Undiagnosed phenylketonuria in parents of phenylketonuric patients, is it worthwhile to be checked?

A. Wiedemann a, B. Leheup a,e, S.-F. Battaglia-Hsu d,e, P. Jonveaux c,e, E. Jeannesson d,e, F. Feillet a,b,e,*

* Corresponding author at: Centre de Référence des Maladies Héréditaires du Métabolisme, Service de Médecine Infantile et de Génétique Clinique, CHU Brabois Enfant, Allée du Morvan, 54500 Vandoeuvre les Nancy, France.
E-mail address: f.feillet@chu-nancy.fr (F. Feillet).

1. Introduction

Phenylketonuria (PKU, MIM# 261600) is an inborn error of metabolism, characterized by mutations of the phenylalanine hydroxylase (PAH) gene [1]. This enzyme converts phenylalanine into tyrosine and the loss of its activity increases the phenylalanine blood concentration which may lead to severe developmental delay in infants and cause progressive neurological troubles as well as psychiatric symptoms in adults [2]. The severity of the illness depends on the genotype, whose variability determines the residual activity of the enzyme[1]. Consequently, some patients with mild hyperphenylalaninemia (mHPA) or mild PKU (mPKU) can have rather mild clinical manifestations and have thus normal social and familial lives. In France, the diagnosis of PKU is achieved via a systematic neonatal screening program established already for more than 40 years with excellent outcomes for patients treated from birth [3]. Aside from the success for the pediatric patients, untreated adult PKU patients (undiagnosed or loss of follow-up) are at risk of neurologic problems [4] and in addition, for women the risk of hyperphenylalaninemic embryofetopathy if these mPKU or mHPA women are pregnant without strict metabolic control [5]. As we recently identified two cases of undiagnosed (or forgotten) mPKU or mHPA in the mothers of PKU children through the neonatal screening process, we wanted to determine the relative risk of having also mHPA/mPKU in parents of the PKU children. As this estimation needs the knowledge of the frequency of PAH heterozygosity and of mild mutation among the population studied, which are known for many countries [6–15], we propose here a formula for such calculation. The results of this calculation show that the prenatal risk of mHPA/mPKU is not negligible if their children are PKU positive. It can even be relatively high in countries with either a high PKU frequency, or with a high mild mutation rate and in countries where the PKU systematic neonatal screening has only just been implemented [16,17].

2. Subjects and methods

We will describe first the two relevant cases to illustrate the importance of diagnosis in the parental population of PKU patients, and then explain in detail the method of calculating the risk of mHPA/mPKU in parents with PKU-positive kids.

2.1. Case reports

In our center, the PKU cohort is composed of a group of 120 patients, whose size increases every year — with 2 to 3 new cases diagnosed by neonatal screening. We report here two parental cases of mHPA/mPKU identified among the mothers of the 120-member cohort.
2.1.1. Patient 1

The first case concerns a boy who was born full-term with intrauterine growth retardation (weight: 2.75 kg, height: 47 cm and head circumference: 32 cm). The PKU neonatal screening test right after birth had revealed his blood Phe level at 1100 μmol/L. He had been treated by diet with a regular metabolic and nutritional follow-up, but despite the excellent metabolic control (98% of Phe levels under 600 μmol/L during the first 10 years of life), he presented microcephaly and development delay (IQ done by WISC IV: 68). We examined this child when he was 11 years old as he was prepared to move out of town for family reasons. With the fact that the PKU metabolic follow-up was excellent during infancy and childhood (till 10–11 years old) and that at birth he already showed microcephaly, we looked for other causes to explain his antenatal microcephaly including maternal PKU. Consequently, we examined his mother and measured her blood Phe level. As her Phe level in blood stood around 852 μmol/L, we suspected thus that the mother also suffered from the same disease. This diagnosis was confirmed by DNA sequencing: the mother bore compound heterozygous mutations for both a mild p.Y414C mutation, with a residual PAH activity at 36% [18] and a classic severe p.R408W mutation. Consequently, we concluded that the child suffered from both PKU and maternal PKU syndrome. The screening of PKU had not been performed on this particular mother before because she was born in 1970, just before the beginning of the systematic neonatal screening in France (1972).

After her diagnosis, this woman was fully evaluated and subsequently followed up in our hospital. We were able to identify in her further investigation that the sister of this mother also has mPKU and that the two children of her sister both have maternal PKU syndromes. The screening of PKU had not been performed on this particular mother before because she was born in 1970, just before the beginning of the systematic neonatal screening in France (1972).

2.1.2. Patient 2

The second patient is a little girl, screened at birth with a Phe level at 2460 μmol/L. She was classified as classic PKU, BH4 unresponsive, and was treated by diet. Faced with the diagnosis, the mother recalled herself being screened for hyperphenylalaninemia (HPA). Going back to the mother’s file, we found that she had at birth a Phe level of 540 μmol/L, classifying her as mHPA. As the policy in France is to treat only patients having Phe levels above 600 μmol/L, she was not treated and was rapidly forgotten without further medical attention. Despite this, she appeared to enjoy a completely normal life and was completely asymptomatic at 26 years old. The DNA analysis showed that the baby girl was homozygous for the severe p.R408W mutation while her mother was compound heterozygous for a very mild mutation p.V245A which is known to have a high residual activity of 50% [18] and the classical severe mutation p.R408W. We checked and found the blood Phe level of the mother at 480 μmol/L, and we did not detect any sign of embryofetopathy in her PKU baby who exhibited normal neonatal anthropometric parameters and who had a strictly normal development at three years of age. Finally, we discovered that the mother’s sister was also screened positive in the neonatal period for mHPA and was also lost from medical follow up since infancy.

2.2. Undiagnosed PKU frequency calculation

2.2.1. Principles of calculation

Alerted by these two cases, we wanted to determine the frequency of undiagnosed PKU in parents who have given birth to PKU children. It is worth noting that our calculation below applies mainly to those parents who are asymptomatic or poorly symptomatic because those with severe undiagnosed PKU are so severely neurologically impaired that they are not able to have children. The genotypes of the mHPA or mPKU patients often contain at least one mild mutation since patients who have two severe mutations typically exhibit a severe form of PKU.

To calculate the frequency of undiagnosed PKU in the parent of a PKU child, we must assume that the PKU parent has a mHPA or a mPKU which is clinically silent for at least 20 years [3] and has at least one mild mutation. We also need the information about the carrier frequency for mutations in PAH gene (using the Hardy–Weinberg Law) and the percentage of the mild mutations in the studied population. The characteristic of a mild mutation can be described by the known in-vitro residual activity in the published database [18].

2.2.2. Calculation

To calculate the risk for a PKU patient to have at least one of his parents with undiagnosed mHPA or mPKU, we need to know the PAH heterozygosity rate and the percentage of the mild PAH mutations in the studied population.

As HPA frequency is known for many countries from the literature, we calculate the carrier frequency (C) by the Hardy–Weinberg law and the risk of transmission is C/2. The mild PAH mutations rates (B) are also known from the literature. With all these parameters we were able to calculate the risk for one PKU child to have at least one parent with undiagnosed mPKU or mHPA (Fig. 1).

As we can assume that one grandparent obligatorily gives one mutation which can be either mHPA or mPKU, there are two possible scenarios.

- First scenario: the first grandparent gave the mild mutation (mild mutation frequency) and the second grandparent gave any other kind of mutation (carrier frequency).
- Second scenario: The first grandparent gave the severe mutation (1–mild mutation frequency) and the second grandparent must have given a mild mutation (mild mutation frequency).

The risk for one parent to be mHPA or mPKU is the sum of these two scenario probabilities.

We can resume the risk with the following formula in which:

- C represents the carrier frequency = \(2\sqrt{A} (1-\sqrt{A})\) in which A is PKU frequency
- B represents the percentage of mild mutations in all PAH mutations identified.

Risk for one parent to be a mPHA or a mPKU = \((B \times C / 2) + ((1 - B) \times C / 2 \times B)\).

As there are two parents, this risk is multiplied by two for one patient.

Final risk for a PKU child to have a mHPA or mPKU parent = R.

\[ R = ((B \times C /2) + ((1-B) \times C/2 \times B)) \times 2. \]

3. Results

In the French population, the frequency of PKU is 1/8557 (2011 report of the French Association for Newborn Screening: AFDPHE) [19]. As the Hardy–Weinberg law allows us to calculate the carrier frequency to be 1/47, and by estimating that the frequency of mild PAH gene mutations is about 34% in France (unpublished data from 697 genotyped PKU patients), we are able to calculate the risk to be 1/83 for a HPA patient to have one of his parents touched by mHPA or mPKU. Since for many countries, the frequency for mild mutations in PAH gene and the carrier frequency for PKU are known from the literature, we also are able calculate this risk for these countries (Table 1).

4. Discussion

Our study here demonstrates that even in countries where a systematic neonatal screening for PKU has been implemented for more than
40 years [20], there are still important risks in finding patients with undiagnosed PKU, in particular those having PKU kids. In our cohort, we observed two cases of non- (or forgotten) diagnosed PKU in the mothers of two PKU children previously identified through the neonatal screening. These two cases are complementary because one of the mothers was born in 1970 — before the initiation of mandatory neonatal PKU screening in France, and the other mother was born in 1983 and identified then at birth as a baby with mild HPA during the neonatal PKU screening but forgotten until the PKU diagnosis of her first child. These findings further permitted the diagnosis of mPKU in the sister of the first mother (and maternal PKU syndrome in her two children who were mentally retarded) and mild HPA for the sister of the second mother. In France, a survey done in 2002 (unpublished data) showed that nearly 25% of women screened positive for hyperphenylalaninemia were lost to follow-up. The percentage is much higher in men, even though the precise percentage has not been established. Our present study shows that the prevalence of undiagnosed (or forgotten diagnosis) mHPA or mPKU can be rather important in the parents of the PKU patients whose genotype analyses revealed a frequency of 34.1% for mild PAH gene mutations (unpublished data). This mild mutation frequency is probably underestimated because the last (2011) report from AFDPHE (French Association for Neonatal Screening) showed that mHPA and mPKU patients represent 63% of all PKU patients (50 mild HPA + 9 mild PKU for 35 classic PKU) while we had only 37% of mHPA and mPKU in the cohort of genotyped patients. If we recalculate this value based on this higher national mild mutation frequency (at 60%, which is more realistic considering the percentage of mHPA and mPKU detected by the neonatal screening), the risk value would increase to 1/56. In any case, the minimal probability of 1/83 to have a mHPA or mPKU parent for all children screened positive in PKU neonatal test makes very relevant a procedure of systematic examination for HPA in the parents of PKU-positive babies. The calculations done for other countries mentioned here also give an estimate for the minimal risk in detecting parents of PKU patients with mHPA or mPKU as the published HPA frequencies are not always considering mild HPA cases. Effectively, the mild mutation rates depend on the type of patients who were genotyped, but in published data, the degree/detail of severities of the HPA populations is often not mentioned. Nevertheless, we can see that the risk can vary widely from one country to another as a function of HPA frequency and mild mutation rate, which is a crucial determinant.

We suggest that the implementation of this type of reciprocal detection protocol is necessary since there is a rather high probability to find parents of any HPA-positive child with mHPA or mPKU. This procedure can be justified by the benefits obtained by such detection protocol.

Using the French data, we were able to calculate that the risk for a HPA child to have one of his parents with mHPA or mPKU is about 1/83. This calculation was based on the data obtained from a cohort of 697 French patients, whose genotype analyses revealed a frequency of 34.1% for mild PAH gene mutations.

### Table 1

<table>
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<th>Country [ref]</th>
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<th>B</th>
<th>R</th>
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<td>Croatia [14]</td>
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<td>1/103</td>
</tr>
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</table>

A: PKU frequency.
B: % of mild mutations.
R: risk for a HPA child to have one of his parents with mHPA or mPKU.

- First, the affected parents, despite their age, can be properly treated. There is today a worldwide consensus for treating PKU for life [21,22]. The detection of mPKU in patients such as that reported in our first observation allowed us to propose to her a low phenylalanine diet as her Phe levels were regularly above 1200 μmol/L. This applied similarly to all adult patients. As we are likely to detect various forms of mild HPA in these parent patients, some of these adult patients may be BH4 responsive, the proper diagnosis will allow them either to be treated by BH4 [23] or to be assisted by specialized medical staff to begin a low phenylalanine diet often too challenging to initiate during adulthood. Additionally, through this reciprocal detection procedure proper medical follow-up can now be done in some of these forgotten patients, as in the case 2 reported above. This is especially important for certain patients who were initially followed up by some medical centers where the follow-up for the PKU patients was stopped after these patients reached adolescence (before 1990).

Fig. 1. Pedigree of a PKU patient who has an undiagnosed mHPA or mPKU parent.

![Pedigree of a PKU patient who has an undiagnosed mHPA or mPKU parent.](image-url)
- The second major point is the prevention of maternal PKU syndrome in case of further pregnancies. This is obvious for the potential siblings of the positively-screened child but it is also relevant for the sisters of the mother. If we had diagnosed mPKU in the mother of our first case report, we would be able to diagnose it in the mother’s sister and we could have avoided the maternal PKU syndrome of her two children who were born after our patient.

- Third, a different genetic counseling to the patient’s family will be needed after taking into account the finding of mHPA or mPKU in the parents of the PKU-positive child. A genetic counseling to the parents is normally given after the diagnosis of PKU in a new born baby. But, if the parents are also found affected by mHPA or mPKU, their PAH gene will also be sequenced and their mutations will be taken into account during the genetic counseling. It is important to convey to these parents the following information. First, the recurrence rate for risk of PKU is 50% and not 25% as in the case of two heterozygous parents. Second, the second PKU child of this family (if there is any) can have a different form of PKU than the first case. This idea is illustrated in our second case report: the mHPA-positive mother has both a very mild mutation p.V425A and a classic severe mutation p.R408W while the father is heterozygous for the severe mutation p.R408W; her children can be heterozygous (50% of cases), severe PKU (25%: homozygous for the p.R408W mutation) or mHPA (25% with the same genotype as the mother).

One may argue against this parental detection procedure since in most countries where the PKU neonatal screening has been implemented from the seventies, the new parents these days have gone through the neonatal screening themselves. It is said that in these countries, the women lost to these events, then the parents these days have gone through neonatal screening in both parents of any child having PKU, especially if this child is born in a country where the implementation of the systematic neonatal screening has been done in the whole country. We think that a systematic search for hyperphenylalaninaemia should be done in both parents of any child having PKU, especially if this child is born in a country where the implementation of the systematic neonatal screening process is only rather recent. We further recommend the implementation of this procedure in countries with high PKU frequency as well as in countries with high immigration rate, such as most European countries.

References