The GTP Cyclohydrolase I Gene in Russian Families With Dopa-Responsive Dystonia

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Objective: To search for mutations in the GTP cyclohydrolase I (GCH-I) gene in a set of Russian families with dopa-responsive dystonia (DRD).

Design: Six large families with 54 affected family members and 2 patients with sporadic DRD were examined. Mutation screening was performed using single-strand conformation polymorphism analysis followed by direct sequencing of the presumably mutated exons; in patients whose results showed a normal pattern on single-strand conformation polymorphism analysis, the entire coding region of the GCH-I gene was sequenced.

Results: Three new heterozygote point mutations located within exons 1, 2, and 4 of the GCH-I gene were identified in 3 families with autosomal-dominant inheritance. All these mutations are predicted to cause amino acid changes in the highly conserved regions of the gene. In patients from 3 other families and in both patients with sporadic DRD, no alterations in the translated portion of the GCH-I gene were observed.

Conclusions: Mutations in the coding region of the GCH-I gene account for a significant fraction (up to half) of the patients with a typical clinical picture of DRD. None of the mutations in the GCH-I gene described so far were detected more than once, which precludes the possibility of creating simple DNA testing procedures for routine clinical practice.

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HEREDITARY dystonias are a clinically and genetically heterogeneous group of disorders characterized primarily by sustained muscle contractions, twisting and repetitive movements, and abnormal postures. One clinical form of hereditary dystonia, originally described as “hereditary dystonia with marked diurnal fluctuations” (HPD), or “rigid form of torsion dystonia,” is now increasingly recognized as a distinct nosologic entity within a spectrum of familial dystonic syndromes. The hallmark of this disorder is a “dramatic” and sustained response to relatively small doses of levodopa. To emphasize this specific clinical feature of the condition, Nygaard et al in 1988 introduced the term “dopa-responsive dystonia” (DRD). In most familial DRD cases, autosomal-dominant transmission of the trait has been reported. In these families, the disease is characterized by low penetrance of an abnormal gene, marked sex predominance for females (reported to be 3:1-4:1), and an early age of onset, typically in the first decade of life. Patients first exhibit dystonic postures of the foot that usually affect gait, followed by gradual progression of the illness to generalized dystonia. Diurnal fluctuations of symptoms, with aggravation toward the evening and improvement in the morning after sleep, are frequent, but not essential, clinical features, being observed in approximately 70% of patients with DRD.

The gene for autosomal-dominant DRD was initially mapped in 3 families to a 22-centimorgan region on chromosome 14q (14q11-q24.3). Further analysis in families of different ethnic origin confirmed linkage of a mutant gene to 14q12-13 and suggested the genetic identity of DRD and HPD.

Using a candidate gene approach, Ichinose et al studied the gene for GTP cyclohydrolase I (GCH-I), the rate-limiting enzyme in biosynthesis of tetrahydrobiopterin, which, in turn, serves as an essential cofactor for tyrosine hydroxylase (TH) and is involved in converting L-tyrosine to levodopa. The GCH-I gene was mapped to chromosome 14q22.1-q22.2, just within the DRD/HPD locus. Four independent heterozygous mutations of the GCH-I gene were associated with the disease in patients from different families with DRD/HPD, and, correspondingly, in these patients the GCH-I activity in mononuclear blood cells was markedly decreased. Several further mutations of the GCH-I gene were identified in British, Japanese, Spanish, and Korean families with DRD, which may serve...
PATIENTS AND METHODS

We examined 6 large, multigenerational families (comprising 54 affected members) and 2 patients with sporadic DRD. The main criterion for inclusion was a marked and sustained response to low doses of levodopa. Such a response was observed in all the familial cases examined, as well as in 1 patient with a negative family history; in 1 sporadic case, levodopa treatment caused only a moderate improvement. Thirty-seven patients, including 35 familial cases, were examined by 2 of us (E.D.M. and I.A.I.-S.), and their clinical features are reported elsewhere. Segregation analysis in the familial cases (Figure 1) suggested autosomal-dominant inheritance with reduced penetrance in all the pedigrees, except family DRD-6, for which a mode of transmission could not be determined with certainty (see the “Comment” section).

Blood samples were obtained with informed consent from 16 individuals, and genomic DNA was extracted by standard procedures. Our strategy of mutation screening was based on single-strand conformation polymorphism (SSCP) analysis, followed by direct sequencing of the presumably mutated exons. Six exons of the GCH-I gene, including splicing junctions, were amplified using a polymerase chain reaction. Primer sequences used for amplification of exon 1 were as follows: 5’ portion of the exon, 5’-GGCCACCGGCGGAGGTTTACCCGAGC-3’ and 5’-GGGAAGGGAGCTCGCTTTATCCCT-3’; and 3’ portion of the exon, 5’-CCGCGAGCGGCTGGAAGGGCGAG-3’ and 5’-CCCCGCCGCCGCGAGCCTACG-3’. For exons 2 through 6, primers were used as reported elsewhere.

For SSCP analysis, the GCH-I gene exons were amplified in a 5-µL volume containing 50 ng of DNA, 50 mmol of potassium chloride, 10 mmol of Tris-hydrochloride (pH, 8.4), 1.5 mmol of magnesium chloride, 25 pmol of each deoxynucleotidetriphosphate, 0.074 MBq of [α-32P]dCTP, 2 pmol of each primer, and 0.125 U of AmpliTaq polymerase (Perkin-Elmer Cetus, Nashville, Tenn). For amplification of exons 3 through 6 and portions of exon 1, the reaction mix was denatured at 94°C for 5 minutes followed by 25 cycles of denaturation (at 94°C for 30 seconds) and annealing and extension (at 60°C for 1 minute), with a final extension at 72°C for 10 minutes. For amplification of exon 2, the mixture was denatured at 94°C for 5 minutes followed by 25 cycles of denaturation (at 94°C for 1 minute), annealing (at 53°C for 30 seconds), and extension (at 72°C for 1 minute), with a final extension at 72°C for 10 minutes. Polymerase chain reaction products were diluted 1:10 with a standard stop solution, boiled for 3 minutes, and immediately chilled on ice before loading. One microliter of each sample was loaded onto a 0.5× mutation detection enhancement gel (FMC BioProducts, Rockland, Maine) containing 0.6× Tris-borate/ethylenediaminetetraacetic acid buffer and 10% glycerol, and electrophoresis was carried out at 4°C for 18 to 24 hours; the gel was exposed to x-ray film (Fuji, Tokyo, Japan) for 24 hours at −70°C.

The second set of polymerase chain reactions was carried out for direct sequencing of exons that showed abnormally migrating bands on SSCP analysis or (in several cases) for direct sequencing of the entire coding region. Each 50-µl polymerase chain reaction contained 400 ng of genomic DNA, 50 mmol of potassium chloride, 10 mmol of Tris-hydrochloride (pH, 8.4), 1.5 mmol of magnesium chloride, 200 pmol of each deoxynucleotidetriphosphate, 8 pmol of each primer (1 primer being biotinylated), and 2.5 U of AmpliTaq polymerase. A temperature profile was the same as for SSCP analysis, except that annealing temperature was 52°C and 30 cycles were performed. Polymerase chain reaction products were purified and concentrated with SUREC-02 (Takara, Tokyo, Japan) and sequenced directly with the same primers as for amplification. Sequencing was performed using an automated ALF DNA Sequencer II (Pharmacia, Uppsala, Sweden), an AutoRead Sequencing kit (Pharmacia) and streptavidin-coated beads for producing single-stranded DNA (Dynal, Oslo, Norway), or conventional autoradiography and a Sequenase Version 2.0 kit (U.S.B., Cleveland, Ohio). Sequences were confirmed on identical and complementary strands.

as a confirmation of the causative role of the GCH-I gene in autosomal-dominant DRD. In view of these findings, the pathogenesis of dystonia in patients with DRD may be related to the secondary dopamine deficiency in the nigrostriatal dopaminergic pathways, resulting from low GCH-I activity, the lack of tetrahydrobiopterin, and the disturbed function of TH. This hypothesis is supported by the decreased levels of tetrahydrobiopterin in cerebrospinal fluid from patients with DRD/HPD and by the decrease in the TH activity in the striatum on postmortem study in a patient with DRD. Homozygous mutations of the GCH-I gene lead to a completely different condition, autosomal-recessive GCH-I deficiency, characterized by severe retardation of development, muscular hypotonia, and convulsions.

With the detailed molecular analysis in families with DRD, it became clear that only some autosomal-dominant and sporadic cases are caused by mutations in the coding region of the GCH-I gene. The discovery of an autosomal-recessive form of DRD, caused by mutations in the TH gene, further complicated the matter and provided evidence for true genetic heterogeneity of DRD. Therefore, studies of additional families with DRD are of crucial importance for clarifying the molecular genetics of DRD. In this article, we present our results of molecular analysis of the GCH-I gene in a set of Russian families with DRD.

RESULTS

Three new heterozygote mutations identified in our families are shown in the Table. The presence of these mutations was presumed on the basis of altered bands on SSCP analysis and was confirmed by direct sequencing of the corresponding exons in one affected member from each family (not shown). In family DRD-4, a T→A transition in exon 1 of the gene resulted in a methionine-to-lysine substitution at residue 102. This mutation destroys a restriction site for the enzyme NtAlIII, and restriction analysis confirmed that all the affected family members are heterozygous carriers of the mutation (Figure 2). In family DRD-1, an abnormally migrating band on SSCP analysis of exon 2 was found in 2 affected persons and 1 obligate carrier (Figure 1); sequencing of
the exon revealed a T→G transversion resulting in a cysteine-to-tryptophan change at residue 141. In family DRD-2, an altered SSCP band of exon 4 in 2 affected persons (Figure 1) suggested the existence of a disease-causing change, and direct sequencing of the sample revealed a G→C transversion that is predicted to cause a serine-to-threonine change at residue 176.

All these sequence alterations are located within highly conserved regions of the gene and are likely to disturb activity of the GCH-I. Moreover, because their presence was confirmed by either SSCP or restriction analysis in several patients in each family (including an obligate carrier in family DRD-1), this may serve as additional evidence for the pathogenic role of the mutations identified.

In patients from 3 families, and in both patients with sporadic DRD, no abnormally migrating bands were observed on SSCP analysis; direct sequencing of the entire coding region and splicing junctions of the GCH-I gene in these patients revealed no variations compared with the control sequence.

**Figure 1.** Pedigrees of the families with dopa-responsive dystonia (DRD). Squares indicate males; circles, females; white symbols, unaffected individuals; black symbols, affected individuals; slashed symbols, deceased individuals; arrows, probands (sequenced family members); arrowheads, cases studied by single-strand conformation polymorphism analysis; and asterisks, cases studied by restriction analysis.

**Figure 2.** Restriction-enzyme analysis of exon 1 in family DRD-4. A T→A transversion destroys a restriction site for NlaIII. The mutation is visualized by the presence of a nondigested polymerase chain reaction product (arrow) from a 3′ portion of exon 1. M indicates size marker; lane 1, normal control; and lanes 2 and 3, affected family members.

<table>
<thead>
<tr>
<th>Family</th>
<th>Exon</th>
<th>Base-Pair Change</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD-1</td>
<td>2</td>
<td>TG→TG</td>
<td>Cys141Trp</td>
</tr>
<tr>
<td>DRD-2</td>
<td>4</td>
<td>AGT→ACT</td>
<td>Ser176Thr</td>
</tr>
<tr>
<td>DRD-4</td>
<td>1</td>
<td>AG→AG</td>
<td>Met102Lys</td>
</tr>
</tbody>
</table>

*Nucleotide substitutions are underscored and boldfaced.*

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**Mutations of the GTP Cyclohydrolase I Gene in Russian Families With Dopa-Responsive Dystonia (DRD)**

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Our results confirm that heterozygous mutations of the 
GCH-I gene are associated with autosomal–dominantly
inherited DRD. We identified 3 new point mutations
distributed throughout the coding region of the 
GCH-I gene, all being missense mutations resulting in protein
amino acid sequence alterations. Several previous
groups also observed disease-causing changes in GCH-I
exons 1, 2, and 4, including transitions within highly
conserved regions of the gene.12,16,18 None of the muta-
tions of the GCH-I gene reported in these Russian fami-
lies with DRD or described so far in other series of
molecularly studied DRD cases12,15-19 were detected
more than once. We agree with Bandmann and al18 that
these findings may preclude the possibility of creating
simple DNA testing procedures in families with DRD
for routine clinical practice.

We found no mutations in the translated portion of
the GCH-I gene in 3 families with typical autosomal-
dominant DRD or in 2 patients with sporadic DRD. These
data are in agreement with those of other groups.12,16 There
are several possible explanations for these findings. First,
some sequence alterations within the noncoding region
of the gene may account for the disease phenotype in these
patients. Second, because the number of available DNA
samples from affected members of families DRD-3 and
DRD-5 was limited and did not allow us to confirm link-
age to chromosome 14q, we cannot exclude a causative
role of another, as yet unidentified, gene(s) in autosomal-
dominant DRD. Finally, our sporadic cases may repre-
sent examples of the autosomal-recessive DRD caused by
mutations of the TH gene.23,24 As shown in Figure 1, a
visual inspection of the pedigree chart DRD-6 does not
allow differentiation between autosomal-dominant and
autosomal-recessive inheritance in this family, taking into
account low penetrance of the mutant gene.8 Therefore,
these patients also could have an autosomal-recessive form
of DRD. Further data collection with detailed clinical,
molecular genetic, and biochemical studies will help
characterize involvement of different genes in the patho-
genesis of DRD.

Our experience shows that in the case of the normal
pattern on SSCP analysis, the possibility of identi-
fying mutations of the GCH-I gene through sequencing
of the entire coding region seems unlikely. We con-
clude that the strategy used in the present study for the
purpose of mutation screening is the most appropriate in
families with autosomal-dominant 14q-linked DRD and
in sporadic cases with the presumed clinical diagnosis of
DRD.

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