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Phenylketonuria in the Latvian population: Molecular basis, phenylalanine levels, and patient compliance

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ABSTRACT

Introduction: Phenylketonuria (PKU) is an inborn error of metabolism characterized by pathogenic variants of the phenylalanine hydroxylase (*PAH*) gene with a resulting accumulation of phenylalanine (Phe) to neurotoxic levels. Diagnosis of PKU in the Latvian population began in 1985 and the present study's aim was to evaluate the available data on all PKU patients in Latvia.

Materials and methods: The medical records of 116 - DNA sample was available in 110 patients (102 nonrelated individuals) diagnosed with PKU in Latvia were obtained. Phe concentrations were measured in dried blood spots. Genomic DNA was analyzed for pathogenic variants in the *PAH* gene. Biochemical data were available through follow-up visits of the 83 patients.

Results: In 97% of patients (99 of 102), pathogenic variants were detected on both alleles. With an occurrence of 69.6%%, the most common pathogenic variant was the severe pathogenic variant p.Arg408Trp. The available data for 83 patients revealed that metabolic control was better in younger age groups and worse in adults.

Conclusion: Latvia exhibits a relatively homogeneous pool of disease-causing PKU alleles with a high prevalence of the classical severe form of PKU. Dietary compliance in all patients' groups is lower than expected, especially it is poor in adult age group.

1. Introduction

Phenylketonuria (PKU) is an inborn error of metabolism characterized by pathogenic variants of the phenylalanine hydroxylase (*PAH*) gene (OMIM#612349, gene ID: 5053) with a resulting accumulation of phenylalanine (Phe) to neurotoxic levels [4]. The most severe phenotype, often termed "classical PKU", is defined based on untreated blood Phe concentrations >1200 μmol/L. This phenotype is also the most common one worldwide. The distribution of metabolic phenotypes varies with the frequency of regional genotypes. While classical PKU is more common in Eastern Europe (approximately 68% of all PKU patients), Mediterranean countries in Europe report milder phenotypes [3]. In 2017, following substantial work by a group of experts, European guidelines for the diagnosis and management of patients with PKU were agreed upon. They advise the maintenance of blood Phe levels between 120 and 360 μmol/L in children aged <12 years and between 120 and 600 μmol/L in children 12 years and above. Furthermore, they recommend the frequency of blood Phe measurements to be at least once a week for the age group 0 to 1 year, fortnightly for children aged 1 to 12 years, and monthly for patients above 12 years [17].

PKU diagnosis through newborn screening programs allows early introduction of a Phe-restricted diet therapy, which prevents the neurotoxic effects of Phe and its metabolites [6]. Key dietary behaviors associated with optimal control of blood Phe concentrations include avoidance of high-protein foods consumption and even distribution of protein substitute throughout the day, and adequate energy intake [13]. Even though this treatment has been available for decades, adherence to recommended dietary restrictions remains an issue of concern. The vast majority of patients have to follow very strict diets and may tolerate only 250–350 mg of Phe per day [18]. Also, social and economic factors may cause patients to deviate from the ideal practice of maintaining their diet every day [11].

Dietary compliance continues to be the main obstacle as patients approach adolescence and adulthood. This results in poor control of

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blood Phe levels, leading to suboptimal outcomes in psychosocial and cognitive assessments [1]. It is well documented in the literature that acceptance of a PKU diagnosis by patients and their subsequent adherence to a low natural-protein diet can be problematic [5]. However, in this regard, very little is known about Latvian patients.

The aim of this study was to obtain a generalized overview from Latvian PKU patients of their observed pathogenic variants in the *PAH* gene, maintenance of dietary therapy, and established practice of medical observation.

2. Materials and methods

The medical records of 116 patients diagnosed with PKU in Latvia were obtained. Although the oldest patient diagnosed with PKU in Latvia was born in 1967, formal diagnosis of PKU in the Latvian population only began in 1985. All clinical and laboratory analyses were conducted during routine clinical work-ups.

2.1. Genomic DNA analysis

For 110 cases, a DNA sample was available for analysis; 102 of these cases were unrelated.

Genomic DNA was extracted from whole blood samples. The presence of the most common pathogenic variants in the *PAH* gene, p. Arg408Trp, was verified using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) [14]. Custom Sanger sequencing of the coding part of the *PAH* gene for "non p. Arg408Trp" was performed on an automated system for sequencing (ABI Prism 310 Genetic Analyzer; Applied Biosystems, USA) according to the manufacturer's protocol. The sequences of the primers used in this study are available upon request. All variants were aligned to reference sequence NM_000277.1. In patients where second variant was not identified were performed hyperphenylalaninemia panel (genes *PAH, DNAJC12, GCH1, PCBD1, PTS, QDPR* - Blueprint Genetics' approach) and if necessary additional copy number variation/ deletion/duplication analysis was conducted. Identified variants were classified as null/severe or mild alleles according to the BioPKU database [www.biopku.org], with genotypes written according to Human Genome Variation Society nomenclature. Additionally to BioPKU database to characterize pathogenicity were used ClinVar allele ID [10], pathogenicity according to ACMG criteria [15] using Varsome website [9]. Pathogenic variant and genotype frequency were calculated only in unrelated individuals. Genotypes for all patients are shown in Appendix A.

2.2. Phe level analysis

At the time of the study, 83 PKU patients were being actively followed by the Clinic of Medical Genetics and Prenatal Diagnostics, Latvian Rare Disease Coordination Center, with 78 maintaining a low protein diet and five receiving tetrahydrobiopterin (BH4) treatment.

A fluorometric assay measured patients' Phe concentrations in dried blood spots (DBS) collected onto filter paper. The assay was based on a modification of the McCaman and Robins quantitative fluorometric method. Briefly, Phe eluted from DBS formed a fluorescent complex with ninhydrin enhanced by the dipeptide L-leucyl-L-alanine. During the reaction, the pH was controlled by succinate buffer. Copper reagent was added to stabilize the ninhydrin-Phe complex and increase the signal before measuring the fluorescence. Fluorescence was measured using a Neonatal Phenylalanine kit (Labsystems Oy, Finland) and a microplate fluorometer (Fluoroskan Ascent; Labsystems Oy).

The study included all the available entries of Phe level in age groups <18 years in 2019; specifically, 46 patients and 945 recordings were analyzed. In the adult group (>18 years of age), the previous five years provided entries from 37 patients with a total of 1145 recordings. The following age groups were created: ≤1 year; 1 year 1 day to 4 years (520 entries from 10 patients); 4 years 1 day to 7 years (212 entries from 12 patients); 7 years 1 day to 12 years (123 entries from 10 patients); 12 years 1 day to 18 years (90 entries from 11 patients); >18 years. Results from the age group ≤1 year were excluded from the study as the children were too small and none of them had started to wean.

All data are reported as means ± SD or numbers (%) unless otherwise indicated. Phe values are reported as means ± SD, median, maximum and minimum for each group. Also, the frequency of blood sampling was counted using descriptive statistics.

2.3. Compliance with ethical standards

Written informed consent was obtained from all participating individuals. The study was performed according to the Declaration of Helsinki and the protocol was approved by the Central Medical Ethics Committee of Latvia.

3. Results

3.1. PAH gene pathogenic variant spectrum

In total, 110 patients of whom 102 were unrelated samples with an incoming diagnosis of "PKU" were analyzed for the presence of pathogenic variants of the *PAH* gene.

In 97% of patients (99 of 102 patients), pathogenic variants were detected on both alleles, and the diagnosis of "PKU caused by variants in the *PAH* gene" was confirmed. Only one pathogenic variant was found in 1.47% of probands (3 patients of 102 unrelated samples). The allele frequencies in the *PAH* gene are presented in Table 1. With an occurrence of 69.61%, the most common pathogenic variant was the severe variant p.Arg408Trp. Patients with the p.[Arg408Trp];[Arg408Trp] genotype accounted for 49% of the samples (50 patients of 102 unrelated samples).

In the Appendix A is showed all patients – their genotype, Phe tolerance, Phe, median Phe level, number of visits per year and Phe measurements, and compliance.

3.2. Metabolic control

Measurements of the level of Phe were available for 83 patients, of which 37 were older than 18 years. Analysis of the identified *PAH* pathogenic variant spectrum and Phe tolerance revealed that 13 patients (15.7%) had a mild form of PKU while the remaining ones had classical PKU with a low Phe tolerance. Throughout the last 20 years, the median age of patients when they were first diagnosed with PKU was 18.47 ± 14.63. Year to year over these 20 years, the age when the diagnosis was first made did not significantly differ. However, one late diagnosis at the age of 21 years did occur in the last five years.

All Phe concentrations measured in DBS presented in relation to the recommended cut-off values are shown in Fig. 1. Only in the 1 year 1 day to 4 years age group less than 75% of the measurements are above the recommended range. It was found that the older the patient, the worse the metabolic control.

For the 22 patients with two null alleles between the ages of 1 year 1 day and 12 years– when the human brain is at its most vulnerable – 15 had a median Phe level within the recommended range (Fig. 2).

3.3. Results from the various age groups

3.3.1. Age group 1 year 1 day to 4 years

This age group had 10 patients, with 7 having two null alleles. The median Phe level at diagnosis was 37.5 mg% and the mean age at the time of first consultation was 14.3 days. The mean frequency of Phe control was 52 ± 25 times per year and the number of annual visits was 6.2 ± 2.6. It was found that 58% of all measurements were within the reference range, 12% were above 360 μmol/L, and the remainder were lower than 120 μmol/L. The median Phe level was 162 μmol/L, with

Table 1
Characterization of identified PKU alleles and their distribution in the Latvian population.

Variation nomenclature		Pathogenicity	Number of all PKU chromosomes ($n = 204$)[e]	Allele frequency %
Nucleotide position in coding sequence[a]	Nucleotide position in protein sequence[b]	ClinVar allele ID or ACMG criteria[c]		
c.1222C>T	p.Arg408Trp	VCV000000577.1[d] – pathogenic	142	69.61
c.838G>A	p.Glu280lys	VCV000000580.8 – pathogenic	11	5.39
c.473G>A	p.Arg158Gln	VCV000000587.7[d] – pathogenic	8	3.92
c.782G>A	p.Arg261Gln	VCV000000582.12[d] – pathogenic	5	2.45
c.842C>T	p.Pro281Leu	VCV000000589.11[d] – pathogenic	3	1.47
c.1315+1G>A	p.?	VCV000000576.14[d] – pathogenic	3	1.47
c.143T>C	p.Leu48Ser	VCV000000608.11[d] – pathogenic	2	0.98
c.311C>A	p.Ala104Asp	VCV000102650.5[d] – pathogenic	2	0.98
c.781C>T	p.Arg261Ter	VCV000000610.7[d] – pathogenic	2	0.98
c.960G>C	p.Lys320Asn	VCV000102910.4 – pathogenic	1	0.49
c.1066-11G>A, p.?	p.?	VCV000000607.12[d] – pathogenic	2	0.98
c.1208C>T	p.Ala403Val	VCV00002731.14[d] – pathogenic	2	0.98
c.1316-1G>A	p.?	VCV000635216.2[d] – pathogenic	2	0.98
c.168+5G>C	p.?	VCV000102606.10 - pathogenic	1	0.49
c.331C>T	p.Arg111Ter	VCV00000058.1.5[d] – pathogenic	1	0.49
c.441+5G>T	p.?	VCV000092742.8[d] – pathogenic	1	0.49
c.506G>A	p.Arg169His	VCV000102706.12[d] – pathogenic	1	0.49
c.533A>G	p.Glu178Gly	VCV000092746.6[d] – pathogenic	1	0.49
c.664_665delGA	p.Asp222Ter	VCV000133249.6 – pathogenic/ likely pathogenic	1	0.49
c.688G>A	p.Val230Ile	VCV000102784.10[d] – likely pathogenic	1	0.49
c.814G>T	p.Gly272Ter	VCV000000596.9[d] – pathogenic	1	0.49
c.874C>A	p.Pro292Thr	ACMG – likely pathogenic – PM1, PM2, PM5, PP2, PP3, PP4	1	0.49
c.886G>A	p.Asp296Asn	ACMG – likely pathogenic – PM1, PM2, PP2, PP3, PP4	1	0.49
c.898G>T	p.Ala300Ser	VCV000092751.12[d] – pathogenic	1	0.49
c.916A>G	p.Ile306Val	VCV000000618.8[d] – pathogenic	1	0.49
c.1066-3C>T	p.?	VCV000000623.5 – pathogenic/ likely pathogenic	1	0.49
c.1111A>G	p.Lys371Glu	ACMG – likely pathogenic – PM1, PM2, PP2, PP3, PP4	1	0.49
c.1241A>G	p.Tyr414Cys	VCV000000593.10 [d] – pathogenic	1	0.49
c.(446+1_447-1)_(509+1_510-1) del	p.?	ACMG – pathogenic – PVS1, PM2, PP3	1	0.49
ND			3	1.47

*ND - no pathogenic variant was found; [a] position in the sequence NM_000277.1; [b] position in the sequence NP_000268.1; [c] ClinVar allele ID, if allele were not reported there, ACMG criteria abbreviations are mentioned ([9]; [15]); [d] reviewed by expert panel – FDA recognized database, [e] frequency calculated in nonrelated patients – from families with multiple patients, there were included only one patient.

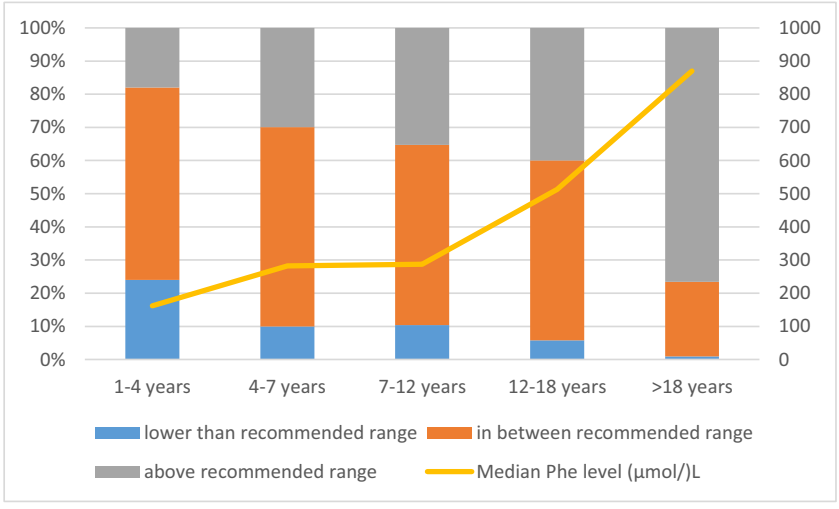

Fig. 1. Measurements of the Phe level in various age groups of PKU patients.

none of the patients having a median Phe level above the recommended level.

3.3.2. Age group 4 years 1 day to 7 years

This age group had 12 patients, with 7 having classical PKU with a low Phe tolerance. Only 3 of the patients delivered samples within the recommended time frame. The mean frequency of Phe control was 14.5

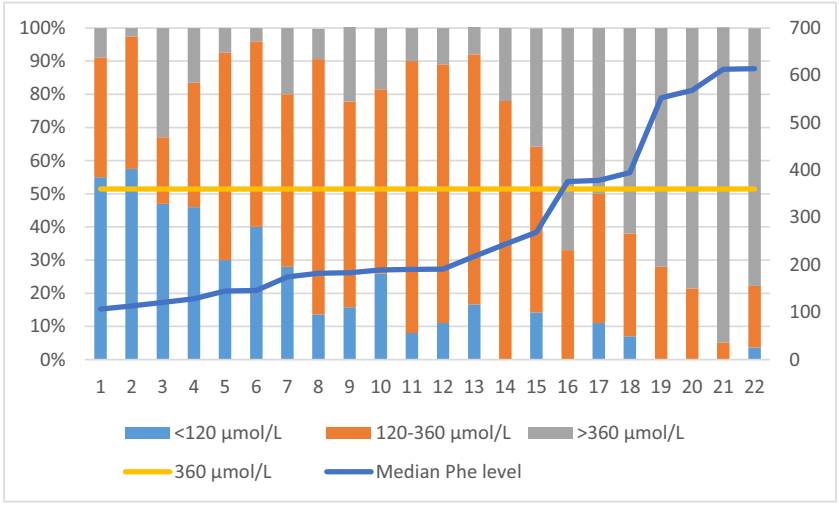

Fig. 2. Phe level control in patients with two null alleles between the ages of 1 year 1 day and 12 years ($n = 22$).

± 8.3 times per year. Although the median Phe level, 268.2 µmol/L, was within the reference range, one patient had a median Phe level of 613.8 µmol/L and another registered 568.2 µmol/L. It was found that 60% of all Phe measurements were within the reference range and 30% were above 360 µmol/L.

3.3.3. Age group 7 years 1 day to 12 years

This age group had 10 patients, with 8 having classical PKU with a low Phe tolerance and 2 receiving BH4 therapy. Phe sampling in this group was unsatisfactory; the average Phe level control was 12.3 ± 3.5 times per year, two times less frequent than recommended. Furthermore, none of the patients in this group followed the guidelines. The median Phe level was 207 µmol/L; however, only 54% of all Phe measurements were in the reference range, while 35% were higher than the recommended level.

3.3.4. Age group 12 years 1 day to 18 years

This age group had 11 patients, with 9 having classical PKU with a low Phe tolerance and 2 receiving BH4 therapy. The total sample count per year was 90, resulting in an average sampling frequency of 8.2 ± 4.4. The median Phe level, 538.2 µmol/L, was within the reference range, although one patient had a median Phe level of 1440 µmol/L. It was found that 54% of all Phe measurements were within the reference range and 40% were above 600 µmol/L.

3.3.5. Age group > 18 years

Although this age group had 67 registered patients, only 35 visited our clinic. Unfortunately, we do not know the reason(s) why patients fail to attend outpatient clinics and forgo therapy. To the best of our knowledge, 3 have died, 6 have left the country, some decided not to continue therapy because of a late diagnosis, and some stopped attending follow-up visits as far back as the 1990s. Of the patients attending follow-up visits, only some followed a strict diet – most of them had poor or no diet control. The number of samplings was 7 ± 5. In 20 of the 35 cases (57%), all the Phe measurements recorded during the last year were above the recommended level. Only 9 of the 35 exhibited a median Phe level below 600 µmol/L (Fig. 3).

4. Discussion

We present here the genotypic data from an updated cohort of Latvian PKU patients. By doubling the patient number in our study from 2003 [14], the frequency of the major disease causing variant p. Arg408Trp decreased from 76% to 69.61%. Despite this decrease, the p. Arg408Trp variant is still a leading cause of PKU with 49% (50 of 102 unrelated patient) of our PKU patients being homozygous for this mutation. These new data now establish Latvia as having the lowest frequency of the p.Arg408Trp variant of all the Baltic countries (Estonia 80% [12] and Lithuania 73% [8]). A recently published study from

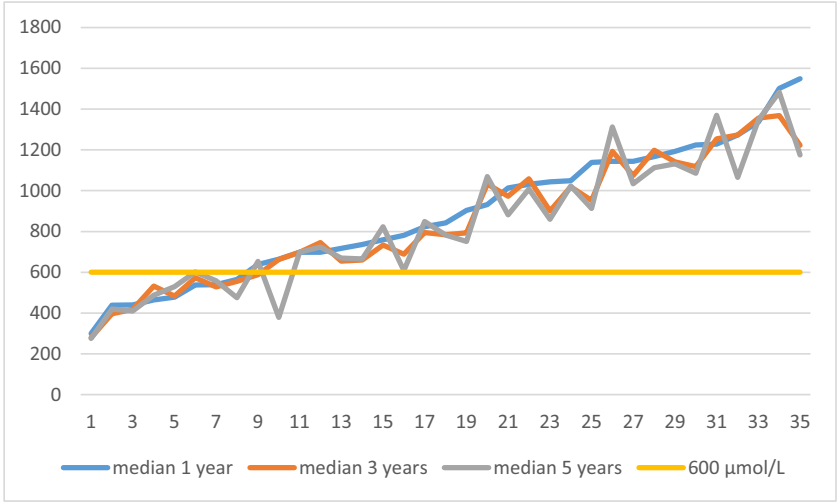

Fig. 3. Phe level control in the last five years in PKU patients older than 18 years.

Russia reported the frequency of this variant to be 50.9%. This finding was derived from a genotype analysis of a cohort consisting of 2579 patients [6], probably more closely reflecting the true frequency. The exceptionally high prevalence of this variant reported almost two decades ago in the Latvian population may have been a consequence of the small group of patients studied in which less common variants were underrepresented. The prevalence reported in the present study is closer to the figure obtained from the Russian study's large cohort. This may be due to the fact that a proportion of Latvian inhabitants are of Russian origin. As Latvia is a small country, a persistent major limitation of population studies is restricted patient numbers.

Three patients had allelic variants p.Pro292Thr, p.Lys371Glu and c.1315-1G>A which our group reported in 2003. To date, to the best of our knowledge, two variants have not been reported in other populations, but c.1315-1G > A reported also Su Y with colleagues [16]. Hyperphenylalaninemia panel consisting of 6 genes (Blueprint, Finland) is performed in all cases when diagnose is doubtful based on clinical symptoms. However we could not identify a second pathogenic variant in three patients.

Unidentified PKU causing variants in the *PAH* gene is also reported in other populations e.g. in Estonia in 2 of 94 patients were not identified none of pathogenic variants and in 2 of 94 patients were not identified second pathogenic variant [12]. It is proposed that PCR amplification and sequencing of all exons and adjacent introns achieves pathogenic variant detection in case of PKU - > 95–99%, and large deletion/duplication analysis <0.05% of variants [2].

Interestingly in Latvia there is only one confirmed case of BH4-HPA. Patient was heterozygous for two variants in *PTS*: c.200C>T, p.(Thr67Met) and c.315-1G>A, but unfortunately patient died and as he was not resident of Latvia his genetical background was different.

According to the pathogenic variant spectrum, there should have been at least six more patients receiving BH4 treatment; however, in reality, only five were receiving it. Two of them were doing very well with their relaxed diet therapy and their tolerance to natural protein was very high (1.0–1.2 g/kg/day). The three other patients, from the same family, were experiencing a delay to the start of their therapy due to social issues. One patient was just about to start BH4 therapy.

This article is the first to attempt to compile the molecular and biochemical data of Latvian PKU patients. Similar studies have previously been conducted in other European countries. For instance, in 2019 Lilleväli et al. published a report on the biochemical control of PKU patients in Estonia, one of Latvia's closest neighbors. In line with our study, their study found that younger children had a better metabolic control than school-aged children. However, we found that metabolic control worsened with age, whereas Lilleväli et al. observed that metabolic control improved and even stabilized after the age of 12 years [11]. Furthermore, an article published by Jurecki et al. in 2017 detailed that adherence to recommendations was age-dependent in PKU patients in the United States, decreasing from 88% in the 0–4 years age group to 33% in adults over 30 years of age [7]. As recently discussed [5], the main reason for this finding of metabolic control deteriorating with age might be that during infancy and childhood it is under the complete control of parents/guardians, whereas during adolescence – when teenagers seek autonomy – it is more difficult for parents/guardians to maintain control of it. It should be noted that although in childhood most of the patients had a median Phe level within the recommended range, the frequency of sampling was lower than recommended. Consequently, it is possible that the statistical results are more positive than reality.

The adult PKU patients in our study had a very different childhood diet experience to the one encountered by PKU children today. Funded by the government, we are now able to offer numerous low-protein products and Phe-free L-amino acid supplements. However, just two or three decades ago, far fewer low-protein products – which were generally unpalatable – were available in Latvia. This limited diet of unpleasant foods that our adult patients experienced in childhood may explain why they can struggle to follow a low-protein diet in adulthood.

The mental state of patients can affect their adherence to strict diets and clinic attendance for regular Phe level checking. However, this becomes less of an issue if patients with poor cognitive function are strictly supervised by a friend/relative. Of our adult patients, 17 (49%) suffer from mild or severe mental retardation. Of the nine (26%) with severe mental retardation, four live in social care housing and five live with their relatives and attend daily care centers. The patients with mild mental retardation either live with partial relative supervision or take care of themselves. It is this last group that is the most problematic and time-consuming for the PKU team with respect to diet and regular Phe level control.

Unfortunately, we have experienced awkward interactions with our PKU children and their relatives. It is normal practice to collect DBS at home then send them to the laboratory for Phe level checking. However, it has been observed that despite a normal Phe level measured by the laboratory, a child visiting the clinic can behave aggressively or appears very annoyed. In such situations, we often ask to take a second sample during the visit. Frequently, the Phe level in the second sample significantly differs from the level measured in the DBS collected at home. Parents sometimes refuse to consent to a second Phe level measurement during the visit, citing an excuse, like, for example, the child is afraid. Another situation that is difficult to explain is when a child has a median Phe level within the reference range but wildly inconsistent Phe level measurements, with some being extremely high (>360 μmol/L–600 μmol/L for small children), others being low (<120 μmol/L), and only a very few being normal. In some cases, it is possible that the parents are sending in DBS that have not been collected from their PKU child.

Taken together, our data have revealed that Phe level control is lacking but fortunately there are numerous ways to improve the situation.

5. In conclusion

Latvia exhibits a relatively homogeneous pool of disease-causing PKU alleles with a high prevalence of the classical severe form of PKU.

Our data reflect similar tendencies previously observed in other studies from different PKU management centers; specifically, an increase in cases of elevated Phe levels in parallel with age. We have ascertained that metabolic control and compliance with dietary treatment in teenager/adult PKU patients are poor. The main factor affecting compliance appears to be psychological difficulties in coping with dietary restrictions in society and the intrinsic negative features of amino acid supplements. Furthermore, the parents of late diagnosed patients can adopt a very negative attitude towards diet adherence. Unfortunately, as our PKU team is very small and we are unable to provide home visits, social workers, and medical nurses, this already adverse situation may be exacerbated. However, we are hopeful that our patients' metabolic control will improve in the future as more low-protein/protein-free products become available. We are also increasing our efforts to educate our patients on the importance of continuous Phe level checking by frequently inviting them to visit the clinic.

Author statement

Kreile M. – wrote a manuscript in consultation with Rita Lugovska, consulted patients.

Lubina O. - developed the theory and performed the computations.

Ozola-Zalite I. – wrote a manuscript in consultation with Rita Lugovska, consulted patients.

Lugovska R. – consulted patients since 1970ies.

Pronina N. – performed DNA analysis.

Sterna O. – performed DNA analysis.

Vevere P. – performed Phe level measurement from DBS.

Konika M. – performed Phe level measurement from DBS.

Malniece I. – was involved in planning and supervised the work.

Gailite L. – contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

All authors discussed the results and contributed to the final manuscript.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2020.100671.

References

[1] N. Al Hafid, J. Christodoulou, Phenylketonuria: a review of current and future treatments, Transl. Pediatr. 4 (4) (2015) 304–317, https://doi.org/10.3978/j.issn.2224-4336.2015.10.07.

[2] L. Birk Møller, A.O.H. Nygren, P. Scott, P. Hougaard, J. Bieber Nielsen, C. Hartmann, F. Güttler, L. Tyfield, J. Zschocke, Low proportion of whole exon deletions causing phenylketonuria in Denmark and Germany, Hum. Mutat. 28 (2) (2007) 207, https://doi.org/10.1002/humu.9481.

[3] N. Blau, Genetics of phenylketonuria: then and now, Hum. Mutat. 37 (6) (2016) 508–515, https://doi.org/10.1002/humu.22980.

[4] N. Blau, F.J. van Spronsen, H.L. Levy, Phenylketonuria, Lancet (London, England) 376 (9750) (2010) 1417–1427, https://doi.org/10.1016/S0140-6736(10)60961-0.

[5] A. Clark, C. Merrigan, E. Crushell, J. Hughes, I. Knerr, A.A. Monavari, E. Treacy, A. Coughlan, Ten-year retrospective review (2003−2013) of 56 inpatient admissions to stabilize elevated phenylalanine levels, JIMD Rep. 46 (1) (2019) 70–74, https://doi.org/10.1002/jmd2.12019.

[6] P. Gundorova, A.A. Stepanova, I.A. Kuznetsova, S.I. Kutsev, A.V. Polyakov, Genotypes of 2579 patients with phenylketonuria reveal a high rate of BH4 non-responders in Russia, PLoS One 14 (1) (2019), https://doi.org/10.1371/journal.pone.0211048 e0211048.

[7] E.R. Jurecki, S. Cederbaum, J. Kopesky, K. Perry, F. Rohr, A. Sanchez-Valle, K. S. Viau, M.Y. Sheinin, J.L. Cohen-Pfeffer, Adherence to clinic recommendations among patients with phenylketonuria in the United States, Mol. Genet. Metab. 120 (3) (2017) 190–197, https://doi.org/10.1016/j.ymgme.2017.01.001.

[8] J. Kasnauskiene, S. Giannattasio, P. Lattanzio, L. Cimbalistiene, V. Kucinskas, The molecular basis of phenylketonuria in Lithuania, Hum. Mutat. 21 (4) (2003) 398, https://doi.org/10.1002/humu.9113.

[9] C. Kopanos, V. Tsiolkas, A. Kouris, C.E. Chapple, M. Albarca Aguilera, R. Meyer, A. Massouras, VarSome: the human genomic variant search engine, Bioinformatics (Oxford, England) 35 (11) (2019) 1978–1980, https://doi.org/10.1093/bioinformatics/bty897.

[10] M.J. Landrum, J.M. Lee, M. Benson, G.R. Brown, C. Chao, S. Chitipiralla, B. Gu, J. Hart, D. Hoffman, W. Jang, K. Karapetyan, K. Katz, C. Liu, Z. Maddipatla, A. Malheiro, K. McDaniel, M. Ovetsky, G. Riley, G. Zhou, D.R. Maglott, ClinVar: improving access to variant interpretations and supporting evidence, Nucleic Acids Research 46 (D1) (2018) D1062–D1067, https://doi.org/10.1093/nar/gkx1153.

[11] H. Lilleväli, K. Reinson, K. Muru, S. Saarsalu, K. Künnapas, T. Kahre, Ü. Murumets, K. Õunap, The evaluation of phenylalanine levels in Estonian phenylketonuria patients during eight years by electronic laboratory records, Mol. Genet. Metab. Rep. 19 (2019) 100467, https://doi.org/10.1016/j.ymgmr.2019.100467.

[12] H. Lilleväli, K. Reinson, K. Muru, K. Simenson, Ü. Murumets, T. Möls, K. Õunap, Hyperphenylalaninaemias in Estonia: genotype-phenotype correlation and comparative overview of the patient cohort before and after nation-wide neonatal screening, JIMD Rep. 40 (2018) 39–45, https://doi.org/10.1007/8904_2017_61.

[13] A. MacDonald, H. Gokmen-Ozel, M. van Rijn, P. Burgard, The reality of dietary compliance in the management of phenylketonuria, J. Inherit. Metab. Dis. 33 (6) (2010) 665–670, https://doi.org/10.1007/s10545-010-9073-y.

[14] N. Pronina, S. Giannattasio, P. Lattanzio, R. Lugovska, P. Vevere, A. Kornejeva, The molecular basis of phenylketonuria in Latvia, Hum. Mutat. 21 (4) (2003) 398–399, https://doi.org/10.1002/humu.9114.

[15] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (5) (2015) 405–424, https://doi.org/10.1038/gim.2015.30.

[16] Y. Su, H. Wang, N. Rejiafu, B. Wu, H. Jiang, H. Chen, X. A, Y. Qian, M. Li, Y. Lu, Y. Ren, L. Li, W. Zhou, The molecular epidemiology of hyperphenylalaninemia in Uygur population: incidence from newborn screening and mutational spectra, Ann. Transl. Med. 7 (12) (2019) 258, https://doi.org/10.21037/atm.2019.05.16.

[17] A.M.J. van Wegberg, A. MacDonald, K. Ahring, A. Bélanger-Quintana, N. Blau, A. M. Bosch, A. Burlina, J. Campistol, F. Feillet, M. Giżewska, S.C. Huijbregts, S. Kearney, V. Leuzzi, F. Maillot, A.C. Muntau, M. van Rijn, F. Trefz, J.H. Walter, F. J. van Spronsen, The complete European guidelines on phenylketonuria: diagnosis and treatment, Orphanet J. Rare Dis. 12 (1) (2017) 162, https://doi.org/10.1186/s13023-017-0685-2.

[18] E. Vieira Neto, F. Laranjeira, D. Quelhas, I. Ribeiro, A. Seabra, N. Mineiro, L. M. Carvalho, L. Lacerda, M.G. Ribeiro, Genotype-phenotype correlations and BH(4) estimated responsiveness in patients with phenylketonuria from Rio de Janeiro, Southeast Brazil, Mol. Genet. Genomic Med. 7 (5) (2019) e610, https://doi.org/10.1002/mgg3.610.