

Neonatal dopa-responsive extrapyramidal syndrome in twins with recessive GTPCH deficiency

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Abstract—The authors report two twin sisters, age 15 years, with recessive GTP cyclohydrolase deficiency, who presented with neonatal onset of rigidity, tremor, and dystonia but with no other symptoms suggestive of a diffuse CNS involvement. The plasma phenylalanine levels were normal. Treatment with L-dopa/carbidopa, started at age 1 year, was associated with sustained recovery from all neurologic signs. The patients were homozygous for a new recessive mutation in the *GCH1* gene.

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GTP cyclohydrolase I (GTPCH) is the limiting enzyme for the synthesis of tetrahydrobiopterin (BH₄), the essential cofactor for phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase.¹ GTPCH is encoded by the *GCH1* gene, located on chromosome 14q22.1–22.2.¹ Patients heterozygous for *GCH1* mutations develop dopa-responsive dystonia (DRD), also called dominantly inherited GTPCH deficiency, whereas patients who are homozygous or compound heterozygous for *GCH1* mutations develop classic recessive GTPCH deficiency.² Recessive GTPCH deficiency is a rare disease, with only 17 patients listed in the international database of BH₄ deficiency.² The clinical picture is characterized by neonatal onset of poor sucking and swallowing difficulties, severe hypotonia, seizures, and psychomotor retardation. In the course of the disease, recurrent hyperthermia and abnormal ocular movements have been observed. Abnormal movements, mainly described as rigidity and hyperkinesias of the limbs, have been reported, but these appeared late and in only a few patients.² Plasma hyperphenylalaninemia, ranging from 300 to 1,200 μM, is usually detected with newborn screening, with a few patients having normal levels up to age 5 months. Treatment with L-dopa/carbidopa, BH₄, and 5-hydroxytryptophan can improve the neurologic symptoms but does not prevent development of severe encephalopathy.²

A patient with atypical presentation of recessive GTPCH deficiency has been described previously.³ Here, we report twins with recessive GTPCH deficiency due to a new homozygous mutation, presenting

with a neonatal onset of an isolated extrapyramidal syndrome with normal plasma phenylalanine levels. They were completely responsive to L-dopa/carbidopa treatment, further supporting the evidence of a broader clinical spectrum of the disease.

Case report. The monozygotic twin sisters, now aged 15, are the second and third children of healthy unrelated parents. A maternal aunt was reported to have PD, with onset after age 60.

Pregnancy was complicated by frequent uterine contractions. Delivery was at term by caesarean section due to fetal distress. The birth weights were 2,330 g and 2,400 g. A mild birth anoxia was reported.

Since the first month of life, rigidity and tremors of the extremities, which worsened toward evening, were noted. From age 4 months, one of the patients also showed prolonged generalized dystonic spasms, with opisthotonus, hyperextension of lower limbs, and hyperpronation of the arms, with diurnal fluctuation. Cognitive development was normal. At age 6 months the children were referred to a pediatric department for diagnostic investigation. The neurologic examination in both children revealed delayed motor development with normal cognitive abilities, rigidity, continuous, small amplitude, irregular and arrhythmic hyperkinesias involving the limbs, exacerbated by crying and passive movements, and symmetric hyperreflexia, without extensor plantar response. The abnormal movements were absent during sleep. Laboratory and instrumental findings included plasma and urinary amino acids, urinary organic acids, EEG, visual and brainstem evoked potential, and results were normal.

At age 1 year, the two sisters were seen by one of the authors (F.A.E); the neurologic picture was unchanged and a trial of L-dopa/carbidopa (5 mg/kg/d) was started. The response to treatment was dramatic, with disappearance of the neurologic signs within 2 days. The subsequent psychomotor development was normal, and the two girls were able to lead normal lives. When we saw the two patients at age 15, they were still taking L-dopa/carbidopa (2 mg/kg/d), and no side effects had been reported since the beginning of the treatment. The neurologic examination was unrevealing, except for a slight generalized hyperreflexia. Cerebral

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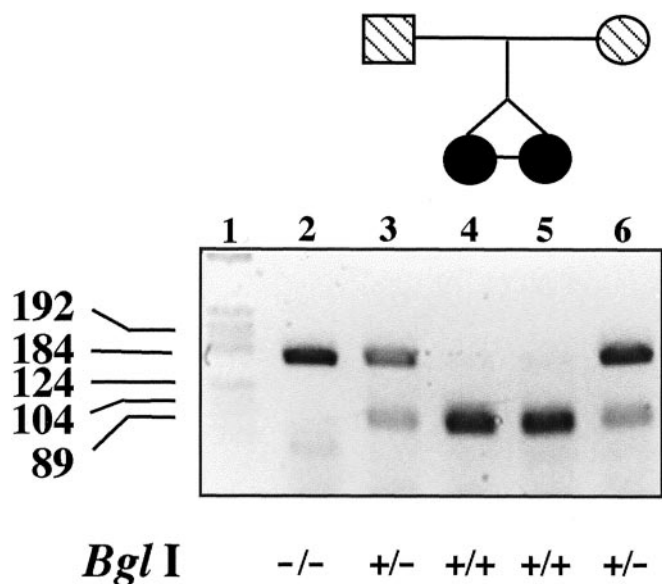


Figure. Restriction site analysis with *Bgl*I enzyme. Lane 1: markers; lane 2: control; lane 3: father; lane 4 and 5: patients; lane 6: mother. The digestion of 175-bp PCR product with *Bgl*I generated two fragments of 91 bp and 84 bp (not separated in the gel) in mutated homozygous individuals, while the heterozygous parents showed both the wild-type fragment of 175 bp and the mutated 91/84-bp fragments.

MRI, performed in one of the two sisters, revealed a mild posterior periventricular leukomalacia. Plasma phenylalanine level was normal. IQ was at the lower limit of the normal range (75 and 76 on the Wechsler Intelligence Scale for Children—Revised).

Neither parent had any signs or symptoms suggesting a GTPCH deficiency, and the neurologic examination was normal.

Genetic studies. After informed consent, genomic DNA of the patients and their parents was extracted from peripheral leukocytes using standard techniques. For rapid detection of mutations, nonradioactive, single-stranded conformation polymorphism analysis of the six *GCHI* exons was performed as described.⁴ PCR amplifications were carried out as previously described.⁵ Direct sequence analysis of the mutated fragments were performed by an automated sequencing system (3100 Genetic Analyzer ABI Prism, Foster City, CA), using the Thermo Sequenase dye terminator cycle sequencing premix kit, v2.0 (Amersham Pharmacia Biotech, Piscataway, NJ).

The twin sisters were found to be homozygous for a novel missense mutation C595G in exon 5, resulting in a Pro199Ala substitution, whereas the parents were found to be heterozygous. This mutation created one restriction enzyme site for *Bgl*I (figure). This novel mutation was not present in 100 normal individuals.

GTPCH-I assay. Skin fibroblasts were obtained from the patients. Cell culturing, production of neopterin and biopterin in fibroblasts after stimulation with cytokines for 24 hours, and measurement of the GTPCH activity were performed as described previously.⁶

Neopterin and biopterin production in fibroblasts from both patients were low, indicating a failure in BH4 synthesis, and GTPCH activity was below the normal range (0.35 and 0.36 μ U/mg; control values 5 to 95 percentile: 1.4 to 6.5 μ U/mg, $n = 35$; age range 1 week to 45 years).

Discussion. Here we report two patients with neonatal onset of an extrapyramidal syndrome completely responsive to L-dopa treatment with normal blood phenylalanine levels due to a recessive GTPCH deficiency. Our patients are different from the classic phenotype of the disease: they presented with a

movement disorder with rigidity and tremor and later, with dystonic movements and postures but without any signs or symptoms suggestive of a diffuse CNS involvement.² Phenylalanine levels were normal in the neonatal period and during follow-up, and the response to L-dopa treatment was dramatic, with complete and persistent remission of the clinical symptoms and without any side effects.

To date, there is only one report of an atypical clinical presentation of recessive GTPCH deficiency, which differs from our two patients by a later onset (2 years and 8 months) of the symptoms, described as posture dystonia, posture rigidity, posture tremor, and neck tilting with diurnal fluctuations.³ This patient, like our two cases, showed a complete responsiveness to L-dopa treatment and had no hyperphenylalaninemia.

The clinical spectrum of recessive GTPCH deficiency is wider than reported previously and includes neonatal or later onset of an isolated extrapyramidal syndrome with no hyperphenylalaninemia. GTPCH deficiency can therefore present the picture of a central form of BH4 deficiency, similar to DRD and sepiapterin reductase deficiency.⁷

In our two patients GTPCH activity measured in cultured fibroblasts was decreased to 9.1% and 8.8% of mean control values. These values are significantly lower than those detected in cytokine-stimulated fibroblasts of other recessive GTPCH deficiency patients.⁶ Indeed, the enzyme activity in peripheral tissues may not reflect accurately the dysfunction in the nigrostriatal system, which is selectively involved in the pathogenesis of the disease. Nevertheless, even if it is not possible to make a correlation between the enzymatic activity and the clinical phenotype, the measurement of GTPCH activity is of high diagnostic value.⁶

Including our cases, six different autosomal-recessive mutations associated with GTPCH deficiency have now been detected. Interestingly these mutations are different from those corresponding to DRD, where more than 80 different mutations have been described or those in a few patients who are compound heterozygous.⁸ Some of these mutations have been studied with coexpression experiments in eukaryotic cells. The mutant proteins associated with DRD are enzymatically inactive and seem to exert an inhibitory effect on the wild-type protein owing to a dominant negative effect.⁹ In contrast, the mutant proteins associated with recessive GTPCH deficiency have a slight residual activity and no inhibitory effect.^{9,10} This would explain why DRD patients usually have lower levels of GTPCH activity compared with patients carrying recessive mutations in the *GCHI* gene.⁶ The recessive mutation R249S found in a patient with a dopa-responsive extrapyramidal syndrome has a higher residual activity, and it was postulated that the clinical phenotype associated with a recessive mutation depends on the residual enzyme activity and thus the R249S mutation induces symptoms only in homozygosity.^{3,9} According to this observation we hypothesize a similar effect of

the mutation found in our two patients displaying the same phenotype.

While the molecular mechanisms involved in the manifestation of the clinical features await further characterizations, our results indicate that autosomal-recessive GTPCH deficiency can also present as dopa-responsive extrapyramidal syndrome in the neonatal period, thus expanding the clinical spectrum of recessive GTPCH deficiency. A trial with L-dopa/carbidopa should therefore be recommended in patients with extrapyramidal symptoms with onset in the neonatal period.

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Toxic neuropathy in patients with pre-existing neuropathy

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Abstract—The authors report significant worsening of a pre-existing neuropathy in six patients who received “non-toxic” dosages of known neurotoxic agents. Before treatment, baseline total neuropathy score (TNS) averaged 9.5 (range 0 to 19). After chemotherapy (Taxol [125 to 175 mg/m² × 4]; vincristine [2 to 5 mg]; cisplatin [40 mg/m² × 8]; and thalidomide [60 g]), the TNS averaged 22 (range 13 to 29). The authors conclude that functionally disabling toxic neuropathy can occur in patients with pre-existing neuropathy at standard doses.

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Functionally disabling peripheral neuropathy is a major dose-limiting side effect of many chemotherapeutic agents.¹ The severity of neuropathy depends on the type of chemotherapy, the dose, and the duration of administration. For most chemotherapeutic agents, a generally safe dose to avoid peripheral neuropathy has been identified.² This study reports the early development of severe neuropathy in six patients with pre-existing neuropathy receiving “safe-dosages” of chemotherapy.

Methods. The patient demographics are given in table 1. All patients underwent a directed history, examination, and nerve conduction testing. A modified total neuropathy score (TNS) was calculated in all patients (see table 1).³

Results. Details of the diagnosis and changes in pre- and post-chemotherapy components of the TNS evaluation are given in table 2. Five patients' baseline evaluations (including nerve conduction studies) documented the presence of pre-existing neuropathy (1 idiopathic small fiber, 1 ataxia telangiectasia, 2 diabetic neuropathies, and 1 Charcot-Marie-Tooth). The last patient, who was a known diabetic and gave a history of heavy alcohol use, was asymptomatic. The mean TNS score was 9.5 (range 0 to 19). Chemotherapy was initiated for lung cancer (2), breast cancer (1), lymphoma (1), oropharyngeal cancer (1), and discoid lupus (1). The chemotherapeutic agents were vincristine (2), Taxol (3), cisplatin (1), and thalidomide (1). Doses of chemotherapy were as follows (Taxol [3] 125 to 175 mg/m² × four cycles; vincristine [2] 2 mg 1 patient, 5 mg 1 patient; cisplatin [1] 40 mg/m² × eight cycles; and thalidomide [1] 100 mg daily × 16 months and 50 mg daily for 8 months). All patients developed significant worsening of their neuropathy while on chemotherapy that continued to progress for up to 8 weeks (range 1 to 24 weeks) after discontinuing therapy. In three patients the neuropathic symptoms were

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