

Brief communication

Neonatal hyperphenylalaninemia, perinatal hemochromatosis, and renal tubulopathy: A unique patient or a novel metabolic disorder?

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Abstract

A neonate presented with hyperphenylalaninemia (HPA), with a persistently elevated phenylalanine/tyrosine ratio. The HPA was responsive to tetrahydrobiopterin (BH₄). His clinical course was dominated by liver failure, associated with perinatal hemochromatosis. He also developed renal tubulopathy. HPA has not previously been reported in association with any of these features. We investigated the etiology of his condition, and discuss the possibility that this represents a novel single-gene disorder.

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Introduction

A neonate presented with persistent hyperphenylalaninemia (HPA), which was responsive to tetrahydrobiopterin (BH₄). His clinical picture was dominated by liver failure. He also developed cardiomyopathy and renal tubulopathy. Autopsy findings were consistent with perinatal hemochromatosis.

HPA is most commonly caused by a defect in phenylalanine hydroxylase (PAH; EC1.14.16.1) causing phenylketonuria (PKU) [1], while about 1–2% of patients with persistent HPA have an enzymatic defect in the synthesis or recycling of the cofactor BH₄ [2]. These disorders are all autosomal-recessive, with the relevant genes well delineated. Perinatal (also known as neonatal) hemochromatosis (OMIM 231100) is quite stereotypical in presentation but heterogeneous in cause [3,4]. In a substantial proportion of cases there is strong evidence for a genetic etiology. Most pedigrees suggest autosomal recessive inheritance, but different modes of inheritance and hence locus heterogeneity have been invoked in

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other families [3,4]. The primary genetic and biochemical causes of perinatal hemochromatosis (PH) remain unknown. Renal tubulopathy (Fanconi syndrome) is a feature of several inherited metabolic disorders and also of some non-genetic conditions [5].

HPA has never previously been reported in association with PH or any of the other clinical problems seen in this patient. Renal tubulopathy has been reported in only a handful of PH patients, a specific subset who actually had co-existing overt renal dysgenesis [3]. We investigated the possible cause(s) of our patient's clinical condition.

Patient history and initial investigations

This male infant was born at 35 weeks' gestation, the first child of non-consanguineous Filipino parents with unremarkable medical and family histories. He was admitted to the neonatal intensive care unit because of prematurity, intra-uterine growth retardation and mild respiratory distress. He was clinically stable initially but over the next few days developed severe gastro-intestinal, pulmonary and renal tract hemorrhages, necessitating multiple transfusions and requiring feeding by total parenteral nutrition (TPN). Multiple coagulation factor deficiencies were identified, attributable to defective hepatic synthesis.

Routine laboratory testing showed hepatocellular dysfunction: severely impaired synthetic functions (albumin, total protein, α 1-antitrypsin, clotting factors), elevated α fetoprotein, and mild conjugated hyperbilirubinemia. Alanine and aspartate transaminases and γ -glutamyltransferase were persistently normal. Alkaline phosphatase and ammonia were initially normal, only showing mild increases in the later course of disease. Two episodes of hypoglycemia were noted within the first few days of life.

He also developed hypertrophic cardiomyopathy and renal tubulopathy. The latter was evidenced by massive generalized aminoaciduria along with significant losses of potassium and phosphate, although glucosuria was not observed. He displayed hyperphenylalaninemia (described in detail below).

Further metabolic testing revealed no evidence of hepatorenal tyrosinemia, citrin deficiency, galactosemia, α 1-antitrypsin deficiency, peroxisomal biogenesis disorders or congenital disorders of glycosylation (CDGS). Extensive testing for other inherited metabolic disorders yielded negative or non-specific findings. A karyotype (350–400 band resolution) was normal. The urine bile acid profile showed significant amounts of unsaturated oxo bile acids. These were most likely secondary to hepatocellular damage [6] but studies to rule out the remote possibility of a primary defect in the gene encoding Δ^4 -3-oxosteroid 5 β -reductase [7,8] are ongoing.

Serum iron parameters measured on day 5 were unremarkable but on day 15 showed moderate alterations (increased serum iron and ferritin, decreased transferrin and increased transferrin saturation) suggestive of PH. Buccal biopsy did not show evidence of iron storage. A liver biopsy was not performed in view of the severe coagulopathy and high risk of bleeding.

The patient died at 6 weeks. Autopsy findings were dominated by multi-organ iron deposition, notably in the liver (hepatocytes, not Kupffer cells), heart (myocytes) and epithelium of pancreas, thyroid and adrenal cortex. The liver showed cirrhotic changes. This overall pattern, which is typical for PH, was consistent with the clinical course of liver failure, the profile of liver function test results, and the cardiomyopathy. Patchy low-level iron deposition seen in the renal tubular epithelium did not appear sufficient to cause the tubulopathy; but microvesicular changes which were noted (of unknown etiology) were consistent with this phenomenon. There was no evidence of renal dysgenesis.

Amino acid profile

On day 4, plasma amino acid analysis by automated anion-exchange chromatography showed markedly elevated phenylalanine, 546 μ mol/L (reference range 16–71) and decreased tyrosine, 18 μ mol/L (32–124). The phe/tyr ratio, 30, was markedly elevated (PKU newborn screening cutoff value 2.2). Methionine and most other amino acids were moderately increased. This is not a typical liver dysfunction profile. Rather, the pattern strongly suggested PKU superimposed on a background of liver dysfunction and TPN. Bloodspot amino acid analysis by tandem mass spectrometry provided an independent validation (in our laboratory numerical values agree closely between the two methods). The newborn screening bloodspot, collected on day 3, showed phe 409 μ mol/L, tyr 21 and a ratio of 20. The HPA persisted, phe climbing to over 2000 μ mol/L by day 10 with the phe/tyr ratio consistently remaining >6 (Fig. 1A). A BH_4 -loading test (22 mg/kg) was then performed (Fig. 1B), and showed BH_4 -responsiveness; phe decreasing by 39% over 24 h, with a concomitant increase in tyr. Phenylalanine restriction was introduced on day 12 (using IV phe-free formula) and brought phe into the therapeutic range (Fig. 1C). Meanwhile tyrosine and other amino acids continued to climb relentlessly, reflecting the progressive liver failure.

Further investigations and discussion

We considered multiple hypotheses and pursued relevant investigations, as listed below.

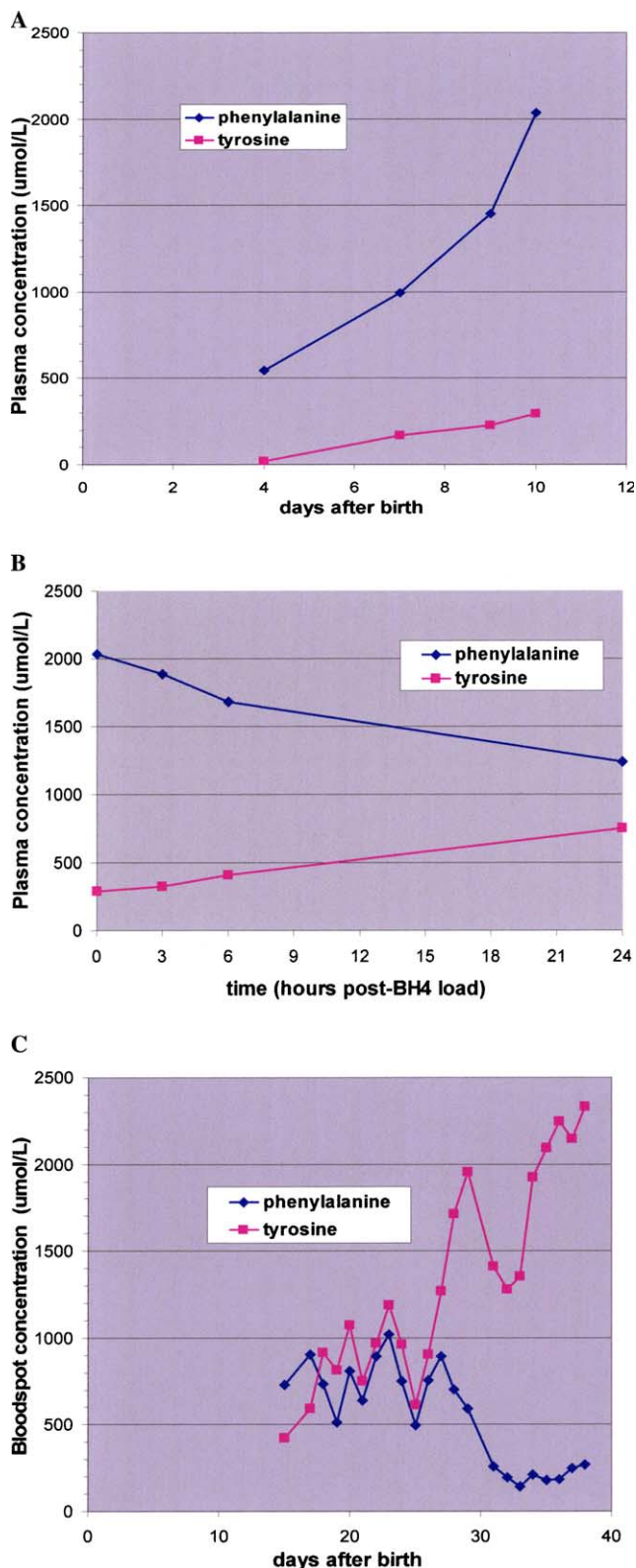


Fig. 1. Longitudinal tracking of phenylalanine and tyrosine concentrations in the patient's blood. (A) Plasma phe and tyr prior to specific intervention. (B) Response of plasma phe and tyr to an oral BH_4 -loading test (22 mg/kg) on day 10. (C) Monitoring response to restriction of phe intake, by analysis of bloodspots. (Measured plasma and bloodspot amino acid values can be regarded as equivalent.)

Two independent disorders, one being PKU

All 13 exons of the *PAH* gene, including intron–exon boundaries, were amplified and sequenced. This approach detects approximately 95% of mutant alleles. No mutations were found in this patient. Since all exons amplified successfully, the possibility of a homozygous deletion affecting the coding region was excluded. A limitation of the analysis is that the promoter and intronic regions were not studied, nor could heterozygous deletions be excluded. Overall, however, these results mean that a primary deficiency of PAH enzyme in this patient is very unlikely.

Two independent disorders, one being a defect of BH_4 synthesis or recycling

Pterin analysis was performed by HPLC, essentially as previously described [9], on urine collected with ascorbate as antioxidant. The ratio of neopterin/total biopterin was normal: 2.4 (0.2–6.0). BH_4 as a percentage of total biopterin was markedly reduced, at 11% (52–86%). 7-biopterin was not identified. These results suggested possible dihydropteridine reductase (DHPR) deficiency, but bloodspot DHPR activity was normal: 15 U/gHb (7–22). Artefactual causes of the low % BH_4 such as improper handling of the urine specimen and secondary inhibition of DHPR in vivo by drugs were excluded, though there remained a possibility that the large volume of transfused blood masked DHPR deficiency.

Studies of neopterin and biopterin production by cytokine-stimulated cultured fibroblasts [10] showed normal neopterin synthesis at 38 pmol/mg (18–98), and biopterin production of 148 pmol/mg, marginally below the reference range (154–303). Enzyme assays on fibroblasts [10] confirmed that DHPR was not deficient, with activity of 11.0 mU/mg (4.5–8.3), and showed normal activity of GTP cyclohydrolase I, 2.2 μ U/mg (1.4–6.5). Studies on the other enzymes of BH_4 synthesis and recycling (6-pyruvoyltetrahydropterin synthase, sepiapterin reductase and pterin 4- α -carbinolamine dehydratase) were not performed because the patterns of fibroblast pterin production and urine pterins were not consistent with an inherited disorder in any of these enzymes.

Secondary BH_4 deficiency

Hepatocellular damage (and/or other tissue damage) associated with hemochromatosis might be expected to impair BH_4 synthesis, with a theoretical possibility of causing BH_4 -responsive HPA. However, BH_4 synthetic enzymes are considered to be present in all organs, the BH_4 recycling and salvage pathways provide some redundancy, and BH_4 appears to be ubiquitous in all tissues and body fluids [2]. HPA has not been reported in association with hepatocellular damage of any cause nor

in association with PH. Therefore clinically significant BH₄ deficiency secondary to cellular damage alone is unlikely.

However, there are other putative causes of BH₄ deficiency within tissues. Recent cultured cell studies and whole-body animal studies [11,12] imply that biopterin circulates predominantly in the oxidized form 7,8-BH₂ rather than BH₄, that BH₂ is taken up by liver much more efficiently than is BH₄, and that BH₂ is then converted to BH₄ within the liver and other tissues by a salvage pathway dependent on dihydrofolate reductase (DHFR, E.C. 1.5.1.3). No primary defect in DHFR has yet been convincingly documented [13], while the proteins responsible for biopterin uptake and transport have not been characterized. The effects of a primary or secondary defect in one of these processes could explain a decreased % BH₄ in blood [11] and BH₄-responsive HPA.

A contiguous gene (microdeletion) syndrome

The possibility of loss of function of two or more adjacent genes responsible for different aspects of the clinical phenotype must be considered. However, we found no evidence of any of the known single-gene causes of HPA which would focus attention on a particular region. Moreover, if considering inheritance to be probably autosomal-recessive, one would need to invoke a defect affecting the same two adjacent genes on both the maternal and paternal chromosomes, which is unlikely given the absence of consanguinity. The patient's karyotype appeared normal, but could only be analysed at moderate resolution (350–400 BR; poorer than 10 Mb resolution). To better investigate the small possibility of a contiguous gene syndrome, array comparative genomic hybridisation (at 1 Mb resolution) is being performed on the patient's DNA.

A novel single-gene disorder providing a unifying diagnosis

No known human single-gene disorder can explain the complex phenotype seen in this patient. However, the weight of evidence points towards an inherited disorder of metabolism due to a single-gene defect. Any plausible candidate would have to impact the synthesis of multiple hepatic proteins including PAH, either directly or indirectly, and also affect renal tubular function.

As one example, we consider the possibility of a defect in hepatocyte nuclear factor 1 α (HNF1 α). Mice with a homozygous knockout of the gene display reduced hepatic synthesis of many proteins including complete silencing of *PAH* gene transcription, renal tubulopathy and growth retardation [14]. Humans with homozygous loss of function of this gene have not been described. Heterozygous mutations in the HNF1 α gene have been found to cause both maturity onset diabetes of the young

(MODY) and glucosuria due to decreased expression of a renal tubular transporter [15,16]. The facts that glucosuria was not noted in our patient, that neither parent has a known family history of MODY, that both parents had normal random and fasting blood glucose values, and that iron deposition was not reported in the mouse model, argue against homozygous loss of function of HNF1 α being the primary cause of his clinical phenotype but they do not completely rule it out.

Conclusions

The complex clinical phenotype of this patient appears to be unique. Perinatal hemochromatosis is a rare condition of heterogeneous etiology, but if HPA were specifically associated with it one would expect this to be previously reported, since neonatal screening for PKU is widespread. However it is feasible that the association could be overlooked when neonatal screening is based on phe concentration alone. Inclusion of the phe/tyr ratio in first-line screening algorithms spectacularly decreases the false-positive rate [17]. Without this, pediatricians may be liable to dismiss the frequent observation of elevated phe in acutely ill newborns as a non-specific finding due to liver dysfunction. Vigilance in appropriate reporting and interpretation of newborn screening results is important in this context.

This patient's phenotype provides an opportunity to explore the genetic bases of perinatal hemochromatosis and gain insight into new aspects of phenylalanine and BH₄ metabolism.

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