SHORT COMMUNICATION

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Preliminary analysis of oral and gut microbiome of an elderly patient with late-diagnosed phenylketonuria

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Abstract

Phenylketonuria (PKU) is a metabolic and genetic disorder caused by a phenylalanine hydroxylase (PAH) gene deficiency that raises Phe levels in organs. Dietary therapy involves an elimination diet and Phe-free items, which may alter microbiota. The study examined the oral and intestinal microbiomes of a 63-year-old PKU patient and a control man, living in rural areas. iSeq100 (Illumina) sequenced the stool and oral 16S rRNA gene V3-V4 region. PKU guts had more Firmicutes and fewer Bacteroidetes than control. Clostridia predominated in PKU, while Bacteroidia dominated in control. Oral Bacteroidetes. Firmicutes, Proteobacteria, and Fusobacteria phyla were similar in both men. The microbiome may differ from those fed a Phe-free diet from birth due to late diagnosis and treatment of PKU. Due to the age of the 63-year-old patient's and late therapy, the results differ from earlier studies. No study has compared an older PKU patient's gut and oral microbiomes.

Key words

diet, gut microbiome, 16S rRNA, oral microbiome, phenylketonuria (PKU)

INTRODUCTION

Phenylketonuria (PKU) is a metabolic and genetic disease in which plasma Phe levels increase due to a phenylalanine hydroxylase (PAH) gene defect. Since May 1994, all Polish newborns must be tested for phenylketonuria, which affects 1 in 7.000 children [1, 2]. The condition can be treated by limiting Phe intake, supplementing necessary amino acids and microelements, and administering large neutral amino acids (LNAAs) or glycomacropeptide (GMP) [3]. Untreated patients frequently suffer mental disorders, seizures, or other neurological problems. Even severely intellectually disabled patients with untreated PKU may benefit from a low-Phe diet [4].

The gut microbiome of PKU patients treated with a Phe-restricted diet has been studied in comparison with that of healthy individuals [5]. Plasma Phe concentration modulates microbiota differences, according to Pinheiro de Oliveira et al, (2016), the PKU group of microorganisms synthesized fewer amino acids than the control group. Inborn errors of metabolism (IEM), such as PKU, exhibit considerable changes in microbial diversity or relative abundance. The diet of PKU patients can change their microbial composition [6].

To improve human health, the oral microbiome and its connections with other microbiomes and health statuses need to be studied [7]. Understanding IEM's gut microbiome composition and which niche probiotics are therapeutic is needed [8]. During adolescence and maturity, many PKU patients loosen their low-Phe diet, which raises Phe levels and causes neuropsychiatric issues like depression and anxiety [9]. These concerns can be alleviated by returning to controlled Phe levels, which is an extremely difficult prospect for older patients.

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OBJECTIVE

The aim of the study was to investigate the microbiota of a 63-year-old PKU patient in comparison with a control, and to compare PKU patients' oral and intestinal microbiomes with controls. Dietary analysis showed study case differences as well as how their age and phenylalanine-free diet affect PKU patients' oral and gut microbiomes.

MATERIALS AND METHOD

The sample collection process and flow diagram of the study are shown in Figure 1. Table 1 presents the list of primers and its sequences used in the study.

RESULTS

Dietary Analysis. Proteins and n-3 fatty acids showed significant diet differences ($p < 0.05$) (Tab. 2). Although consumption of sugar was not statistically significant, PKU patients gained twice as much energy from sugary foods.

PKU patients had reduced protein intake, resulting in amino acid levels that differed significantly (Tab. 3). Only magnesium, manganese, and potassium intake showed no significant differences.

Gut microbiota composition in PKU and control. In the PKU, 32 phylum-level taxonomic categories were identified, compared to 25 in the control: Firmicutes, Bacteroidetes,

Table 1. List of primers used in metagenetic PCR reaction (16S rDNA V3-V4 hypervariable region)

Primer	Primer's sequence
16S_F	5'-(TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCA)-3'
16S_R	5'-(GTCTCGTGGGCTCGGAGATGTGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC)-3'

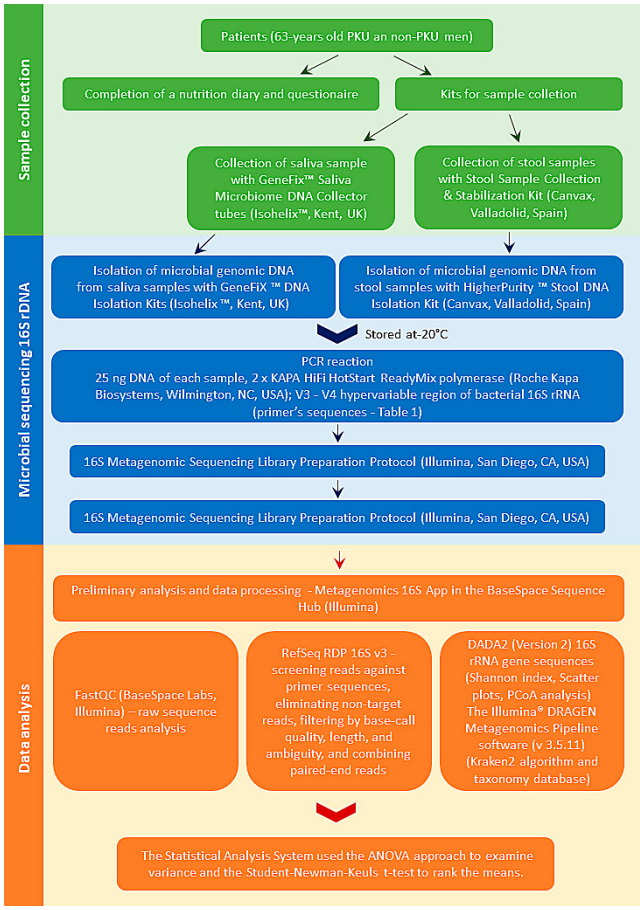

Figure 1. Sample collection process and flow diagram of the study (with materials)

and Proteobacteria. In PKU, Clostridia and Bacteroidia predominated, whereas in the control, Bacteroidia and Clostridia occurred. PKU's gut microbiota was dominated by Clostridiales, while the control's was Bacteroidales. The PKU sample was 157 family-level taxonomic groups and the control 138. The PKU contained greater and lower abundances of *Ruminococcaceae* and *Prevotellaceae*. respectively. The PKU and control had similar *Lachnospiraceae* levels. *Prevotella* was the most prevalent genus in control. Other genera include *Faecalibacterium*, *Lachnospiracea_incertae_sedis*, *Alistipes*, and others. *Prevotella_copri* was the most prevalent species in PKU. followed by *Catabacter_hongkongensis* and *Faecalibacterium_prausnitzii*.

Oral Microbiota Composition in PKU and Control. The total phylum-level taxonomic categories identified were 19 in PKU and 20 in the control. Bacteroidetes. and Firmicutes phyla showed similar abundance in PKU and the control. Bacteroidia predominated in both samples. along with Betaproteobacteria and Clostridia. Bacilli and Actinobacteria were more abundant in PKU than in control. respectively. Bacteroidales were similar in PKU and in control. The most prevalent family in PKU and control was *Prevotellaceae*. *Prevotella* was the most prevalent genus in both samples.

Table 2. Energetic value and macronutrients content in the diet of a PKU patient and control

	Control		PKU patient		Significance of difference at $p \le 0.05$
	Average	SD	Average	SD	
Energy (kcal)	2494	373	1743	451	
Total proteins (g)	109	11	32	9	*
Fat (g)	110	52	74	28	
Total carbohydrates (g)	269	59	253	64	
Fibre (g)	29.9	11.5	24.1	1.4	
Starch (g)	159	30	108	27	
Sugars (g)	52	7	76	36	
Digestible carbohydrates (g)	239	47	228	63	
Total monounsaturated FAs¥ (g)	31.9	23.9	8.4	1.3	
Total polyunsaturated FAs (g)	29.8	15.6	4.9	0.6	
n-3	3.0	1.4	0.4	0.1	*
n-6	25.7	13.7	4.3	0.5	
Total saturated FAs (g)	28.8	21.2	26.3	28.3	
Trans acids	0.87	0.76	0.10	0.10	
Cholesterol (mg)	290.0	131.4	31.3	27.2	
n-6/n-3	8.6	0.8	9.9	0.7	
Energy from protein (%)	17.4	2.6	7.3	3.2	*
Energy from fat (%)	39.6	13.2	38.1	6.4	
Energy from digestible carbohydrates (%)	38.3	9.3	52.2	4.3	
Energy from saturated FAs (%)	10.4	6.0	13.6	10.3	
Energy from sugars (%)	8.3	2.3	17.5	3.8	*

¥ FAs – fatty acids

Rothia_mucilaginosa was most abundant in PKU, while *Porphyromonas_pasteri* was most abundant in control.

The DRAGEN programme also identified the following species in the oral microbiome PKU: *Prevotella intermedia*, *Prevotella denticola*, *Veilonella*.

Gut and Oral Microbiome Composition. The dendrogram (Fig. 2) shows samples clustered by genus. The PKU gut microbiomes had more *Phascolarctobacterium*, *Catabacter*, *Alloprevotella*, *Clostridium* IV, *Blautia*, and *Ruminococcus* than the control. *Prevotella*, *Bacteroides*, *Lachnospiracea_incertae_sedis*, *Ruminococcus*, and *Coprococcus* predominated in the control gut microbiota compared to PKU. *Streptococcus*, *Porphyromonas*, *Alloprevotella*, *Rothia*, and *Fusobacterium* were more abundant in PKU oral microbiomes than in controls. The control oral microbiome had more *Prevotella*, *Neisseria*, *Bacteroides*, *Veillonella*, *Eubacterium*, and *Saccharibacteria species incertae sedis* than in PKU.

DISCUSSION

PKU diets focus on maintaining plasma Phe levels to promote healthy physical and mental development. Screening adult and elderly PKU patients for osteoporosis and cardiovascular

Table 3. Amino acids. mineral and vitamin content in the diet of PKU patient and control

	Control		PKU patient		Significance of difference at $p \leq 0.05$
	Average	SD	Average	SD	
Cystine (g)	1.43	0.29	0.33	0.15	*
Histidine (g)	2.67	0.75	0.70	0.30	*
Isoleucine (g)	3.90	1.14	1.00	0.46	*
Leucine (g)	6.97	1.53	1.77	0.86	*
Lysine (g)	6.37	1.53	1.47	0.75	*
Methionine (g)	2.03	0.46	0.50	0.26	*
Phenylalanine (g)	4.17	1.02	1.03	0.42	*
Threonine (g)	3.57	0.84	0.90	0.40	*
Tryptophan (g)	1.10	0.17	0.27	0.06	*
Tyrosine (g)	2.97	0.67	0.80	0.36	*
Valine (g)	4.43	1.07	1.20	0.40	*
Calcium (mg)	795	143	396	19	*
Copper (mg)	1.6	0.4	0.9	0.1	*
Iron (mg)	18.0	3.7	8.0	0.4	*
Magnesium (mg)	316	57	235	12	
Manganese (mg)	5.1	0.9	3.5	0.8	
Phosphorus (mg)	1414	92	635	22	*
Potassium (mg)	2818	593	2190	194	
Selenium (µg)	141.1	23.4	38.4	4.7	*
Sodium (mg)	4860	351	1477	364	*
Zinc (mg)	13.4	1.0	5.7	0.4	*
Vitamin A (IU)	2664	1045	4920	3883	
Vitamin D (IU)	174	55	279	28	*
Vitamin E (mg)	7.2	2.4	7.0	0.9	
Vitamin K (µg)	78	20	104	63	
Thiamine (B1) (mg)	2.5	0.8	1.2	0.1	
Riboflavin (B2) (mg)	2.3	0.5	0.8	0.1	*
Niacin (mg)	27.2	1.9	12.7	3.0	*
Pantothenic acid (B5) (mg)	7.0	0.2	3.2	0.3	*
Vitamin B6 (mg)	1.9	0.2	2.1	0.2	
Vitamin B12 (µg)	3.3	1.3	0.7	0.2	*
Folates (µg)	611	215	244	29	*
Vitamin C (mg)	26	23	107	27	*

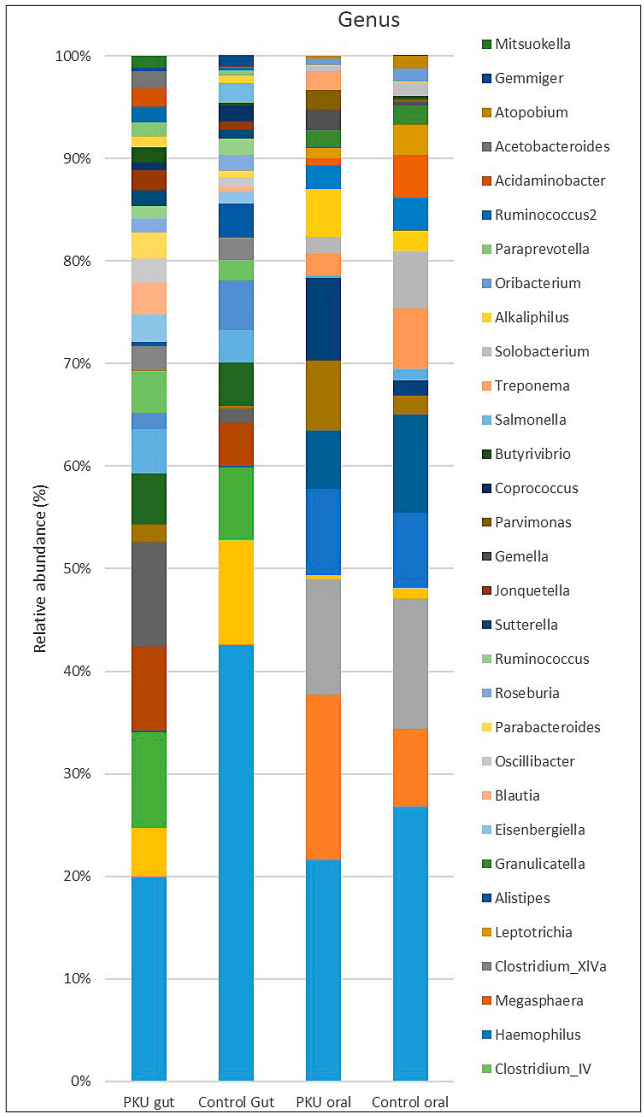

Figure 2. Genus-level comparison between PKU and control samples. Dendrogram representing a hierarchical clustering of samples based on genus-level classifications (>1% relative abundance)

disease is recommended [10]. Nowadays, tetrahydrobiopterin, GMP therapy, and LNAA supplementation could make PKU diets flexible [11]. The Woodcock-Johnson test showed that the PKU patient's IQ following diet therapy was 101±11, an average IQ, proving that metabolic management by food therapy promotes intellectual development [10].

An analysis of the diets of the healthy control and PKU patient showed a significant difference in protein intake. Usually, a diet of a PKU patient is similar to controls [12]; however, some studies show that the diet in PKU male patients contains less protein than healthy control [13]. In the analyzed cases, significant differences were recorded for energy levels obtained from sugars. Although sugar intake was not significant, however, in the case of the PKU patient it was approximately 50% higher, which could affect differences in oral microbiota, as it affects gut microbiota [14].

PKU patient studies suggest nutrition affects the microbiota-gut-brain axis. Bacteroidetes and Firmicutes dominated PKU and control gut microbiomes, followed by Proteobacteria and Verrucomicrobia. Bacteroidetes and Firmicutes in PKU individuals aged 33 (±1.98) showed similar results [15]. In PKU patients, Bacteroides genus abundance decreased, similar to earlier studies in children [16]. PKU and control groups showed similar Bacteroidetes levels in adult studies [15, 17].

The control group contained greater *Bacteroides* and *Prevotella* than PKU. *Prevotella* dominates in the gut microbiome in a 'traditional' lifestyle that eats differently from the Western diet [18].

The presented research confirmed that the Firmicutes type has more *Blautia* and *Clostridium* in PKU patients' gut microbiota than in earlier studies [16, 19]. A high-fibre diet and inulin-containing PKU low-protein products may increase this genus [19]. Clostridiales predominated PKU, while Bacteroidales predominated control.

Other adult cohort studies identified that *Lachnospiraceae* was more abundant in PKU patients' microbiomes [20]

or less prevalent [5, 15]. *Lachnospiraceae* produce SCFAs. mostly propionate, that benefit the host organism's health. However, different taxa of the genus are connected to metabolic syndrome, obesity, diabetes, liver disease, inflammatory disease, depression, and multiple sclerosis [21]. *Ruminococcaceae* were slightly more prevalent in PKU than controls, contrasting previous research [20].

The oversight of parents and meal preparation cause diet and microbiota alterations in PKU children and adults. Adults are less restrictive with respect to diets. Some individuals quit the Phe-free diet but return when symptoms increase [4].

Age, food, oral health, diseases, and drugs affect the oral microbiota. Proteobacteria – inflammatory bacteria – increase with ageing [22]. PKU and a Phe-free diet could interrupt microbial homeostasis. Pro-inflammatory cytokines, linked to such neuropsychiatric disorders as anxiety and depression, could be affected by microbiota imbalance [22].

Goot et al. [23] examined gut microbiota and behaviour in mice with reduced Phe intake. Shannon diversity of PKU microbiota changed with a Phe-free diet. The *Entereococcaceae, Erysipelotrichaceae*, *Porphyromonadaceae*, and *Alloprevotella* families were linked to plasma Phe concentrations and may be ideal candidates for PKU metabolic potential and microbiome probiotics. Goot et al. [23] found that non-restricted dieters may not have deficit microbiota.

Although Poland has screened PKU since 1994, recently introduced PKU may go undetected. Late-onset PKU has been described in a 59-year-old man with dementia and parkinsonism who exhibited bilateral diffusely hyperintense parietal and occipital white matter lesions [24]. Phe-restricted diets partially improved cognitive impairment and parkinsonism, but symptomatic PKU patients need a thorough diagnosis.

Isolating species and analyzing metabolic capability are necessary to create efficient probiotics, boost microbiome diversity and resilience, while liberalizing Phe intake and improving metabolic and behavioural outcomes in PKU patients.

CONCLUSIONS

This is the first microbiome investigation in elderly phenylketonuria patients and controls. PKU microbiota varied from controls. Late-diagnosed PKU patients may have different microbiota. The obtained results differ from past research due to the late therapy of the 63-year-old patient. In future, full metagenomic analysis is needed, considering nutrition and geography, and comparing the microbiomes of children, adults, and the elderly.

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