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

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INTRODUCTION

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.

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,

The gut microbiome composition in PKU and control samples revealed differences. 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 contained 157 family-level taxonomic groups, and the control's contained 138. The PKU sample had 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. Bacteroides 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.

**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

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer’s sequence</th>
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<tr>
<td>16S_F</td>
<td>5’-(TCGTCGCCGAGCCGATGCTTGTAAGAGAGCGCTACGGGNGGCGWGA)-3’</td>
</tr>
<tr>
<td>16S_R</td>
<td>5’-(GTCTACGGGCTCGGAGATGTGTATAGAGACAGGACTACHVGGGTATCTAATCC)-3’</td>
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**Figure 1.** Sample collection process and flow diagram of the study (with materials)
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 Bacteroidiales 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.