

Phenotypic heterogeneity of dopa-responsive dystonia in monozygotic twins

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Abstract—The clinical expression of dopa-responsive dystonia (DRD) was found to be different in a pair of affected monozygotic twins. An earlier onset was associated with a more disabling course of disease. Whereas monozygosity was genetically proven, the search for pathogenic mutations in the GTP-cyclohydrolase-1 gene was negative. The contribution of environmental factors appeared minimal. Intrafamilial variability of DRD phenotype may be related to yet unknown non-Mendelian epigenetic or proteomic factors.

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Dopa-responsive dystonia (DRD) is a genetically determined condition characterized by childhood-onset dystonia of the lower limbs progressively evolving into generalized dystonia and parkinsonism, marked worsening toward the evening, sustained response to levodopa (LD), and female predominance. Patients with DRD range from asymptomatic carriers to severely affected, wheelchair-bound individuals. DRD is most commonly a dominant monogenic disorder with incomplete penetrance, resulting from heterozygous mutations in the GTP-cyclohydrolase 1 (GCH1) gene at chromosome 14q11–24.3.¹ More than 70 different mutations have been reported.² Interfamilial phenotypic heterogeneity has been correlated with the nature and position of mutations, based on the observation that pedigrees carrying different mutations within the same exon share similar clinical features. On the other hand, intrafamilial heterogeneity is more difficult to explain. Hypotheses include compound heterozygosity, polymorphisms or additional mutations in modulatory genes for GCH1, variable ratio of mutant to wild-type GCH1 messenger RNA, post-translational modifications of GCH1 gene products, and environmental factors.³ This problem appears even more complex based on the following observation of a major variability of DRD phenotype in monozygotic twins.

Patients. Twin sisters were delivered spontaneously after an uneventful pregnancy and had normal motor and developmental milestones in early childhood. Medical history was unremarkable. They lived in the same household up to their mid-30s and their daily lives were similar as to habits, diet, hobbies, traveling, and physical training. They never took any recreational drugs or relevant medications. They did not marry and never got pregnant. Family history is free of any neurologic disease in 24 relatives.

Twin A developed walking difficulties by age 15 years with stiffness, cramps, and intermittent dystonia in the lower limbs, more marked on the right side. Symptoms worsened toward the evening and following physical effort. Over the years, mild dysto-

nia of the right arm became apparent while foot dystonia progressed slowly, producing contractures of both Achilles tendons, which led to bilateral tenotomy at age 25. She took a job as a cook, working most of the time in a standing position without difficulty. At age 43, prominent neurologic features included intermittent mild dystonic movements of hands and legs, a slow gait with diminished arm swings, and mild equinovarus of both feet. Brain MRI was normal. Treatment with 100 mg LD/25 mg benserazide/day improved symptoms.

Twin B presented at age 12 years with equinovarus deformation of the right foot rapidly progressing toward severe dystonia of both lower limbs with postural instability. Symptoms worsened toward the evening and upon physical effort. Within 3 years dystonia spread to the right arm and writing became impaired, leading to left-handedness. She developed marked flexed posture of the neck and walking became increasingly difficult with falls. At 18, she took a factory job allowing her to sit during work and by age 28 her working capacity was reduced to 50%. She underwent bilateral tenotomy at age 25. By age 42, neurologic examination showed severe parkinsonism with amimia, severe and fixed antecollis (figure), rigidity of the right arm, and severe bradykinesia of all limbs. She walked with severe equinovarus posturing of both feet, abolished arm swing, and impairment of postural reflexes. Brain MRI was normal. Daily treatment with 200 mg LD/50 mg benserazide resulted in considerable improvement of most symptoms, allowing her to resume a full-time job. Antecollis remained virtually unchanged.

Methods. CSF analyses. Lumbar puncture was performed at 8 AM in both twins, before any LD treatment was initiated. Pterins and monoamine metabolite levels were measured as reported.²

Genetic studies. For zygosity analysis, five polymorphic markers, from five autosomes (D1S249, FGA, D12S87, D15S543, D22S264), were analyzed. Their reported heterozygosities are 0.87, 0.79, 0.61, 0.86, and 0.80.⁴ Parental DNA was not available. The search for a GCH1 mutation was performed as follows. Exons 1 to 6, including splice junctions, were PCR amplified and directly sequenced on an ABI 3100 (Applied Biosystems, Rotkreuz, Switzerland). All primer sequences are available on request.

A signed informed consent was obtained from both patients covering all aspects of the study including videotaping and publication.

Results. Routine analyses of both CSF samples were normal. CSF levels of pterins and monoamine metabolites are shown in the table. Reduced levels of neopterin and biopterin in both twins confirmed the diagnosis of GCH1-deficient DRD.

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Figure. Photographic portrait of the dopa-responsive dystonia (DRD) twin sisters (left: twin A, right: twin B) showing striking physical resemblance, which contrasts with the presence of parkinsonism with hypomimia and severe antecollis only in twin B, as differential features of DRD expression.

The two sisters had identical genotypes at all five polymorphic loci tested. The probability that this happens by random segregation is $(1 - \text{heterozygosity}/2)$,² for each marker. The probability that the sisters are identical for all five markers is therefore <0.01 and we concluded that the twins are monozygotic. Direct sequence analysis of the complete coding sequence of GCH1, including 15 intronic bases at each splice site and 30 bases upstream of the initiator ATG, revealed no mutations.

Discussion. We observed striking phenotypic variability of DRD in these two genetically demonstrated monozygotic twins. Significant clinical differences in-

Table Results of the CSF analyses of pterins (neopterin and biopterin) and monoamine metabolites (homovanillic acid [HVA] and 5-hydroxyindol-acetic acid [5HIAA]) in twins A and B

Compound measured	Reference intervals (>16 y of age)*	Twin A	Twin B
Neopterin, nmol/L	9–20	4.0	5.9
Biopterin, nmol/L	10–34	4.1	6.7
HVA, nmol/L	115–488	122	251
5HIAA, nmol/L	66–141	129	274
HVA to 5HIAA ratio	1.5–3.5	0.95	0.92

* Age-adjusted reference intervals.²

involved age at onset, pace of disease progression, clinical features, severity of dystonia, and amount of LD necessary to alleviate symptoms. An earlier onset was associated with a more disabling evolution. The combination of low CSF biopterin and neopterin levels (see the table) is consistent with GCH1-deficient DRD, and cannot result from TH deficiency (normal CSF biopterin and neopterin), nor from early onset parkinsonism due to mutations in the parkin gene (reduced biopterin, normal neopterin), which can masquerade as DRD.⁵

No mutation of GCH1 was found in our patients, as repeatedly reported earlier.¹ In such instances DRD has been related to large deletions of one or several exons of GCH1,⁶ mutations in the noncoding regulatory or intronic regions of GCH1, or mutations in other genes having a regulatory effect on GCH1.³ In our patients, the absence of clinically apparent DRD in any other known relatives suggests a non-dominant mode of inheritance or the spontaneous occurrence of a new mutation. Finally, we have no evidence of different environmental factors, as both twins lived in the same household and led a similar lifestyle up to adulthood.

To our knowledge, four pairs of DRD twins in unrelated families have been reported thus far,^{6–9} three of which might have been identical twins,^{7–9} although monozygosity has been confirmed in none. Unlike ours, these twin pairs seemed to have exhibited a virtually identical phenotype. In yet another report, and reminiscent of our observation, age at onset and disease severity differed in monozygotic twins with generalized dystonia, which might be classified retrospectively as DRD.¹⁰

Previous attempts to explain intrafamilial phenotypic variability mostly rely on the observation that carriers with the same mutation may have different mutant/wild type GCH1 messenger RNA ratios, and that mutant GTP-cyclohydrolase protein might exert a dominant negative effect.³ Although the degree of residual GCH1 activity has been associated with disease severity, this can hardly explain the phenotypic variability in our patients, because the more severely affected twin had the highest CSF concentrations of pterins and HVA. Nonetheless, a possible role of residual GCH1 activity in determining the individual phenotype is not completely excluded, in view of the different doses of LD required to improve symptoms. Polymorphisms or mutations in modulatory genes have also been proposed to explain intrafamilial heterogeneous phenotype.³ In monozygotic twins, however, intergenic effects, such as modulatory gene variants, can be excluded, as the entire genetic background is identical. It is therefore necessary to postulate the existence of an alternative mechanism, an example of which being a proteomic effect of yet to be defined post-translational modifications of the GCH1 gene product generating distinct protein isoforms with different residual enzymatic activity.

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