

*Case report*

# 6-Pyruvoyltetrahydropterin synthase deficiency diagnosed in tandem mass spectrometry-based newborn screening

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## Introduction

6-Pyruvoyltetrahydropterin synthase deficiency (6PTSD) is a relatively rare, autosomal recessive disease. The enzyme deficiency results in impaired synthesis of tetrahydrobiopterin (BH4) which is an obligatory cofactor for hydroxylation of phenylalanine to tyrosine, and synthesis of associated neurotransmitters. Hyperphenylalaninaemia might not be prominent in this disease and it is possible to miss it in regular Guthrie test neonatal screening.

BH4 is a cofactor for a group of aromatic amino acid monooxygenases. Thus, BH4 is required for oxidation of phenylalanine to tyrosine and tyrosine to dihydroxyphenylalanine (dopa) and tryptophan to 5-hydroxytryptophan (5-HTP). BH4 is synthesized from guanosine triphosphate (GTP) via four enzymatic steps [1]. Defects in the biosynthesis or recycling of biopterin cause biopterin-dependent hyperphenylalaninaemias which account for only about 2% of all patients with phenylketonuria (PKU).

The most common biopterin-dependent PKU is 6-pyruvoyltetrahydropterin synthase deficiency (6PTSD; McKusick #

261640). A recent update [2] indicates the presence of 181 patients worldwide, being more common in Saudi Arabia than elsewhere [3]. The files of our hospital indicate 25 infants with 6PTSD. Recently, we were able to diagnose a case of 6PTSD in a newborn 24 hours after birth through a new programme for neonatal screening based on tandem mass spectrometry (MS/MS) [4].

## Patient and results

The patient, a newborn female, small for gestational age, was admitted to the intensive care nursery for 24 hours of observation. Her regular neonatal screening blood spot was analysed by ESI-MS/MS [5] which indicated moderately elevated phenylalanine (Phe) at 340  $\mu\text{mol/L}$  (normal <120  $\mu\text{mol/L}$ ), and phenylalanine/tyrosine (Phe/Tyr) ratio of 3.3 (normal <3 [6]) (Figure 1). This sample was the 2985th blood spot taken from normal newborns delivered at the hospital and screened by the MS/MS approach. The patient was recalled on day 4 of life for repeat testing, and admitted on day 8 for detailed work up. By then, blood

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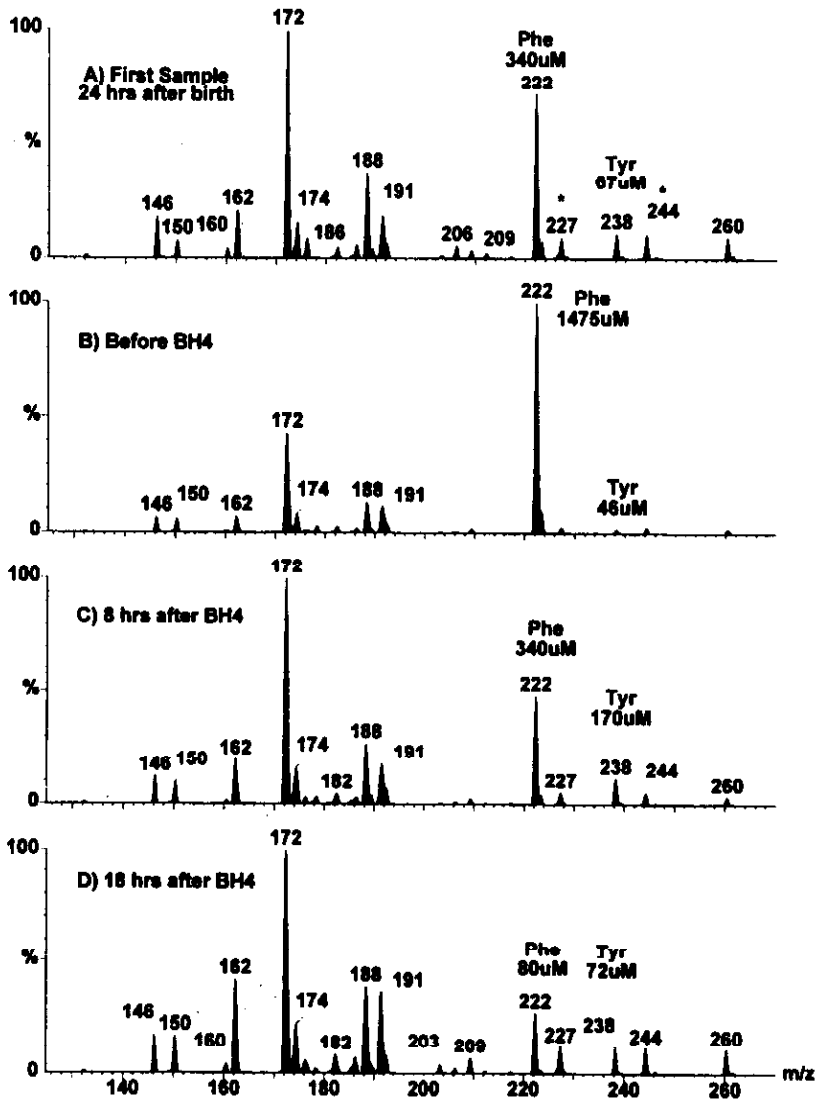


Figure 1 Blood spot amino acid profiles from 6PTSD patient obtained by ESI-MS/MS analysis, scanning for a constant neutral loss of 102 Da. A) first sample collected 24 hours after birth; B) before BH4 treatment; C) 8 hours after BH4; D) 18 hrs after BH4. The signals in the profile correspond to the protonated molecular ions ( $MH^+$ ) of amino acid butyl esters. Their masses are as follows: alanine (146;  $d_4$ -labelled isotope 150), serine (162), proline (172), valine (174;  $d_5$ -labelled isotope 182), threonine (176), pyroglutamic/deammonited glutamine and lysine/pipecolic acid (186), leucine/isoleucine (188;  $d_5$ -labelled isotope 191), glutamine/lysine (203), methionine (206;  $d_5$ -labelled isotope 209), histidine (212), phenylalanine (Phe)(222;  $d_5$ -labelled isotope 227), tyrosine (Tyr)(238;  $^{13}C_6$ -labelled isotope 244), glutamic dibutyl ester (260). \* = Internal standard. The x axis is the mass scale and the y axis is the % intensity of the signal.

Phe was 1210  $\mu\text{mol/L}$  and Phe/Tyr ratio was 20.1. She was an asymptomatic infant with questionable myoclonic activity, but normal EEG and brain MRI. A serum sample was analysed for neopterin, which was significantly elevated to 44 ng/ml (normal 0.0–2.5 ng/ml). Family history indicated that parents were first cousins, and that a 3-year old cousin was also a 6PTSD patient who was diagnosed late, at the age of 3 months.

The patient was placed on 20 mg/kg per day BH4. Blood Phe and Phe/Tyr ratio normalized in less than 24 hours (Figure 1). Her serum neopterin dropped to 8.0 ng/ml at 72 hours after initiation of treatment. She was then placed on dihydroxyphenylalanine (dopa) 15 mg/kg/day, carbidopa 4 mg/kg/day and L-5-hydroxy-tryptophane 5 mg/kg/day, in addition to BH4. She has been developing normally since then.

## Discussion

Although 6-PTSD and other bipterin-dependent hyperphenylalaninaemias cause a severe neurometabolic disease, the associated hyperphenylalaninaemia might not be prominent. It might not even be detected if the patient is kept fasting [3]. A patient with guanosine triphosphate cyclohydrolase I deficiency was missed in normal neonatal screening performed by the Guthrie test [7,8]. It is important to detect the presence of bipterin-dependent PKU as early as possible since it is usually fatal, otherwise causing severe neurologic crippling, and the therapy is usually rewarding [3].

Neonatal screening of blood spots using automated electrospray tandem mass spectrometry (ESI-MS/MS) is a relatively new "broad spectrum" approach to screening [4]. It has been shown to be robust, with high sample throughput (500 samples/day/

instrument), efficient as a diagnostic method, and also a follow-up method for a large number of aminoacidopathies, organic acidaemias, and fatty acid oxidation defects [5]. The ability to use stable isotopically-labelled internal standards makes this approach a definitive method for quantifying many analytes with specificity, sensitivity and accuracy, thereby decreasing false-positive results [9]. These same advantages of MS/MS are crucial when dealing with the modern worldwide problem of early discharge of mother and child from hospital, as the increased sensitivity and specificity of this method reduce false-negative results, and therefore the possibility of missing cases.

Furthermore, some of the diseases identified by this technique have short safety windows; therefore, it is best applied on a blood sample obtained at less than 2 days of age. A case in point is the patient presented here who was identified from a neonatal screening blood spot collected at 24 hours of age, after which the child was discharged. The data from the initial blood spot provided the rationale for recalling the neonate and initiation of a work-up that indicated the presence of a bipterin-dependent PKU.

This report encourages the use of this method as a neonatal screening procedure, both for classical and bipterin-dependent PKU, among other neonatal inborn errors of metabolism.

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