disease risks*.

Резюме

МЕТАГЕНОМНЫЙ АНАЛИЗ КИШЕЧНЫХ БАКТЕРИАЛЬНЫХ СООБЩЕСТВ У КАЗАХСТАНЦЕВ

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Введение. Метагеном человека в 100 раз превышает собственный геном и определяет многие физиологические процессы в нашем организме. Метагеном имеет специфические характеристики для каждой популяции, что определяет маркеры заболеваний, течение и пути профилактики и лечения патологий.

Материалы и методы. Исследования проведены согласно процедурам стандартам IHMC (International Human Microbiome Consortium). Настоящее исследование является первым в мире по изучению микробиома популяции Центральной Азии. Сопоставление казахских образцов кишечного микробиома с образцами других популяций, продемонстрировали основные отличия и сходства и установили что микробиом зависит от питания, климато-географических особенностей, образа жизни, социальных факторов, возраста. Мы сравнили микробиоту дистальной части кишечника 149 казахстанцев в возрасте от 25 до 65 лет.

Результаты. Наши исследования показали, что микробиомы различаются в зависимости от климатических и географических особенностей, образа жизни, социальных факторов, возраста. Анализ на уровне mOTU позволил определить микробиомное ядро, которое ввключает следующие роды Faecalibacterium, *Bacteroides, Dorea, Collinsella, Oscillibacter, Ruminococcus, Subdoligranulum, Coprococcus, Escherichia, Eberichia, Eberichia Roseburia, Parabacteroides* и *Prevotella*. Микробиомное ядро не изменяется в течение жизни, и формирует энтеротип человека, который определяет риски развития заболеваний, метаболизм лекарственных веществ и особенности питания для поддержания здоровья. Казахстанские образцы в основном относятся к энтеротипу 3. Кроме того, на уровне mOTU мы обнаружили значимые (Spearman FDR <0,05) ассоциации со многими категориями питательных веществ, которые были изучены с помощью опросника FFQ. В связи с изучением функциональности бактериальных генов с использованием базы данных KEGG были определены 44 пути KEGG со значительными различиями, в зависимости от клинических и лабораторных характеристик, а также от анамнеза.

Заключение. Определены основные характеристики кишечного метагенома казахстанцев.

Ключевые слова: *метагеном, энтеротип, молекулярные операционные таксономические единицы (mOTU), биоразнообразие, риски заболеваний.*

Түйіндеме

ҚАЗАҚСТАНДАҒЫ ЖЕКЕ АДАМДАРДЫҢ ІШЕК МИКРОБТЫҚ ҚАУЫМДАСТЫҒЫН МЕТАГЕНОМИКАЛЫҚ ТАЛДАУ

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Кіріспе Адамның метагеномы өзінің геномынан 100 есе үлкен және денеміздегі көптеген физиологиялық процестерді анықтайды. Метагеноманың әр популяцияға тән сипаттамалары бар, олар аурулардың белгілерін, патологияны алдын-алу және емдеу жолдарын анықтайды.

Материалдар мен әдістер. Зерттеулер IHMC (Халықаралық адам микробиомасы консорциумы) стандарттарына сәйкес жүргізілді.

Нәтижелер. Бұл зерттеулер Орта Азияда популяция микробиомын зерттеу бойынша дүниежүзіндегі алғашқы зерттеу. Ішек микробиомының қазақстандық үлгілерін басқа популяциялармен салыстыру – негізгі айырмашылықтар мен ұқсастықтарды көрсетті және микробиомның тамақтануға, климаттық және географиялық ерекшеліктеріне, өмір салтына, әлеуметтік факторларға, жасына байланысты екендігі анықталды.

Біз 25 пен 65 жас аралығындағы 149 қазақстандықтың дистальды ішектің микробиоталарын салыстырдық. Біздің зерттеулеріміз микробиомалардың климаттық және географиялық ерекшеліктерге, өмір салтына, әлеуметтік факторларға, жасына байланысты ерекшеленетінін көрсетті. мOTU деңгейіндегі талдау микробиомның ядросын анықтауға мүмкіндік берді, оның құрамына келесі туыстар кіреді: Faecalibacterium, *Bacteroides, Dorea, Collinsella, Oscillibacter, Ruminococcus, Subdoligranulum, Coprococcus, Escherichia, Eberichia, Eberichia Roseburia, Parabacteroides* және *Prevotella* Микробиомның ядросы өмір бойы өзгермейді және денсаулықты сақтау үшін аурулардың даму қаупін анықтайтын, дәрілердің метаболизмі мен тамақтану ерекшеліктерін анықтайтын адамның энтеротипін құрайды. Қазақстандық үлгілер негізінен энтеротип 3-ке енеді. Сонымен қатар, MOTU деңгейінде FFQ сауалнамасы арқылы зерттелген көптеген қоректік заттардың санаттары бар маңызды қауымдастықтар табылды (Spearman FDR <0.05). KEGG деректер базасын қолдана отырып, бактериалды гендердің функционалдығын зерттеуге сай клиникалық және зертханалық сипаттамаларына, сондай-ақ медициналық тарихына байланысты 44 KEGG жолы айтарлықтай айырмашылықтары бар екендігі анықталды.

Қорытынды. Қазақстандықтардың ішек метагеномының негізгі сипаттамалары анықталды.

Негізгі сөздер: *метагеном, энтеротип, молекулалық жұмыс жасайтын таксономиялық бірліктер (MOTU), биоалуантүрлілік, ауру қаупі.*

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Introduction

Microorganisms, make up approximately 50% of the Earth's biomass [4]. Moreover, human microbial communities exceed our human cells by 10 times and contain at least 100 times more genetic information than the human genome [18]. In addition, the human gut has been identified as one of the most densely populated econiches with more bacterial cells than all our other microbial communities combined[8]. It comprises hundreds of microbial species, and contributes substantially to human metabolic processes, about 40 % of small molecules in human blood are of the gut microbiome origin [3]. The gut microbiome influences drug metabolism and defines the diet preferences.

The first comprehensive metagenomic study of the human intestine was carried out in 2005[9]. Mucosal biopsy and fecal samples from three healthy adults were collected and 13,333 16S rRNA sequences were generated, resulting in the largest amount of sequencing data generated in a single study of any environment at that time. Of the 395 phylotypes identified, 48% were Bacteriodetes and 51% were Firmicutes, with the remaining microflora containing members of the Proteobacteria, Verrucomicrobia, Fusobacteria, Cyanobacteria, Spirochetes and VadinBE9V. Two years later, the Human Microbiome Project (HMP) was initiated by the NIH as a worldwide initiative to gain a greater understanding of the genetic information contained within the gut microflora and what effect, if any, this information might have on human health and disease [12]. In 2010, Nelson et al. published the results of an initial reference genome study of 178 genomes from the human gut microflora; this resulted in 30,867 polypeptides, of which 29,987 (~97%) were considered novel, providing a tantalizing glimpse into the potential treasure trove of novel genetic loci that exists within the gut microbiome[6]. In 2020, the IHMC announced the new Million Microbiome Initiative, which will feature all of Central Asia.

Human gut microbiome plays an important role in daily physiological processes in our body. All the impacts of gut microorganisms on human life can be grouped into the following items: digestion, metabolism of endogenous and exogenous compounds, immunological defence mechanisms, and prevent the colonization of the gastrointestinal tract by pathogenetic microorganisms.

The intestine is a giant unique filter that recycles and processes everything that enters our bodies through the mouth. The holoorganism, the joint community of human and microbial cells thus resulting, often mutually benefits both types of cell. Microbial cells produce many of the necessary enzymes for digesting carbohydrates and proteins in the colon, whereas the human host cells cannot produce these enzymes. Digestion thus provides nutrients such as vitamin K, vitamin B12, thiamin and riboflavin. *Alcock J. et al (2014)* asserts that microbes in the gastrointestinal tract are under selective pressure to manipulate host eating [1]. Conversely, host diet influences microbial composition and diversity [10]. This factor plays a greater role than any other, including climatic and geographical characteristics, or host genetics. Therefore, the development of nutritional advice taking into account features and reactivities of the gut microbiome is a promising direction for personalized medicine. This will allow the body to maintain homeostasis, to prevent and even treat diseases through control of intestinal microbial composition and activity by what daily meals are taken.

The traditional diet of Kazakh people is very different from either European or East Asian cuisine. It involves a high intake of red meat (especially horse), following established traditions. Most Kazakh individuals also drink black and green tea, on average 6-10 cups a day, and further common is regular consumption of fermented milk products (kurt, koumiss, shubat, buttermilk, sour cream), and of large amounts butter-fried baked products. All these factors could potentially affect the intestinal microbiota and determine the features of the pathogenesis and course of several diseases, an understanding of the gut microbiome and its role in the etiopathogenesis of diseases and the possibility of its control are a promising tool.

We attempted to characterize the gut microbiomes of Kazakh individuals, to learn any features characteristic of these microbiomes, including the potential influence of diet. We here present the first metagenomic studies of gut microbiota from Kazakh individuals. We compare these microbiomes with other cohorts from around the world, and

likewise, evaluate how these microbiomes shift under variation of clinical and lifestyle parameters.

Methods

Sample collection

This is a non-interventional cohort study. 149 individuals of both sexes aged 25-75 years were recruited from Nur-Sultan, the administrative center of Kazakhstan from 2015 up to 2019 in autumn and winter season months, September - January.

The exclusion criteria were any evidence of taking antibiotics for 3 months or less prior to sampling. A stool sampling kit consisting of a sample collection tube, cotton swabs and sterile tissue papers was given to each subject together with a questionnaire about each individual's consumption behavior and a consent form.

The consent documents were signed by all participants before fecal collection. The study protocol and consent documents were approved by the Ethic Committee of Center for life sciences National Laboratory Astana Nazarbayev University with ethical approval number 311/2537.

Human faecal samples were collected and frozen immediately, all samples were maintained at 80°C until they were used for metagenomic studies.

Sample processing and sequencing

Total DNA was extracted from all fecal samples using an adapted G'NOME kit (BIO 101) protocol as described in [21]. Samples were sequenced at the EMBL GeneCore facility using an Illumina HiSeq 2500. On average 2.7±1.1 Gbp of 100 bp paired-end shotgun sequencing reads was generated for each sample.

Data processing and data analysis

Data processing and data analysis were done as described in [14]. Reads were processed using the MOCAT pipeline [BMJ open], community ecological indices, including taxonomic richness and evenness as well as Shannon diversity, were determined based on rarefaction analysis of the mOTU data. Computer analysis thus provides the taxonomic composition of samples with respect to metagenome-derived taxonomic units (mOTUs), to a reference database of known microbial genomes; taxonomic distance between samples (Bray-Curtis and log-transformed Euclidean distances between. mOTU profiles); ecological diversity of samples; gene richness of samples; enterotypes of samples; functional profiles of samples with respect to KEGG (Kyoto Encyclopedia of Genes and Genomes) modules and pathways.

Dietary data

The habitual dietary intakes of participants during the past 12 month were assessed using a food-frequency questionnaire (FFQ) consisting of 217 questions, of which 132 questions differentiating the summer-winter diet. The analysis of the FFQ was performed using the FETA software [16].

Statistical analysis

For alpha-diversity analysis the abundance of the community (Chao1 and Ace indices) and the measure of biodiversity (Shannon index) was assessed. Non-parametric Mann-Whitney (MW) and Kruskal-Wallis (KW) tests were used when comparing two or more Shannon index comparison groups respectively. To test normal distribution within samples, Shapiro-Wilk normality test was used. The determination of the influence of individual parameters such as gender, vaccination status, and patient age on the relative biodiversity and abundance of taxa was calculated using ANOSIM and PERMANOVA statistical tests upon Bray-Curtis, UniFrac, weighted and unweighted distances using the vegan package (v.2.5.3). The random dispersion affecting beta-diversity statistics was tested using betadisper. All graphs were generated using ggplot2 (v.3.0.0) [14].

Results and Discussion

We compared the distal gut microbiota of 149 Kazakhstan individuals aged 25 - 65 years. Our studies have shown that microbiomes are different depending on climatic and geographical features, lifestyle, social factors, age. The present studies of the microbiome of Kazakhs as representatives of the Central Asian population are the first in the world.

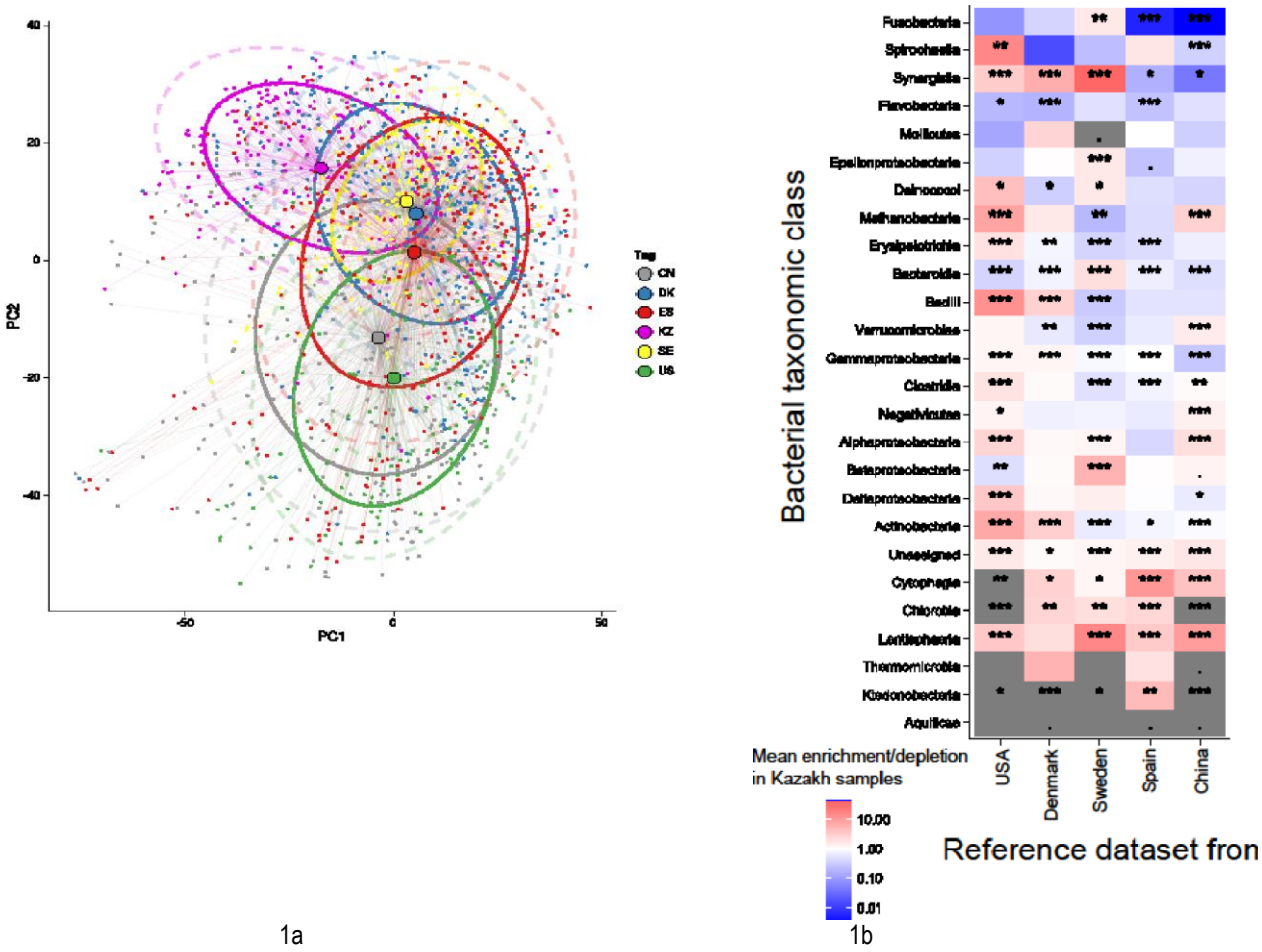
As we see from Figure 1 (detailed describing were done in research on metagenome with metabolic syndrome) [14], a comparison of Kazakh samples of the intestinal microbiome with samples of other populations, USA HMP [7], Spain, Denmark (MetaHIT samples) [5], Sweden [13] and China [17] showed a clear difference.

A breakdown of these results at the level of bacterial taxonomic families is shown in Figure 1b. Along with significant differences, similar characteristics are observed with the intestinal microbiome of the Mongolian population, which is explained by traditionally developed dietary preferences, as well as with the intestinal microbiome of the peoples of Siberia, which in turn is explained by similar climatic and geographical characteristics, lifestyle and eating habits.

The data obtained, as well as the data of other researchers on European countries, countries of East Asia and the American continent, allow us to conclude that the characteristics of the microbiome of Kazakhstanis are the same for all populations of Central Asia.

Microbial DNA sequencing analysis showed that at the phylum level, the most common are *Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes, Archei* and *Verrucomicrobia*. Other bacterial phyla widely represented in the population included *Chlorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Fusobacteria, Lentisphaerae, Spirochaetes, Synergistetes*, and *Tenericutes*. At the genus level, the dominance of *Blautia, Bifidobacterium, Ruminococcus, Bacteroides, Eubacterium, Faecalibacterium, Prevotella*, and *Clostridium* are demonstrated. For all participants, the ratio of *Firmicutes* to *Bacteroidetes* (F/B) ranges from 0,2 to 21.

mOTU analysis (The Molecular Operational Taxonomic Unit) showed that in 90-100% of all analyzed samples microbial cells belonging to the genera Faecalibacterium, *Bacteroides, Dorea, Collinsella, Oscillibacter, Ruminococcus, Subdoligranulum, Coprococcus, Escherichia, Eberichia, Eberichia Roseburia, Parabacteroides* and *Prevotella*. Most of these belong to the *Firmicutes* fil. This group of microorganisms form a microbiome core that does not change throughout life, and their ratio determines the human enterotype. Like typing by blood group, which is genetically determined and does not change throughout life, the enterotype is strictly individual, unchanged regardless of a change in geographical location, diseases, lifestyle, changes in eating habits, etc. Moreover, it is enterotypes that determine the risks of developing microbiome-associated diseases.



1a 1b

Figure 1 - Analysis of samples of the gut microbiome of the Kazakhstan population with data from international databases. 1a – Betta diversity different nationals group; 1b - Heatmap indicates the degree of change. Asterisks indicate statistical significance. FDR <0,1; *: FDR <0,05; **: FDR <0,01; *: FDR <0,001.**

Projecting the Kazakh samples into enterotype principal component space (showing also 110 MetaHIT samples for comparison) reveals Kazakh samples mostly belong to Enterotype 3. Metabolic syndrome case or control samples do not separate strongly in enterotype space. Eleven (6%) of the 172 samples were assigned to enterotype 1, 71% were assigned to enterotype 2 and the rest (23%) were assigned to enterotype 3 (Figure 2).

Table 1 shows the comparative data on the distribution of enterotypes among the studied population and the MetaHIT (METAgenomics of the Human Intestinal Tract) data, which presents data on the European population. The table shows that the distribution of enterotypes among the Kazakh and European populations is significantly different.

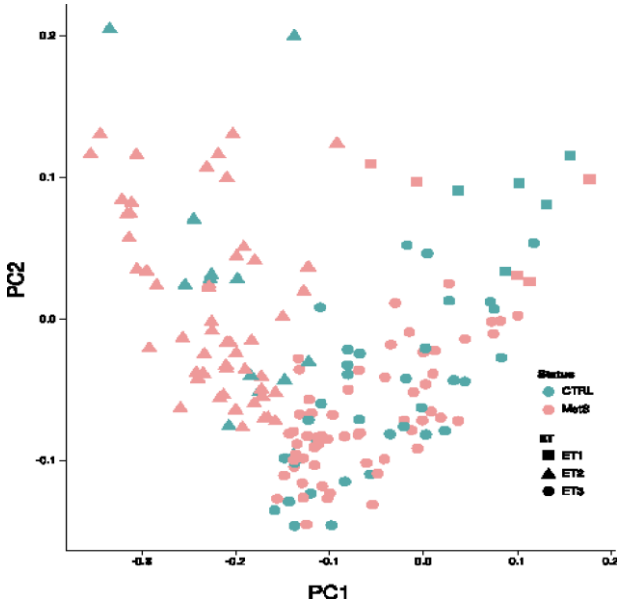


Figure 2. Enterotyping.

Table 1. Comparative analysis of the enterotypes distribution.

Enterotype	Kazakh (%)	MetaHIT (%)
Bacteroidetes-rich	6%	27%
Prevotella-rich	71%	26%
Firmicutes-rich	23%	48%

The compositional structure of the microbiome depends on a number of factors, the leading ones are genotype and nutrition.

An analysis of the Russian cohort *(Tyakht et al.)* showed that the enterotype I Bacteroidetes is rare in this population, and we thus observe the same trend in Kazakh samples. Despite the fact that the first enterotype combines mainly those who prefer red meat in their diet. Moreover, most Europeans belong to the first enterotype, while in our study this number is small. It is generally accepted that in the world red meat is consumed the most in Kazakhstan and Argentina. Why do not Kazakhs belong to the first enterotype? Processing technology matters and what exactly gets into the digestive system. Kazakh cuisine is characterized by careful processing of meat, while in the European cuisine only insignificant heat treatment.

For diet analysis, all study participants completed standard FFQ (Food Frequency Questionnaire) questionnaires. The questionnaire is data on the list of food products and dishes and the frequency of their use by the respondent. All data using a computer program was converted into a quantitative expression of the macro- and microelements consumed. Converting the dietary information available for these samples into a uniform format, the metagenome-derived gut taxonomic composition was tested for significant (Spearman test, BH FDR < 0.1) correlations with dietary features. While more in depth analysis of these results is needed to determine what drives each correlation and to what extent it may be of relevance to any application, already an initial survey reveals significant dependencies of the gut taxonomic composition on host diet, though whether this influence is direct or indirect is unclear. Analysis at the level of large taxonomic groups showed a relatively low level of association. At the same time, at the mOTU level, we found significant (Spearman FDR <0.05) associations to one or more categories of nutrients (Fig. 3).

Figure 3 shows these correlations as a network view at the level of bacterial taxonomic families. In panel, a significant positive correlations are shown, with the clearest features being Prevotellaceae increasing in relative abundance in a mineral- and vegetable-rich diet, as well as several families, increased in relative abundance with higher consumption of various sugars, fats and alcohol. Panel b shows instead negative correlations, with a set of bacterial families anticorrelated with many of the Prevotella-associated nutrients and food groups. These patterns may reflect overarching bimodal distributions in the gut ecosystem such as Prevotella as an enterotype driver bacterium. Beyond these larger patterns, several smaller features are seen such as an anticorrelation between iodine intake and Fusobacteraceae abundance.

This reveals a broad pattern of dependencies wherein many microbial taxa share similar correlations or anticorrelations with dietary features. Given this structure, a clearer view of the network becomes possible in the form of a power graph, wherein nodes are grouped together as “power nodes” based on the similarities of their interactions with other nodes – a power network made from links between nutrient measures and microbial taxa hence will contain power nodes representing groups of taxa with similar nutrient dependencies and groups of nutrients with similar effects on the microbiome. This graph is shown in Figure 3.

Most associations are negative, indicating that higher consumption of certain foods is associated with a decrease in the number of certain types of bacteria. The central node (A) includes information on the main nutritional components: proteins, fats, carbohydrates, a number of minerals, and the total energy value of consumed products. increased consumption of fats, sugars, increased total calorie content of foods is inversely proportional to the content of such groups as *Prevotellas, Bacteroidetes, Clostridiales*. The amount of consumption of fish dishes is negatively correlated with representatives of Clostridia. Consumption of vegetables and vegetable dishes is inversely proportional to cluster 1103: Bacteroides intestinalis. Another block (J), combining representatives of Firmicutes, Methanobrevibacter smithii and Alistipes, correlates on the one hand with the consumption of nuts and seeds, on the other hand with essential fatty acids. Total carbohydrate intake is negatively correlated with the (L) block of Prevotella, Eubacterium, and Bacteroidetes. Only two associations were directly proportional, namely between the consumption of alcohol-containing products (M) and cluster 973: Bifidobacterium catenulatum-Bifidobacterium pseudocatenulatum complex.

To study the functionality of bacterial genes, the KEGG database (Kyoto Encyclopedia of genes and genomes) was used. 44 KEGG pathways (Fig. 7) showed significant differences and allowed to be grouped according to clinical and laboratory characteristics, as well as an anamnesis. These data will allow in the future to develop non-invasive screening tests for early diagnosis. The most significant differences were determined for the CMP-Kdo biosynthesis (lipopolysaccharide biosynthesis) (P≤0.01) and KdpD-KdpE (potassium transport) genes of the two-component regulation system (P≤0.03), gluconeogenesis, oxaloacetate => fructose-6P (P ≤0.04), phosphate transport system (P≤0.053), reduced sulfate assimilation (P≤0.05).

The obtained data and knowledge of the metagenome of the Kazakhstan population allowed us to develop recommendations, diets and functional foods for manipulating the intestinal microbiota, increasing / decreasing certain populations of microorganisms in order to control some physiological processes, prevent the development of diseases and their treatment.

The fact of the absence of significant seasonal changes in the intestinal microbiome is also noteworthy. The city of Nur-Sultan is considered the second coldest capital in the world. In general, the Nur-Sultan climate is sharply continental and is characterized by sharp daily temperature fluctuations of 2-20 degrees throughout the year. Absolute maximum temperature: +41.6 ° C, Absolute minimum temperature: −51.6 ° C [23].

Perhaps the body of Astana residents is so adapted to such changes in temperature and atmospheric pressure that the approach to studying the response of the intestinal microbiome should be different. This is confirmed by the data on the study of the intestinal microbiome of the Mongol among residents of Ulan Bator, characterized by similar climatic conditions (Mongolians core gut microbiota and its correlation with seasonal dietary changes).

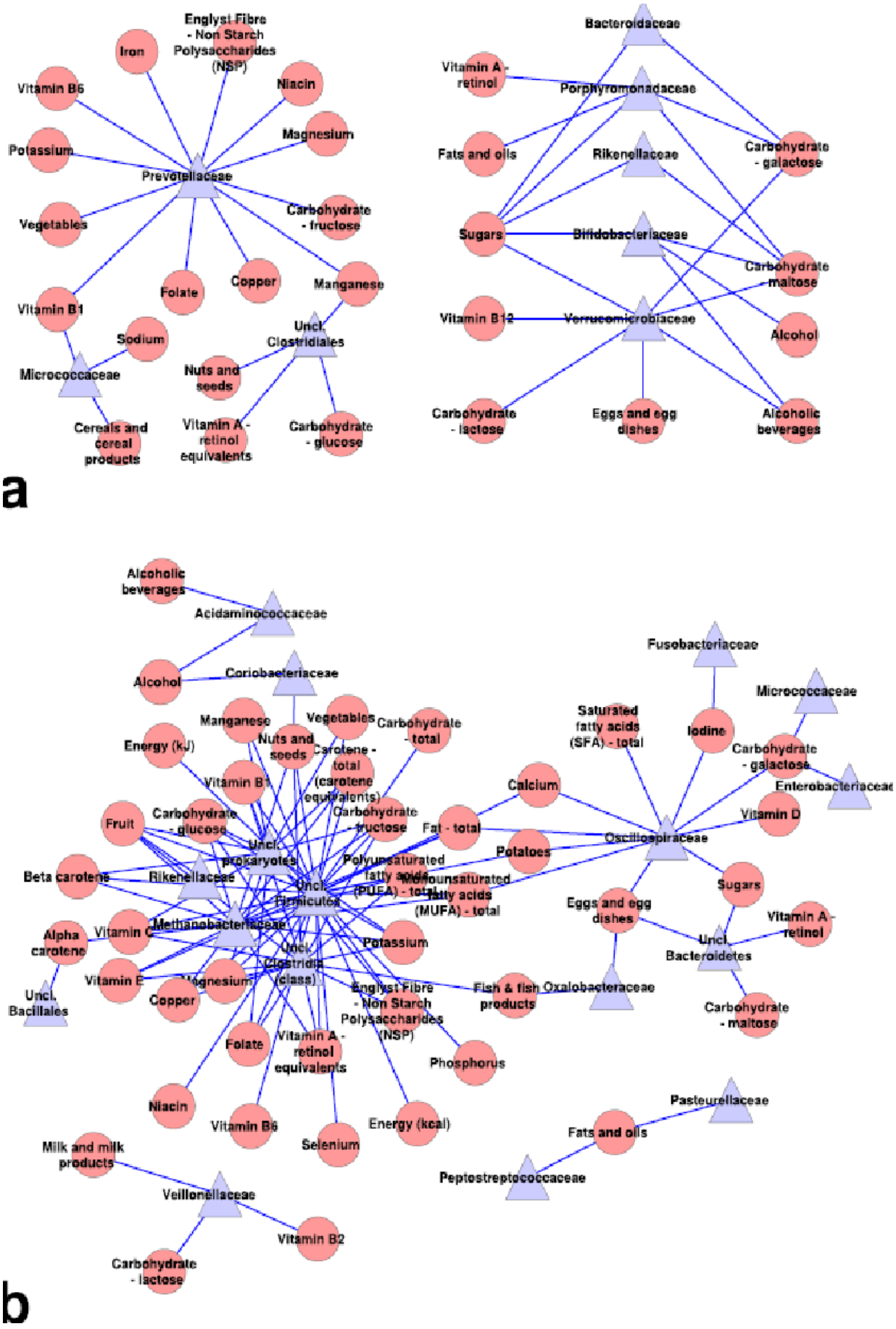


Figure 3. Visualization of the network of consumed micro-macronutrients and the composition of the gut microflora.

The analysis was carried out in comparing data for several months of January, March, June, September and November and little change was noted in their intestinal microbiota composition throughout the year [22]. However, a similar analysis for other Khentii regions of Mongolia, TUW, showed significant seasonal differences. The authors found that the intestinal microbiome in the regions of Khentii, TUW is characterized by a similar pattern in the months of March, June, September and significantly differs from the data for January, November. *Emily R. Davenport et al* in their study of the intestinal microbiome among the Hutterites found seasonal differences in both (i) the abundance of particular taxa (false discovery rate, 0.05), including highly abundant phyla Bacteroidetes and Firmicutes, and (ii) overall gut microbiome diversity (by Shannon diversity; P = 0.001). *Zhang J. et al (2014)* associate it with significant seasonal changes in diet [22].

Today, not only the relationship of the microbiome and pathologies such as diabetes type 2, cardiovascular diseases [14], [15], neurodermatitis [2], bronchial asthma [11], neurodegenerative pathologies[19] are determined, and also identified markers that allow highly accurate detection and prediction of disease. Studies by P. Bork showed that markers are not universal, but specific and depend on the genotype, climatic and geographical characteristics, and nutrition [20]. Along with this, it should be mentioned that the metagen is plastic and easily controlled with the help of diet, biological products, including probiotic, as well as new technologies of fecal transplantation and synthetic stool. However, all of these technologies are applicable with knowledge of the characteristics of the microbiome.

Conclusion

This study is the first metagenome study of representatives of the Central Asian population and demonstrated similarities and differences in the comparison between European and non-European populations. An in-depth study of the microbiome is important for early detection, development of prevention and treatment tools, as well as for the effective selection of probiotic.

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2) Questioning, statistical processing and nutrition analysis: Ayaulym F. Nurgozhina, Laura E. Chulenbayeva, Zhanagul R. Khassenbekova;

3) Sample collection, DNA extraction: Shynggys D. Sergazy, Madiyar A. Nurgaziyev;

4) Llibrary preparation, sequencing, bioinformatics analysis: Samat S. Kozhakhmetov, Altynai K. Tuyakova, Yermek O. Aitenov

5) Statistical processing and data analysis: Alexandr E. Gulyaev, Zhaxybay Sh. Zhumadilov;

6) Preparation of documentation and registration of a clinical trial: Bakytgul A. Yermekbayeva;

7) Study design and manuscript prepairing: Almagul R. Kushugulova.

Conflict of interest

The authors declare no conflict of interest.

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