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# Evaluation of Oral Health and Its Modifying Factors: Oral Microbiome, Genetic Markers, and Patient Knowledge in Phenylketonuria and Type 1 Diabetes

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The Doctoral Thesis is available in RSU Library and on RSU website: https://www.rsu.lv/en/dissertations

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# Abbreviations used in the Thesis

| | |
|---|---|
| CPITN | Community index of periodontal treatment needs |
| DEFB1 | Beta-defensin 1 |
| DNA | Deoxyribonucleic acid |
| EU | European Union |
| IL1B | Interleukin-1 beta |
| OR | Odds ratio |
| pH | Potential of Hydrogen |
| Phe | Phenylalanine |
| PAH | phenylalanine hydroxylase |
| PKU | Phenylketonuria |
| T1D | Type 1 diabetes |
| Tyr | Tyrosine |
| WHO | World Health Organization |

# Introduction

Oral health encompasses a range of diseases and conditions, including dental caries, periodontal disease, and more. While hygiene habits are a primary factor affecting oral health (WHO, 2024), the long-term effects of diet, the oral microbiome, and genetic predisposition still being studied (Ostrowska et al., 2024).

The oral cavity, being open to the environment, serves as a primary entry route for microbes into the human body. Saliva plays a crucial role in maintaining oral homeostasis and contributes significantly to the body's disease-protection mechanisms (Sampaio-Maia et al., 2016). For a considerable time, it has been recognised that diet and oral hygiene strongly affect the composition of oral microorganisms. The majority of reports still associate *Streptococcus mutans* with dental caries. (Dianawati et al., 2020; Simon-Soro et al., 2014) and *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola,* and *Tannerella forsythia* are the primary bacteria identified in periodontitis (Nazir et al., 2020). However, relatively little information exists on the impact of diet on the salivary microbiome, which may in turn affect oral health.

There is evidence that genetic variants in immunity-determining genes (e. g. *IL1B, DEFB1*) and taste receptor genes (e. g. *TAS1R2, TAS2R38*, and *CD36*) predispose individuals to caries and periodontitis (Cogulu & Saglam, 2022; Nibali et al., 2019).

Although the number of patients on medically prescribed diet is increasing, it remains unclear whether these long-term diets affect the human oral microbiome. For patients with certain metabolic conditions, adhering to a strict diet is essential to ensure adequate intake of micro- and macronutrients for normal development. Low-protein diets, for instance, are prescribed for individuals with phenylketonuria (PKU) (van Wegberg et al., 2017). PKU is a metabolic disorder marked by a deficiency or absence of the enzyme

phenylalanine hydroxylase (PAH), which is necessary for converting the amino acid phenylalanine (Phe) into tyrosine (Tyr). Patients with PKU follow a carbohydrate-rich diet supplemented with a low-pH, Phe-free amino acid formula (van Spronsen et al., 2021). This diet increases carbohydrate intake, which heightens the risk of dental caries. The Phe-free amino acid formula is also sweetened and acidic, posing additional risks to oral health (Rocha & MacDonald, 2016).

Type 1 diabetes (T1D) is another heterogeneous disorder characterised by the complete absence of insulin due to the destruction of pancreatic beta cells (Vanderniet et al., 2022). Managing diabetes is a lifelong process that requires constant monitoring, especially as paediatric patients grow. A critical component of treatment is diet therapy, which emphasises meal regularity and precise carbohydrate counting to optimise glycaemic control (American Diabetes Association Professional Practice, 2021). Both the disease itself and dietary adjustments have been associated with oral health issues, including an increased incidence of dental caries and periodontitis (Ferizi et al., 2022; Gunasekaran et al., 2022).

One of the objectives of this research is to investigate the association between diet and oral health among patients with PKU in Latvia still responsive to follow-up visits. T1D patients and a control group (individuals without known chronic diseases or dietary restrictions) will be enrolled to assess oral health status and explore potential influencing factors, such as dietary restrictions, oral microbiome composition, genetic predisposition, and oral hygiene habits. Oral health assessments will be performed for individuals from 12 years of age, as this allows the evaluation of the health of permanent teeth.

**Aim of the Thesis**

The aim of this study was to assess the oral health parameters associated with caries and periodontal disease risk in patients with PKU and T1D, focusing on dental hygiene status, oral microbiome composition, and the effectiveness of tailored oral hygiene recommendations for patients with PKU in Latvia. Additionally, the study examined whether PKU and T1D patients with the *IL1B* rs1143634, *DEFB1* rs11362, *TAS1R2* rs35874116, *TAS2R38* rs1726866 and rs713598, *CD36* rs1761667 genetic variations have a higher incidence of caries and periodontitis compared to healthy controls.

**Objectives of the Thesis**

- To evaluate oral health in patients with PKU andT1D
- To develop oral hygiene recommendations for patients with PKU and via survey results evaluate patient / patient caregiver knowledge and daily habits of oral hygiene and recommendation application in everyday practice within one-month period.
- To investigate the effect of oral microbiome characteristics on caries activity and periodontal disease risk in patients with PKU and T1D, compared to healthy individuals in Latvia.
- To analyse genetic predisposition to caries and periodontal disease in PKU, T1D and a control groups, by detecting the genotypes for *IL1B* rs1143634, *DEFB1* rs11362, *TAS1R2* rs35874116, *TAS2R38* rs1726866 and rs713598, *CD36* rs1761667.

**Hypotheses of the Thesis**

1  In patients with PKU, daily oral care habits and knowledge regarding oral hygiene are insufficient, which results in poorer oral health outcomes.

2   Single nucleotide variants *IL1B, DEFB1, TAS1R2, TAS2R38,* and *CD36* influence oral health in patients with PKU and T1D.

3   In individuals with PKU and T1D, the oral microbiome is altered compared with the control group, and in relation to specific dietary characteristics, this increases the risk of oral diseases.

## Novelty of the Thesis

This is the first study to examine the oral microbiome in patients with PKU and T1D and its influence on oral health; previously, only individual microorganisms had been studied. For the first time, oral hygiene guidelines specifically for patients with PKU have been developed.

In Latvia, no prior research has investigated the oral health of patients with rare diseases or endocrine disorders. Until now, neither oral health nor oral health awareness in these patient groups had been studied, as the primary focus was limited to managing the underlying disease. Additionally, this is the first study in Latvia to investigate the association between single nucleotide variants and oral health in context of a specific disease.

## Personal contribution

The author's own contribution was to organise, coordinate, and carry out the study's primary components. This includes:

- Designing the research concept together with supervisors.
- Recruiting and enrolling participants in all three groups (PKU, T1D, and control group).
- Performing and/or organising the oral health examinations.
- Preparing and administering the oral hygiene questionnaires for patients with PKU and developing the tailored oral hygiene recommendations.
- Collecting saliva samples for microbiome and genetic analyses.

- Participating in laboratory work, including DNA isolation, genetic analysis.
- Carrying out data analysis (oral health indices, questionnaire results).
- Interpreting results in the context of oral health, diet, microbiome, and genetics.
- Writing the manuscript of the Thesis.
- Author of three first-author publications and co-author of one publication.

# Conclusions

1  Patients with PKU and T1D have a higher prevalence of carious teeth and increased risk of periodontal disease, higher Silness-Löe plaque index, CPITN index and Greene-Vermillion index comparing with control group. The PKU group exhibited the worst oral health outcomes among all three study groups.

2  Survey results indicate that knowledge of oral care and hygiene is insufficient both among adult patients and caregivers of paediatric patients. The specifically developed recommendations led to significant improvements only in the group of patients under 18 years of age.

3  No major or systemic differences in overall microbiome composition were found between the groups; however, within the context of these specific diets, certain individual genera showed statistically significant differences that were associated with oral health characteristics.

4  Genetic associations between *DEFB1* rs11362, *ILB* rs1143634, *CD36* rs1761667, and *TAS2R38* rs713598 variants and oral health characteristics were observed in the PKU and T1D groups, suggesting that genetic factors may contribute to oral health differences in these populations.

# Proposals

This study has the potential to serve as a reference for future research aiming to develop recommendations for the prevention of periodontal disease and the reduction of caries risk in patients with PKU and T1D. We have identified the importance of regularly reminding patients about maintaining good oral health. One effective strategy could be the annual administration of a questionnaire that not only monitors oral hygiene behaviours but also reinforces daily oral care recommendations. These reminders could be seamlessly incorporated into the routine annual visits patients with PKU have with their dietitians.

An essential step forward would be the formal inclusion of a dentist in the multidisciplinary care team – specifically one with expertise in PKU – to ensure comprehensive oral health management. Regular encouragement from healthcare professionals, emphasizing the importance of dental check-ups, can significantly enhance patient awareness and adherence to good oral hygiene practices.

To evaluate the long-term impact of these recommendations, it would be valuable to repeat the survey after three years.

# Publications and reports on topics of the Thesis

## Publications:

1.  Abola, I., Emulina, D. E., Skadins, I., Brinkmane, A., Gailite, L., and Auzenbaha, M. 2022. "Dental Status and Periodontal Health of Patients with Phenylketonuria in Latvia," *Acta Stomatol. Croat.*, , doi: 10.15644/asc56/2/2.

2.  Abola, I., Gudra, D., Ustinova, M., Fridmanis, D., Emulina, D. E., Skadins, I., Brinkmane, A., Lauga-Tunina, U., Gailite, L., Auzenbaha, M. 2023. "Oral Microbiome Traits of Type 1 Diabetes and Phenylketonuria Patients in Latvia," *Microorganisms*, doi: 10.3390/microorganisms11061471.

3.  Emulina, D. E., Abola, I., Brinkmane, A., Isakovs, A., Skadins, I., Moisejevs, G., Gailite, L., Auzenbaha, M. 2024. "The Impact of IL1B rs1143634 and DEFB1 rs11362 Variants on Periodontitis Risk in Phenylketonuria and Type 1 Diabetes Mellitus Patients in a Latvian Population," *Diagnostics*, doi: 10.3390/diagnostics 14020192.

4.  Abola, I., Intlere, N. A., Brinkmane, A., Laktina, S., Zarina, A., Vasilevska, L., Skadins, I., Moisejevs, G., Gailite, L., Auzenbaha, M. 2024. "Oral health care knowledge among Phenylketonuria patients in the Latvian population," *Molecular Genetics and Metabolism Reports*, 2024, doi: 10.1016/j.ymgmr.2024.101167

## Reports and theses at international congresses and conferences:

1.  35th ESPKU Virtual Conference, 15–17 October 2021, "Oral health in Latvian patients with Phenylketonuria".

2.  Theses for the 9th Congress of Latvian Doctors, "Orālā mikrobioma raksturojums fenilketonūrijas un 1. tipa cukura diabēta pacientiem Latvijas populācijā".

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