The Complexity of Newborn Screening Follow-Up in Phenylketonuria

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Abstract In the United States, and most developed nations, the newborn screening (NBS) panel covers many primary disorders of metabolism, including phenylketonuria (PKU). When an elevated phenylalanine level is identified, the infant is evaluated for PKU and should also be tested for tetrahydrobiopterin (BH₄) deficiency. A neonate presented with a phenylalanine level of 254 $\mu\text{mol/L}$ (reference range $<138 \mu\text{mol/L}$) on newborn screening. The infant's confirmatory phenylalanine was 118 $\mu\text{mol/L}$ (reference range $<77 \mu\text{mol/L}$). Her urine pterin profile was normal, and initially she had no measurable activity of red blood cell (RBC) dihydropteridine reductase (DHPR). Subsequent study revealed normal levels of CSF tetrahydrobiopterin and neurotransmitter metabolites, and by 18 months of age, her RBC DHPR activity was detectable at 0.5 nmol/min/mgHgb (reference range 0.8–3.9). Sequencing of the QDPR gene for DHPR revealed c.1A>T nucleotide substitution in exon 3 expressed as “p.MET1?” Phenylalanine hydroxylase (PAH) gene sequencing revealed compound heterozygosity for L249F and A300S. Although initial testing suggested the child was affected with DHPR deficiency,

further analysis, finding increasing levels of DHPR activity and PAH compound mutant heterozygosity, indicated that the primary disorder is mild hyperphenylalaninemia with carrier status for DHPR deficiency. This is an example of newborn screening results leading to confusing findings requiring extensive biochemical studies and genotyping in order to arrive at the appropriate diagnosis.

Introduction

It is generally known that newborn screening (NBS) in the United States and many developed nations now includes screening for many primary metabolic diseases. What is not broadly understood are the complexities and uncertainties involved in the follow-up of many abnormal results. We present the case of a neonate detected with a mild elevation of phenylalanine on NBS which usually indicates mild hyperphenylalaninemia (MHP) but can point to a disorder of tetrahydrobiopterin (BH₄) metabolism. Consequently, initial follow-up for an elevated phenylalanine requires not only quantitative confirmation of the elevation and determination of either PKU requiring dietary therapy or MHP, which is benign, but also assessment of the urinary pterin profile and assay of red blood cell (RBC) dihydropteridine reductase (DHPR) activity. Differentiating PKU or MHP from BH₄ deficiency is critical since the management of these conditions is different.

In the case presented, evaluation following the newborn screen required plasma, urine, and CSF studies and full sequencing of both the PAH and QDPR genes in the proband and her parents to achieve a definitive diagnosis.

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Clinical Report

The infant, born at 41 weeks gestation to a G1P0 mother, had a positive NBS suggestive of phenylketonuria (PKU), with a phenylalanine level of 254 $\mu\text{mol/L}$ (reference range $<138 \mu\text{mol/L}$) at 34 h of age. On physical exam, the patient was a vigorous female neonate with a normal exam. Laboratory studies showed a phenylalanine level of 118 $\mu\text{mol/L}$ (reference range $<77 \mu\text{mol/L}$) and 5 days later, 158 $\mu\text{mol/L}$. Since the hyperphenylalaninemia was less than 360 $\mu\text{mol/L}$, no treatment was initiated. At 9 days of age, the patient's RBC DHPR activity was reported as undetectable (reference range 0.8–3.9 nmol/min/mgHgb). Analysis of urine pterins revealed biopterin of 23% (reference range 11.5–50.7). Repeat urine pterins indicated 25.7% biopterin. Repeat RBC DHPR activity at 12 days of age was 0.2 nmol/min/mgHgb (reference range 0.8–3.9). These results suggested that a primary defect in BH_4 recycling was the cause of the initial phenylalanine elevation. Given these findings, a lumbar puncture was performed for the measurement of CSF neurotransmitter metabolites and concentration of BH_4 . The result indicated normal levels of CSF neopterin, tetrahydrobiopterin, 5-methyltetrahydrofolate, and the neurotransmitter metabolites, homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA). However, neonates with DHPR deficiency, likely peripheral, can have normal CSF BH_4 and neurotransmitter metabolites (Opladen et al. 2012). Sequencing of the QDPR gene for DHPR was performed and revealed a single c.1A>T nucleotide substitution in exon 3. This is expressed as “p.MET1?” with the “?” indicating unknown effect on protein translation. Deletion/duplication testing of the gene was negative. Sequencing of the PAH gene revealed two mutations: L249F and A300S, a genotype consistent with MHP (Guldberg et al. 1998). At 18 months of age, the infant's RBC DHPR activity had risen to 0.5 nmol/min/mgHgb (reference range 0.8–3.9). Parental testing revealed that the mother carried both the A>T alteration in the QDPR gene and the L249F mutation in the PAH gene. Her RBC DHPR activity was 0.8 nmol/min/mgHgb, at the lower end of the normal range. No changes were identified in the QDPR gene of the father, and he was found to carry the A300S PAH gene mutation. Deletion/duplication analysis of the DHPR gene was negative in both parents. At 2 years of age, growth and development in the infant has been normal with no signs of clinical disease.

We concluded that the phenylalanine elevation in the infant is due to the hypomorphic PAH genotype (MHP) and that given the increasing RBC DHPR enzyme activity over time and the presence of only a single QDPR mutation in the patient and her mother and no QDPR mutation in the father, the infant is only a carrier for DHPR deficiency.

Discussion

This experience suggests that in the immediate newborn period, a carrier for DHPR deficiency may have no detectable RBC DHPR activity and may be incorrectly diagnosed as having DHPR deficiency unless additional testing and careful follow-up are performed.

The initial NBS result in our patient suggested a diagnosis of mild hyperphenylalaninemia. However, a mild phenylalanine elevation such as this can also indicate a more serious condition caused by a primary defect in synthesis or recycling of BH_4 . BH_4 is the obligatory cofactor not only for the hepatic PAH but also for two other hydroxylases, tyrosine hydroxylase and tryptophan hydroxylase, both required for neurotransmitter synthesis (Hyland et al. 2001). Thus, a deficiency of BH_4 due to a defect in its synthesis or recycling results not only in hyperphenylalaninemia but also in deficiencies of the neurotransmitters dopamine and serotonin with consequences of which can be neurologically devastating. Early diagnosis and treatment with BH_4 as well as with precursors of the neurotransmitters are critical in allowing an optimal neurological outcome (Jäggi et al. 2008).

This case represents several considerations: (1) it demonstrates the value of definitively assessing infants with an elevated phenylalanine level and absent or very low DHPR activity. Without the comprehensive follow-up testing, our patient could have been inappropriately treated for DHPR deficiency. (2) The PAH deficiency in this infant prevents determining whether the initial absence of DHPR activity per se would have led to the neonatal hyperphenylalaninemia. We believe that this is a possibility and may account for some instances of false-positive “PKU” results in NBS. (3) It is possible that our patient harbors a second QDPR mutation in trans as the unidentified mutation may be in the 5' promoter region or deep within an intron. This would be especially plausible if the expression of this lesion were both tissue and time dependent in development resulting in no significant effect in brain tissue, more so in liver and with the highest impact in erythroid progenitors, yet an effect that diminishes with time. This would be akin to a “peripheral” deficiency of DHPR as reported by Opladen et al. (2012), the “peripheral” deficiency associated with 6-pyruvoyl-tetrahydrobiopterin synthase (PTPS) deficiency (Niederwieser et al. 1987). (4) It is also possible that the heterozygous state of DHPR deficiency due to the c.1 A>T gene mutation causes a “peripheral” deficiency. However, carrier status for DHPR deficiency seems unlikely to cause hyperphenylalaninemia since the mother of our patient is also heterozygous for mutant QDPR yet is normophenylalaninemic and was not noted to have a positive “PKU” test when she was born. (5) The combination of MHP and DHPR carrier status in the

patient that we present is presumably extremely rare, given the approximately 1:30,000 frequency of MHP and an approximate incidence of 1:612 for the carrier state of DHPR deficiency, the latter based on the frequency of 2% pterin defects among all hyperphenylalaninemia and DHPR deficiency accounting for approximately 1/3 of all pterin defects (Opladen et al. 2012). This calculates to one in approximately 41,000,000.

Newborn screening has expanded rapidly. As a result, children are being identified who are carriers or have mild variants of conditions once thought to only present as rapidly deteriorating disorders. It is thus critical that in the encounter of the family with the primary health-care provider prior to referral to a metabolic clinic, there be an opportunity to emphasize that further testing of an abnormal initial result may be needed not only to confirm the result but also to determine its significance. Guidance around follow-up of an abnormal result on NBS is essential in helping families better understand the process and anticipate what may be done during their initial encounter with the metabolic team.

Synopsis

This case illustrates the potential for misdiagnosis of DHPR deficiency when evaluating an infant detected with hyperphenylalaninemia on newborn screening.

Compliance with Ethics Guidelines

Conflict of Interest

Leah E. Hecht, Ann E. Wessel, Harvey L. Levy, and Gerard T. Berry declare they have no conflict of interest.

Informed Consent and Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

Leah E. Hecht drafted the initial manuscript and approved the final manuscript as submitted.

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