Wide Expressivity Variation and High But No Gender-Related Penetrance in Two Dopa-Responsive Dystonia Families With a Novel GCH-I Mutation

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Abstract: We describe the clinical and molecular correlates in two Italian families with dopa-responsive dystonia (DRD) and the same novel mutation of GTP-cyclohydrolase I (GCH-I) gene. Thirty-five subjects were examined and the genotype correlated to phenotype. Childhood onset foot dystonia is present in 7 subjects currently under the age of 40. In 1 patient bilateral foot dystonia was evident at birth suggesting that dystonia may be active as early as in utero. In another patient, dystonia spontaneously remitted in adolescence, to relapse 8 years later, as writer’s cramp. Dystonia and parkinsonian signs are present in 5 other patients. In 2 subjects an isolated parkinsonism started over the age of 45. A 5-base pair insertion at codon 242 within exon 6 of GTP-cyclohydrolase I (GCH-I) gene that shifts the reading frame and results in a premature stop at codon 247 with truncation of the polypeptide has been detected in 21 subjects. Considering dystonia and parkinsonism the overall penetrance is 0.71 and not significantly different in men (0.69) and women (0.75). Genealogical studies seem to exclude that these families are related but haplotype analysis suggests a single founder. Our findings in subjects with the same mutation indicate a wide intrafamilial variation in expressivity and high penetrance in DRD but do not confirm the reported influence of gender on GCH-I gene mutation penetrance. © 2004 Movement Disorder Society

Key words: dopa-responsive dystonia; parkinsonism; gender-related penetrance; expressivity; founder effect

Dopa-responsive dystonia (DRD) is a distinct striatal dopamine deficiency disorder characterized by: (1) childhood-onset dystonia with diurnal fluctuations, (2) concurrent or subsequent parkinsonian signs, and (3) marked and sustained response to relatively low doses of levodopa (L-dopa).1,2 About 50% of autosomal dominant and sporadic cases are caused by a mutation of the GTP-cyclohydrolase I (GCH-I) gene.3,5 The gene product catalyzes the first step in the biosynthesis of (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) the cofactor for tyrosine hydroxylase. Mutations of one copy of GCH-I result in reduced striatal tyrosine hydroxylase and dopamine that causes symptoms in some but not all carriers.6 Clinical studies demonstrated penetrance of 0.3 in a large North America family, and predominance of affected women (2 to 6:1).2,7,8 More recent clinical and molecular correlations demonstrated, when minor signs and symptoms were considered, higher penetrance (0.76) and a 1.8 to 2.9 times higher incidence in women than in men.9,10

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We report the clinical and molecular correlates in two Italian families with the same novel mutation of GCH-1 gene.

SUBJECTS AND METHODS

Subjects

We encountered two unrelated subjects (index cases in Fig. 1) with childhood onset leg dystonia who responded dramatically to low doses of L-dopa. Family history and clinical examination revealed that the disorder was familial in both cases (Fig. 1). Thirty-five subjects (5 members of Family 1 and 30 of Family 2) were examined by 2 neurologists before molecular data were obtained. To assess penetrance, a subject was considered affected if he or she had symptoms and signs of dystonia or parkinsonism. Dystonia was defined as a syndrome of sustained muscle contraction frequently causing twisting and repetitive movements. According to Nygaard and colleagues we rated dystonia as definite when there were unequivocal features of dystonia, probable when examination was highly suggestive of dystonia, and possible when examination was abnormal but not diagnostic of.

FIG. 1. Pedigree of the two families studied. Arrow indicates the index case in each family.
dystonia. Parkinsonism was determined by the presence of (1) rest tremor (at least intermittently present at one site), (2) bradykinesia, (3) postural instability (at least three steps required to recover, on repeated pull steps), and (4) rigidity (moderate in at least one site). The presence of two or more of the above features (including either 1 or 2) was scored as definite parkinsonism. The presence of either (1) or (2), alone, was scored as probable parkinsonism. The presence of either (3) or (4) or both was scored as possible parkinsonism.

Comparison of frequencies and percentages were made by Fisher’s exact test and χ2 test with Yates’s correction when indicated. Significance was set at P < 0.05.

Molecular Studies

Genomic DNA was extracted from peripheral blood of 35 subjects using standard techniques. Each of the six exons of GCH 1 gene was amplified by PCR according to Ichinose and colleagues.12 Screening for point mutations was carried out by the single strand conformational polymorphisms (SSCP) approach by running denaturated PCR products on a 0.75% MDE gel at 150 V for 17 hr. Samples showing an abnormal bands pattern were submitted to direct sequencing both on the forward and the reverse strand using an automated sequencer (ABI Prism 310). Sequences were compared with those reported in databases (GenBank accession number: U15256-15259). For Parkin gene, molecular genetic analyses included sequencing for conventional mutation screening and exon dosage to test heterozygous deletion or duplication of one or more exons. The 12 coding exons including the flanking intronic sequences of the parkin gene were amplified by PCR according to Hattori and colleagues.4 The PCR products were visualized on ethidium bromide-stained 2% agarose gels and the presence or absence of the target exons was detected. Moreover, the PCR products were subjected to direct nucleotide sequence analysis using an automated DNA sequencer (AB-373A Perkin-Elmer). Nucleotide positions were determined according to the cDNA sequence published in the DNA Data Bank of Japan (DDBJ accession number: AB009973). Gene dosage analysis was carried out by quantitative duplex PCR using the ABI-7900HT SDS Applera and TaqMan fluoregenic probes.

Genealogical Study and Haplotype Analysis

The two families are from the same district and originally from two villages about 15 km apart. Genealogical information was obtained from the oldest family members three generations back from the index cases. We also genotyped the two families under study with four microsatellite markers flanking the GCH1 gene: D14S989, D14S1057, D14S66, D14S63.

RESULTS

Clinical Findings

Family 1.

Subject IV-2 is a 27-year-old woman index of this family of five living members (Fig. 1). She had difficulty walking from age 6 worsening during the course of the day and after exertion. Examination at 27 years of age showed a stiff gait with knee hyperextension, lumbar hyperlordosis, and supination of the left foot after prolonged exertion. Treatment with L-dopa (100 mg/day) resulted in complete remission of symptoms. A summary of the clinical features of the mutated subjects of Family 1 is reported in Table 1.

Family 2.

Subject IV-13 is a 30-year-old man index case of this family of 40 living members (Fig. 1). In childhood he presented with a waddling gait and tendency to walk on his toes. A muscle dystrophy was suspected and a muscle biopsy was carried out in another institution at the age of 10 with normal results. Examination at 30 years of age showed bilateral leg dystonia (mainly on the left), lumbar hyperlordosis, and dorsal scoliosis. Treatment with L-dopa (200 mg/day) resulted in complete remission of symptoms. A summary of the clinical features of the mutated subjects of Family 2 is reported in Table 1. In the left side of family tree (Fig. 1) Subject III-6, a 52-year-old who lacked the mutation, began at age 35 to show a right hemiparkinson with slow but relentless progression. Currently, he is scarcely responsive to therapy with wearing-off and off-on phenomenon and dyskinesias. Subject (III-8), also lacking the mutation, is currently 56 years old and has had blepharospasm since age 40 consisting chiefly of clonic contractions of eyelids that were rarely sustained.

Molecular Studies and Clinical Correlation

In the two families the GCH-I gene was examined in 35 subjects (21 men and 14 women). Amplification of exon 6 and subsequent SSCP analysis revealed a band shift in five members of Family 1 and 16 members of Family 2 (Figs. 1, 2). Sequencing of this exon demonstrated a 5-base pair insertion at codon 243 (Fig. 2). This mutation results in a frameshift leading to a premature stop at codon 247 with truncation of the polypeptide. A total of 21 subjects (13 men and 8 women) showed the
The mutation frequency was not significantly different between men (13 of 21, 61%) and women (8 of 14, 57%) \( (P = 0.944) \).

**Clinical signs and symptoms of dystonia or parkinsonism (including all classes) are present in 15 of 21 mutated subjects (Fig. 1, Table 1) with an overall penetrance...**

<table>
<thead>
<tr>
<th>Family</th>
<th>Subject/gender</th>
<th>Age at onset (y)</th>
<th>Age at examination (y)</th>
<th>Signs and symptoms at onset</th>
<th>Signs and symptoms at examination</th>
<th>Dopa (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>III-1/M</td>
<td>2</td>
<td>53</td>
<td>Gait disorder; diagnosis of cephalal palsy; surgery for bilateral pes equinovarus at 15</td>
<td>Dystonia in all limbs; severe bradykinesia; generalized rigidity; marked postural instability (DD + DP)</td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>III-2/F</td>
<td>48</td>
<td></td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>IV-1/M</td>
<td>29</td>
<td></td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>IV-2/F</td>
<td>6</td>
<td>27</td>
<td>Difficulty walking worsening after exertion; diurnal fluctuation</td>
<td>Stiff gait with knee hyperextension; lumbar hyperlordosis; supination of the left foot after prolonged exertion (DD)</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>IV-3/F</td>
<td>6</td>
<td>27</td>
<td>Difficulty walking worsening after exertion; diurnal fluctuation</td>
<td>Stiff gait with knee hyperextension; lumbar hyperlordosis; supination of the right foot after prolonged exertion (DD)</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>III-10/M</td>
<td>46</td>
<td>65</td>
<td>Tremor of left hand</td>
<td>Stiff, wide-based gait; rest tremor and rigidity of left hand; bradykinesia; postural instability (DP)</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>III-11/M</td>
<td>Childhood</td>
<td>62</td>
<td>Dystonia more pronounced in right foot; diurnal fluctuation</td>
<td>Dystonia &gt; right foot; rigidity; mild bradykinesia; rest tremor right hemisoma; postural tremor of hands; dorsal scoliosis (DP + DP)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>III-13/F</td>
<td>5</td>
<td>60</td>
<td>Difficulty walking with supination of feet and rigidity worsening after exertion; diurnal fluctuation</td>
<td>Stiff, wide-based, waddling gait; dystonic left hand; rigidity; postural tremor right hand; postural instability; dorsal scoliosis (DD + PoP)</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>III-14/M</td>
<td>50</td>
<td>58</td>
<td>Tremor of right hand</td>
<td>Rest tremor and rigidity of right hand (PrP)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>III-17/F</td>
<td>4</td>
<td>51</td>
<td>Dystonia more pronounced in right foot; diurnal fluctuation (at 10 surgery for right pes equinovarus)</td>
<td>Foot dystonia &gt; right; rigidity; mild bradykinesia; rest tremor; dorsal scoliosis (DD + DP)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>III-18/F</td>
<td>Childhood</td>
<td>47</td>
<td>Dystonia more pronounced in right foot; diurnal fluctuation</td>
<td>Dystonia &gt; right foot; rigidity; mild bradykinesia; rest and postural tremor of hands; left striatal toe; dorsal scoliosis (DD + PrP)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>IV-7/M</td>
<td>39</td>
<td></td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IV-8/F</td>
<td>&lt; 6</td>
<td>30</td>
<td>Foot dystonia; diurnal fluctuation (at 12 complete spontaneous remission)</td>
<td>Writer’s cramp starting at age 20 (DD)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IV-9/M</td>
<td>&lt; 6</td>
<td>33</td>
<td>Dystonia more pronounced in right foot; diurnal fluctuations</td>
<td>Dystonia &gt; right foot (DD)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IV-12/M</td>
<td>Uncertain</td>
<td>36</td>
<td></td>
<td>Walks more on the lateral side of feet; frequent supination of feet while seated (PoD)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IV-13/M</td>
<td>Childhood</td>
<td>30</td>
<td>Waddling gait; tendency to walk on toes</td>
<td>Dystonia &gt; left foot; lumbar hyperlordosis and dorsal scoliosis (DD)</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>IV-14/M</td>
<td>Childhood</td>
<td>22</td>
<td>Mild waddling gait; tendency to walk on toes</td>
<td>Mild dystonia &gt; left foot; lumbar hyperlordosis and dorsal scoliosis (DD)</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>IV-15/M</td>
<td>Childhood</td>
<td>29</td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IV-19/F</td>
<td>25</td>
<td></td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IV-20/M</td>
<td>22</td>
<td></td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>V-1/M</td>
<td>Birth?</td>
<td>5</td>
<td>Bilateral pes equinovarus at birth</td>
<td>Supination of feet; dystonic right hand while running (DD)</td>
<td>—</td>
</tr>
</tbody>
</table>

M, male; F, female; DD, definite dystonia; PoD, possible dystonia; DP, definite parkinsonism; PrP, probable parkinsonism; PoP, possible parkinsonism.
of 0.71. According to the proposed scoring method, 6 patients have definite foot dystonia with onset before the age of 12. Subject IV-12 of Family 2, because of mild nondiagnostic signs, has been classified as possible dystonia. Five other subjects have definite dystonia with parkinsonism (4 definite, 1 possible). Two subjects have a late adulthood-onset parkinsonism (1 definite, 1 probable) not preceded by childhood dystonia, responding to low dosage of L-dopa and without dopa-related complications for at least 7 years. In Family 2, Subject III-6 with young-onset parkinsonism and Subject III-8 with blepharospasm did not show the mutation (Fig. 1). In Subject III-6 molecular analysis of Parkin gene did not show any mutation.

Penetrance was not significantly different between men (9 of 13, 0.69) and women (6 of 8, 0.75) \( (P = 1) \) even when only definite dystonia and parkinsonism were considered (men, 7 of 13, 0.54; women, 6 of 8, 0.75) \( (P = 0.4) \).

**Genealogical Study and Haplotype Analysis**

Genealogical study three generations back to the index cases did not reveal any common ancestor. Haplotype analysis revealed a common haplotype for the four microsatellite markers used in all mutated members of the two families.

**DISCUSSION**

The clinical findings in two DRD families with the same GCH-1 mutation confirm the broad spectrum of symptoms and signs even in the same kindred ranging from childhood onset dystonia to isolated late adulthood-onset parkinsonism (Fig. 1). Some clinical presentations and findings deserve further discussion. The youngest subject (V-1, Family 2) presented a correctable pes equinovarus at birth and at 5 years had inversion of the feet and right hand dystonia while running. This suggests that DRD may be present even at birth, masquerading as an orthopedic problem, and dystonia may be active as early as in utero. Two other subjects (III-1, Family 1; III-17, Family 2) had surgery in their teens for structured pes equinovarus. Scoliosis or lumbar hyperlordosis were evident in 8 of 15 (53%) manifesting carriers of the mutation, all with childhood-onset dystonia. Scoliosis, lumbar hyperlordosis, and torticollis, which may be regarded as axial manifestations of dystonia, have been described previously in 47% of DRD subjects\(^{13}\) and are probably an underestimated manifestation of DRD.

Subject III-1 of Family 1 was reported to have had cerebral palsy and the correct diagnosis was made only at the age of 53. A label of diplegic cerebral palsy has been given frequently to subjects with DRD.\(^{13}\)
Subject IV-13 of Family 2 presented in childhood with a waddling gait and tendency to walk on his toes. He was suspected having a myopathy and received an unnecessary muscle biopsy before the correct diagnosis was made at the age of 30. Recently a boy similarly suspected to have a myopathy because of waddling gait, generalized hypotonia, and proximal weakness has proved to be an atypical presentation of DRD.14

Spontaneous remission of dystonia, as in Subject IV-8 of Family 2 (Table 1), has been reported in 5 patients; only 2 of them confirmed genetically.15-18 In 3 cases dystonia relapsed in 5 to 20 years.16,17 In particular, the subject reported by Bandmann and colleagues17 is very similar to ours in so much as foot dystonia went into complete remission and the relapse was represented by focal dystonia (torticollis and writer’s cramp). Focal dystonia in DRD is rare. The cases described in the literature,9,7,17 however, and our subject with writer’s cramp suggest that DRD should be considered in the differential diagnosis in subjects with focal dystonia and a positive family history for generalized dystonia.

Isolated parkinsonism as a presenting sign of DRD that has been reported in 3 cases belonging to a large North American family (at that time molecular diagnosis was not available)7 and more recently in 4 genetically confirmed cases.18,19 Our study confirms in 2 subjects that late-adulthood parkinsonism (not preceded by childhood dystonia) is part of the spectrum of clinical presentation of GCH-I gene mutations.

Finally, a possibly confounding factor was the presence in Family 2 of a subject with young-onset parkinsonism and another subject with mild blepharospasm who did not have the GCH-I gene mutations. The subject with young-onset parkinsonism did not show any mutation in the Parkin gene. We considered these cases as the occasional occurrence of other movement disorders in a DRD family.

Penetrance of DRD is reported to be low (about 0.3). This value, based primarily on the clinical study of a large North American family, has been calculated only on cases with definite dystonia.7 Inclusion of cases with probable and possible dystonia increased penetrance to 0.42 and 0.62, respectively. Definite parkinsonism alone had a penetrance of 0.25 and penetrance was nearly complete if all categories of dystonia and parkinsonism were included.7 These penetrance values are not certain because molecular correlations were not available at that time.7 In a more recent study of 5 German families with 33 members carrying mutations of GCH-I, 25 subjects had clinical symptoms and signs with an overall penetrance of 0.76 and a range in the individual family from 0.4 to 1.9 In this study, even subtle dystonic signs introduced in 3 subjects by a special writing test were considered.9 In the present study, using the same rating scales for dystonia and parkinsonism proposed by Nygaard and colleagues,7 correlation between clinical and molecular data yielded a penetrance of 0.71. Six subjects (2 women, 4 men) above 22 years of age were asymptomatic carriers. As in other families with DRD, the onset for dystonia is below the age of 12,1 the possibility of other cases of dystonia in our families is unlikely. It cannot be excluded, however, that some or all of the asymptomatic carriers, who did not manifest childhood dystonia, could develop adult parkinsonism with the result of a nearly complete or complete penetrance.

Clinical studies demonstrated in DRD a predominance of affected women (2 to 6:1).1,2 In the study by Furukawa and colleagues10 the penetrance of GCH-I mutations was 0.87 in women and 0.38 in men in 5 families and 4 sporadic cases, all with different mutations of GCH-I, for a total of 23 subjects.10 It should be emphasized that in this study only cases with dystonia were considered. In 5 German families with 33 mutated subjects, penetrance was 1 in women and 0.55 in men considering subtle dystonic signs and pain in the legs only when walking.9 In our two families with the same GCH-I mutation, the penetrance was not significantly different in woman and in men even when only definite cases with dystonia and parkinsonism were considered. This cannot be due to a sampling bias because the frequency of GCH-I mutation in women and men was not different. It is open to debate whether there is a real influence of gender on GCH-I gene mutation penetrance.

Currently, more than 60 different mutations of GCH-I occurring in many regions of the gene, including the 5’ untranslated region, have been reported.17,19,20 A common mutation of the GCH-I has not been found in the Japanese or the British population and some data on sporadic cases suggest a relatively high spontaneous mutation rate in the GCH-I gene.10,21 This is quite surprising in a rare autosomal-dominant gene defect where a founder mutation in the population examined might have been expected. A C544T, Glu182stop was detected in two apparently unrelated German families,9 and an Arg216stop nonsense mutation was demonstrated in two families living in the same region of France.19 Our families come from the same district and both have a 5-base pair insertion at codon 243 resulting in a frameshift and a premature stop at codon 247 with truncation of the polypeptide, suggesting a common origin of the mutated chromosomes. Although genealogical studies seemed to exclude that the families were related, haplotype analysis suggested the possibility that they descend from a single founder.
LEGENDS TO THE VIDEO

Segment 1. Lower limb dystonia. Family 2, Subject V-1: involuntary supination of right foot while standing; supination of feet and mild rigidity of lower limbs more evident while running. Family 1, Subject IV-2: stiff gait with knee hyperextension, supination of the left foot while running. Family 2, Subject III-18: lower limb dystonia, more pronounced in the left foot, rigidity, and mild bradykinesia.

Segment 2. Gait disorders. Family 2, Subject III-13: stiff, wide based, waddling gait, and dystonic posture of the left hand (flexion of the third and fourth with exten-sion of the second and fifth fingers). Family 2, Subject III-10: wide based rigid gait, rest tremor of hands (more of the left hand); hesitancy and instability in change of direction. Family 1, Subject III-1: generalized dystonia and rigidity, severe bradykinesia.

Segment 3. Postural instability. Family 1, Subject III-1.


Segment 5. Rest tremor. Family 2, Subject III-10.

Family 2, Subject III-18.

Segment 6. Rest dystonia. Family 2, Subject III-18: dystonia at rest of the right foot.

Segment 7. Striatal toe. Family 2, Subject III-18.

Segment 8. Pre-therapy and post-therapy. Family 1, Subject III-1.

REFERENCES