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DOKTORI ISKOLA

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Programvezető: Dr. Szabó Attila, egyetemi tanár

Témavezetők: Dr. Reusz György, professor emeritus

Dr. Zsidegh Petra, egyetemi adjunktus

Examination of the quality of life and the effect of COVID-19 pandemic on metabolic control in Hungarian paediatric PKU population

PhD thesis

Dóra Becsei MD

Károly Rácz Doctoral School of Clinical Medicine,

Semmelweis University

Supervisors: György Reusz MD, DSc and Petra Zsidegh MD, PhD

Official reviewers: Gábor Rácz MD, PhD

Nikolette Szücs MD, PhD

Head of the Complex Examination Committee: Barna Vásárhelyi MD, DSc

Members of the Complex Examination Committee: András Szabó MD, PhD

Gergely Kriván, MD, DSc

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List of abbreviations

AA: amino acids

BH_4: tetrahydrobiopterine

Bio: biopterine

BMI: body mass index

CE: COVID-19 era

CLD: Control to lockdown period

CSF: cerebrospinal fluid

CURP: control to unrestricted period

DBS: dried blood spot

DHPR: dihydropteridine reductase

DHPRD: dihydropteridine reductase deficiency

DPR: dietary protein restriction

EMA: European Medical Agency

ETPKU: early-treated PKU

FLAIR: Fluid-attenuated inversion recovery

GMP: glycomacropeptide

GTPCHD: guanosine triphosphate cyclohydrolase I deficiency

HPA: hyperphenylalaninaemia

HRQoL: Health-related quality of life

HVA: homovanillic acid

IQ: intelligence quotient

IQR: interquartile range

LAAD: L-amino acid deaminase

LNAA: large neutral amino acid

MRI: magnetic resonance imaging

MS/MS: tandem mass spectrometry

NCE: Non-COVID-19 era

Neo: neopterin

NBS: newborn screening

PAH: phenylalanine-hydroxylase

PAL: phenylalanine-ammonia-lyase

Pc: percentile

PFC: prefrontal cortex

Phe: phenylalanine

PKU: phenylketonuria

Prim: primapterin

PTPSD: 6-pyruvoyltetrahydropterin synthase deficiency

REMS: Risk Evaluation and Mitigation Strategies

QoL: quality of life

SARS: Severe Acute Respiratory Syndrome

SRD: sepiapterin reductase deficiency

Tyr: tyrosine

URP: unrestricted period

US FDA: United Stated Food and Drug Administration

WMA: white matter abnormalities

5-HIAA: 5-hydroxyindoleacetic acid

5-MTHF: 5-methyltetrahydrofolate

1. Introduction

1.1. History and prevalence of PKU

Phenylketonuria (PKU, OMIM 261600) was first described by Asbjorn Folling in 1934, who identified patients by abnormal excretion of phenylpyruvic acid in their urine (1,2). The term phenylketonuria was coined by Penrose in 1937 (3). Mentally retarded children in institutions all over the world were assessed by urinary ferric chloride test globally, and tests turned out to be positive in 1% to 2% of seriously retarded patients in most western countries. For years, PKU was considered an unfortunate disorder of mental retardation for which nothing could be done (4) .

Jervis identified the enzyme defect of PKU, which obstructed the normal metabolism of phenylalanine to tyrosine in 1947 (5). In 1953, however, Bickel developed a special low phenylalanine (Phe) diet, which has been the gold standard therapy of PKU to date, and the levels of phenylalanine in plasma and urine fell dramatically (6). Newborn screening for PKU is based on the landmark discovery by Robert Guthrie (7) and has been widely used in clinical practice since the early 1960s. This bacterial inhibition test had been introduced in 32 US states and throughout Europe by the late 1970s (8). Over time the Guthrie test has been replaced by modern methods, such as chromatography, fluorometry and more recently tandem mass spectrometry. The introduction of multiplex methods-detection of several metabolites in one test- lead to the expansion of screening programmes for metabolic disorder in newborns (8,9).

The prevalence of phenylketonuria varies widely around the world (9). In Europe, the prevalence is about 1 per 10,000 livebirths (10). Northern Ireland, Spain and Turkey have a particularly high prevalence of mild hyperphenylalaninaemia (HPA) (11–13).

In Hungary, the prevalence is 1 per 8,500 births (14). The children with PKU are screened and treated by two metabolic centers: Pediatric Center of Semmelweis University, Budapest and Department of Pediatrics and Pedriatic Health Center of Albert Szent-Györgyi Centre, Szeged. About two-thirds of the patients are referred to the Budapest center, where about 180 PKU patients are cared for.

1.2. Pathophysiology of PKU

Phe is an essential amino acid, used for protein synthesis, as well as for the production of tyrosine (Tyr) and its derivatives, such as dopamine, norepinephrine, and melanin. Phe is hydroxylated to Tyr by the phenylalanine-hydroxylase enzyme (PAH) (15,16). The PAH enzyme requires the tetrahydrobiopterine (BH_4) cofactor, molecular oxygen and iron. Numerous mutations of PAH gene have been described (17). Patients with PKU have high Phe levels due to an inborn error of PAH, leading to alternative metabolic pathway activations (15,16,18,19). High Phe and the alternative metabolites are toxic to oligodendrocytes (20). Phe metabolism is summarized in Figure 1.

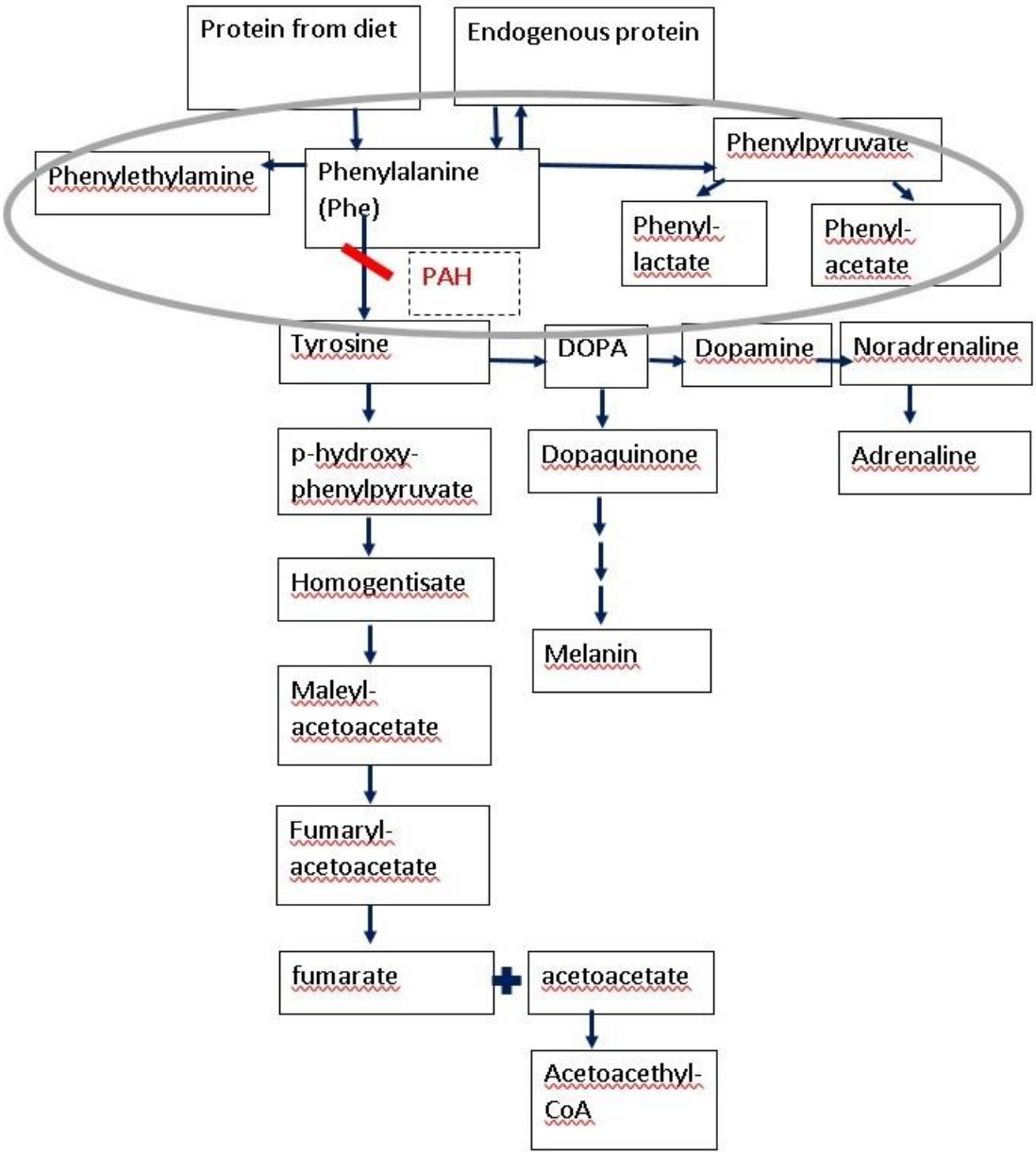

Figure 1. Phe metabolism. Normal Phe metabolism (black pathway). Phenylketonuric pathways are shown in grey circle. Adapted by Schuck et al (16).

Hoeksma et al. found a negative relationship between plasma Phe concentration and the cerebral protein synthesis. High Phe levels may cause abnormal cerebral protein metabolism, which may affect both myelin and dopamine synthesis pathways (21). Reduced myelination associated with PKU leads to aberrant brain neurosignaling (20) and neurocognitive deficits including decreased speed of information processing (22).

According to another hypothesis, depletion of neurotransmitters in the brain, especially dopamine deficiency in the prefrontal cortex, is thought to be responsible for abnormal executive functions, which may explain some of the neurocognitive deficits observed in PKU (23).

1.3. Clinical presentation

1.3.1. Classic symptoms

Before the era of diet therapy, patients with PKU had very severe symptoms, such as microcephaly, severe mental retardation, hyperactivity, attention and perceptual-motor problems, aggressiveness, negative mood and motor disturbance (24). Epilepsy and quadriparesis have been reported, if the treatment was delayed (25–28). Patients with untreated PKU lack melanin formation due to reduced tyrosine metabolism. This is manifested in the majority of the patients with fair skin, blond hair, and blue eyes (29). Dermatitis occurs in 20 to 40% of untreated PKU patients (29).

1.3.2. Cognitive and psychiatric symptoms

Children with treated PKU show overall intellectual functioning within the normal range, but lower than the general population and their siblings (30,31). A meta-analysis involving 43 studies showed a 1.8 to 3.8 point reduction in IQ predicted by each 100 μmol/l increase in lifetime blood Phe level (32). Cognitive impairment is well described in more domains: slowed information processing speed (33), motor planning (24), strategy (23,34), working memory (35), motor control and sustained attention (36,37). Executive dysfunctions have also been described in working memory, inhibitory control, conceptual reasoning, mental flexibility and organizational strategy (22,38,39).

Patients with PKU can demonstrate decreased social competence, autonomy, and self-esteem, they can also present phobias, depressed mood, and anxiety (40).

1.3.3. Growth and nutritional outcomes

Growth retardation including height and weight was reported in children and adolescents following Phe-restricted diet (41,42). However, numerous retrospective studies have reported a higher body mass index (BMI) and a higher prevalence of overweight in patients with PKU compared to the normal population, especially in females (43–46),

presumably caused by higher energy, fat and carbohydrate content of special low-protein foods (47).

These patients also demonstrate a lack of intake of various nutrients with low blood levels of preformed long-chain polyunsaturated fatty acids (48,49). These essential fatty acids are vital for normal brain and retinal development and their deficiencies can result in visual and cognitive impairment (49). Micronutrient deficiencies including coenzyme Q10, catalase and selenium have been documented by Gassio et al., who have revealed that selenium deficiency and neuropsychological deficits are associated with PKU (50).

Bone formation and resorption markers have been found to be significantly reduced in children on PKU diet compared to healthy controls (51). Osteopenia and osteoporosis have been reported in the adult PKU population (52,53). Possible outcomes are summarized in Figure 2, published by Enns (23).

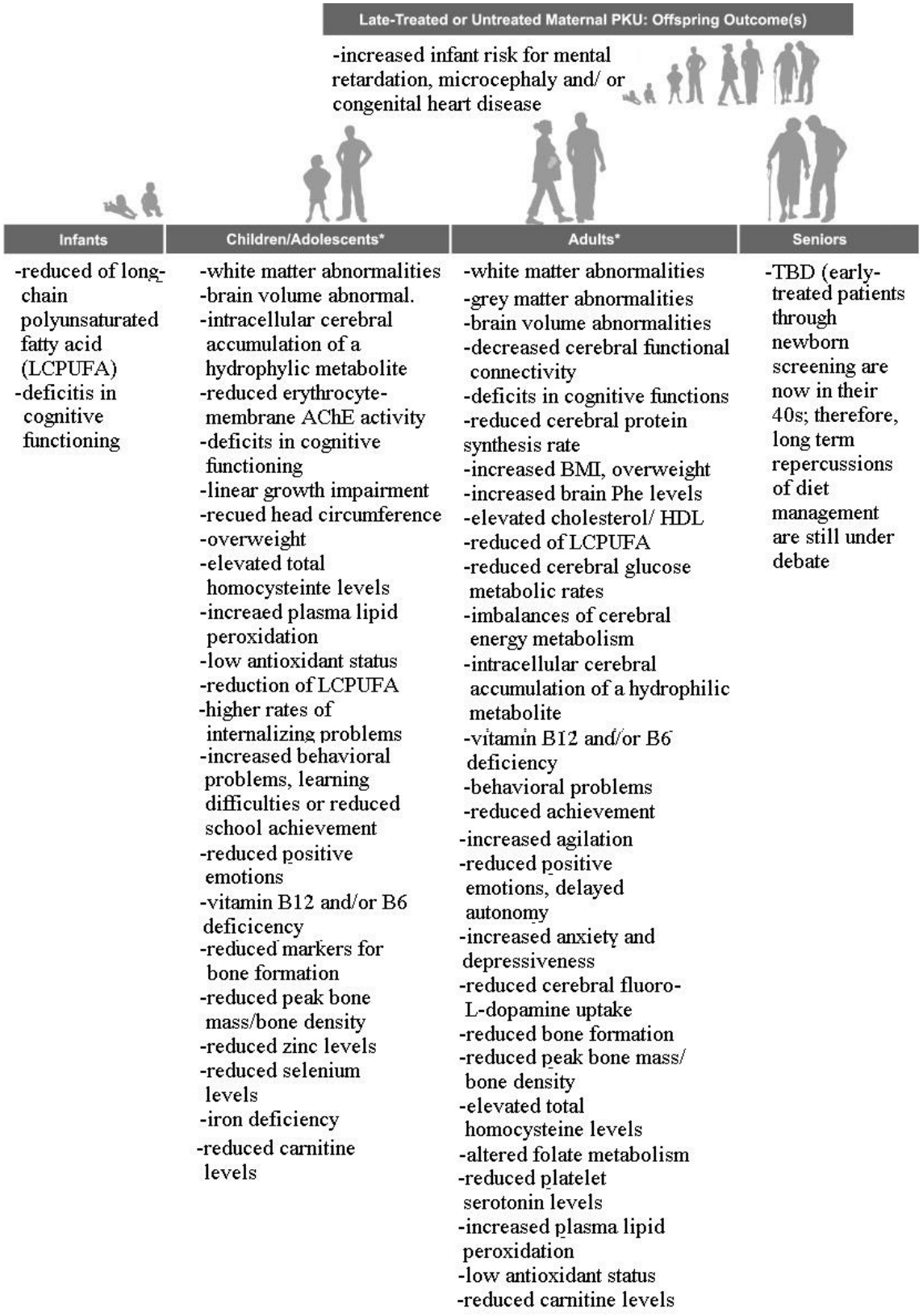

Figure 2 Summary of suboptimal outcomes of PKU in four age groups edited by Enns et al (23).

1.3.4. MRI findings in PKU

White matter abnormalities (WMAs) are presented on magnetic resonance imaging (MRI) in both early and late treated patients (54–56). The pattern of WM involvement in early-treated PKU (ETPKU) is characterized by patchy or diffuse symmetrical lesions of deep and periventricular WM (occipito-parietal, frontal, temporal) appearing as signal hyper-intensity on T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences and in a minority of subjects, as signal hypo-intensity in T1-weighted sequences. Several studies have shown that the extent and severity of WMAs appear to be moderated by patient age and/or blood Phe levels, with older age and/or higher Phe levels associated with increased white matter involvement (54,55).

In subjects with blood Phe levels <300–600 μmol/l, no WMAs have been reported (57,58). However, other factors are involved as suggested by the occurrence of WM variation (improvement or worsening) in patients with constant Phe values, and the wide variability of WM involvement under similar values of blood and brain Phe (59,60).

WMAs are reported to be reversible. Two controlled studies (61,62) showed an improvement of WMAs (3 to 6 months) after reduction of blood Phe levels. With functional MRI technique, atypical prefrontal cortex neural activity was shown in PKU patients during N-back working memory tasks. Decreased connectivity within and between the prefrontal cortex (PFC) and other brain regions was also described in PKU patients(63). Abbgotspon et al. showed a lower PFC activation compared to normal controls (64).

1.4. Subtypes of PKU

Since there is no consensus on phenotype classification (65), a simplified classification scheme is proposed, derived from Blau (66). Elevated Phe levels can result from either a deficient PAH activity, or from defects of enzymes involved in the pterin cycle. About 2% of all Phe level elevations detected by the newborn screening program are due to disorders in BH_4 metabolism, highlighting the importance of considering differential diagnosis in all cases of even slightly elevated blood Phe level. After a repeated positive newborn screening results, an outpatient evaluation is required with a special attention to neurological symptoms. In order to discriminate among different subtypes neonatal BH_4-loading test, analysis of urine pterin profile and measurement of dihydropteridine

reductase (DHPR) activity in DBS are essential. A precise diagnosis should be obtained for the initiation of the optimal therapy as early as possible (66).

PAH-dependent PKU forms (classic PKU and the milder form, HPA) can be distinguished by Phe tolerance. Phe tolerance is determined by the amount of daily Phe intake that a patient can tolerate without exceeding the upper target range of blood Phe.

1.4.1. Classic PKU

Pterins and DHPR activity are normal and neonatal BH_4 loading does not lead to any significant decrease in Phe. Lifelong Phe-restricted diet is needed to avoid or minimalize the classic symptoms mentioned above (chapter 1.3.).

1.4.2. HPA

Mild hyperphenylalaninemia is considered when there is no need of a Phe-restricted diet in order to achieve Phe levels below 360 uM.

1.4.3. BH_4-deficient PKU

In these forms of PKU, the PAH gene is normal, and it is based on a defect in enzymes involved in metabolism of cofactor BH_4. Four subtypes can be differentiated by pterin levels and DHPR activity (Fig. 3). BH_4 deficiency also affects the synthesis of catecholamines, and serotonine in the central nervous system (CNS), leading to multiple and usually more severe neurological symptoms than in classic PKU (67). Symptoms contain abnormal muscle tone, poor head control, seizures, and delayed motor development. In the treatment of BH_4-deficiencies, the replacement of BH_4 and/ or neurotransmitters are essential (67).

1.4.4. BH_4-responsive PKU

In cases where BH_4 loading test is positive and analysis of pterin profile and DHPR activity is normal BH_4-responsive PKU is considered. In BH_4-responsive patients, therapy with BH_4 increases residual PAH activity, which results in a decrease in blood Phe concentrations and/or an increase in natural protein tolerance (68). As not all PKU patients respond to BH_4 supplementation, it is important to appropriately select patients with PKU who benefit from long-term BH_4 treatment. Eligibility for treatment with BH_4 is typically assessed by performing a BH_4 test period or treatment trial to assess its

effects. All identified PAH-mutations associated with BH_4-responsiveness are tabulated in the BIOPKU database (http://www.biopku.org) and compared with existing information.

1.5. Screening and follow-up

1.5.1. Newborn screening

The newborn screening for PKU in Hungary started in 1975, preceded by regional pilot studies (69). The Hungarian NBS panel contains 27 inherited disorders, the recommended sampling age is 48-72 hours. DBS aminoacid and acylcarnitine profile is determined by TANDEM mass spectrometry (MS/MS). Phe levels above 179.64 umol/l (>99.98 pc) are considered abnormal.

1.5.2. BH_4 loading and therapy start

After a positive screening result, patients are carried out in a metabolic centre. After medical evaluation through several consultations with a physician and a dietitian the family is informed and educated. The newborn will also undergo a BH_4 loading test to differentiate between subtypes of PKU. In most cases, after the confirmatory and diagnostic testing, breast milk and Phe-free formula are titrated according to the Phe levels to determine the maximum phenylalanine tolerance (70).

1.5.3. Follow-up and treatment goals

Patients with PKU need lifelong follow-up and treatment. Metabolic control is achieved by monitoring Phe levels (dried blood spot, DBS self-sampling). The target range for Phe is 120-360 umol/l under 12 years of age, and 120-600 umol/l above this age (65). The patient is also need systematic outpatient follow up to monitor long-term complications at any age. The minimum DBS samplings and outpatient visits recommended by the European PKU guidelines are summarised in Table 1.

Table 1. Minimum frequency of DBS sampling and outpatients visits recommended by the complete European guidelines on phenylketonuria (65).

Age	Frequency of DBS	Frequency of visits
0-1 year	Weekly	Every 2 months
1-12 years	Fortnightly	Twice per year
12-18 years	Monthly	Twice per year
>18 years	Monthly	Once per year
Pregnancy	Weekly before conception, twice per week during pregnancy	Once per trimester
Increased frequency depending on indication: treatment changes, social circumstances, clinical ground, and adherence issues		

A medical and dietary history, an assessment of anthropometry including body mass index estimation, and a physical and neurological examination, with particular attention to clinical signs of Phe toxicity and nutrient (including Phe) deficiency (65,71,72) should be performed at each outpatient visit. Clinic reviews should always include a discussion on treatment issues and mental and physical health. Guidelines recommend annual testing for biochemical nutrients such as homocysteine, methylmalonic acid, haemoglobin, MCV and ferritin. All other micronutrients and hormones should be tested during childhood if clinically indicated. Bone densitometry and neurocognitive functions should be measured firstly in adolescence. Psychological problems should be screened clinically. Psychosocial functioning and quality of life (QoL) should be assessed once in childhood and once in adolescence. MRI should be performed in case of unexpected clinical course (65).

1.6. Therapeutic approaches

1.6.1. Diet therapy

Diet therapy is the first described therapy and is still the gold standard management option for PKU. The optimal diet for PKU should provide the target Phe ranges, be nutritionally complete and help normal growth. It should be also palatable, flexible and easy to follow. The diet has changed very little since the 1950s. It is based on natural protein restriction,

protein supplementation, calorie supplementation and a free-food system (73). Restricted foods are for example chicken, fish, eggs, cheese, nuts, seeds, quinoa, oats, soya, lentils, gelatine and aspartame (sweetener). The amount of dietary Phe tolerated will vary between individuals, depending on the severity of PKU. Adequate energy intake from very low protein sources is essential to meet energy requirements. There are a number of traditional foods that are naturally very low in protein and can be eaten without measurement. They include vegan cheese, butter, margarine, vegetable oils, sugar, jams, honey, and most fruits and vegetables. Although there is a wide range of specially produced 'starch based' low protein foods such as bread, flour, pasta and biscuits, access to these varies across Europe. These products are generally expensive if not funded by government/insurance or supplied by hospitals. They can provide up to 50% of energy intake (70). Providing adequate doses of protein, usually based on phenylalanine-free amino acids supplements, is essential to promote normal growth, prevent protein deficiency, provide a source of tyrosine, and help optimise blood phenylalanine control (65,70). In classic PKU group, protein supplements are likely to provide at least 75% of daily nitrogen requirements. When the dose of protein replacement is determined, body weight, age, growth and the prescribed amount of phenylalanine/natural protein are considered. Protein supplements are available in the form of amino acid powders, capsules, tablets, bars and liquids and may contain added carbohydrate, fat, vitamins and minerals (70). Problems may arise with the taste of the supplements (74) and the dietary compliance (65,73). Diet becomes difficult in adolescence with its social aspects (73).

Glycomacropeptide (GMP) is a low Phe protein and can be used for protein supplementation in PKU. It is a by-product of cheese whey, and although theoretically Phe-free, some residual phenylalanine remains in the product due to the extraction process (70). Studies showed safe GMP usage above 12 years of age, reduction in Phe levels and good acceptance of the product (75–77).

A relatively new treatment option is the use of large neutral amino acids (LNAA), such as Tyr, valine, tryptophan, leucine, isoleucine, arginine, histidine, methionine and threonine. In 1953, Christensen et al. reported on the role of LNAA and their transport to the brain in patients with PKU, describing that high Phe levels could block the transport of other LNAAs to the brain on the blood-brain carrier (78). Two studies found a significant short-term reduction in blood Phe levels(79,80), but other authors did not

suggest this effect (81,82). Schindeler et al. found LNAA had a specific impact on verbal generativity and flexibility (83). Burlina reported high levels of adherence to medication in adults after 12 months of LNAA treatment, with 96% reporting complete adherence. Phe levels remained unchanged, whereas Tyr levels increased in most patients. All patients experienced significant improvement in the QoL. LNAAs may provide patients with further opportunities to improve medication adherence and, consequently, their QoL (84).

1.6.2. Tetrahydrobiopterin

Blood Phe concentrations are reduced by tetrahydrobiopterin (BH_4) treatment, a cofactor and natural chaperone for PAH, in some patients with PKU (85). The United States Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) approved the medical form of BH_4 (Kuvan®) for the treatment of PKU in responders (86,87). The addition of BH_4 is well tolerated, and leads to reduced and stabile Phe levels in responsive patients (88,89). However, more than a half of patients with classic PKU do not respond to BH_4 (65,90).

1.6.3. PAL

Enzyme substitution therapy has recently become a treatment option for PKU. Pegvaliase contains phenylalanine ammonia lyase (PAL), which converts Phe to harmless metabolites, trans-cinnamate and ammonia. Pegvaliase reduces Phe concentrations independently of PAH activity. However, the majority of the patients experienced mild to moderate side effects. Due to the risks of anaphylaxis, Pegvaliase is only available through the Risk Evaluation and Mitigation Strategies (REMS) program, which includes requirements for prescriber and pharmacy certification and patient educational enrolment (91,92).

Pegvaliase (Palynziq®) was approved by the US FDA in 2018 and by the EMA in 2019 only for the therapy of adult patients with uncontrolled blood Phe administered as a subcutaneous injection (93,94). Most patients were motivated to continue treatment despite the adverse effects and achieved efficacy long-term (95–97).

Further studies should be conducted to examine the efficacy and safety profile in children.

1.6.4. Synthetic bionic

In 2018, a synthetic biotic strain of *E. coli* Nissle 1917, called SYNB1618 was introduced. SYNB1618 consumed Phe in the human gastrointestinal tract by two different mechanisms (98). One mechanism is based on the PAL enzyme, and the other is the enzyme L-amino acid deaminase (LAAD), which converts Phe to phenylpyruvic acid. A recent first-in-human study in healthy volunteers and PKU patients demonstrated that SYNB1618 was generally well tolerated. All participants cleared the bacteria within 4 days of the last dose. Adverse events were mostly mild to moderate in severity and gastrointestinal (98).

1.6.5. Liver transplantation and cell-based therapy

About 95% of PAH protein is expressed in the liver. Successful liver transplantation can restore somatic PAH activity and plasma Phe levels to essentially normal (99). Liver transplantation in a child with PKU was first described in the 1990s. The 10-year-old boy had unrelated end-stage chronic liver disease. His blood phenylalanine levels dropped dramatically with a normal diet after the transplantation (99).

After almost thirteen years, a woman with classic PKU who was unable to maintain a diet and was unresponsive to pharmacological therapies underwent a domino liver transplantation from a donor with classical branched chain ketoacid dehydrogenase deficiency. Phe levels normalized two days after the surgery. Her mood, symptoms and quality of life improved. Liver transplantation is obviously not a realistic therapy strategy for the majority of patients with PKU because of its risks and the limited number of donors. Women with classic PKU of childbearing age may be potential recipients in some well-selected cases, because of the danger of maternal PKU syndrome. The use of domino allografts from patients with maple syrup urine disease offers the potential for liver transplantation without losing the traditional donor allograft liver from the population (100).

After PAH-positive hepatocyte transplantation into PAH-deficient Pah mice, a significant reduction in Phe levels was described when liver repopulation exceeded 5% (101). Stéphenne et al. reported liver cell transplantation in a 6-year-old boy with severe tetrahydrobiopterin nonresponsive phenylketonuria after failing to comply with dietary restrictions. Transplanted hepatocytes were partially obtained from an explanted

glycogen storage type 1b liver. After two infusions, Phe levels dropped to the therapeutic range. Liver function tests showed a transitional increase, but normalized within 2-3 days, showing the safety of the procedure. Cell-based therapy seemed to be a promising therapeutic option for PKU, and the domino concept may solve the issue of cell sources for hepatocyte transplantation (102). Further research is needed on long-term effects and safety.

1.6.6. Gene therapy

PAH is a monogenetic disorder, and blood Phe level is easily measured biomarkers, making PKU a prime candidate for gene therapy (103). A gene therapy model of PKU was demonstrated in a mouse model in 1994 (104). Developments in the design of viral vectors for gene therapy have contributed to translating gene therapy into clinical research (103,105–107), as adeno-associated and lentiviral vectors do not cause strong immune reactions. In July 2023, three human studies are listed on Clinicaltrials.gov (108–110).

1.7. Quality of life of patients with PKU

The subjectively perceived impact of PKU on daily lives of patients is receiving increasing attention. The Health-related quality of life (HRQoL) questionnaire is an easy-to-use strategy to measure QoL of patients with PKU. The HRQoL has been described as a multidimensional, self-reported questionnaire that provides subjective perceptions of physical, psychological, social functioning, and overall well-being (111). Although most studies have found normal QoL in adult PKU patients (112–115), but Demirdas et al. reported a significantly lower HRQoL for cognitive functioning (116). Landolt et al. found fewer positive emotions in children with PKU (117). No differences were observed for age, type, and sex, but lower scores were reported for adolescents on family cohesion and time of parental impact (118). Poor dietary adherence tended to worsen with age. No significant differences were observed in HRQoL scores for dietary adherence (119).

Regnault et al. first developed and validated PKU-specific HRQoL questionnaires for patients with PKU and their parents in 2018 (120). An increasing trend was observed in the overall impact of diet and guilt when diet was not followed according to disease severity. Patients with severe PKU enjoyed their meals less than patients with mild to moderate PKU. Similar observations were made by parents of patients (121).

The emotional effects of PKU were most pronounced in the age group of 9-11-year-olds. Slow thinking was the most common symptom, but the majority of the domains studied reached moderate/high frequency in children. Problems with their general health, emotional effects of PKU, adherence to supplements, and dietary protein restriction were reported by adolescents. All age groups found the taste of supplements unsavoury (122).

Eighteen mothers of children with PKU reported the greatest impact of PKU on the anxiety of their children during blood tests on their own HRQoL, as well as guilt associated with poor adherence to dietary restrictions and supplementation regimens. Higher scores were also found for the emotional, social and overall impact of PKU (123).

Hungarian adults had better HRQoL scores after ten years of good metabolic control. The highest scores (indicating larger burden) were mostly related to the emotional impact of PKU and disease management. Weak to fair positive correlations were found in several domains, either short or long term, after performing correlation analysis between Phe levels and QoL scores for all patients (74).

In Hungary, there has been no systematic analysis of the QoL in the paediatric population.

1.8. COVID-19 pandemic and PKU

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused a new pandemic, many countries had to implement quarantine measures ("lockdown", LD) to reduce the number of new COVID-19 cases (124,125). A health emergency was declared in Hungary on March 11, 2020, after which a strict closure was introduced (126,127). Restrictions were lifted on June 16, 2020. Rules on partial closure were reintroduced on September 1 of the same year, as the spread decreased (partly due to the vaccination programme), restrictions were lifted on April 19, 2021.

Measures implemented during the two lockdown periods differed. Initially, there was a total ban on leaving home and attending school. During the second closure, upper secondary education was conducted online, and community programmes were suspended. The COVID-19 pandemic emerged as a new healthcare challenge in the routine follow-up care of patients with chronic disorders (128–130) and also with PKU (131–133). Patients were asked to opt for telemedicine visits instead of regular visits, when possible. This new context brought about a number of changes that can have both positive

and negative implications for the effectiveness of newborn screening and metabolic control of individuals treated.

2. Objectives

In the research projects constituting the basis for the doctoral dissertation, quality of life indicators of Hungarian children with PKU were assessed in terms of severity, frequency, and metabolic control. The impact of the COVID-19 pandemic on the metabolic status of Hungarian children with PKU was also investigated, including the comparison of age at diagnosis and metabolic control in different lockdown periods.

The principal questions of the research were:

1. Severity/ frequency of HRQoL domains in our patients

2. Which indicators had the highest and the lowest impacts on QoL among Hungarian children and adolescents in each module (symptoms module, general module, supplement registration module, dietary protein restriction module)?

3. The most severe/frequent impacted domains according to the adherence

4. Was there an observable difference in QoL between patients with good and poor metabolic control?

5. Was there an observable difference in QoL between patients with HPA vs. classic PKU?

6. Neonatal study: Was there any delay in the diagnosis and early initiation of treatment in neonates with PKU who were born in the COVID-19 era (CE)?

7. Did home blood sampling frequency change during the pandemic?

8. Were there any changes in blood Phe levels during CE vs NCE?

9. Assessment of metabolic control in both lockdown periods (LD1: 25/03/2020–16/06/2020 and LD2: 01/09/2020–19/04/2021) vs. unrestricted period (URP, 17/06/2020–31/08/2020).

10. Correlations between DBS sampling frequencies and median Phe levels

3. Methods

3.1. Patient selection

3.1.1. Quality of life in Hungarian children with PKU

In our 1st observational study, adolescents with HPA and classic PKU aged between 12 and 18 years as well as parents of the 0–18-year-old children were included. From the patient pool, we excluded children with BH_4-treatment (n = 6) prior to statistical analysis. The PKU-QoL questionnaire was completed anonymously.

After exclusions, answers of 59 parents about their children with PKU/HPA were analysed. Our study also included 11 self-reported responses of adolescents. Details of the included patients are shown in Table 2.

Good or poor compliance were defined on the basis of regularly sent Phe levels. For each patient, all Phe levels were considered from their birth and annual mean Phe levels were calculated. Adherence was deemed poor if more than 50% of the annual Phe levels were above the target range.

Table 2. Enrolled patients in the QoL study. HPA: hyperphenylalaninaemia, IQR: interquartile range

Data of patients	Number	Age (median IQR)	Lifetime Phe (median IQR)	On diet (%)	Supplement users (%)
a) adolescents					
HPA	1	15	294.18	0	0
Classic PKU with good adherence	9	16.5 (15-17)	327.64 (250.9-429.8)	100	100
Classic PKU with poor adherence	1	14	516.54	100	100
b) children whose parental questionnaire was about					
HPA	20	9 (2.5-11)	207.9 (148.3-261.1)	0	0
Classic PKU with good adherence	30	9 (6-14)	242.3 (201.1-272.71)	100	100
classic PKU with poor adherence	9	8 (5-14)	378 (338.4-508.7)	100	100

3.1.2. A comparison of metabolic control in the COVID-19 era vs. NCE and an assessment of metabolic control in both lockdown periods (LD1 and LD2) vs. unrestricted period (URP)

The following exclusion criteria were used: switch in therapy (n = 3), death (n = 1) and expatriation (n = 7). The selection of children is presented in Figure 3. Given the different recommended target Phe ranges for children and adolescents, patients were divided into two age subgroups: 2–12 years (51 patients) and 13 to 18 years (21 patients). Children younger than two years of age were excluded as a comparison of NCE and CE could not be performed in these cases.

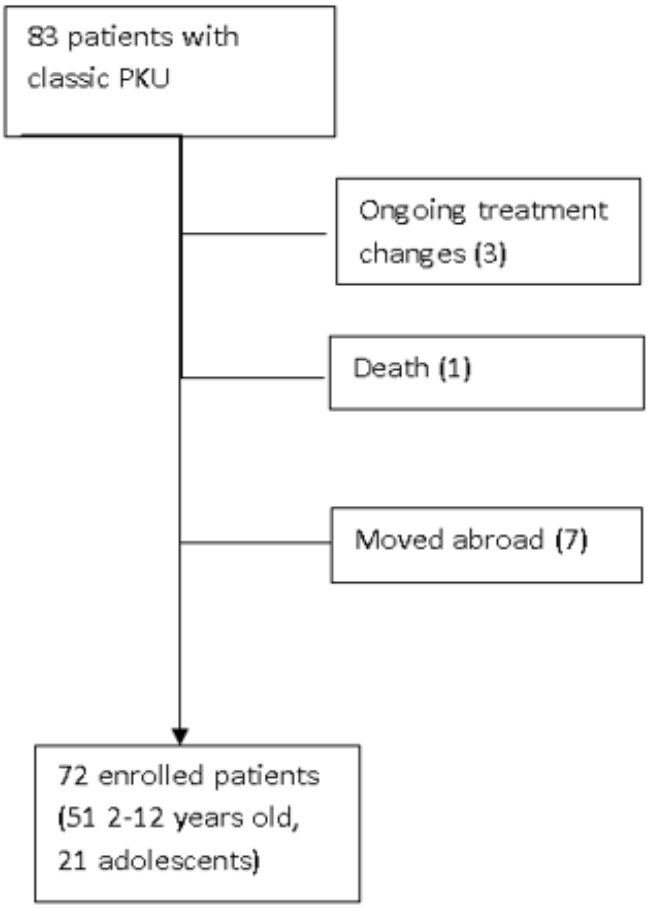

Figure 3. Patient selection in the metabolic study. Exclusion criteria were treatment changes, death and expatriation.

3.1.3. Neonatal study

Patients were included with pathological Phe levels and completed the neonatal BH_4 loading test during the CE (6 patients) vs. the NCE (8 patients). No patients had to be excluded because of unknown neonatal Phe levels.

3.2. Metabolic control- DBS

DBS Phe levels were determined by mass spectrometry (API2000; Perkin-Elmer Sciex, Toronto, ON, Canada) at the 1st Department of Paediatrics, Semmelweis University, Budapest.

For newborn screening, population-based cut-off values were calculated in different percentages. The entire data sets (approx. 650,000 newborn samples between 2011 and

2021) were systematically reviewed, and statistical parameters calculated. All DBS samples that met pre-analytical and analytical requirements (i.e., sample collection, storage, transportation, and quality control) were included. Phe levels above 179.64 uM (>99.98 pc) were considered abnormal. In order to reach the therapeutic target range, the third day of Phe measurement was taken within the target limit (120–360 μmol/l).

Groups with good and poor adherence were defined on the basis of regularly sent Phe levels. For each patient, all Phe levels were considered from their birth and annual mean Phe levels were calculated. Adherence was deemed poor if more than 50% of the annual Phe levels were above the target range.

3.3. The PKU-QoL questionnaire

PKU-QoL questionnaires are freely available for use in non-funded academic research from their website: https://eprovide.mapi-trust.org/instruments/phenylketonuria-impact-and-treatment-quality-of-life-questionnaire. Adolescent and parent versions were translated following the steps recommended by Jurecki (134) with the permission of the owner. The adolescent version features 58 questions, whereas the parental version is composed of 54 questions. Both versions contain four modules, detailed in Table 3.

Table 3. The structure of the PKU-HRQoL questionnaire. DPR: dietary protein restriction

Modules	Domains
Symptoms	self-health rated status, headaches, stomach aches, tiredness, lack of concentration, slow thinking, irritability, aggressiveness, moodiness, sadness, anxiety
PKU in General	emotional impact, practical impact, social impact, anxiety about Phe levels and blood test
Supplement administration	adherence to supplements, practical impact of supplements, social impact of supplements, taste, guilt if poor adherence to supplements
Dietary Protein Restriction module	guilt if the diet is not followed, management of dietary protein restriction (DPR), food temptation, adherence to DPR, practical impact of DPR, the social impact of DPR, the overall impact of DPR, overall difficulty following DPR, taste, food enjoyment

The recall period focused on the last one week for all sections except for ‘patient's general feeling’ where the recall period was ‘in general’. The following interpretation rules were applied for all domain scores in a range from 0 to 100:

- for symptom scores, a higher score is associated with more frequent symptoms,
- for adherence scores, a higher score is associated with lower adherence,
- for other scores, a higher score is associated with a more significant impact (120).

Once items were scored, a domain score was calculated for each domain, with more than 70% of the items scored using the formula below:

Domain score= Sum of item scores within the domain/Number of non-missing item scores within the domain * 25

According to the developers, the severity of domain scores can be interpreted as follows: a score between 0 and 25 indicates little/no impact or symptoms; between 26 and 50 moderate impact or symptoms; between 51 and 75 major impact or symptoms, and scores over 75 indicate very severe impact or severe/frequent symptoms (121).

3.4. Periods examined in the COVID-19 metabolic study

We compared metabolic control during the CE (11.03.2020 to 19.04.2021) and a similar period of one-year prior (01.02.2019 to 10.03.2020 (non-COVID-19 era, NCE). Our study also assessed metabolic control in both lockdown periods (LD1: 25.03.2020 – 16.06.2020 and LD2: 01.09.2020 – 19.04.2021.04) vs. unrestricted period (URP, 17.06.2020 – 31.08.2020). The periods examined were defined following the Hungarian lockdown regimens. Given possible seasonal changes, metabolic control was also analysed in similar periods of NCE: 1) control period to LD1 (CLD1 15.03.2019 – 16.06.2019), 2) control period to LD2 (CLD2, 17.06.2019 – 31.08.2019) and 3) control period to URP (CURP, 01.09.2019 – 10.03.2020). The periods are presented in Figure 4.

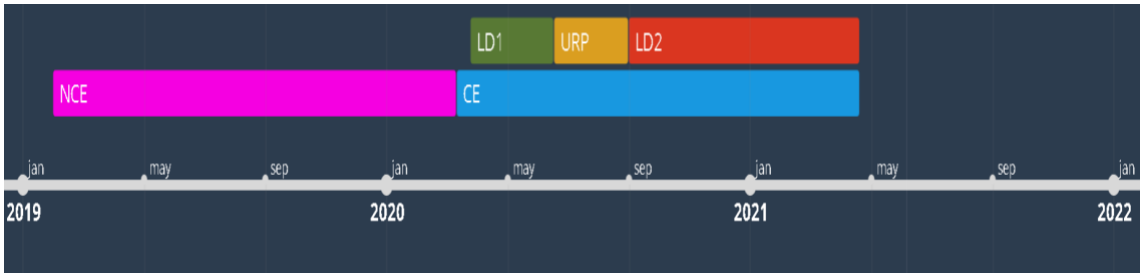

Figure 4. The examined periods. NCE: CE COVID-19 era, NCE: non-COVID-19 era, LD: lockdown period, URP: unrestricted period

3.5. Statistics

Data were tabulated using Microsoft Excel 2016 and analysed with the IBM® SPSS® 23 statistical software package. Normality of the data was tested using the Kolmogorov-Smirnov and Shapiro–Wilk tests, with $p > 0.05$ accepted as normal distribution. Non-normally distributed data are reported as the median and interquartile range (IQR). Parameters, which followed normal distribution, were expressed as mean and standard variation levels. Wilcoxon signed-rank tests were performed to assess related groups according to different time point. A Mann-Whitney test was performed to analyse unrelated groups. The level of significance was set at $p < 0.05$.

3.6. Ethical consideration

The studies followed the guidelines of the World Medical Association Declaration of Helsinki of 1975. Both studies were approved by the institutional Ethics Committee. All participants in our observational study on the quality of life signed the informed consent form.

4. Results

4.1. Severity/ frequency of HRQoL domains among patients with PKU

Seventy-eight percent of the domains (25/32) had little/no impact. Moderate severity was reached for irritability (adolescent median 50, IQR 25–75), emotional impact of PKU (adolescent median 35, IQR 30–45), parental median 31.3 (IQR 14.1–43.8), guilt if poor adherence to supplements (adolescent median 37.5, IQR 25–62.5), adherence to dietary protein restriction in adolescents (median 28.1, IQR 18.8–46.9) and practical impact of dietary protein restriction (adolescent median 32.1, IQR 14.3–42.9). Only food enjoyment of adolescents reached a major impact on QoL (median 62.5, IQR 25–75).

4.1.1. Symptoms module

Ten out of 11 (90.9%) domains had little or no frequency (median score < 25).

Irritability was the most frequent symptom among adolescents (median 50, IQR 25–75), stomach aches and anxiety reported as rarest (medians 0, IQRs 0–25). Among parental answers, the most frequent symptom was lack of concentration and irritability (medians 25, IQRs 0–50), the rarest was headache (median 0, IQR 0–0). The impact of all symptom domains is detailed in Table 4.

Table 4. Impact of symptoms module. IQR: interquartile range. Significant results are underlined. The * sign indicates border of significance. (135)

	ado. Med. (IQR)	parental med. (IQR)	Ado. vs. Parental (p)	PKU median (IQR)	PKU-good adh. Med. (IQR)	PKU-poor adh. Med. (IQR)	PKU good vs. poor adh. (p)	HPA	HPA vs. good adh. of PKU (p)
Symptoms module									
General health	25 (0-50)	12.5 (0-25)	0.295	25 (0-25)	0 (0-25)	25 (0-25)	0.715	25 (0-25)	0.927
Headache	25 (0-25)	0 (0-0)	0.063	0 (0-0)	0 (0-0)	0 (0-25)	0.2	0 (0-0)	0.986
Stomach-aches	0 (0-25)	0 (0-25)	0.513	0 (0-25)	0 (0-25)	25 (0-25)	0.176	0 (0-25)	0.87
Tiredness	25 (25-25)	25 (0-25)	0.285	25 (0-25)	25 (0-25)	25 (25-50)	<u>0.030</u>	25 (0-50)	0.053*
Lack of concentration	25 (0-50)	25 (0-50)	0.769	25 (0-25)	25 (0-25)	50 (25-50)	0.125	25 (0-50)	0.859
Slow thinking	25 (0-25)	0 (0-25)	0.127	0 (0-25)	0 (0-0)	0 (0-25)	<u>0.018</u>	0 (0-25)	0.205
Irritability	50 (25-75)	25 (0-50)	0.059	25 (0-50)	25 (0-31.25)	50 (25-75)	0.226	25 (0-50)	0.235
Aggressive-ness	0 (0-25)	0 (0-25)	0.689	0 (0-0)	0 (0-0)	0 (0-25)	0.355	0 (0-18.75)	0.08
Moodiness	25 (0-25)	25 (0-25)	0.964	25 (0-25)	25 (0-25)	25 (25-50)	0.125	25 (0-50)	0.399
Sadness	25 (0-25)	0 (0-25)	0.176	0 (0-25)	0 (0-25)	25 (0-25)	0.109	0 (0-25)	0.798
Anxiety	0 (0-25)	0 (0-25)	0.631	0 (0-25)	0 (0-0)	25 (0-50)	<u>0.015</u>	0 (0-25)	0.181

PKU in general module

Almost all domains (8/9, 88.9%) had little/no impact on QoL. The emotional impact of PKU was the most affected domain in terms of answers from adolescents (median: 35, IQR 30–45) and parental questionnaires (median: 31.3, IQR 14.1–43.8). The least affected areas were practical and social impacts among parental data (medians 0, IQRs 0–10) and anxiety about blood tests among adolescents (median 12.5, IQR 0–25). The results are detailed in Table 5.

Table 5. Impact of general domains. IQR: interquartile rage, NA: not asked. Significant results are underlined. (135)

	ado. Med. (IQR)	Paren-tal med. (IQR)	Ado. vs. Paren tal (p)	PKU median (IQR)	PKU-good adh. Med. (IQR)	PKU-poor adh. Med. (IQR)	PKU good vs. poor adh. (p)	HPA	HPA vs. good adh. of PKU (p)
Emotional impact of PKU	35 (30-45)	31.3 (141-43.8)	0.40	28.2 (18.8-37.5)	28.13 (18.8-39)	25 (12.5-31.3)	0.14	25 (6.3-53.1)	0.32
Practical impact of PKU	16.7 (0-33.3)	0 (0-10)	0.084	4.2 (0-12.5)	4.2 (0-15.6)	4.2 (3.1-8.8)	0.894	0 (0-5)	0.20
Social impact of PKU	16.6 (10.4-33.3)	0 (0-10)	<u>0.05</u>	10 (5-20)	10 (5-19.1)	10 (5-20)	0.5	10 (0-15)	0.83
Financial impact of PKU	NA.	10 (3.75-20)		25 (0-25)	25 (0-25)	0 (0-25)	0.286	0 (0-0)	<u>0.02</u>
Overall impact of PKU	20.5 (15.9-31.8)	11.7 (7.2-22.4)	0.10	12.5 (8.9-21.7)	13.8 (10.7-21.7)	8.6 (7.9-13.8)	0.164	16.7 (8.3-27.7)	0.88
Child anxiety – Blood test	12.5 (0-25)	25 (6.25-50)	0.12	12.5 (0-37.5)	12.5 (0-37.5)	37.5 (12.5-50)	0.165	43.8 (25-75)	<u>0.02</u>
Impact of blood test	NA	25 (12.5-50)		25 (9.4-37.5)	12.5 (0-37.5)	25 (18.8-62.5)	0.207	43.8 (12.5-82)	0.068
Child anxiety on Phe levels	25 (25-50)	25 (25-68.8)	0.730	25 (25-75)	25 (25-56.3)	25 (0-75)	0.715	25 (18.8-50)	0.303
Inform. about PKU	NA	25 (25-50)		25 (25-50)	25 (25-50)	25 (0-50)	0.613	50 (31.5-50)	0.045

4.1.2. Supplement registration module

Four out of six domains (66.66%) had little or no effect on QoL. The guilt of poor adherence to supplements domain reached a moderate impact on QoL in 12–17-year-old children (median 37.5, IQR 25–62.5) and the highest impact in parental data (median 25, IQR 0–75). The less affected domain was impact of supplements on family among adolescents (medians 0, IQR 0–12.5 and 0–25) and adherence to supplements from parental data (0, IQR 0–0).

Table 6. Impact of supplements module on QoL. IQR: interquartile range, NA: not asked, n.r.: not relevant. Significant results are underlined. Suppl.: supplements (135)

	ado. Med. (IQR)	parental med. (IQR)	Ado. vs. Paren-tal (p)	PKU median (IQR)	PKU-good adh. Med. (IQR)	PKU-poor adh. Med. (IQR)	PKU good vs. poor adh. (p)
Guilt if poor adherence to suppl.	37.5 (25-62.5)	25 (0-75)	0.335	25 (0-75)	25 (0-75)	25 (0-56.3)	0.853
Adherence to suppl.	12.5 (0-18.75)	0 (0-0)	0.052	0 (0-0)	0 (0-0)	0 (0-0)	0.684
Impact of suppl. on family	0 (0-12.5)	0 (0-25)	0.58	0 (0-25)	0 (0-25)	25 (18.8-56.3)	<u>0.047</u>
Manage-ment of suppl.	NA	0 (0-25)		0 (0-25)	0 (0-0)	25 (0-75)	0.073
The practical impact of suppl.	15.6 (6.3-23.4)	0 (0-8.3)	<u>0.025</u>	0 (0-8.3)	0 (0-8.3)	0 (0-8.3)	0.641
Taste-suppl.	37.5 (12.5-50)	NA		NA	NA	NA	

Dietary protein restriction module

Seven out of 10 domains (70%) reached little or no impact on QoL. Dietary protein restriction seems to have the most severe impact on QoL among Hungarian children. Food enjoyment showed the highest impact in both adolescent (median 62.5, IQR 25–75) and parental (median 25, IQR 0–43.75) groups. The least impacted domain was adherence to dietary protein restriction in parental answers (median 0, IQR 0–0) and the social impact of dietary protein restriction in adolescents (median 10, IQR 3.75–25). Results in the dietary protein restriction (DPR) module are shown in Table 7.

Table 7. Results of the QoL questionnaire in adolescents and in children according to their parents. Ado: adolescents, DPR: dietary protein restriction, g. vs. p. adh: good vs. poor adherence. IQR: interquartile range, med: median, NA: not asked, n.r.: not relevant.

	ado. Med. (IQR)	parental med. (IQR)	Ado. vs. Parental (p)	PKU median (IQR)	PKU-good adh. Med. (IQR)	PKU-poor adh. Med. (IQR)	PKU g. vs. p. adh. (p)	HPA	HPA vs. good adh. of PKU (p)
Guilt if DPR was not followed	25 (25-25)	25 (0-68.8)	0.805	25 (0-50)	25 (25-50)	12.5 (0-25)	0.176	62.5 (25-100)	0.314
Management of DPR	NA.	12.5 (8.3-25)		12.5 (6.3-22. 9)	12.5 (5.2-20.6)	16.7 (12.5-25)	0.36	12 (0-21)	0.856
Food temptation	25 (12.5-56.2)	NA		NA	NA	NA		NA	
Adherence to DPR	28.1 (18.8-46.9)	0 (0-0)	<0.001	0 (0-0)	0 (0-0)	0 (0-25)	0.59	0 (0-0)	0.576
Practical impact of DPR	32.1 (14.3-42.9)	25 (20.5-39.3)	0.921	25 (21.4-39.3)	25 (21.4-39.3)	21.4 (21.4-32.1)	0.282	42.8 (42.8-51.8)	0.056
Social impact of DPR	10 (3.75-25)	12.5 (0-18.8)	0.468	12.5 (0-21.9)	0 (0-12.5)	12.5 (0-37.5)	0.412	6.25 (0-12.5)	0.942
Overall impact of DPR	21.88 (11.9-31.3)	NA		NA	NA	NA		NA	
Overall difficulty following DPR	25 (0-25)	NA		NA	NA	NA		NA	
Taste- low protein food	25 (18.75- 50)	NA		NA	NA	NA		NA	
Child food enjoyment	62.5 (25-75)	25 (0-43.8)	0.016	25 (0-25)	25 (25-43.8)	0 (0-6.3)	0.023	50 (37.5-50)	0.328

4.2. The severity of domains according to metabolic adherence

In adolescents with good compliance, emotional impact and taste of supplements were moderate, whereas food enjoyment had a severe impact. Only one patient completed the questionnaire in the adolescent group with poor compliance; several domains were marked as moderately/very impacted. Answers of parents indicated that the emotional impact was moderate in children with good compliance. Three moderately prominent domains were found in families with poor adherence according to parental responses: anxiety about blood test, lack of concentration, and irritability (Table 8).

Table 8. Most severe/frequent impacted domains of patients according to clinical compliance. DPR: dietary protein restriction (135)

	Adolescents with good adherence (n=11)	Adolescents with poor adherence (n=1)	Parents of children with good adherence (n=55)	Parents of children with poor—adherence (n=9)
Severe/frequent impact (score > 75)	1: food enjoyment (75)	6: lack of concentration, irritability, sadness, moodiness, impact of supplements on family, guilt if poor adherence to Supplements	-	-
Major impact (score 51-74)	-	7: social imp. of DPR, practical imp. of DPR, the overall impact of DRP, practical imp. of PKU, adherence to diet, pract. imp. of supplements, the emotional impact of PKU	-	-
Moderate impact (score 26-50)	2: the emotional impact of PKU, the taste of supplements		1: the emotional impact of PKU	3: anxiety of blood test, lack of concentration, irritability

4.3. QoL in groups with “good” vs. “poor” adherence

In a subgroup analysis of 30 parents of children with classic PKU children with good adherence and nine parents of children with poor adherence, significant differences were found in the domains: tiredness, slow thinking, anxiety, food enjoyment, management of supplements, and impact on family supplements (Table 3).

Tiredness (median 25, IRQ 0–25 vs. median 25, IQR 25–50, p = 0.03), slow thinking (median 0, IQR 0–0 vs. median 0, IQR 0–25, p = 0.018) and anxiety (median 0, IQR 0–0 vs. median 25, IQR 0–50, p = 0.015) were less frequent symptoms with good adherence.

Children with good adherence enjoyed their food less (median 25 vs. 0, p = 0.025). Supplements generated more arguments in families of children with poorer adherence (median 25 vs. 0, p = 0.047).

A noteworthy result is that the two groups do not differ in general health status (p = 0.72) and had similar information on PKU (p = 0.61).

4.4. QoL results in HPA vs. classic PKU

The two groups did not differ in the frequencies of symptoms. Analysing data only for children with good adherence and the HPA group, we found that only tiredness showed a nearly significant difference (p = 0.053), and interestingly, the latter group was more affected. The financial effect of PKU had a greater impact on patients with good compliance than those in the HPA group (p = 0,002). Patients with HPA had greater anxiety before the blood test than children with good compliance the (*p* = 0.002).

Typically, as in the current study, patients with HPA do not need to take protein supplements. Seven patients in the HPA group needed a protein-restricted diet, but not as strict as in the classic PKU (135).

4.5. Time needed to reach the therapeutic Phe range (neonatal study)

Six children were born with classic PKU during the CE. Data of eight patients born with classic PKU during the NCE were analysed for the time required to reach the therapeutic

Phe target range. Neonates born during CE needed time to reach the therapeutic Phe range (median 25 days, IQR: 23.25–26) similar to that of patients born during NCE (median 23.5 days, IQR 22.5–24, $p = 0.59$).

There was no clear trend between initial Phe level and days to reach the target metabolic range, although earlier confirmation of the diagnoses had some positive effect on reaching the target Phe range (Table 9).

Table 9. Detailed values of the first DBS sample in newborns (studied). CE: COVID-19 era, DBS: dried blood spot, NCE: non-COVID-19 era. (136)

Patient list	Diagnosis confirmed (age in days)	Therapeutic Phe range reached (age in days)	Phe level of the first DBS (umol/l)
Patients who were born in the CE (n = 6)			
P1	4	12	606.28
P2	16	23	615.83
P3	11	24	473.29
P4	13	26	544.85
P5	10	26	359.91
P6	20	33	260.77
Patients who were born in the NCE (n = 8)			
P1	5	19	606.28
P2	11	21	579.61
P3	19	23	699.48
P4	9	23	884.27
P5	13	24	600.11
P6	16	24	615.88
P7	10	24	359.91
P8	11	29	890.06

4.6. Frequency of sampling in CE vs. NCE

Sampling frequency of 51 children aged 2–12 years and 21 adolescents aged (13–18 years old were analysed over a 13-month normal (NCE) and COVID-19 pandemic period (CE). The younger age group showed an increasing trend in their cumulative and median DBS sampling frequency (9% and 17%) during the CE. Four percent of patients sent fewer samples, 35.2% sent the same number of samples, whereas 60.8% of the children sent more samples than in a similar unaffected period (Table 10).

Table 10. DBS monitoring frequencies in children with classical PKU DBS: dried blood spot, NCE: non-COVID-19 era, IQR: interquartile range, CE: COVID-19 era (136)

Parameter	2-12 years old	>13 years old
Median DBS NCE [IQR]	17 [12.5-26.5]	11.5 [7-16.3]
Median DBS CE [IQR]	20 [11-28]	11.5 [8.8-14.5]
p value	0.158	0.917
Patients, with increased frequency (%)	31 (60.8%)	8 (36.4%)
Patients, with stable frequency (%)	18 (35.2%)	9 (40.9%)
Patients, with decreased frequency (%)	2 (4%)	5 (22.7%)
Cumulative frequency NCE	1131	266
Cumulative frequency CE	1243	266

In the older age group, cumulative frequencies were similar in the two study periods. The incidence of DBS sampling remained relatively unchanged in most patients (40.9%), although two presented no samples at all. Eight (36.4%) adolescents sent more samples compared to the previous year and 5 (22.7%) decreased their frequency (Table 10) during CE. Neither difference was significant.

As the examination of children under two years of age was affected by certain distorting factors, such as potentially changing examination duration and fluctuating Phe levels during therapy adjustment, their group was treated separately (Table 11). DBS frequency was increased, but not significantly.

Table 11. DBS frequencies in the youngest children. All patients increased their DBS frequency. (136)

Parameter	*< 2 years old*
median DBS 2019/20 (IQR)	39 (33.25-44)
median DBS 2020/21 (IQR)	42 (32.75-48.25)
p value	0.144
patients, with increased frequency (%)	4 (100%)
patients, with stable freq. (%)	0
patients, decreased freq. (%)	0
cumulative freq. 2019/20	153
cumulative freq. 2020/21	384

4.7. Assessing Phe levels during NCE vs. CE

Median Phe level significantly increased in both age groups (Table 12). The younger age group had a 5.3% higher Phe level during CE, and it was even higher (7.5%) in the adolescent group. Of the 51 younger patients, 30 had Phe levels within the recommended range, whereas 20 results were outside the range. One patient who did not complete Phe tests during NCE submitted 14 samples during CE, all of which were within the reference range. Of 21 adolescents, 12 submitted samples in the recommended range during NCE, whereas 7 had Phe values outside the range. In both periods studied, two patients did not submit DBS samples for the Phe test.

Table 12. DBS Phe levels (umol/l) of the patients studied according to age groups. Phe: phenylalanine, IQR: interquartile range, NCE: non-COVID-19 era, CE: COVID-19 era, rec.: recommended (136)

Parameters	2-12 years old	>13 years old
Median Phe [IQR] in NCE (umol/l)	321.3 [237.5-461.7]	505.8 [377.2-659.7]
Median Phe [IQR] in CE (umol/l)	338.6 [247-453.1]	544.0 [438.5-724.8]
p value (NCE vs. CE)	**0.036**	**0.009**
Patients in rec. Phe range in NCE	30 (58.8%)	12 (57.1%)
Patients in rec. Phe range in CE	26 (51.0%)	11 (52.3%)

There was a decreasing trend in the number of patients within the target Phe range in both age groups during CE.

The results of the youngest patients are detailed in Table 13. The median Phe decreased significantly in CE.

Table 13. Median Phe parameters and number of patients in recommended Phe range in children younger than two years.

Parameters	< 2 years
median Phe (IQR) in 2019/20	240.06 (198.33-344.12)
median Phe (IQR) in 2020/21	200.45 (169.57-235.66)
p value	0.273
patients in rec. Phe range in 2019/20	3
patients in rec. Phe range in 2020/21	10
patients out of the rec. Phe range in 2019/20	1
patients out of the rec. Phe range in 2020/21	0

4.8. Changes in Phe levels during CE (LDP vs. URP)

Phe levels in the two age groups during CE are presented in Figure 5.

Median Phe levels varied differently in the two subgroups when separated according to the two lockdown periods due to government restrictions. Children aged 2-12 years had the highest median Phe (386.5 μmol/l) in the second lockdown (LD2) period with the strictest lockdown rules. Phe in LD2 was significantly higher than in the unrestricted period (URP) Phe level (312.2 μmol/l) in the younger group (p=0.001), although it did not differ from Phe in LD1 (345.3 μmol/l, p= 0.196). Phe levels in LD1 and URP were also similar (p=0.414).

The highest median Phe level in adolescents (553.3 μmol/l) was measured in URP, without any lockdown regulations. It was significantly higher than the median Phe level in LD1 (529.0, p=0.049), but not significantly higher than Phe level in LD2 (525.1, p=0.8). Phe values were also examined in the corresponding NCE periods, although differences were not significant (136).

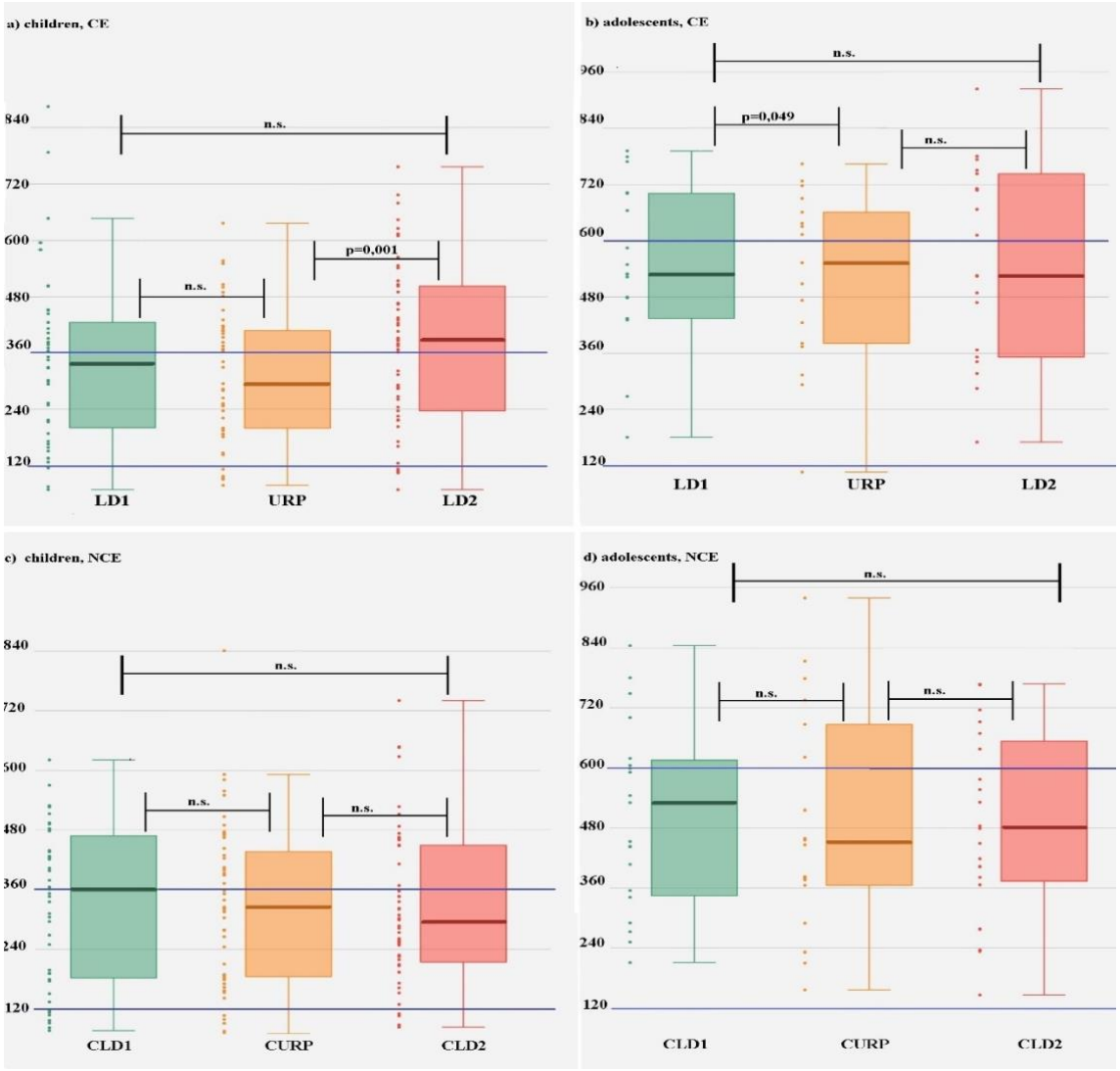

Figure 5. Phe levels of patients with PKU during CE and NCE. CE: COVID-19 era (2020/21), NCE: Non-COVID-19 era (2019/20). CLD1: control to lockdown period 1, CLD2: control to lockdown period 2, CURP: control to unrestricted period, LD1: lockdown period one, UPR: unrestricted period, LD2: lockdown period two. Blue horizontal lines indicate the recommended Phe ranges in PKU. n.s.: not significant. Single dots represent median Phe levels for each patient. (136)

Children younger than 2 years had the highest median Phe in LD2. There was no significant difference in any periods between CE and the periods of one year before (Table 14).

Table 14. Median Phe levels of children <2 years in different periods of CE and NCE. LD: lockdown period, URP: unrestricted period

Parameters	< 2 years
Median Phe LD1	151.32 (56.9-237.64)
Median Phe Period1	393.41 (299.46-487.4)
P	0.665
median Phe URP	150.45 (89.48-254.97)
Median Phe Period2	411 (249.25-626.09)
P	0.068
Median Phe LD2	245.34 (174.46-276.02)
Median Phe Period 3	267.53 (237.62-341.54)
P	0.456

4.9. Correlations of DBS sampling frequencies and median Phe levels

Correlations between DBS sampling frequencies and Phe levels were also analysed. There were significant negative correlations between the DBS frequencies and Phe levels in both age groups during NCE (children r=-0.43, p= 0.002; adolescents r=-0.37, p=0.012), and in the adolescent group during CE (r=-0.6, p= 0.006), but it was not significant in the children group during CE (r=0,214 p=0,144).

Detailed results can be seen in Table 15.

Table 15. Correlations between frequency and Phe levels

Age group	Examined period	Correlation	Significance
Children	2019/20, P1	-0.15	0.325
Children	2019/20, P2	-0.11	0.45
Children	2019/20, P3	-0.15	0.293
Adolescents	2019/20, P1	-0.4	0.088
Adolescents	2019/20, P2	-0.48	0.046
Adolescents	2019/20, P3	-0.25	0.283
Children	COVID-19 era, LD1	-0.15	0.317
Children	COVID-19 era, RP	-0.36	0.011
Children	COVID-19 era LD2	-0.26	0.066
Adolescents	COVID-19 era, LD1	-0.37	0.134
Adolescents	COVID-19 era, RP	-0.62	0.008
Adolescents	COVID-19 era, LD2	-0.28	0.239

5. Discussion

5.1. Severity of domains among age and metabolic compliance

Our study found that PKU had a significant impact on the lives of adolescents; the median score of food enjoyment reached major severity (median score: 62.5, IQR: 25–75). The only adolescent patient with poor adherence reported six severe/frequent and seven significant domains. However, further data are ultimately needed to draw accurate conclusions.

No median score reached significant or severe impact/frequent symptoms (>50) based on parental answers. In the group with good adherence, the emotional impact of PKU reached a moderate impact on QoL. In the low adherence group, three modules reached a moderate impact on QoL: anxiety about blood test, lack of concentration, and irritability (135).

Moderate emotional impact was observed in both adolescent and parental questionnaires, compared with previous studies that described emotional impact in children (123), adults (74) and in all age groups (121). The age group 9-11 years was found to be most affected by emotional impact in the study by Alptekin et al. (122). Emotional effects appear to be a typical, moderate/severe impacted PKU symptom, in which further support should be considered.

Our data were also compared with the results of a study of patients from seven European countries. Tiredness as the highest observed median symptom score for all self-reported age groups (median score 50, IQR 25–50 in adolescents) was below the moderate symptom score (median 25, IQR 0.0–50.0) as judged by parents according to Bosch et al. Our patients reported that tiredness had little or no impact on QoL and scored the highest in the group with poor compliance, although it failed to reach moderate severity (median 25, IQR 25–50). The emotional impact of PKU (adolescent median 30.0 IQR 20.0–40.0, parental 37.5 (25.0–62.5), and the acceptance of supplement taste (adolescent median 50.0 IQR 25.0–50.0) had similarly strong impacts on the Bosch's cohort and current study. Adolescents in the study by Bosch et al. rated their low-protein diets higher than our participants (121).

For patients with PKU and their relatives, low Phe diets and nutritional supplements pose a huge challenge, especially with regard to maintaining lifelong adherence, as mentioned before by other authors (137–140). Glycomacropeptide (GMP) containing low Phe provides new management options for PKU, with improved support for psychological well-being and organic function (141). Subjects reported that GMP-supplemented regimens were superior in sensory qualities to their usual amino acid formulas (77,142).

5.2. QoL in children with PKU with good vs. poor adherence.

If more than 50% of phenylalanine concentrations fall outside the target range over a period of 6 months, European guidelines recommend increasing the frequency of blood phenylalanine monitoring and outpatient visits, and re-education, and it is considered poor metabolic compliance (65). Barta et al. separated "good" and "suboptimal" adherence groups based on individual mean Phe levels over the examined period (last 10 years) (74). In our QoL study, metabolic compliance refers to lifetime Phe levels in children.

In Hungarian adults, better HRQoL was found in case of good compliance (74). Conversely, our study investigated the differences in QoL between good and suboptimal dietary adherence in the paediatric population. It is very important to clarify: our study did not include children who did not follow the necessary diet at all. Although children in the poor metabolic compliance group had Phe values outside the target range, they nonetheless maintained at least partial dietary adherence and regularly attended metabolic care.

Our findings showed that children with poorer adherence were more likely to be tired, anxious, and slow-minded. The only adolescents in our study group with poor adherence reported symptoms more frequently than the adolescent group with good adherence; however, these domains differed (135).

Children with poor compliance were more likely to enjoy their food because of a relaxed diet.

Correlations between PKU severity and tiredness (123), and the financial impact of PKU (121,123) were also reported by children in earlier studies.

5.3. QoL in our HPA group vs. classic PKU

Bosch et al. compared classical vs. mild-to-moderate PKU. Scores of parents were higher in the classic PKU group for general health status, emotional, financial, overall, and practical impacts of PKU, practical impact of supplements, and food enjoyment by children (121). For these domains, we found a difference only in economic effects. It should to be noted that our HPA patients did not require taking a strict special diet and did not consume any supplements.

In our cohort, HPA patients showed more anxiety before blood tests, which may be due to their lower occurrence and thus patients were not accustomed to the sampling procedure. Patients with classic PKU had more information about PKU. This finding can be explained by regular clinical and dietary visits and involvement in the social life of PKU community. Our HPA patients were relatively significantly more tired, although none of them correlated the domain with their disease (135).

5.4. COVID-19 pandemic and PKU

The COVID-19 pandemic has emerged as a new healthcare challenge in the routine follow-up care of patients with chronic illnesses (128–130) and PKU (131–133). Patients were asked not to travel for regular outpatient visits and/or to opt for telemedicine visits instead. This new context led to a number of changes that can have both positive and negative implications for the effectiveness of newborn screening and the metabolic stability of patients treated.

5.4.1. No delay in treatment

Our metabolic screening centre operated without interruption during the COVID-19 pandemic. Postal delivery of DBS samples may have been an external limiting factor, although neither the postal service nor patients reported any delays. Our results show that infants with PKU born during COVID-19 reached their therapeutic Phe range in the same time frame as neonates born before the pandemic. This is consistent with reports from other countries (133). However, no previous studies reported the necessary period to

reach the therapeutic Phe range in neonates born during the COVID-19 era. To the best of our knowledge, the current study is the first to compare the effectiveness of PKU screening in neonates born during the COVID era vs. infants born before it. It is also important to note that there was no clear trend between the initial Phe level and the required days to reach the therapeutic range. This may also be due to the fact that the Phe target range for neonates is adjusted by titrating breast milk and Phe-free formula, tailored to the individual. Thus, for very high Phe, e.g., infants initially fed by formula only, a marked decrease in Phe levels was observed in such instances. Earlier diagnosis appeared to have some positive effect on the achievement of the therapeutic levels (136).

5.4.2. Frequencies of DBS sampling

Rovelli et al. reported an overall decrease (5.9%) in monitoring frequency during CE, although 39% of their patients sent more DBS samples during CE. Of those who increased their sampling frequency, 85% were older than 12 years. Their study included data on adults; however our study focused exclusively on patients aged 2–18 years old (131). Herle et al. also reported that more school-aged patients sent fewer samples during CE, and patients aged 16 years and older sent significantly fewer DBS samples in 2020 (132).

During CE, our patients forwarded 1,397 DBS samples, i.e. numerically 8% more than the previous year, although the increase was not significant. This indicates that our patients were well-prepared for home blood sampling, and that the vast majority (70 out of 72) did not neglect metabolic monitoring during the pandemic. Furthermore, we registered one child who started sending DBS samples after three years during CE without any Phe monitoring (136).

5.4.3. Changes in Phe levels during NCE vs. CE

In a study by Rovelli et al., Phe levels in a group of 4–12-year-olds were similar in the COVID-19 era (median: 278.8 μmol/l) compared to the previous year (median 315.4 μmol/l, $p = 0.771$). However, in the older age group, including adolescents and adults, median Phe levels decreased by 22.5% during the pandemic ($p < 0.0001$) (131).

In the present study, the median Phe level increased significantly (5.4% and 7.5%) from NCE to CE in both age groups; however, the median remained within the recommended

range, and the number of patients outside the target range did not change significantly (136).

5.4.4. Changes in Phe levels during lockdown and non-lockdown periods

Walkoviak et al. found that, median Phe levels during the lockdown and non-lockdown periods did not differ between the different age groups, which were divided into three age subgroups (0–6 years, 7–12 years and 13–18 years) (133). In our patients, the group of 2–12-year olds had significantly higher median Phe levels in LD2 (with the strictest regulatory measures) than in the unrestricted period (URP) (368.8 umol/l vs. 281.8 umol/l, $p = 0{,}001$). In contrast, the group of 13–18 years old had the highest median Phe levels in URP.

Metabolic control can be impacted by a number of factors, such as reduction in physical activity (143–146) or changes in eating habits. Increased consumption of junk food and sweets, continuous snacking and a reduced consumption of fresh foods have also been highlighted (147,148). Lopez-Moreno et al. reported an increase in emotional eating (desire to consume a certain type of food) and eating to compensate for boredom in adults during the pandemic (143). Similar changes may also occur in childhood. Isolation at home may further impact daily routines, such as shifts in bedtime (145), with a possible effect on blood sampling and Phe levels (daily blood Phe levels typically vary by ≤50% in healthy subjects, although the variation can be much higher in patients with PKU (149)). Symptoms of anxiety, depression or ADHD exacerbation as well as other mental problems have also been reported by other authors (149). The pandemic situation may also negatively impact the economic status of families affected (150,151). Metabolic formulas (Phe-free protein substitutions) are supplied free of charge in Hungary on the one hand, low-Phe dietary products, on the other hand, are typically non-reimbursable, expensive, and not readily available. Some stores also had to change their opening hours and experienced delays in supply. All these factors presumably played different roles in the two age-groups observed. Further studies are needed to assess the leading factor underpinning the changes in metabolic compliance in each age group. As stated in the European guidelines, more frequent blood Phe testing and/or visits may be necessary in case of therapeutic or social changes. Should another COVID-19 closure occur, a higher level of DBS sampling may be recommended.

Similar periods of the NCE years were also analysed herein, namely CLD1: control to lockdown periods 1 and 2 (CLD1, CLD2) and control to the unrestricted period (CURP). In contrast to CE, no significant changes were observed during these periods. As no significant changes were observed in NCE, the differences between URP and LD periods during the pandemic period are likely to have been independent of seasonal fluctuations and confirm the effect of the pandemic situation (136).

5.4.5. Correlations of DBS sampling with Phe levels

In both years examined, DBS sampling frequencies correlated negatively with Phe levels (more DBS samples resulted in better compliance). Thus, if a child or adolescent sends fewer DBS samples than before, closer monitoring and an outpatient visit may be recommended.

5.5. Limitations

5.5.1. Quality of life

The strength of the study is that, to the best of our knowledge, this is the first study to compare subjective HRQoL in adherent and poorly adherent children using the MAPI PKU-specific QoL questionnaire.

However, the study has certain significant limitations. We did not use any alternative QoL measurements, such as reports from close family members or teachers, structured interviews by experts, or patient-generated indexes of QoL (152) to further explore QoL in this patient group.

The questionnaires were completed anonymously; thus, correlations with Phe levels could not be assessed, although good vs. poor/suboptimal compliance analyses were examined.

Patients who abandoned the diet completely and regularly skipped their outpatients visit could not be investigated.

Our study did not include healthy control patients. As developers of the PKU-QOL have emphasised, the questionnaire only addresses issues relevant to patients with PKU , so comparisons with a control group from the general population are not possible (121).

Finally, the number of enrolled adolescents is relatively low, although we were able to recruit one third of our adolescents with PKU. Corresponding subgroup analyses could not be performed due to the low enrolment , despite the fact that our centre provides care for children and adolescents with inborn errors of metabolism in the northern half of Hungary (135).

5.5.2. Metabolic control in children with PKU in the COVID-19 era

Despite the fact that we are a national screening centre, where more than half of Hungarian children with PKU are in our care, the number of current cases is still relatively low due to the low prevalence. The relatively small sample size may bias results obtained.

The current study focused only on the effect of the pandemic on newborn screening and metabolic control of patients with PKU. Therefore, the prevalence, effect and long-term consequences of COVID-19 disease with its psychological, physical and potential quality of life changes were not assessed.

Although our patients did not report COVID-19 disease or long-COVID symptoms, the prevalence of COVID-19 disease was not formally assessed.

Our centre only monitors Phe from DBS on a regular basis, as this is much less of a burden for the child. In reviewing the international literature, this can be considered routine. Plasma amino acid measurements are performed annually as recommended by current European guidelines (65). During the COVID-19 era, in order to minimise human contact, outpatient visits were postponed if our patients with PKU had no complaints and their Phe levels were in the recommended range. Under these circumstances, comparison of plasma Phe levels during CE and NCE was not possible.

The type and exact amount of low-protein diet products consumed were not recorded during the study (136).

6. Conclusion

6.1. QoL

Overall, our patients showed good HRQoL; most of the domains investigated (29/36) were marked with little/no impact. On the basis of parental answers, no median score reached significant or severe impact/frequent symptoms (>50). The most common symptom was irritability in adolescents, lack of concentration and irritability in children. Moderately impacted domains were the emotional impact of PKU and food enjoyment. Dietary protein restriction seems to have the most severe impact on QoL of Hungarian children. HPA patients have better QoL than patients with classic PKU patients, which was also the case when we compared only children with PKU with good metabolic controls. Increased (worse) HRQoL was found in patients with suboptimal metabolic control, indicating that regular clinical visits, dietary consultations, and regular monitoring of Phe levels are essential. Special attention should also be paid to improving the emotional health of patients with PKU. The finding that parents of children with poor adherence did not report difficulties with dietary protein restriction rules may suggest a lack of, or unrealistic perception of the severity of PKU and the need for a lifelong Phe-restricted diet. A noteworthy result is that the groups with good and poor adherence did not differ in their general health and have similar information on PKU.

6.2. Metabolic control in children with PKU in the COVID-19 era

Our neonates born with PKU during the COVID-19 pandemic were effectively screened and there was no delay in the initiation and efficiency of dietary management. DBS frequency did not increase significantly in CE in the children or in the adolescents. Median Phe levels increased significantly in 2-12-year-old's and >13 year-old's over the COVID-19 period, although they still remained in the recommended range, and the number of patients outside the range did not change significantly. In the COVID-19 era, Phe levels changed differentially in the two age groups. The 2–12-year-old children had the highest median Phe (386.5 μmol/l) in the second lockdown (LD2) period with the strictest lockdown rules. The highest median Phe level in adolescents (553.3 μmol/l) was measured in URP, without any lockdown regulations. Further studies are needed to analyse the role of age-specific factors influencing compliance. There was no difference between the groups in 2019/2020 (NCE). Significant negative correlations were found

between the frequency of sent DBS samples and Phe levels in the two years studied, underlining the importance of special attention in the presence of reduced DBS frequency.

7. Summary

Assessing the quality of life of patients is receiving an increasing attention. We were the first to assess the quality of life of Hungarian children with PKU and, to our knowledge, we were the first in the world to use the MAPI PKU-specific questionnaire to compare the quality of life of children with good and poor adherence.

We establish that our patients enjoyed a good quality of life, as 80% of the domains assessed had little or no or impact on their quality of life. The most important factor in the of quality-of-life domains examined was consumption of diet food. Moderate effects were achieved by emotional impact and food enjoyment. Patients with classic PKU had a poorer quality of life than those with HPA, and poor metabolic compliance was associated with a poorer QoL. The most commonly reported symptom was irritability. Based on the recognised effects, special attention should be paid to the emotional life of people with PKU.

We were also among the first to examine the impact of restrictions caused by the COVID-19 pandemic on the metabolic status of PKU patients.

We observed that the pandemic caused by COVID-19 did not delay the diagnosis and initiation of treatment of babies born with PKU during this period in Hungary.

Our study showed that the median Phe levels of children and adolescents with PKU increased during the first year of the COVID-19 pandemic, but overall remained within the reference range. In the year of the pandemic, eight per cent more dried blood drop samples were sent compared to the previous year. During the period of the pandemic, Phe levels in children and adolescents under 12 years of age shifted in different directions: children had the highest levels during the restrictive period, whereas adolescents had the highest levels during the summer period without restrictions. Factors influencing metabolic status (e.g. financial situation, exercise, change in eating habits, snacking, eating out of boredom, changes in daily rhythm) may have played a different role in the two age groups.

We observed a significant negative relationship between the number of samples sent and the median Phe level, supporting the knowledge that a decreasing number of samples should be given special attention.

8. Összefoglalás

A betegek életminőségét egyre nagyobb figyelem övezi. Mi voltunk az elsők, akik felmérték a magyar PKU-s gyermekek életminőségét, és tudomásunk szerint a világon elsőként használtuk a MAPI PKU-specifikus kérdőívet, hogy összehasonlítsuk a jó és rossz adherenciájú gyermekek életminőségét.

Eredményeink azt mutatják, hogy betegeink általában jó életminőségben élnek, mivel az értékelt területek 80%-a kevés vagy semmilyen hatással nem volt életminőségükre. Az életminőségi területek közül a legjelentősebb tényező a diétás ételek fogyasztása volt. Mérsékelt hatásokat észleltünk az érzelmi hatások és az ételek élvezete terén. A klasszikus PKU-s betegek rosszabb életminőséggel rendelkeztek, mint azok, akiknek HPA-ja volt, és a rossz anyagcsere státusz rosszabb életminőséggel társult. A leggyakrabban jelzett panasz az ingerlékenység volt. Ezen megállapítások alapján különös figyelmet kell fordítani a PKU-val élők érzelmi jólétére.

Elsők között vizsgáltuk a COVID-19 világjárvány okozta korlátozások hatását is a PKU-s betegek anyagcsere státuszára. Tanulmányunk megállapította, hogy a világjárvány nem késleltette a PKU-val született csecsemők diagnózisát és kezelésének megkezdését Magyarországon ebben az időszakban.

Megállapítottuk, hogy a PKU-s gyermekek és serdülők medián Phe-szintje nőtt a COVID-19 világjárvány első évében, de összességében a referencia tartományon belül maradt. A világjárvány évében nyolc százalékkal több szárított vércsepp mintát küldtek be az előző évhez képest. A pandémia idején a 12 év alatti gyermekek és serdülők Phe-szintje különböző irányokba mozdult el: a gyermekek a legmagasabb szinteket a korlátozások időszakában érték el, míg a serdülők a korlátozások nélküli nyári időszakban. Az anyagcsere állapotát befolyásoló tényezők (pl. pénzügyi helyzet, testmozgás, étkezési szokások változása, nassolás, unalomból való evés, napi ritmus változása) eltérő szerepet játszhattak a két korcsoportban.

Szignifikáns negatív összefüggést figyeltünk meg a beküldött minták száma és a medián Phe-szint között, ami alátámasztja azt a tudást, hogy a minták számának csökkenésére különös figyelmet kell fordítani.

9. References

1. Folling A. Uber Ausscheidung von Phenylbrenztraubensaure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillitat. Hoppe-Seylers 2 Physiol Chem. 1934;227:169–76.

2. Folling A. Utskillelse av fenylpyrodruesyre i urinen som stoffskifteanomali i forbindelse med imbecillitet. Nord Med Tidskr. 1934;8:1054–9.

3. Penrose L, Quastel JH. Metabolic studies in phenylketonuria. Biochem J. 1937;31(2):266–74.

4. Centerwall SA, Centerwall WR. The discovery of phenylketonuria: the story of a young couple, two retarded children, and a scientist. Pediatrics. 2000 Jan;105(1 Pt 1):89-103.

5. Jervis GA. Studies on phenylpyruvic oligophrenia; the position of the metabolic error. J Biol Chem. 1947;169(3):651–6.

6. Bickel H, Gerrard J, Hickmans EM. The influence of phenylalanine intake on the chemistry and behaviour of a phenyl-ketonuric child. Acta Paediatr. 1954;43(1):64–77.

7. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics. 1963;32:338–43.

8. Groselj U, Tansek MZ, Battelino T. Fifty years of phenylketonuria newborn screening - A great success for many, but what about the rest? Mol Genet Metab. 2014;113(1–2):8–10.

9. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. Lancet (London, England). 2010;376(9750):1417–27.

10. Loeber JG. Neonatal screening in Europe; the situation in 2004. J Inherit Metab Dis. 2007;30(4):430–8.

11. Zschocke J, Mallory J, Eiken H, Nevin N. Phenylketonuria and the peoples of Northern Ireland. Hum Genet. 1997;100:189–94.

12. Desviat LR, Pérez B, Gámez A, Sánchez A, García MJ, Martínez-Pardo M,

Marchante C, Bóveda D, Baldellou A, Arena J, Sanjurjo P, Fernández A, Cabello ML, Ugarte M. Genetic and phenotypic aspects of phenylalanine hydroxylase deficiency in Spain: molecular survey by regions. Eur J Hum Genet. 1999;7:386–92.

13. Ozalp I, Coşkun T, Tokatli A, Kalkanoğlu HS, Dursun A, Tokol S, Köksal G, Ozgüc M, Köse R. Newborn PKU screening in Turkey: at present and organization for future. Turk J Pediatr. 2001;43:97–101.

14. Bókay J, Kiss E, Simon E, Szőnyi L. [Maternal phenylketonuria]. Orv Hetil. 2013;154(18):683–7.

15. Kaufman S. The phenylalanine hydroxylating system in phenylketonuria and its variants. Biochem Med. 1976;15(1):42–54.

16. Schuck PF, Malgarin F, Cararo JH, Cardoso F, Streck EL, Ferreira GC. Phenylketonuria Pathophysiology: on the Role of Metabolic Alterations. Aging Dis. 2015 Oct 1;6(5):390-9.

17. Scriver CR, Kaufman S. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: The Metabolic and Molecular Bases of Inherited Disease (8 ed). 2001. p. 1667–724.

18. Williams RA, Mamotte CD, Burnett JR, Prof C, Burnett J. Phenylketonuria: An Inborn Error of Phenylalanine Metabolism. Clin Biochem Rev. 2008;29(1):31.

19. Flydal MI, Martinez A. Phenylalanine hydroxylase: Function, structure, and regulation. IUBMB Life. 2013;65(4):341–9.

20. Dyer CA. Pathophysiology of phenylketonuria. Ment Retard Dev Disabil Res Rev. 1999;5(2):104–12.

21. Hoeksma M, Reijngoud DJ, Pruim J, de Valk HW, Paans AMJ, van Spronsen FJ. Phenylketonuria: High plasma phenylalanine decreases cerebral protein synthesis. Mol Genet Metab. 2009;96(4):177–82.

22. Anderson PJ, Wood SJ, Francis DE, Coleman L, Anderson V, Boneh A. Are neuropsychological impairments in children with early-treated phenylketonuria

(PKU) related to white matter abnormalities or elevated phenylalanine levels? Dev Neuropsychol. 2007;32(2):645–68.

23. Enns GM, Koch R, Brumm V, Blakely E, Suter R, Jurecki E. Suboptimal outcomes in patients with PKU treated early with diet alone: Revisiting the evidence. Vol. 101, Molecular Genetics and Metabolism. Academic Press; 2010. p. 99–109.

24. Welsh MC, Pennington BF, Ozonoff S, Rouse B, McCabe ER. Neuropsychology of early-treated phenylketonuria: specific executive function deficits. Child Dev. 1990;61(6):1697–713.

25. Bechar M, Bornstein B, Elian M, Sandbank U. Phenylketonuria presenting an intermittent progressive course. J Neurol Neurosurg Psychiatry. 1965;28(2):165–70.

26. Malamud N. Neuropathology of phenylketonuria. J Neuropathol Exp Neurol. 1966;25(2):254–68.

27. Villasana D, Butler IJ, Williams JC, Roongta SM. Neurological deterioration in adult phenylketonuria. J Inherit Metab Dis. 1989;12(4):451–7.

28. Thompson AJ, Smith I, Brenton D, Youl BD, Rylance G, Davidson DC, Kendall B, Lees AJ. Neurological deterioration in young adults with phenylketonuria. Lancet (London, England). 1990;336(8715):602–5.

29. Fisch RO, Tsai MY, Gentry WC. Studies of phenylketonurics with dermatitis. J Am Acad Dermatol. 1981;4(3):284–90.

30. Gassió R, Artuch R, Vilaseca MA, Fusté E, Boix C, Sans A, Campistol J. Cognitive functions in classic phenylketonuria and mild hyperphenyl-alaninaemia: experience in a paediatric population. Dev Med Child Neurol. 2005;47(7):443–8.

31. Moyle JJ, Fox AM, Arthur M, Bynevelt M, Burnett JR. Meta-analysis of neuropsychological symptoms of adolescents and adults with PKU. Neuropsychol Rev. 2007;17(2):91–101.

32. Waisbren SE, Noel K, Fahrbach K, Cella C, Frame D, Dorenbaum A, Levy H.

Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. Mol Genet Metab. 2007;92(1–2):63–70.

33. Channon S, German E, Cassina C, Lee P. Executive functioning, memory, and learning in phenylketonuria. Neuropsychology. 2004;18(4):613–20.

34. Antshel KM, Waisbren SE. Timing is everything: Executive functions in children exposed to elevated levels of phenylalanine. Neuropsychology. 2003;17(3):458–68.

35. White DA, Nortz MJ, Mandernach T, Huntington K, Steiner RD. Deficits in memory strategy use related to prefrontal dysfunction during early development: Evidence from children with phenylketonuria. Neuropsychology. 2001;15(2):221–9.

36. Brunner RL, Berry H. Phenylketonuria and sustained attention: the continuous performance test. Int J Clin Neuropsychol. 1987;9:68–70.

37. Huijbregts SCJ, De Sonneville LMJ, Licht R, Van Spronsen FJ, Verkerk PH, Sergeant JA. Sustained attention and inhibition of cognitive interference in treated phenylketonuria: Associations with concurrent and lifetime phenylalanine concentrations. Neuropsychologia. 2002;40(1):7–15.

38. Leuzzi V, Pansini M, Sechi E, Chiarotti F, Carducci C, Levi G, Antonozzi I. Executive function impairment in early-treated PKU subjects with normal mental development. J Inherit Metab Dis. 2004;27(2):115–25.

39. Anderson PJ, Wood SJ, Francis DE, Coleman L, Warwick L, Casanelia S, Anderson VA, Boneh A. Neuropsychological functioning in children with early-treated phenylketonuria: impact of white matter abnormalities. Dev Med Child Neurol. 2004;46(4):230–8.

40. Brumm VL, Bilder D, Waisbren SE. Psychiatric symptoms and disorders in phenylketonuria. Mol Genet Metab. 2010;99 Suppl 1:S59-63

41. Aldámiz-Echevarría L, Bueno MA, Couce ML, Lage S, Dalmau J, Vitoria I, Andrade F, Blasco J, Alcalde C, Gil D, García MC, González-Lamuño D, Ruiz M, Peña-Quintana L, Ruiz MA, González D, Sánchez-Valverde F. Anthropometric

characteristics and nutrition in a cohort of PAH-deficient patients. Clin Nutr. 2014;33(4):702–17.

42. Arnold GL, Vladutiu CJ, Kirby RS, Blakely EM, DeLuca JM. Protein insufficiency and linear growth restriction in phenylketonuria. J Pediatr. 2002 Aug 1;141(2):243–6.

43. Walkowiak D, Kaluzny L, Bukowska-Posadzy A, Oltarzewski M, Staszewski R, Moczko JA, Musielak M, Walkowiak J. Overweight in classical phenylketonuria children: A retrospective cohort study. Adv Med Sci. 2019;64(2):409–14.

44. Albersen M, Bonthuis M, de Roos NM, van den Hurk DA, Carbasius Weber E, Hendriks MM, de Sain-van der Velden MG, de Koning TJ, Visser G. Whole body composition analysis by the BodPod air-displacement plethysmography method in children with phenylketonuria shows a higher body fat percentage. J Inherit Metab Dis. 2010;33(Suppl 3):283.

45. Gokmen Ozel H, Ahring K, Bélanger-Quintana A, Dokoupil K, Lammardo AM, Robert M, Rocha JC, Almeida MF, van Rijn M, MacDonald A. Overweight and obesity in PKU: The results from 8 centres in Europe and Turkey. Mol Genet Metab reports. 2014;1:483–6.

46. Burrage LC, McConnell J, Haesler R, O'Riordan MA, Sutton VR, Kerr DS, McCandless SE. High prevalence of overweight and obesity in females with phenylketonuria. Mol Genet Metab. 2012;107(1–2):43–8.

47. Pena MJ, Almeida MF, van Dam E, Ahring K, Bélanger-Quintana A, Dokoupil K, Gokmen-Ozel H, Lammardo AM, MacDonald A, Robert M, Rocha JC. Special low protein foods for phenylketonuria: availability in Europe and an examination of their nutritional profile. Orphanet J Rare Dis. 2015;10(1).

48. Koletzko B, Beblo S, Demmelmair H, Müller-Felber W, Hanebutt FL. Does dietary DHA improve neural function in children? Observations in phenylketonuria. Prostaglandins Leukot Essent Fat Acids. 2009;81(2–3):159–64.

49. Beblo S, Reinhardt H, Muntau AC, Mueller-Felber W, Roscher AA, Koletzko B. Fish oil supplementation improves visual evoked potentials in children with

phenylketonuria. Neurology. 2001;57(8):1488–91.

50. Gassió R, Artuch R, Vilaseca MA, Fusté E, Colome R, Campistol J. Cognitive functions and the antioxidant system in phenylketonuric patients. Neuropsychology. 2008;22(4):426–31.

51. Ambroszkiewicz J, Gajewska J, Laskowska-Klita T. [Markers of bone formation and resorption in prepubertal children with phenylketonuria]. Med Wieku Rozw. 2003;7(1):89–95.

52. Pérez-Dueñas B, Cambra FJ, Vilaseca MA, Lambruschini N, Campistol J, Camacho JA. New approach to osteopenia in phenylketonuric patients. Acta Paediatr. 2002;91(8):899–904.

53. Modan-Moses D, Vered I, Schwartz G, Anikster Y, Abraham S, Segev R, Efrati O. Peak bone mass in patients with phenylketonuria. J Inherit Metab Dis. 2007;30(2):202–8.

54. Peng H, Peck D, White DA, Christ SE. Tract-based evaluation of white matter damage in individuals with early-treated phenylketonuria. J Inherit Metab Dis. 2014;37(2):237–43.

55. Camp KM, Parisi MA, Acosta PB, Berry GT, Bilder DA, Blau N, Bodamer OA, Brosco JP, Brown CS, Burlina AB, Burton BK, Chang CS, Coates PM, Cunningham AC, Dobrowolski SF, Ferguson JH, Franklin TD, Frazier DM, Grange DK, Greene CL, Groft SC, Harding CO, Howell RR, Huntington KL, Hyatt-Knorr HD, Jevaji IP, Levy HL, Lichter-Konecki U, Lindegren ML, Lloyd-Puryear MA, Matalon K, MacDonald A, McPheeters ML, Mitchell JJ, Mofidi S, Moseley KD, Mueller CM, Mulberg AE, Nerurkar LS, Ogata BN, Pariser AR, Prasad S, Pridjian G, Rasmussen SA, Reddy UM, Rohr FJ, Singh RH, Sirrs SM, Stremer SE, Tagle DA, Thompson SM, Urv TK, Utz JR, van Spronsen F, Vockley J, Waisbren SE, Weglicki LS, White DA, Whitley CB, Wilfond BS, Yannicelli S, Young JM. Phenylketonuria Scientific Review Conference: state of the science and future research needs. Mol Genet Metab. 2014;112(2):87–122.

56. Anderson PJ, Leuzzi V. White matter pathology in phenylketonuria. Mol Genet Metab. 2010;99 Suppl 1:S3-9

57. Manara R, Burlina AP, Citton V, Ermani M, Vespignani F, Carollo C, Burlina AB. Brain MRI diffusion-weighted imaging in patients with classical phenylketonuria. Neuroradiology. 2009;51(12):803–12.

58. Weglage J, Pietsch M, Feldmann R, Koch HG, Zschocke J, Hoffmann G, Muntau-Heger A, Denecke J, Guldberg P, Güttler F, Möller H, Wendel U, Ullrich K, Harms E. Normal clinical outcome in untreated subjects with mild hyperphenylalaninemia. Pediatr Res. 2001;49(4):532–6.

59. Hood A, Antenor-Dorsey JA, Rutlin J, Hershey T, Shimony JS, McKinstry RC, Grange DK, Christ SE, Steiner R, White DA. Prolonged exposure to high and variable phenylalanine levels over the lifetime predicts brain white matter integrity in children with phenylketonuria. Mol Genet Metab. 2015;114(1):19–24.

60. Leuzzi V, Tosetti M, Montanaro D, Carducci C, Artiola C, Carducci C, Antonozzi I, Burroni M, Carnevale F, Chiarotti F, Popolizio T, Giannatempo GM, D'Alesio V, Scarabino T. The pathogenesis of the white matter abnormalities in phenylketonuria. A multimodal 3.0 tesla MRI and magnetic resonance spectroscopy (1H MRS) study. J Inherit Metab Dis. 2007;30(2):209–16.

61. Cleary MA, Walter JH, Wraith JE, Jenkins JP, Alani SM, Tyler K, Whittle D. Magnetic resonance imaging of the brain in phenylketonuria. Lancet (London, England). 1994;344(8915):87–90.

62. White DA, Antenor-Dorsey JA, Grange DK, Hershey T, Rutlin J, Shimony JS, McKinstry RC, Christ SE. White matter integrity and executive abilities following treatment with tetrahydrobiopterin (BH4) in individuals with phenylketonuria. Mol Genet Metab. 2013 Nov 1;110(3):213–7.

63. Christ SE, Moffitt AJ, Peck D. Disruption of prefrontal function and connectivity in individuals with phenylketonuria. Mol Genet Metab. 2010;99 Suppl 1:S33-40

64. Abgottspon S, Muri R, Christ SE, Hochuli M, Radojewski P, Trepp R, Everts R. Neural correlates of working memory and its association with metabolic parameters in early-treated adults with phenylketonuria. NeuroImage Clin. 2022;34.

65. van Wegberg AMJ, MacDonald A, Ahring K, Bélanger-Quintana A, Blau N, Bosch AM, Burlina A, Campistol J, Feillet F, Giżewska M, Huijbregts SC, Kearney S, Leuzzi V, Maillot F, Muntau AC, van Rijn M, Trefz F, Walter JH, van Spronsen FJ. The complete European guidelines on phenylketonuria: Diagnosis and treatment. Vol. 12, Orphanet Journal of Rare Diseases. BioMed Central Ltd.; 2017.

66. Blau N, Hennermann JB, Langenbeck U, Lichter-Konecki U. Diagnosis, classification, and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. Mol Genet Metab. 2011;104 Suppl:S2-9

67. Opladen T, López-Laso E, Cortès-Saladelafont E, Pearson TS, Sivri HS, Yildiz Y, Assmann B, Kurian MA, Leuzzi V, Heales S, Pope S, Porta F, García-Cazorla A, Honzík T, Pons R, Regal L, Goez H, Artuch R, Hoffmann GF, Horvath G, Thöny B, Scholl-Bürgi S, Burlina A, Verbeek MM, Mastrangelo M, Friedman J, Wassenberg T, Jeltsch K, Kulhánek J, Kuseyri Hübschmann O; International Working Group on Neurotransmitter related Disorders (iNTD). Consensus guideline for the diagnosis and treatment of tetrahydrobiopterin (BH4) deficiencies. Orphanet J Rare Dis. 2020;15(1):1–30.

68. R.A.F. Evers, A.M.J. van Wegberg, K. Ahring, S. Beblo, A. Bélanger-Quintana, A.M. Bosch, A. Burlina, J. Campistol, T. Coskun, F. Feillet, M. Giżewska, S.C.J. Defining tetrahydrobiopterin responsiveness in phenylketonuria: Survey results from 38 countries. Mol Genet Metab. 2021 Apr 1;132(4):215–9.

69. Schuler A, Somogyi C, Toros I, Nagy A, Kiss E, Varadi I, Fekete G. Twenty years of experience with phenylketonuria in Hungary. Int Pediatr. 1996;11:114–7.

70. MacDonald A, van Wegberg AMJ, Ahring K, Beblo S, Bélanger-Quintana A, Burlina A, Campistol J, Coşkun T, Feillet F, Giżewska M, Huijbregts SC, Leuzzi V, Maillot F, Muntau AC, Rocha JC, Romani C, Trefz F, van Spronsen FJ. PKU dietary handbook to accompany PKU guidelines. Orphanet J Rare Dis. 2020 Jun 30;15(1):171.

71. Pode-Shakked B, Shemer-Meiri L, Harmelin A, Stettner N, Brenner O, Abraham S, Schwartz G, Anikster Y. Man made disease: clinical manifestations of low

phenylalanine levels in an inadequately treated phenylketonuria patient and mouse study. Mol Genet Metab. 2013;110 Suppl:S66-70.

72. van Spronsen FJ, van Rijn M, van Dijk T, Smit GP, Reijngoud DJ, Berger R, Heymans HS. Plasma Phenylalanine and Tyrosine Responses to Different Nutritional Conditions (Fasting/Postprandial) in Patients With Phenylketonuria: Effect of Sample Timing. Pediatrics. 1993;92(4):570–3.

73. MacDonald A. Diet and compliance in phenylketonuria. Eur J Pediatr. 2000;159 Suppl 2(2).

74. Barta AG, Sumánszki C, Turgonyi Z, Kiss E, Simon E, Serfőző C, Reismann P. Health Related Quality of Life assessment among early-treated Hungarian adult PKU patients using the PKU-QOL adult questionnaire. Mol Genet Metab Reports. 2020 Jun 1;23:100589.

75. Lim K, van Calcar SC, Nelson KL, Gleason ST, Ney DM. Acceptable low-phenylalanine foods and beverages can be made with glycomacropeptide from cheese whey for individuals with PKU. Mol Genet Metab. 2007 Sep-Oct;92(1-2):176-8.

76. Ney DM, Gleason ST, van Calcar SC, MacLeod EL, Nelson KL, Etzel MR, Rice GM, Wolff JA. Nutritional management of PKU with glycomacropeptide from cheese whey. J Inherit Metab Dis. 2009;32(1):32–9.

77. van Calcar SC, MacLeod EL, Gleason ST, Etzel MR, Clayton MK, Wolff JA, Ney DM. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. Am J Clin Nutr. 2009;89(4):1068–77.

78. Christensen HN. Metabolism of amino acids and proteins. Annu Rev Biochem. 1953;22:233–60.

79. Matalon R, Michals-Matalon K, Bhatia G, Burlina AB, Burlina AP, Braga C, Fiori L, Giovannini M, Grechanina E, Novikov P, Grady J, Tyring SK, Guttler F. Double blind placebo control trial of large neutral amino acids in treatment of PKU: effect on blood phenylalanine. J Inherit Metab Dis. 2007;30(2):153–8.

80. Concolino D, Mascaro I, Moricca MT, Bonapace G, Matalon K, Trapasso J, Radhakrishnan G, Ferrara C, Matalon R, Strisciuglio P. Long-term treatment of phenylketonuria with a new medical food containing large neutral amino acids. Eur J Clin Nutr. 2017;71(1):51–5.

81. Yano S, Moseley K, Azen C. Large neutral amino acid supplementation increases melatonin synthesis in phenylketonuria: a new biomarker. J Pediatr. 2013;162(5):999–1003.

82. Koch R, Moseley KD, Yano S, Nelson M, Moats RA. Large neutral amino acid therapy and phenylketonuria: A promising approach to treatment. Mol Genet Metab. 2003;79(2):110–3.

83. Schindeler S, Ghosh-Jerath S, Thompson S, Rocca A, Joy P, Kemp A, Rae C, Green K, Wilcken B, Christodoulou J. The effects of large neutral amino acid supplements in PKU: an MRS and neuropsychological study. Mol Genet Metab . 2007;91(1):48–54.

84. Burlina AP, Cazzorla C, Massa P, Loro C, Gueraldi D, Burlina AB. The Impact of a Slow-Release Large Neutral Amino Acids Supplement on Treatment Adherence in Adult Patients with Phenylketonuria. Nutr 2020, Vol 12, Page 2078. 2020;12(7):2078.

85. Blau N, Erlandsen H. The metabolic and molecular bases of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. Mol Genet Metab. 2004 Jun 1;82(2):101–11.

86. Administration UF and D. US Prescribing Information 2020 [Internet]. 2020. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/022181s020,205065s006lbl.pdf

87. European MA. European Public Assessment Report – Product Information 2020 [Internet]. 2021. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/kuvan

88. Muntau AC, Burlina A, Eyskens F, Freisinger P, De Laet C, Leuzzi V, Rutsch F,

Sivri HS, Vijay S, Bal MO, Gramer G, Pazdírková R, Cleary M, Lotz-Havla AS, Munafo A, Mould DR, Moreau-Stucker F, Rogoff D.Efficacy, safety and population pharmacokinetics of sapropterin in PKU patients <4 years: results from the SPARK open-label, multicentre, randomized phase IIIb trial. Orphanet J Rare Dis. 2017 Mar 9;12(1).

89. Levy HL, Milanowski A, Chakrapani A, Cleary M, Lee P, Trefz FK, Whitley CB, Feillet F, Feigenbaum AS, Bebchuk JD, Christ-Schmidt H, Dorenbaum A; Sapropterin Research Group. Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a phase III randomised placebo-controlled study. Lancet (London, England). 2007 Aug;370(9586):504–10.

90. Vockley J, Andersson HC, Antshel KM, Braverman NE, Burton BK, Frazier DM, Mitchell J, Smith WE, Thompson BH, Berry SA; American College of Medical Genetics and Genomics Therapeutics Committee. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. Genet Med. 2014;16(2):188–200.

91. Harding CO, Amato RS, Stuy M, Longo N, Burton BK, Posner J, Weng HH, Merilainen M, Gu Z, Jiang J, Vockley J; PRISM-2 Investigators. Pegvaliase for the treatment of phenylketonuria: A pivotal, double-blind randomized discontinuation Phase 3 clinical trial. Mol Genet Metab. 2018;124(1):20–6.

92. Hydery T, Coppenrath VA. A Comprehensive Review of Pegvaliase, an Enzyme Substitution Therapy for the Treatment of Phenylketonuria. Drug Target Insights. 2019;13(1).

93. European MA. European Public Assessment Report – Product Information [Internet]. 2020. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/palynziq#product-information-section

94. Administration UF and D. US Prescribing Information 2018 [Internet]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761079s000lbl.pdf

95. Sacharow S, Papaleo C, Almeida K, Goodlett B, Kritzer A, Levy H, Martell L, Wessel A, Viau K. First 1.5 years of pegvaliase clinic: Experiences and outcomes. Mol Genet Metab Reports. 2020 Sep 1;24:100603.

96. Adams D, Andersson HC, Bausell H, Crivelly K, Eggerding C, Lah M, Lilienstein J, Lindstrom K, McNutt M, Ray JW, Saavedra H, Sacharow S, Starin D, Tiffany-Amaro J, Thomas J, Vucko E, Wessenberg LB, Whitehall K. Use of pegvaliase in the management of phenylketonuria: Case series of early experience in US clinics. Mol Genet Metab reports. 2021;28.

97. Hollander S, Viau K, Sacharow S. Pegvaliase dosing in adults with PKU: Requisite dose for efficacy decreases over time. Mol Genet Metab. 2022 Sep 1;137(1–2):104–6.

98. Isabella VM, Ha BN, Castillo MJ, Lubkowicz DJ, Rowe SE, Millet YA, Anderson CL, Li N, Fisher AB, West KA, Reeder PJ, Momin MM, Bergeron CG, Guilmain SE, Miller PF, Kurtz CB, Falb D. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. Nat Biotechnol. 2018 Oct;36(9):857-864.

99. Vajro P, Strisciuglio P, Houssin D, Huault G, Laurent J, Alvarez F, Bernard O. Correction of phenylketonuria after liver transplantation in a child with cirrhosis. N Engl J Med. 1993;329(5):363.

100. Raghu VK, Dobrowolski SF, Sindhi R, Strauss KA, Mazariegos GV, Vockley J, Soltys K. Domino liver transplant from a donor with maple syrup urine disease into a recipient with phenylketonuria. Mol Genet Metab Reports. 2022 Jun 1;31:100866.

101. Hamman K, Clark H, Montini E, Al-Dhalimy M, Grompe M, Finegold M, Harding CO. Low Therapeutic Threshold for Hepatocyte Replacement in Murine Phenylketonuria. Mol Ther. 2005;12(2):337.

102. Stéphenne X, Debray FG, Smets F, Jazouli N, Sana G, Tondreau T, Menten R, Goffette P, Boemer F, Schoos R, Gersting SW, Najimi M, Muntau AC, Goyens P, Sokal EM. Hepatocyte transplantation using the domino concept in a child with tetrabiopterin nonresponsive phenylketonuria. Cell Transplant. 2012;21(12):2765–

70.

103. Grisch-Chan HM, Schwank G, Harding CO, Thöny B. State-of-the-Art 2019 on Gene Therapy for Phenylketonuria. Hum Gene Ther. 2019 Oct 1;30(10):1274–83.

104. Fang B, Eisensmith RC, Li XH, Finegold MJ, Shedlovsky A, Dove W, Woo SL. Gene therapy for phenylketonuria: Phenotypic correction in a genetically deficient mouse model by adenovirus-mediated hepatic gene transfer. Gene Ther. 1994;1(4):247–54.

105. Phillips AJ. The challenge of gene therapy and DNA delivery. J Pharm Pharmacol. 2010 Feb 18;53(9):1169–74.

106. Ates I, Rathbone T, Stuart C, Hudson Bridges P, Cottle RN. Delivery Approaches for Therapeutic Genome Editing and Challenges. Genes 2020, Vol 11, Page 1113. 2020;11(10):1113.

107. Li H, Xu LX, Yu J, Tan L, Miao P, Yang X, Tian Q, Li M, Feng CX, Yang Y, Sha N, Feng X, Sun B, Gong M, Ding X. The role of a lncRNA (TCONS_00044595) in regulating pineal CLOCK expression after neonatal hypoxia–ischemia brain injury. Biochem Biophys Res Commun. 2020 Jul 12;528(1):1–6.

108. Clinicaltrials.gov N. AAV Gene Therapy Study for Subjects With PKU [Internet].. Available from: https://clinicaltrials.gov/ct2/show/NCT04480567?term=gene+therapy&cond=phenylketonuria&draw=2&rank=1

109. Clinicaltrials.gov NCT03952156. Gene Therapy Clinical Study in Adult PKU [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT03952156?term=gene+therapy&cond=phenylketonuria&draw=2&rank=2

110. Clinicaltrials.gov N. Safety and Efficacy of HMI-103, a Gene Editing Development Candidate in Adults With Classical PKU Due to PAH Deficiency [Internet]. Available from: https://clinicaltrials.gov/ct2/show/NCT05222178?term=gene+therapy&cond=phenylketonuria&draw=2&rank=3

111. European Medicines Agency. Reflection paper on the regulatory guidance for the use of healthrelated quality of life (HRQL) measures in the evaluation of medicinal products [Internet]. 2004. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003637.pdf

112. Simon E, Schwarz M, Roos J, Dragano N, Geraedts M, Siegrist J, Kamp G, Wendel U. Evaluation of quality of life and description of the sociodemographic state in adolescent and young adult patients with phenylketonuria (PKU). Health Qual Life Outcomes. 2008;6(1):25.

113. Bosch AM, Tybout W, van Spronsen FJ, de Valk HW, Wijburg FA, Grootenhuis MA. The course of life and quality of life of early and continuously treated Dutch patients with phenylketonuria. J Inherit Metab Dis. 2007;30(1):29–34.

114. Mütze U, Roth A, Weigel JF, Beblo S, Baerwald CG, Bührdel P, Kiess W. Transition of young adults with phenylketonuria from pediatric to adult care. J Inherit Metab Dis. 2011;34(3):701–9.

115. Cazzorla C, Cegolon L, Burlina AP, Celato A, Massa P, Giordano L, Polo G, Daniele A, Salvatore F, Burlina AB. Quality of Life (QoL) assessment in a cohort of patients with Phenylketonuria. BMC Public Health. 2014;14(1):1–9.

116. Demirdas S, Maurice-Stam H, Boelen CC, Hofstede FC, Janssen MC, Langendonk JG, Mulder MF, Rubio-Gozalbo ME, van Spronsen FJ, de Vries M, Grootenhuis MA, Bosch AM. Evaluation of quality of life in PKU before and after introducing tetrahydrobiopterin (BH4); a prospective multi-center cohort study. Mol Genet Metab. 2013 Jan 1;110(SUPPL.):S49–56.

117. Landolt MA, Nuoffer JM, Steinmann B, Superti-Furga A. Quality of life and psychologic adjustment in children and adolescents with early treated phenylketonuria can be normal. J Pediatr. 2002 May 1;140(5):516–21.

118. Cotugno G, Nicolò R, Cappelletti S, Goffredo B, Dionisi Vici C, Di Ciommo V. Adherence to diet and quality of life in patients with phenylketonuria. Acta Paediatr. 2011;100(8):1144–9.

119. Vieira E Neto, Maia HS Filho, Monteiro CB, Carvalho LM, Tonon T, Vanz AP, Schwartz IVD, Ribeiro MG. Quality of life and adherence to treatment in early-treated Brazilian phenylketonuria pediatric patients. Brazilian J Med Biol Res. 2018;51(2).

120. Regnault A, Burlina A, Cunningham A, Bettiol E, Moreau-Stucker F, Benmedjahed K, Bosch AM. Development and psychometric validation of measures to assess the impact of phenylketonuria and its dietary treatment on patients' and parents' quality of life: The phenylketonuria - Quality of life (PKU-QOL) questionnaires. Orphanet J Rare Dis. 2015;10(1):1–18.

121. Bosch AM, Burlina A, Cunningham A, Bettiol E, Moreau-Stucker F, Koledova E, Benmedjahed K, Regnault A. Assessment of the impact of phenylketonuria and its treatment on quality of life of patients and parents from seven European countries. Orphanet J Rare Dis. 2015;10(1).

122. Alptekin IM, Koc N, Gunduz M, Cakiroglu FP. The impact of phenylketonuria on PKU patients' quality of life: Using of the phenylketonuria-quality of life (PKU-QOL) questionnaires. Clin Nutr ESPEN. 2018;27:79–85.

123. Morawska A, Mitchell AE, Etel E, Kirby G, McGill J, Coman D, Inwood A. Psychosocial functioning in children with phenylketonuria: Relationships between quality of life and parenting indicators. Child Care Health Dev. 2020;46(1):56–65.

124. Qun L, Xuhua G, Peng W, Xiaoye W, Lei Z YT. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. N Engl J Med. 2020;382(13):1199–207.

125. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 [Internet]. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

126. Communicable disease threats report, 1-7 March 2020, week 10 [Internet]. Available from: https://www.ecdc.europa.eu/en/publications-data/communicable-disease-threats-report-1-%0A7-march-2020-week-10.

127. Röst G, Bartha FA, Bogya N, Boldog P, Dénes A, Ferenci T, Horváth KJ, Juhász A, Nagy C, Tekeli T, Vizi Z, Oroszi B. Early phase of the COVID-19 outbreak in hungary and post-lockdown scenarios. Viruses. 2020;12(7).

128. Coskun F, Hanta I, Cilli A, Ozkaya G, Ursavas A, Sevinc C. Effects of the COVID-19 pandemic on the follow-up and treatment of patients with idiopathic pulmonary fibrosis: a cross-sectional, multicentre phone call survey. BMJ Open. 2021;11(8).

129. Chen G, Zhou Y, Xia J, Yao J, Zheng K, Qin Y, Li X. When the COVID-19 pandemic changed the follow-up landscape of chronic kidney disease: a survey of real-world nephrology practice. Ren Fail. 2020;42(1):733–9.

130. Karatas S, Yesim T, Beysel S. Impact of lockdown COVID-19 on metabolic control in type 2 diabetes mellitus and healthy people. Prim Care Diabetes. 2021;15(3):424–7.

131. Rovelli V, Zuvadelli J, Ercoli V, Montanari C, Paci S, Dionigi AR, Scopari A, Salvatici E, Cefalo G, Banderali G. PKU and COVID19: How the pandemic changed metabolic control. Mol Genet Metab Reports. 2021 Jun 1;27:100759.

132. Herle M, Brunner-Krainz M, Karall D, Goeschl B, Möslinger D, Zobel J, Plecko B, Scholl-Bürgi S, Spenger J, Wortmann SB, Huemer M. A retrospective study on disease management in children and adolescents with phenylketonuria during the Covid-19 pandemic lockdown in Austria. Orphanet J Rare Dis. 2021;16(1):367.

133. Walkowiak D, Mikołuć B, Mozrzymas R, Kałużny Ł, Didycz B, Jaglowska J, Kurylak D, Walkowiak J. The Impact of the First 2020 COVID-19 Lockdown on the Metabolic Control of Patients with Phenylketonuria. Nutr 2021, Vol 13, Page 2024. 2021;13(6):2024.

134. Jurecki E, Cunningham A, Birardi V, Gagol G, Acquadro C. Development of the US English version of the phenylketonuria - quality of life (PKU-QOL) questionnaire. Health Qual Life Outcomes. 2017;15(1):46.

135. Becsei D, Hiripi R, Kiss E, Szatmári I, Arató A, Reusz G, Szabó AJ, Bókay J, Zsidegh P. Quality of life in children living with PKU - a single-center, cross-sectional, observational study from Hungary. Mol Genet Metab reports. 2021;29.

136. Becsei D, Kiss E, Szatmári I, Arató A, Reusz G, Szabó AJ, Bókay J, Zsidegh P. A retrospective analysis of metabolic control in children with PKU in the COVID-19 era. Mol Genet Metab Reports. 2022;32:100897.

137. MacLeod EL, Ney DM. Nutritional Management of Phenylketonuria. Ann Nestlé. 2010;68(2):58.

138. MacDonald A, Ferguson C, Rylance G, Morris AA, Asplin D, Hall SK, Booth IWAre tablets a practical source of protein substitute in phenylketonuria? Arch Dis Child. 2003;88(4):327–9.

139. Alves Vieira T, ele Nalin T, Correa Krug B, Matzenbacher Bittar C, Brinckmann Oliveira Netto C, Vanessa Doederlein Schwartz I. Adherence to Treatment of Phenylketonuria: A Study in Southern Brazilian Patients. J inborn errors metab screen. 2015;3.

140. Jurecki ER, Cederbaum S, Kopesky J, Perry K, Rohr F, Sanchez-Valle A, Viau KS, Sheinin MY, Cohen-Pfeffer JL. Adherence to clinic recommendations among patients with phenylketonuria in the United States. Mol Genet Metab. 2017 Mar 1;120(3):190–7.

141. Ney DM, Etzel MR. Designing medical foods for inherited metabolic disorders: why intact protein is superior to amino acids. Curr Opin Biotechnol. 2017 Apr 1;44:39–45.

142. Zaki OK, El-Wakeel L, Ebeid Y, Ez Elarab HS, Moustafa A, Abdulazim N, Karara H, Elghawaby A. The Use of Glycomacropeptide in Dietary Management of Phenylketonuria. J Nutr Metab. 2016;2016.

143. López-Moreno M, López M, Miguel M, Garcés-Rimón M. Physical and Psychological Effects Related to Food Habits and Lifestyle Changes Derived from Covid-19 Home Confinement in the Spanish Population. Nutrients. 2020;12(11):1–17.

144. Calcaterra V, Iafusco D, Carnevale Pellino V, Mameli C, Tornese G, Chianese A, Cascella C, Macedoni M, Redaelli F, Zuccotti G, Vandoni M. "CoVidentary": An online exercise training program to reduce sedentary behaviours in children with

type 1 diabetes during the COVID-19 pandemic. J Clin Transl Endocrinol. 2021;25.

145. Burkart S, Parker H, Weaver RG, Beets MW, Jones A, Adams EL, Chaput JP, Armstrong B. Impact of the COVID-19 pandemic on elementary schoolers' physical activity, sleep, screen time and diet: A quasi-experimental interrupted time series study. Pediatr Obes. Obes. 2022 (1):e12846.

146. Paterson DC, Ramage K, Moore SA, Riazi N, Tremblay MS, Faulkner G. Exploring the impact of COVID-19 on the movement behaviors of children and youth: A scoping review of evidence after the first year. J Sport Heal Sci. 2021 Jul 6;

147. Zemrani B, Gehri M, Masserey E, Knob C, Pellaton R. A hidden side of the COVID-19 pandemic in children: the double burden of undernutrition and overnutrition. Int J Equity Health. 2021 Dec 1;20(1).

148. Rolland B, Haesebaert F, Zante E, Benyamina A, Haesebaert J, Franck N. Global Changes and Factors of Increase in Caloric/Salty Food Intake, Screen Use, and Substance Use During the Early COVID-19 Containment Phase in the General Population in France: Survey Study. JMIR Public Heal Surveill. 2020;6(3).

149. Cleary M, Trefz F, Muntau AC, Feillet F, van Spronsen FJ, Burlina A, Bélanger-Quintana A, Giżewska M, Gasteyger C, Bettiol E, Blau N, MacDonald A.Fluctuations in phenylalanine concentrations in phenylketonuria: A review of possible relationships with outcomes. Mol Genet Metab. 2013 Dec 1;110(4):418–23.

150. Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, Agha M, Agha R. The socio-economic implications of the coronavirus pandemic (COVID-19): A review. Int J Surg. 2020;78:185.

151. Carroll N, Sadowski A, Laila A, Hruska V, Nixon M, Ma DWL, Haines J, On Behalf Of The Guelph Family Health Study. The Impact of COVID-19 on Health Behavior, Stress, Financial and Food Security among Middle to High Income Canadian Families with Young Children. Nutrients. 2020;12(8):1–14.

152. Ruta D, Garratt A, Leng M, Russell I, MacDonald L. A new approach to the measurement of quality of life. The Patient-Generated Index. Med Care. 1994;32(11):1109–26.

Bibliography of the candidate's publications

Publications related to the thesis:

1. Becsei D, Hiripi R, Kiss E, Szatmári I, Arató A, Reusz G, Szabó AJ, Bókay J, Zsidegh P. Quality of life in children living with PKU - a single-center, cross-sectional, observational study from Hungary. *Mol Genet Metab Rep*. 2021 Nov 16;29:100823. doi: 10.1016/j.ymgmr.2021.100823.
   IF: 2.1
2. Becsei D, Kiss E, Szatmári I, Arató A, Reusz G, Szabó AJ, Bókay J, Zsidegh P. A retrospective analysis of metabolic control in children with PKU in the COVID-19 era. *Mol Genet Metab Rep*. 2022 Jul 8;32:100897. doi: 10.1016/j.ymgmr.2022.100897.
   IF:1.9

Publications not related to the thesis:

1. Barta AG, Becsei D, Kiss E, Sumánszki C, Simonová E, Reismann P. The Impact of Phenylketonuria on Body Composition in Adults. *Ann Nutr Metab*. 2022;78(2):98-105.
   IF: 3.9
2. Becsei D; Pajtók C*; Körner A, Szabó A. A COVID-19 gyermekgyógyászati vonatkozásai. ORVOSKÉPZÉS 2020, 95 (3): 532-538.
3. Becsei D ; Erni I ; Kiss E ; Simonova E ; Szatmari I ; Szabo AJ ; Zsidegh P ; Bokay J. Cognitive functions in children with PKU. *J Inherit Metab Dis*. 2018 41 (1):99.

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