

Brief Communication

Autosomal recessive GTP cyclohydrolase I deficiency without hyperphenylalaninemia: Evidence of a phenotypic continuum between dominant and recessive forms

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Abstract

We describe a unique presentation of autosomal recessive (AR) GTP cyclohydrolase I (GTPCH) deficiency, with severe CNS involvement but without hyperphenylalaninemia. A male infant presented with progressive spasticity, dystonia and oculogyric episodes. Blood phenylalanine levels were persistently normal: whereas an oral phenylalanine loading test revealed impaired phenylalanine clearance. CSF neopterin and tetrahydrobiopterin (BH₄) were low, homovanillic acid marginally low and 5-hydroxyindoleacetic acid normal. Fibroblasts showed decreased GTPCH enzyme activity. A homozygous novel mutation of *GCH1*, p.V206A, was identified. On treatment (BH₄, L-Dopa/Carbidopa and 5-hydroxytryptophan), motor development improved. Mutational analysis provided neonatal diagnosis of a younger brother who, after 18 months on treatment, shows normal development. AR GTPCH I deficiency can present without hyperphenylalaninemia and with normal or subtle CSF neurotransmitter profiles. Testing for GTPCH deficiency should be considered for patients with unexplained neurological symptoms and extrapyramidal movement disorder.

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GTP cyclohydrolase I (GTPCH: EC 3.5.4.16) deficiency (OMIM 600225) is an inborn error of tetrahydrobiopterin (BH₄) synthesis. BH₄ acts as a cofactor of the phenylalanine-, tyrosine- and tryptophan hydroxylases and its deficiency may result in hyperphenylalaninemia (HPA) and decreased production of the neurotransmitters dopamine and serotonin [1]. GTPCH deficiency occurs in autosomal recessive (AR) and autosomal dominant (AD) forms.

While the AR form presents with complex neurological dysfunction, including global developmental delay, spasticity and seizures, the AD form presents as Dopa-responsive dystonia (DRD). Patients with the AR form are usually recognized because of HPA; elevated blood phenylalanine (phe) is typically evident on routine newborn screening [2], though onset of HPA can occur much later [3]. Diagnosis is inferred from abnormally low urinary pterins and low neurotransmitter metabolites and pterins in CSF. In patients with the AD form, broadly similar CSF changes occur, but HPA is absent under physiological conditions.

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AR and AD GTPCH deficiencies have long been considered as two clinically distinct disorders (OMIM 233910 and 128230, respectively). A few isolated reports [4–6], however, have shown that the division is not absolute, as some degree of phenotypic overlap can occur.

We describe two male siblings with AR GTPCH deficiency and persistently normal phenylalanine levels in the presence of early-onset complex neurological dysfunction; illustrating the lack of direct correspondence between neurological phenotype, HPA and mode of inheritance in GTPCH deficiency.

Case report

Patient 1

This three-year-old male was the first offspring of consanguineous Pakistani parents, after uneventful pregnancy and delivery. He had poor suck and tremulous movements since birth. Within the first year of life he developed lower limb dystonia and spasticity, excessive drooling, oculogyric crises, poor sleep and progressive microcephaly (head circumference <2nd percentile). At the age of 12 months he was unable to roll over or sit without support and had no speech development. Severe feeding difficulties required insertion of a gastrostomy tube. MRI, MRS and EEG were normal. Metabolic laboratory investigations included two full plasma amino acid profiles in which all values were well within normal reference ranges, including phe and tyr (phe 42 and 44 $\mu\text{mol/L}$, reference range 34–101). Retrospective inspection of newborn screening bloodspot data confirmed normal phe and tyr (phe 51 $\mu\text{mol/L}$, reference range 36–81). CSF amino acid profile was unremarkable, including a phenylalanine value of 8 $\mu\text{mol/L}$ (4–33). Analysis of neurotransmitter metabolites in CSF showed marginally low homovanillic acid (HVA), 284 nmol/L (294–1115) and normal 5-hydroxyindoleacetic acid (5HIAA), 177 nmol/L (129–520). CSF pterin analysis showed low neopterin, <5 nmol/L (7–65) and low BH₄, 8 nmol/L (18–58), a pattern suggestive of GTPCH deficiency. An oral phe-loading test (100 mg phe/kg) revealed an abnormally sustained elevation of plasma phenylalanine (556 $\mu\text{mol/L}$ at 4 h post-load), with phe/tyr ratios persistently greater than 16 (19.1 at 4 h post-load; healthy controls <2, reference [7]). Urine pterins were borderline-low at baseline, with minimal increase post-load and unremarkable neopterin/biopterin ratios (data not shown). Analysis of bloodspots [8] collected at all four time points found virtually no pterins detectable. Studies on cytokine-stimulated cultured fibroblasts [9] showed very low production of pterins: neopterin <1 pmol/mg protein (18–98), biopterin 13 pmol/mg protein (154–303). GTPCH enzyme activity in cultured fibroblasts was almost undetectable: 0.1 $\mu\text{U/mg}$ protein (1.4–6.5). These results confirmed the diagnosis of GTPCH deficiency.

Sequencing of all exons and intron–exon boundaries of the *GCHI* gene (GenBank Accession No. NM_000161)

demonstrated homozygosity for a c.617T>C nucleotide substitution in exon 5. This corresponds to a missense mutation predicting substitution of valine 206 by alanine (p.V206A), not previously reported [10–12]. No other nucleotide sequence variations were detected. Both parents, who were asymptomatic, were heterozygous for this mutation. V206 is a highly conserved amino acid. BLASTP analysis of the surrounding sequence GVGVVVEATHM returned complete identity in GTPCH from many diverse species. In a few species, the equivalent of human V206 is substituted by isoleucine. A BLASTP search using the sequence GVGVVVEATHM, simulating the p.V206A mutation, did not identify any matching sequences. These results imply that a branched-chain amino acid residue is required at position 206, and that substitution by alanine (a straight-chain amino acid) would have deleterious effects on protein function. The sequence alteration seen in our patient is not listed in the NCBI SNP database. Several studies involving systematic sequencing of *GCHI* did not identify this change in a total of over 150 ethnically-diverse individuals (>300 chromosomes) [13–15]. The above data jointly imply that p.V206A is pathogenic, and that homozygosity for this mutation is the underlying cause of the observed severe loss of enzyme activity and thus of the biochemical abnormalities and of the severe clinical disease phenotype inherited in an autosomal recessive manner.

The patient was started on treatment with BH₄ (2 mg/kg/day), and L-Dopa/10%Carbidopa and 5-hydroxytryptophan (both initially at 1 mg/kg/day then increasing gradually to final dosages of 10 and 8 mg/kg/day L-Dopa and 5-hydroxytryptophan, respectively). Folinic acid treatment, 5 mg daily, was added to prevent depletion of cerebral folate by L-Dopa/Carbidopa treatment (methyl group trapping by L-Dopa, forming 3-*O*-methyl-dopa [16]). After 10 months of treatment, spasticity and dystonia had largely resolved. At three years of age, he can walk and run. He has normal mental development. His sleep has normalized. He is gaining weight and his head circumference is close to the 10th percentile.

Patient 2

The younger brother of the index patient was diagnosed with GTPCH deficiency in the neonatal period by targeted mutation analysis. He had tremulous movements since birth. CSF analysis showed decreased pterins: neopterin 5 nmol/L (7–65), and BH₄ 13 nmol/L (18–58), but all neurotransmitter metabolites were well within their reference ranges. Urine pterins were borderline-low (data not shown). Phenylalanine, measured four times during the first 3 weeks of life, was always within reference range: plasma phe 41 and 59 $\mu\text{mol/L}$ (16–71), blood spot phe 44 and 47 $\mu\text{mol/L}$ (36–81). At age 4 weeks he was started on treatment with increasing dosages of BH₄ (1–2.5 mg/kg/day), L-Dopa/10%Carbidopa (1–6 mg/kg/day), 5-hydroxytryptophan (1–4 mg/kg/day) and folinic acid (5 mg/day). At age 18 months his neurological development is age-appropriate.

Discussion

We have described a unique case of an autosomal recessive form of GTPCH deficiency, presenting without HPA, and caused by homozygosity for a novel missense mutation in the *GCHI* gene. The classic constellations of features seen in the AR and AD forms of GTPCH deficiency are presented in Table 1. Between those two phenotypically “mild” and “severe” extremes we place the profiles from the few previous reports describing patients with atypical or intermediate presentations, and we illustrate how our patients occupy a novel niche in a continuous spectrum of GTPCH deficiency phenotypes. A few other atypical presentations of AR GTPCH deficiency have been described: with DRD [4]; with a neonatal Dopa-responsive extrapyramidal syndrome [5] and with an intermediate phenotype [6].

HPA, or plasma phe > 120 μmol/L [1,2,6], is considered a cardinal feature of classic AR GTPCH deficiency. Excluding our own patients, of the remaining 20 patients with AR GTPCH deficiency reported in the BIODEF database [10], HPA was a consistent finding in 17 cases, while phe values were not reported for the other three patients. Most patients (14 out of 17) were diagnosed as a direct result of newborn screening, having neonatal plasma phe levels between 300 and 1200 μmol/L, while in three patients HPA was recognized later in life. Overt HPA has never been reported in any patient with dominantly-inherited

GTPCH deficiency (classic DRD), nor in the Dopa-responsive recessive phenotypes [4,5]. This led to the conclusion that “it is likely that complete GTPCH deficiency produces HPA, while partial deficiency produces DRD” [4]. Our case (in addition to those in [6]) clearly shows that the situation is not so simple; and also shows that the mode of inheritance is not reliably predictable from clinical or biochemical phenotypes.

Mutations have been reported for only a minority of the patients with AR GTPCH deficiency listed in BIODEF, but in each family disease was attributed to homozygosity for a “private” missense mutation [11], as in our patient. In patients with AD disease and DRD, more than 85 independent heterozygous *GCHI* mutations have been observed including “null” alleles and missense mutations [11,12]. Genotype analysis and *in vitro* expression studies together imply that at least two distinct molecular mechanisms underlie the different forms of GTPCH deficiency. A dominant-negative effect of the heterozygous mutations seen in AD patients has been inferred: from observations that DRD patients have residual activities much lower than the theoretical 50%, from the multimeric nature of the active GTPCH enzyme providing opportunity for intersubunit interactions, and from *in vitro* evidence of interactions at the protein and RNA levels [1,2,18]. In contrast, it is assumed that the mutations causing AR GTPCH deficiency do not have significant adverse effects on the products of the normal allele, so that heterozygotes are asymptomatic.

Table 1

The phenotypic spectrum of GTPCH deficiency disorders: clinical, biochemical and molecular genetic features compared between the classic dominant and recessive forms, reported atypical patients, and the patients described in our present report

	Dopa-responsive dystonia (DRD), classic phenotype, including expanded psychiatric phenotype: autosomal dominant	Dopa-responsive extrapyramidal phenotype, and intermediate phenotype: autosomal recessive	Our patients: autosomal recessive	GTPCH deficiency with HPA, classic phenotype: autosomal recessive
References	[1,10,17,18,20,21]	[4–6]	The present report	[1,2,10,22]
Number of patients/families reported	>>100 patients/ >100 families	5 patients/ 4 families	2 patients/ 1 family	>20 patients/ >15 families
<i>GCHI</i> genotypes	Heterozygous (various classes of mutation)	Homozygous missense mutations [4,5], compound heterozygous [6]	Homozygous missense mutation (p.V206A)	Homozygous missense mutations
Hyperphenylalaninemia	–	+/- ^a	–	+
Mental retardation	–	–	+	+
Delayed motor development	–	+	+	+
Psychiatric symptoms	+/- ^b	–	–	–
Seizures	–	–	–	++
Limb dystonia	++	++	+	–
Oculogyric crises	–	+/- ^c	+	+
Truncal hypotonia	–	+	++	++
Limb spasticity	+/- ^b	–	++	++
Parkinsonism	+/- ^b	+	+	+

Key to symbols: +, denotes feature present; –, denotes feature absent; +/-, denotes an inconsistent or uncertain finding.

Notes:

^a “Normal plasma phenylalanine” (no details) in one patient in [4], twin patients in [5] and one patient in [6]; inconstant HPA observed in one patient in [6].

^b Observed in some but not all patients within this group.

^c Reported in one patient in this group [6].

This case highlights practical issues regarding diagnosis of GTPCH deficiency. The absence of HPA must not be allowed to deter further investigations of this possibility when the clinical phenotype is suggestive. Plasma amino acid analysis, using tighter phe reference ranges than those used in newborn screening, can help diagnose some patients with BH₄ synthesis disorders who have mild HPA [19]. In our index patient, however, only an oral phe loading test yielded any indication of impaired phe metabolism. A sobering feature of this case investigation was the fact that CSF neurotransmitter analysis on the index patient showed only a marginal decrease in HVA and normal 5HIAA while low pterins were the only clearly abnormal finding in CSF. This demonstrates the importance of analysing CSF pterins in addition to neurotransmitter metabolites when the clinical phenotype warrants. Urine pterin results on both of our patients were not strikingly abnormal, and of limited diagnostic value. Patients with unexplained neurological symptoms associated with extrapyramidal movement disorders should still be considered at risk for GTPCH deficiency or other treatable disorders of BH₄ synthesis or recycling, even if they have normal blood phe levels and/or equivocal or unremarkable CSF neurotransmitter profiles, and further appropriate laboratory investigations should be pursued. In such patients, it is not advisable to use a treatment trial with L-Dopa as a diagnostic tool, because while this may somewhat improve the movement disorder it can also lead to an inaccurate diagnosis: thus precluding appropriate treatment with full neurotransmitter replacement and BH₄ supplementation. Instead, in clinically suspicious cases we recommend performing a second tier of tests, comprising (1) measurement of pterins as well as neurotransmitters in CSF and (2) an oral phenylalanine loading test. If the results of either or both of those tests are suggestive of GTPCH deficiency, confirmation of diagnosis must then be sought, using enzyme assay in fibroblasts and/or molecular analysis.

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