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# **PhD THESIS**

**THE MANAGEMENT OF PHENYLKETONURIA: CLINICAL,  
NUTRITIONAL AND GENETIC CORRELATIONS**

**- ABSTRACT-**

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# ABSTRACT

## INTRODUCTION

Phenylketonuria (PKU) is a rare autosomal recessive disorder caused by mutations in the PAH gene, which impairs the enzyme that breaks down phenylalanine (Phe). This leads to Phe accumulation, causing severe intellectual disability, autistic behaviors, motor impairments, rashes, and seizures. The highest incidence is in Turkey (38 per 100,000 neonates) and the lowest in Thailand (0.3 per 100,000 neonates). Globally, PKU affects about 6 out of 100,000 infants. In Romania, the forecasted prevalence is 1 per 100,000, with higher rates in Bihor County and North-Western Romania. While there is no cure, dietary management is crucial. This involves minimizing Phe intake and using specialized metabolic formulas, starting ideally within the first five days of life. Strict adherence to this diet is essential to prevent irreversible neurological damage therefore newborn screening programs are crucial for early detection. Dietary management proves significant challenges due to attitudes like food neophobia, pickiness, and neophilia, which must be addressed when considering a personalized treatment for these patients.

The present work aimed to conduct a comprehensive examination of patients diagnosed with PKU by exploring the genetic, clinical, and nutritional aspects. The study sought to uncover the complex relationships between genetic mutations, clinical manifestations, and dietary management, focusing on growth and food neophobia. A significant part of the research involved validating and translating the Food Neophobia Scale (FNS) to understand food neophobia deeply. Additionally, it investigated the mutations in the PAH gene, the progression of PKU symptoms, and the nutritional status of children with PKU, comparing their growth trajectories with standard growth charts and quantifying the prevalence of food neophobia in this population.

The PhD thesis incorporates three studies, two cross-sectional and one retrospective-longitudinal, carried out in accordance with the Principles of the Declaration of Helsinki (1975, revised in 2013), and with approval from the ethics committee. The doctoral research includes the following studies:

- Study 1 – the factors impacting the reduction of neophobia prevalence in PKU patients
- Study 2 – the prevalence of eating behaviors and food neophobia in a national study
- Study 3 – the contribution of dietary therapy to the growth of children with PKU

Published in 2024 in ISI journals (Nutrients, Foods, and Medicina), the research offers insights to improve clinical management and well-being for PKU patients.

# PERSONAL CONTRIBUTIONS

## STUDY I. Factors impacting the reduction in neophobia prevalence in Phenylketonuria patients

### Material and method

The first study included 34 patients aged between 7 months and 40 years with PKU, from our department at the Emergency Hospital for Children "Louis Țurcanu" in Timișoara, and members of the PKU Life Romania Organization, between November 1, 2018, and December 31, 2023. Data were collected through an interview questionnaire, partially developed by the researcher after reviewing relevant literature and translated into Romanian, which was offered to participants. Respondents completed the online survey using Google Forms. The questionnaire was divided into three parts: participant characteristics, familial characteristics and eating behaviors utilizing the Food Neophobia Scale. FNS, a validated 10-item questionnaire developed by Pliner and Hobden, measures willingness to try new foods, acceptance of exotic cuisines, and expectations about novel foods. It includes five items related to neophobic behavior and five related to neophilic behavior, with higher scores indicating greater neophobia (range 10 to 70). The study was conducted with the approval of the ethics committee of the Children's Emergency Hospital "Louis Țurcanu" and the University of Medicine and Pharmacy, as well as with the approved consent of the relatives.

### Results

Sixty-eight participants (34 PKU patients and 34 controls) were included in the study. The sex ratio M/F = 2:1, and the age groups between the PKU group and the control group were comparable. Patients with PKU were diagnosed in the following ways: 29.41% within the first 15 days of life, 44.11% between days 16 and 30, 14.70% between day 31 and three months, 2.94% by the end of the first year, and 8.82% beyond the first year. Of the patients, 88.23% were diagnosed using screening. The individuals within the PKU group had a substantial prevalence of malnutrition, with 35.30% displaying underweight, 23.53% being overweight, and 23.53% displaying severe obesity.

Upon analyzing the questionnaire data, a significant percentage of both PKU group and control group respondents exhibited FNS scores exceeding 35. Notably, all subjects in the over-18-year-old group scored above this threshold. Importantly, no statistical differences in the incidence of neophobia were observed between males and females. The responses to each

question indicated significant disparities for several items, even though the overall FNS scores were relatively close.

The data reveal statistically significant correlations between the FNS score, current age, and the period between birth and diagnosis. Notably, as age increased, the FNS score tended to be higher, indicating a greater risk of neophobia with advancing age. Additionally, a correlation was observed between the length of time from birth to diagnosis and FNS scores, with earlier diagnoses being associated with a lower risk of high FNS scores. To further validate our findings and provide a more robust statistical analysis, we conducted additional tests focusing on the observed correlations. We provided a detailed comparison of data between the PKUG and the CG, including variables such as developmental milestones, FNS scores, primary caregiver's educational level, and Phe levels at birth and currently. Additionally, while FNS scores showed significant variation between groups, the differences were more pronounced when considering the educational level of the primary caregiver. These findings suggest that higher educational levels among primary caregivers are associated with better management and possibly lower levels of food neophobia in PKU patients. Furthermore, the analysis underscores the importance of early diagnosis and continuous monitoring of Phe levels in improving dietary compliance and reducing the risk of neophobia.

Given the findings that highlighted a significant variation in the parents' educational levels based on whether they came from urban or rural environments, we conducted a follow-up inquiry. This follow-up involved comparing the correlation outcomes between the FNS score and various parameters investigated within the PKU group (PKUG), differentiated by their environment of origin (rural/urban). The results indicated no significant differences in any of the investigated items based on the rural or urban origin of the participants. This suggests that the environment of origin does not play a significant role in influencing the FNS scores or the associated parameters within the PKU group.

Synthesizing the results of the statistical analyses conducted we found a correlation between the FNS score and age, showing that as age increases, the risk of food neophobia also increases and also between the FNS score and the interval between birth and the diagnosis of PKU, indicating that earlier diagnosis is associated with lower neophobia scores.

## **STUDY II. The prevalence of eating behaviors and food neophobia: a Romanian study**

### **Material and method**

The second study focused on translating and validating the Food Neophobia Scale, involving 59 medical students in a pilot group and 385 respondents from the general Romanian population. Data collection occurred between November 1, 2023, and February 29, 2024, via Google Forms. Participants rated statements on a Likert scale from 1 (strongly disagree) to 7 (strongly agree), with certain items reverse-scored. The national survey sample size was calculated using OpenEpi for a 90% confidence level and a 10% margin of error. After excluding incomplete data, 359 respondents were included in the final analysis. The questionnaire's reliability was evaluated using Cronbach's alpha for internal consistency and Spearman's rho for robustness. These methods ensured a thorough assessment of the questionnaire's reliability and validity, offering insights into food neophobia among participants. The study was conducted with the approval of the ethics committee of the Children's Emergency Hospital "Louis Țurcanu" and the University of Medicine and Pharmacy.

### **Results**

The national group consisted of 359 respondents, with a mean age of 38.07 years and a standard deviation of 10.75. The female-to-male sex ratio was 4:1. There is a consistent pattern in both the pilot group (PG) and the national group (NG) regarding the standard deviation, mean scores, and skewness for each questionnaire item. This uniformity across both groups indicates robustness in the data collected and analyzed.

The confidence intervals obtained align consistently with the Cronbach's alpha coefficients. PG demonstrated a strong correlation with a Cronbach's alpha of 0.803 (95% CI: 0.772, 0.831), while NG exhibited a slightly higher Cronbach's alpha coefficient of 0.837 (95% CI: 0.811, 0.861).

Both figure 1 and 2 provide comprehensive descriptive statistics for each item on the FNS. The mean FNS score for the NG was calculated to be 31.86. With neophilia categorized by an FNS score below 25 and neophobia by a score above 35, it is notable that the majority of respondents (69.20%) fell within the neutral category, scoring between 25 and 35 on the FNS. Additionally, the proportions of individuals classified as low in food neophobia and neophobes were distinct, comprising 12.59% and 18.21% of the respondents, respectively. In contrast, in PG, 42.10% of respondents were categorized as neophobic, while 10.52% were identified as neophilic.

ANOVA analysis among individuals with an FNS score greater than 35, based on demographic and comorbidity status, revealed no significant differences in FNS scores across the NG for most factors. The exception was educational level, where the difference approached significance ( $p = 0.078$ ).

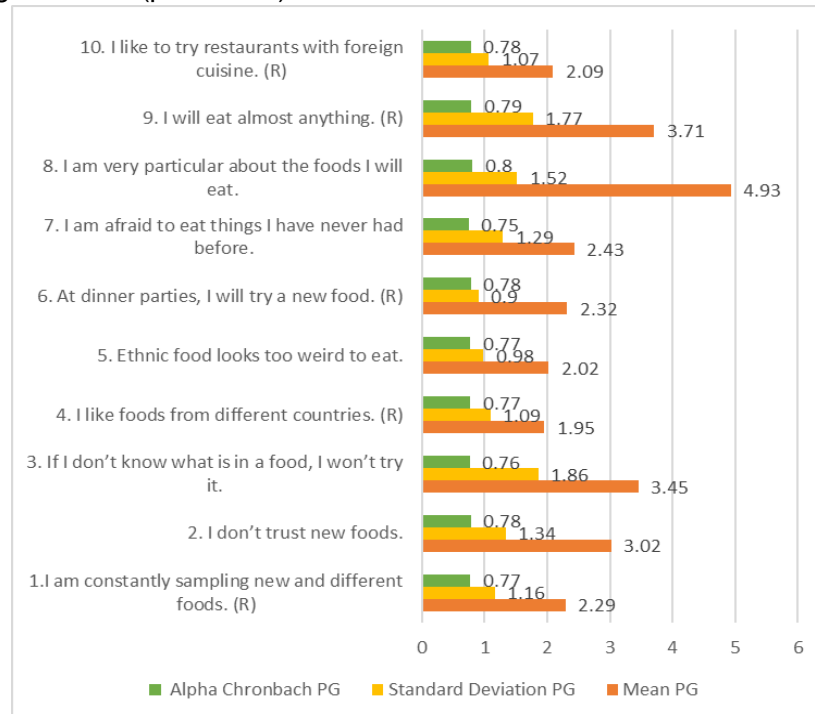


Figure 1. The statistical interpretation of the results from the questionnaire administrated to the pilot group

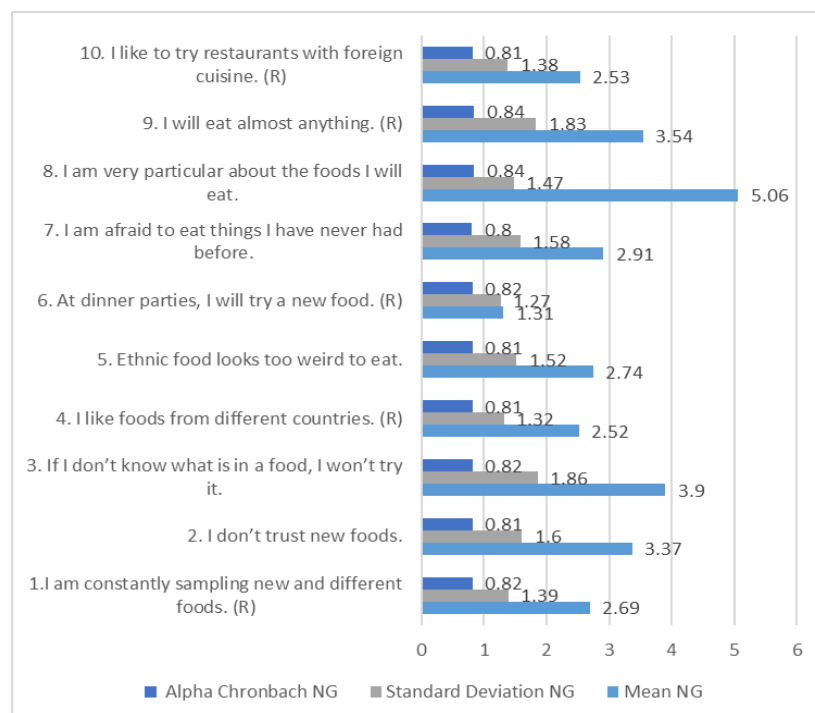


Figure 2. The statistical interpretation of the results from the questionnaire administrated to the national group



# **STUDY III. Evaluating therapy and growth in children with Phenylketonuria: a retrospective longitudinal study from two Romanian centers**

## **Material and method**

In this retrospective longitudinal study, we examined the growth trajectories of children with PKU from two Romanian pediatric referral centers, Timisoara and Oradea, covering the period from January 2010 to March 2024. Inclusion criteria were ages one month to three years, confirmed PAH deficiency, adherence to a Phe-restricted diet, and the regular clinical check-ups. Exclusion criteria included major congenital anomalies, additional chronic disorders, or incomplete data. The study included 18 children (14 from Timisoara and 4 from Bihor) and a control group of non-PKU children matched by age and gender. The control group excluded those with conditions affecting growth, such as malnutrition, growth hormone deficiency, genetic disorders, and gastrointestinal diseases. The study was conducted with the approval of the ethics committee of the Children's Emergency Hospital "Louis Țurcanu" and the University of Medicine and Pharmacy.

Retrospective data were collected, including demographic details (age, gender), genotype, growth progression, Phe blood levels, and dietary intake information from diagnosis up to three years of age. The study utilized the child's weight, length, and height measurements to determine growth indicators including weight-for-age z score (WAZ), height-for-age z score (HAZ), and BMI-for-age z score (BAZ). These scores provided insights into the child's growth trajectory relative to established norms.

## **Results**

All PKU patients received dietary oversight from the same dietitian at each center. The cohort consisted of 9 boys and 9 girls, with a median follow-up duration of 36 months (interquartile range [IQR] = 9.75).

The diagnosis of PKU in nearly all patients was facilitated through newborn screening using the Guthrie test, with only one patient was identified later in infancy. The median age at diagnosis was 21 days (range: 4 to 390 days), and all children promptly initiated their prescribed diet within 24 hours of diagnosis. The majority of patients (72.2%) were diagnosed with classical PKU, comprising 8 males and 5 females, while one female patient had moderate PKU, and four patients exhibited mild hyperphenylalaninemia (mHPA), consisting of one male and three females.

In PKU patients, the mean birth weight was  $3184 \pm 527$  g (females:  $2889 \pm 479$  g; males:  $3430 \pm 395$  g), all falling within the normal birth weight range (2500 – 4000 g). The average body length at birth was  $49.4 \pm 2.1$  cm (females:  $48.6 \pm 2.5$  cm; males:  $50.2 \pm 0.8$  cm). When compared to controls, PKU patients exhibited a statistically borderline lower birth length ( $p=0.04$ ), while birth weight did not significantly differ. Notably, breast milk served as a source of natural protein for 83.3% of infants during infancy.

Treatment involved a Phe-restricted diet for 14 children, overseen by the same dietary team, aimed at maintaining Phe blood levels below  $242 \mu\text{mol/L}$  (4 mg/dl). Conversely, four children followed a liberalized diet approach.

Protein intake ranged from 1.3 to 3.22 g/kg per day (mean  $2.17 \pm 0.36$  g/kg per day), which corresponds to 90% to 292% of the Recommended Dietary Allowance (RDA). Caloric intake ranged from 71.9 to 116.7 kcal/kg per day (mean  $91.5 \pm 10.3$  kcal/kg per day), also representing 90% to 292% of RDA. These values surpass the RDAs and exceed the recommended safe intake levels as outlined by the World Health Organization.

The median phenylalanine (Phe) concentrations over the entire study duration served as the basis for evaluating metabolic control. Effective metabolic control was defined as Phe levels below  $242 \mu\text{mol/L}$ . In total, 15 patients (83.3%) achieved effective metabolic control.

We conducted a comparative analysis of growth trajectories between the PKU group and Romanian growth standards, assessing z-scores for weight, height, and BMI in both PKU patients and controls. The most notable distinctions were observed in weight-for-age z-scores, indicating that PKU patients consistently exhibited lower weights compared to controls across various age assessments. Height trajectories remained largely similar between the two groups over the three-year period, except at birth. Additionally, despite PKU children showing lower WHZ and BAZ values compared to controls at different age points, the median z-scores for each age fell within the range of -2 to +1.

Based on the BMI-for-age z-score and length/height-for-age z-score, the majority of PKU patients maintained normal weight and height throughout the entire study period.

Finally, Spearman correlation analysis was conducted to explore potential relationships between growth parameters, blood Phe levels, and dietary variables. The findings indicated positive correlations between WAZ, HAZ, and BAZ with dietary Phe tolerance ( $p=0.441$ ,  $p=0.244$ , and  $p=0.188$ , respectively). Additionally, WHZ, HAZ, and BAZ were found to correlate with the protein/calory ratio ( $p=0.376$ ,  $p=-0.248$ , and  $p=0.383$ ).

## **ELEMENTS OF ORIGINALITY AND PERSONAL CONTRIBUTIONS**

Interest in nutrition and its influencing factors has surged, particularly in managing rare diseases like phenylketonuria. Food neophobia significantly impacts diet adherence and food selection among PKU patients. This thesis is the first in Romania to translate and validate the Food Neophobia Scale into Romanian, assessing food behaviors and identifying neophobia risk factors in PKU patients. Also, it contributes to future research on neophobia in other pathologies. The findings highlight the importance of closely monitoring children with PKU to improve disease control and patient outcomes.

This research represents the first evaluation of food neophobia and the assessment of therapy and growth in young Romanian PKU patients. The results demonstrate the impact of food neophobia on quality of life and treatment adherence, emphasizing the need for continuous monitoring and adjustment of nutritional therapies to ensure optimal development.

The comprehensive approach of this research addresses medical, nutritional, psychosocial, and dietary adaptation aspects, crucial for improving the management and quality of life for PKU patients in Romania.

## **LIMITATIONS OF THIS DOCTORAL THESIS**

This PhD thesis which provides important insights into PKU management also has several limitations. The studies had small sample sizes and a short three-year observation period, which may not fully capture age-related changes in food neophobia and long-term growth trends. The retrospective data collection introduced potential biases, as it focused only on children with PKU who attended medical check-ups. Self-reported data on dietary behaviors also posed interpretation challenges. Control groups were selected from hospitalized children for routine check-ups or commune pediatric diseases rather than a broader population. Additionally, the research did not explore body composition, micronutrient status, and trace elements, which are important for understanding nutritional impacts. Future research should use larger, more diverse samples and prospective designs to better assess dietary interventions' long-term effects on growth and health.

## GENERAL CONCLUSIONS

In conclusion, the studies encompassed in this doctoral thesis spanning six years of research have provided crucial insights into managing phenylketonuria and related aspects:

1. The first study advances our understanding of food neophobia in the management of PKU. We've identified various factors influencing neophobic tendencies, such as parental education and early diagnosis. This emphasizes the need for comprehensive support strategies to assist PKU individuals in adhering to their diets and improving their overall quality of life (**Study 1**).
2. The translation and validation of the FNS in Romanian for assessing food behaviors among individuals with PKU represent pioneering efforts in Romania. This foundational work not only aids in understanding food neophobia in PKU but also serves as a blueprint for studying neophobia in various other pathologies. The insights gained can inform tailored interventions, products, and public health initiatives aimed at promoting diverse and nutritious eating habits (**Study 2**).
3. Our study on the growth trajectories of Romanian children with PKU during early childhood highlighted adequate growth across a diverse population, including classical PKU and mild hyperphenylalaninemia. Despite overall good metabolic control, persistent lower weight-for-age z-scores compared to controls underscore the need for ongoing monitoring and potential adjustments in dietary strategies. Future multicenter studies are essential to validate these findings and explore additional factors influencing growth and development in this specific population (**Study 3**).

In summary, this doctoral thesis contributes significantly to the field by addressing critical aspects of PKU management, including food neophobia, growth patterns, and the translation of assessment tools. These findings provide a foundation for advancing personalized care strategies and enhancing outcomes for individuals living with PKU.

## **FUTURE STUDIES**

In the future, I plan to advance research in rare metabolic disorders, with a particular focus on phenylketonuria. My research will explore innovative approaches to managing food neophobia in PKU patients and investigate new therapeutic strategies and specialized nutritional supplements aimed at optimizing growth and neurocognitive development in children with PKU. This approaches aim to enhance dietary treatment protocols and improve the quality of life for PKU patients. Through these efforts, I seek to advance management practices and make a meaningful contribution to the field of PKU, benefiting both patients and the global medical community.