

Late diagnosis of phenylketonuria with p.L48S/p.R408W genotype and late positive response to tetrahydrobiopterin – case presentation and literature review

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Abstract

Phenylketonuria (PKU) is an autosomal recessive disease with important consequences on nervous system development, if not properly treated. Decrease of the antioxidative mechanisms, altered transport of amino acids through the blood-brain barrier, low brain protein synthesis, hypomyelination are the main contributors to the neural damage and central nervous system functional impairment. Diagnosis of PKU and hypothyroidism are part of the neonatal screening program for inborn diseases and an early diagnosis should be provided in order to prevent complications.

We are reporting here a case of a late-diagnosed PKU with a p.L48S/p.R408W genotype, with a clinical evolution more severe than in cases with similar genotype, despite a particularly good late response to tetrahydrobiopterin (BH4) on the metabolic status. The severity of the evolution with persistent neuro-psychic impairment could be related to the associated subclinical hypothyroidism and the attention deficit hyperactivity disorder (ADHD) syndrome. Hypertrophic and hyperplastic obesity, cutis laxa and inverted nipples were also diagnosed and the BMI over the 99th percentile was maintained at those levels afterwards. In the discussion section, we review the literature covering the complexity of the mutual influences of these medical conditions in PKU patients.

Through this case presentation, we underline the importance of neonatal screening as a major preventive action of neurological impairment. We also highlight the importance of performing genotyping in PKU and of a complete (48h) sapropterin test in order to avoid missing the late responders, in PKU patients with p.L48S/p.R408W genotype.

Keywords

Phenylketonuria, subclinical hypothyroidism, attention deficit syndrome, obesity, tetrahydrobiopterin, neonatal screening.

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How to cite

Moldovanu F, Calin RB, Nanu MI, Otelea MR. Late diagnosis of phenylketonuria with p.L48S/p.R408W genotype and late positive response to tetrahydrobiopterin – case presentation and literature review. *J Pediatr Neonat Individual Med.* 2020;9(1):e090117. doi: 10.7363/090117.

Case presentation

We report a case of a 7-year-old boy diagnosed with phenylketonuria (PKU) at the age of 3 years, with a severe psychomotor disability, an intelligence quotient (IQ) score between 20 and 30 and a global development quotient (QD) score of 29.23.

The patient's first medical records date from around the age of 3, when the result of the blood Phe level (1,301.36 $\mu\text{mol/L}$) raised the suspicion of PKU. At that age, his height was 105 cm and his weight was 18 kg. The boy showed autistic behaviour and was unable to communicate with his parents. No cardiovascular, respiratory and urinary system modifications were found on the clinical examination. Urine and stool examination offered no abnormal findings.

He was born in the 37th gestation week with a lower than normal weight of 2,300 g. During pregnancy, his mother followed a treatment with a uterine muscle relaxant.

MRI scans made visible a slightly dilated ventricular system and a hypointense T2 signal in the brain stem. Blood analysis showed high levels of Phe (989 $\mu\text{mol/L}$). A classic PKU diagnosis was assumed based on the high value of Phe/Tyr ratio (39.38) and on the high levels of ammonia (116 $\mu\text{mol/l}$). Blood count, liver and renal functions were within the normal range. Genetic evaluation was performed, showing 2 heterozygote mutations of the phenylalanine hydroxylase (PAH) gene (PAH ENST00000553106 C. [1222C>T]/[143T>C],

ENSP00000448059 P. [Arg408Trp]/[Leu48Ser]). Both mutations are described in the BIOPKU database [1]. Genetic examination of parents was unavailable, so it is not known whether the mutations are on the same allele or not.

The tetrahydrobiopterin (BH4) oral overload test was performed with four tablets of a synthetic sapropterin (sapropterin dihydrochloride, Kuvan®); blood tests were collected at 0, 4, 8, 12 and 16 hours. The Phe levels slightly decreased and a diagnosis of classic PKU was given. Because there was no collection of blood after 24 and 48 hours, detection of late BH4 response was missed.

A low life-long Phe diet was recommended in order to keep the Phe blood levels within the accepted range of 120-360 $\mu\text{mol/L}$ [2]. The diet consisted of low Phe foods and Tyr and L-amino acids as supplements. Values of Phe, Tyr decreased afterwards. Despite diet and amino acid supplementation, 2 years after the confirmation of PKU, symptoms did not significantly improve.

In December 2015, the neuropsychiatric examination revealed an attention deficit hyperactivity disorder (ADHD) syndrome; psychological evaluation showed moderate retard and a cognition development at pre-operational level. The IQ was between 20 and 30 and the QD was 25. He was easily distracted, unable to maintain attention for more than 2-3 seconds and did not have object permanence. There was no usage of verbal communication and the basic needs were transmitted to the parents by non-verbal means. The general psychomotor developmental age was around 2.5 years and the imagination and memory capacities were below 2 years old, although he was 5 years old at that time. He had underdeveloped gross and fine motor skills; the orthostatic walking and equilibrium were at a normal level for 25 months of age. Imitation, grip and handling, tool usage, visual motor skills measured were at around 20 months of development. An irritative diagram (higher power of all frequency bands) of the EEG, predominantly in the frontal areas, was found. Amelioration in the EEG diagram was observed after 1.5 years of dietary treatment.

The latest psychological examinations detected some progress in mental development and in social behaviour, such as syllabification, increased attention to the surroundings, and focus up to 10 seconds.

Fifteen months after the PKU diagnostic, the patient was also identified with subclinical

hypothyroidism, with high TSH (8.18 μ IU/mL) and normal FT4 and FT3 hormones (18.69 pmol/L, respectively 7.38 pmol/L). Levothyroxine 25 μ g/day was prescribed and, under this treatment, the TSH, FT3 and FT4 hormones were kept within normal values ever since.

The hypertrophic and hyperplastic obesity, cutis laxa and inverted nipples were first diagnosed in 2015. The BMI was over the 99th percentile (according to the WHO charts) and was maintained at those levels afterwards. In 2016, elevated blood total cholesterol (1.54 mmol/L) and triglycerides (1.22 mmol/L) were noticed. The cholesterol level remained high (above 4.4 mmol/L), but the triglycerides values returned to normal at the following measurements. Furthermore, a high insulin response (63.17 μ IU/L) was detected during the first hour of a glucose load test in 2016; at the end of the test (two hours from the glucose intake), the insulin level decreased to normal. The hypertrophic and hyperplastic obesity was interpreted as related to the imbalanced nutrition and, possibly, in association with the hypothyroidism. Parents admitted to having difficulties in managing the recommendations to maintain normal weight and that the child had a low level of physical activity.

As diet treatment has not given significant results, in 2015 sapropterin dihydrochloride therapy was tested again. The positive effect became manifest only after 48 hours, when the blood Phe value dropped from 5.18 mg/L to 1.42 mg/L. The Phe intake tolerance rose from 550 mg/day to 800 mg/day, decreasing the strictness of the diet. These findings classified the patient as late responder and explained why the results for the previous test were negative. From there, 300 mg per day of sapropterin dihydrochloride was prescribed. In accordance, the primary diagnosis was changed to PKU with BH4 deficiency. After 4 years from the diagnosis of PKU, the sapropterin dihydrochloride dose was increased to 400 mg per day, further augmenting the daily Phe tolerance to 1,000 mg. Subsequently to the introduction of the sapropterin dihydrochloride therapy, the values of the Phe varied between 237.77 and 75.58 μ mol/L, with one exception when it was 326.94 μ mol/L.

Over the years, the patient received various secondary diagnoses that could be associated with the diet treatment: hypercalcemia, eosinophilia, hypozincemia, eating disorders. High values of ammonia, vitamin B12, vitamin B6, taurine and tryptophan were also linked to medication.

Eosinophilia, with values between 600-800 eosinophils/ μ l persisted since 5 years old; parasitic diseases were constantly searched for, but all tests were negative.

Up until now, the child has been kept under observation with regular blood tests and hospitalizations. The permanent treatment is sapropterin dihydrochloride and levothyroxine (Euthyrox®, 25 μ g) with intermittent amino acid supplementation. Despite the normal levels of Phe and of the thyroid hormones, he still has a severe psychomotor impairment. His parents are advised to seek genetic counselling before conceiving another child.

Discussion

We report a case of late treated PKU with a p.L48S/p.R408W genotype. The p.L48S is a missense mutation rather frequent in the Balkans, with a variable phenotype, from severe [3] to moderate or mild [4]. The protein produced in subjects with this mutation is more rapidly catabolised than normal [5]. The R408W mutation causes structural distortion and aggregation of the PAH, with a total loss of the protein functionality [6]. The increased predisposition of mental illness in patients with R408W mutation was assumed in some studies [7] but was not confirmed by others [8]. Besides being a cofactor for PAH in the transformation of Phe in Tyr, BH4 binds to both unstable enzymes and protects them from the degradation, eventually increasing their biological effect. Therefore, these mutations are generally classified among the BH4 responders [9]. According to BIOPKU [1], this genotype was reported in only 72 cases, of which 40 had classic PKU and 32 had a mild form of the disease.

Our patient distinguishes from previously reported cases with the same genotype in both aspects: first, he has a severe, persistent, neuro-psychic impairment, most probably due to the delay in diagnosis (after the age of 3) and possibly to the co-morbidities (ADHD, subclinical hypothyroidism); second, he had a rather good but late response to BH4 therapy, a finding communicated only in 6 cases registered in this database.

The severity of the disease in late-diagnosed patients is the main argument for neonatal screening. However, improvement in the intelligence score [10, 11], attention, happiness

and energy levels [12] in late-diagnosed classic PKU were also reported after the introduction of the proper diet and treatment. The presumed mechanisms explaining this lack of response are presented in **Fig. 1**.

The coexistence of the ADHD and the subclinical hypothyroidism have probably influenced this poor response. PKU is considered one of the “organic” causes of autism [13]. Apparently, autistic symptoms are present also in moderate and mild cases [14], but are more frequent in the late-diagnosed ones [14, 15], as this case is. After the BH₄ treatment was started, we have recorded some benefit on the attention scale, in parallel with the decrease in the Phe levels, as found in a randomized clinical trial [16], but this child remained by now with a severe neuro-psychic impairment.

Screening for hypothyroidism was not performed at birth and there is not any documented proof for a congenital form. The mother had no thyroid disorder

and the boy was eutrophic at birth; therefore, we can only presume that at birth the thyroid function was normal. In children and adolescents, subclinical hypothyroidism progression to overt hypothyroidism is rare [17]. There is no consensus on how the subclinical hypothyroidism affects the neuro-psychic development with studies that found negative effects such as attention deficits [18] or spatial and verbal memory impairment [19] and others that found the intellectual development and verbal communication not to be affected even in those who never received levothyroxine [20]. Considering these observations, the contribution of the subclinical hypothyroidism to the IQ and QD impairment is difficult to estimate. Treatment in subclinical hypothyroidism is not advisable; however, our patient cumulated several risk factors, including proatherogenic metabolic abnormalities that balanced the decision towards substitution therapy and monitoring, in line with current recommendations [21].

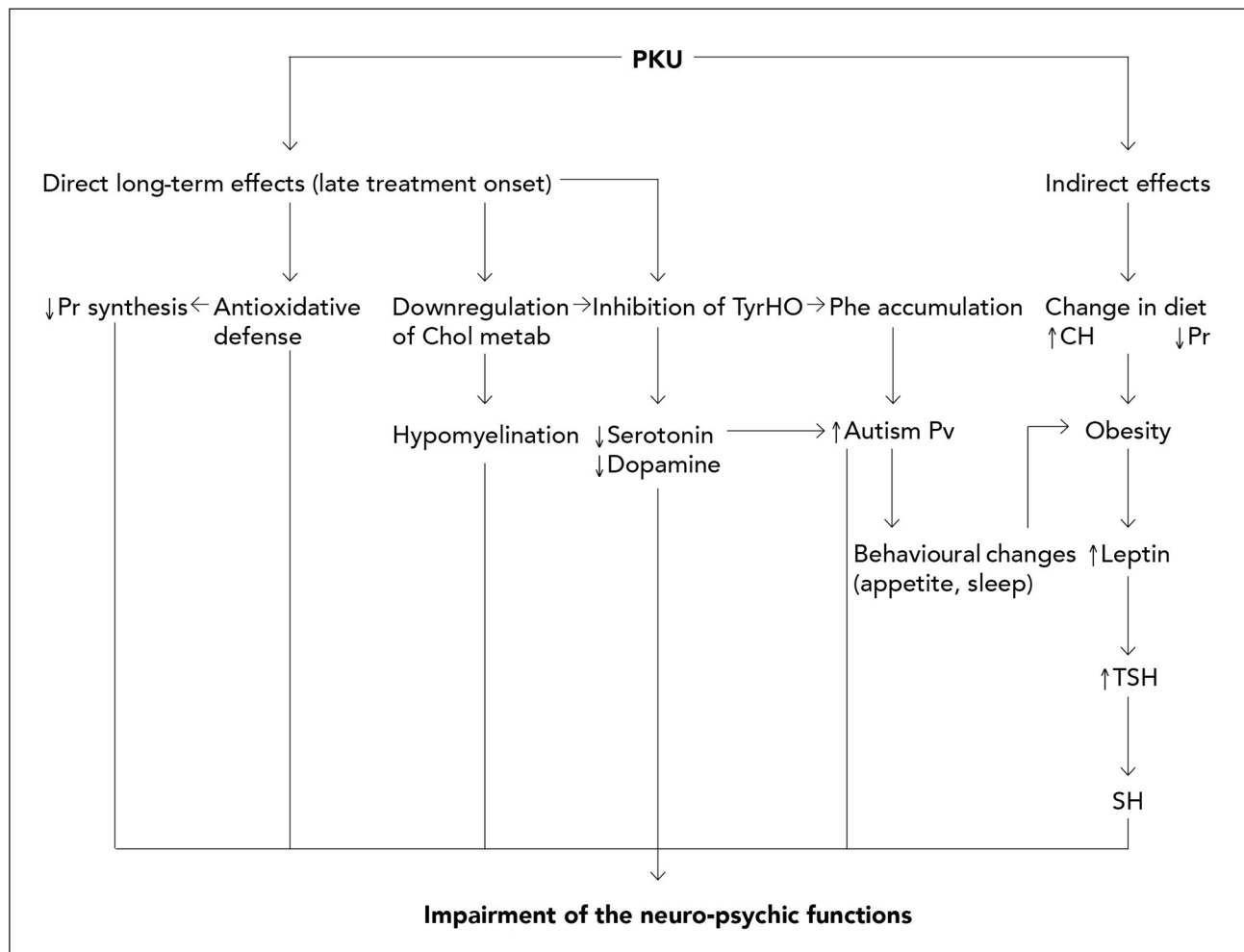


Figure 1. Presumed mechanisms for the persistence of the neuro-psychic symptoms.

PKU: phenylketonuria; Pr: protein; TyrHO: tyrosine hydroxylase; Phe: phenylalanine; Chol: cholesterol; metab: metabolism; CH: carbohydrates; TSH: thyroid stimulating hormone; SH: subclinical hypothyroidism; Pv: prevalence.

A possible explanation for the subclinical hypothyroidism is obesity itself. The association between subclinical hypothyroidism and obesity was found in a large cross-sectional study [22]; another proof of this close relation came from prospective research showing significant reduction in the plasma TSH and of the thyroid volume after weight loss program became efficient [23]. This direct relation is biologically supported by the positive feedback between TSH and leptin, an adipokine secreted in excess from hypertrophic adipocytes [22, 24]. TSH promotes the differentiation of preadipocytes into adipocytes [25]. Activation of the TSH receptors in the adipose tissue induces an increase in the leptin production [26], while leptin stimulates the TSH release [27].

In general, PKU patients have a down-regulated cholesterol metabolism that predisposes them to low serum cholesterol levels. The boy presented here had intermittent modifications of the serum metabolic markers (total cholesterol, triglycerides, hyperinsulinemia and impaired glucose tolerance). These modifications could all be related to overweight, which becomes an important issue in PKU patients nowadays [28]; the lipid metabolism impairment could be a “silent” effect of the subclinical hypothyroidism [29]. Boys diagnosed with PKU are not more frequently overweight and obese [30], even with the increasing prevalence of high weight. In children with autism spectrum disorders, the changes in diet preferences, sleep and affective problems are known risk factors for obesity [31]. In our patient, the glucose tolerance and the triglycerides normalized after BH4 and levothyroxine treatment were started, but the total cholesterol remained above the limits and the BMI persisted in the obesity range. Loose diet as consequence of the positive response to the therapy and low activity level contributed to these effects.

Conclusions

Complying with the current neonatal screening program is an effective tool for the prevention of the PKU and congenital hypothyroidism complications. Delayed intervention (after 3 years old) had minimal benefit in 3 years of follow up, even in a genotype considered responsive to treatment.

A complete (48h) BH4 test should be performed in PKU patients with a p.L48S/p.R408W genotype in order to avoid missing the late responders.

The diet supplements should be monitored to prevent possible side effects or overdose of micronutrients that could additionally affect inflammation and metabolism.

Declaration of interest

The Authors declare no conflict of interest. There was no financial funding for writing this manuscript.

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