

High mutation rate in dopa-responsive dystonia: Detection with comprehensive *GCHI* screening

Abstract—Mutations in *GTP cyclohydrolase I (GCHI)* are found in 50 to 60% of cases with dopa-responsive dystonia (DRD). Heterozygous *GCHI* exon deletions, undetectable by sequencing, have recently been described in three DRD families. We tested 23 individuals with DRD for the different mutation types by conventional and quantitative PCR analyses and found mutations, including two large exon deletions, in 87%. The authors attribute this high mutation rate to rigorous inclusion criteria and comprehensive mutational analysis.

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Dopa-responsive dystonia (DRD) is a childhood-onset movement disorder characterized by excellent sustained response to low-dose levodopa.¹ Most DRD is autosomal dominantly inherited with reduced penetrance.¹ Sequence alterations in the *GTP cyclohydrolase I (GCHI)* gene have been found by single-strand conformation polymorphism (SSCP)/sequence analysis in about 50 to 60% of clinically typical cases.^{1–3} This relatively low mutation detection rate has remained one of several unresolved questions associated with DRD.^{1,4} Recently, a heterozygous exon deletion was identified by Southern Blot analysis in a single family that was undetectable by conventional sequencing methods.⁵ To test for the significance of large heterozygous deletions/multiplications, our group developed a quantitative duplex PCR assay and found exon deletions in two of four DRD families.⁶ We here report the results of a systematic mutational screen of 23 unrelated individuals with DRD, using both sequence analysis and quantitative PCR.

Methods. *Patients.* Twenty-three individuals with DRD were identified through tertiary referral movement disorders centers (Beth Israel Medical Center, Columbia-Presbyterian Medical Center, University of Lübeck) or in response to research advertisements. Informed consent was obtained from all patients. The criterion for diagnosis of DRD was definite dystonia with marked and sustained response to levodopa therapy.⁷ Neurologic examinations and dystonia sites were rated by movement disorder special-

ists as previously described.⁸ Age at onset was determined using patient history or medical records. Family history of a relative with dystonia or parkinsonism was verified by examination when possible. Continuous variables were compared using nonparametric methods (Mann-Whitney), and categorical variables using the Fisher exact test (STATA, V.8). None of the patients underwent a measurement of CSF tetrahydrobiopterin.

Mutational analysis. Mutational analysis was performed as previously described including sequence analysis and quantitative PCR to test for large exon deletions.⁶ Novel missense mutations and two sequence alterations that occurred in one patient were tested in 100 chromosomes from a European control population. Mutation-negative individuals were screened for *Parkin* mutations by sequencing and quantitative PCR.

Results. *Patients.* We included 23 unrelated patients (three males, 20 females) with clinically definite DRD. Mean age at onset was 7.0 ± 3.5 years (range 1 to 12), and all had onset in the limbs. Clinical details and family background are listed in the table.

Mutational analysis. We detected 20 mutations in the 23 patients (87%) (see table). Missense mutations were found in 10 and nonsense mutations in 2 individuals. In addition, three splice site mutations, three small deletions/insertions, and two large exon deletions were identified (figure 1 and 2). One of the mutations (IVS5 + 3insT) was found in two unrelated patients. None of the seven novel missense mutations was detected in 100 European control chromosomes. One patient carried two alterations that both resulted in amino acid changes (see table). One of the alterations (68C>T) previously identified in three other patients,^{3,9} however, was found in one of our controls. The other novel change was not found in our controls. Relatives to test for segregation of the sequence changes were not available.

In addition to these alterations, we initially considered Patient D4022 to carry a heterozygous deletion of exon 1, suggested by a ratio of the concentration of exon 1 of *GCHI* to *beta globin* of 0.52. However, sequence analysis with primers flanking the initial PCR product revealed a heterozygous mismatch mutation at the annealing site of the last 3'-nucleotide of the reverse primer.

None of the three *GCHI* mutation-negative individuals carried a *Parkin* mutation.

The mutation-positive patients (Group A, n = 20) and mutation-negative patients (Group B, n = 3) did not vary in their average age at onset (Group A, median 7.5 years and Group B, 6.0 years). They did not differ significantly in whether there was an affected family member with dystonia (13/20 in A and 3/3 in B) or with adult-onset parkin-

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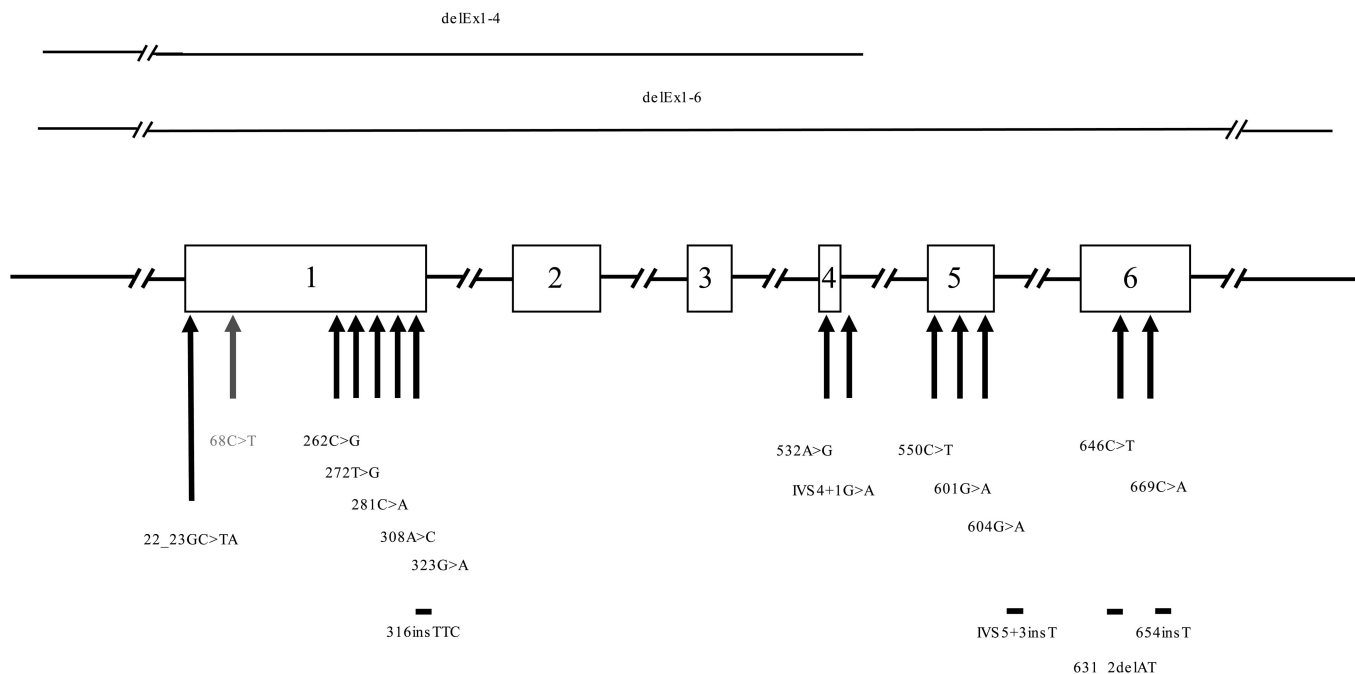


Figure 1. Mutations in GTP cyclohydrolase I (GCHI) detected in this study. A schematic representation of the GCHI gene with the six exons (squares) is given. Mutations found by sequence analysis are shown below the gene (substitutions as arrows and small deletions/insertions as bars) and large deletions of several exons are depicted above the gene. A single base-pair substitution that is considered to represent a rare polymorphism is indicated with a gray arrow.

sonism (6/18 in A [two cases in group A were unknown] and 2/3 in B). The average daily dose of levodopa was higher in Group A (median 225 mg, range 25-700) vs. Group B (150 mg, range 100-300); however, this difference was not significant.

Discussion. We present a comprehensive mutational analysis, including sequencing and quantitative PCR analyses, of a well characterized cohort of individuals with DRD and found mutations in a very

high percentage of patients (>85%). This is much greater than reported in previous studies, which showed mutation rates of 50 to 60%.¹⁻³

We attribute the high mutation detection rate in our study sample to two factors: 1) the use of quantitative PCR to identify large heterozygous deletions of whole exons and 2) rigorous clinical inclusion criteria. Large exon deletions were previously found in two of the four described Turkish families⁶ and in two of the 23 cases in the present investigation. Thus, this mutation type accounts for approximately 10% of the GCHI mutations in our sample of DRD cases, emphasizing the importance of quantitative PCR as previously shown for other genes, such as *Parkin* or *alpha-synuclein*. Our quantitative duplex PCR assay serves as a simple diagnostic test to screen for exon deletions and should be a standard in the molecular diagnosis of DRD in cases without any changes detectable by sequencing. Interestingly, we identified a deletion of all six exons of GCHI in Patient A27674, which is undetectable by Southern blot analysis previously used to detect exon deletions in GCHI.⁵ We confirmed the sensitivity of our quantitative PCR assay by the detection of a false-positive deletion due to a mutation at the last 3'-nucleotide of the annealing site for the reverse primer. Mutations of this nucleotide are critical because the PCR reaction starts at this point and needs immediate contact with the template. Both of the large exon deletions detected in the present study should result in haploinsufficiency of the gene product.

In addition, we adopted rigorous clinical criteria

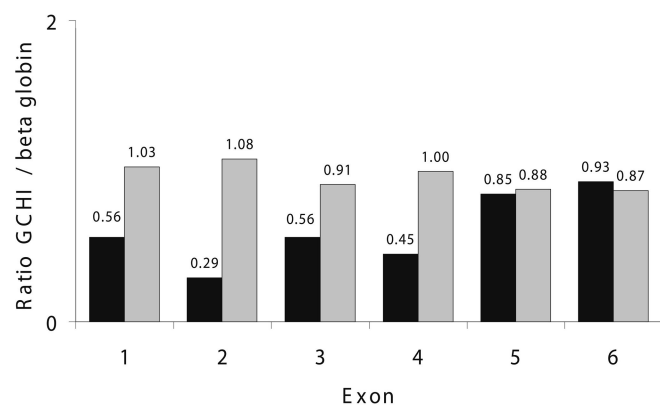


Figure 2. Results of quantitative PCR analysis for Patient B7143 (solid). The diagram shows the ratio of each exon of GTP cyclohydrolase I (GCHI) compared with a reference gene (beta globin). Ratios between 0.25 and 0.70 indicate a heterozygous deletion, whereas ratios between 0.80 and 1.20 exclude a deletion. Ratios for exons 1 to 4 of Patient B7143 suggest a deletion of these exons, whereas the other exons and all exons of the control (gray) are not deleted.

Table Clinical and genetic data on the 23 patients

Patient	<i>GCHI</i> mutations (DNA)	<i>GCHI</i> mutations (protein)	Sex/age, y/age at onset, y	Site of onset	Levodopa dose, mg	Sites affected	Family history of PD	Family history of dystonia	Origin
A27674	Ex1-6del	Loss of protein	M/17/5	Legs	25	Arms, legs	No	Yes	Germany, England
B7143	Ex1-4del	Loss of protein	F/37/12	Arm, leg	100	Leg, arm	Unknown	No	Unknown
C859	262 C>G	Arg88Gly	F/21/12	Legs	200	Legs	No	Yes	Germany
D4022	272T>G	Leu91Arg	F/14/10	Leg	100	Leg	No	No	AJ: Poland, Russia
E3721	281C>A	Thr94Lys	F/25/11	Leg	150	Trunk, legs	Yes	Yes	Scotland, Ireland, France, England
F27258	308A>C	Gln103Pro	F/40/3	Limbs	600	Arms, legs	No	Yes	Unknown
T2424	323G>A	Gly108Asp	F/16/9	Legs	100	Arms, legs	Yes	Yes	England, Germany, Holland
G4460	532A>G	Arg178Gly	F/36/8	Leg	100	Legs	Unknown	Yes	Norway, Germany
H3942	550C>T	Arg184Cys	F/25/1	Legs	100	Neck, trunk, arms, legs	Yes	No	AJ: Poland, Ukraine, Russia, Austria
I1275	601G>A	Gly201Arg	F/35/11	Leg	350	Arms, legs	No	No	Germany
J7671*	604G>A	Val202Ile	F/28/5	Leg	150	Leg	No	Yes	AJ: Poland, Hungary
K180	669C>A 68C>T	Ser223Arg Pro23Leu	M/38/7	Legs	500	Arms, legs	Yes	No	Italy
L546	22_23GC>TA	Ala8Stop	F/30/3	Legs	700	Arms, legs	No	Yes	Germany, England, Cuba, Spain
M26296*	646C>T	Arg216Stop	F/31/4	Leg, trunk	300	Arm, legs	Yes	Yes	Germany, Switzerland
N8819	316insTTC	106Thr/ins106Phe	F/17/12	Leg	500	Leg	Yes	Yes	Germany, England, Ireland
O8548*	631_632delAT	Met 211/Stop248	F/17/9	Leg	250	Neck, arm, leg	No	No	Italy, Sweden, Caribbean
P5664	654ins T	Val218/stop249	F/37/5	Leg	300	Arms, legs	No	Yes	Italy
Q8306	IVS4+1G>A	Premature truncation	M/34/6	Leg	500	Lower face, neck, arm, leg	No	Yes	Portugal, Scotland
R3793	IVS5+3insT	Premature truncation	F/44/1	Arms, legs	400	Trunk, legs	No	No	AJ: Poland, Germany, Russia
S5852	IVS5+3insT	Premature truncation	F/30/10	Limbs	100	Arm, legs	No	Yes	Japan, Korea
U4834	None detected		F/54/6	Arm, leg	150	Arm, leg	Yes	Yes	Ireland, Native North America
V27259	None detected		F/41/5	Leg	100	Arm, trunk, legs	No	Yes	Germany, Ireland, partly AJ
W8829	None detected		F/41/7	Leg	300	Arms, legs	Yes	Yes	England

* No DNA for proband, mutation identified in a family member.

GCHI = GTP cyclohydrolase 1; PD = Parkinson disease; AJ = Ashkenazi Jewish.

and did not include individuals with clinical features of DRD who had never tried levodopa, regardless of diurnal fluctuations and sustained response to anticholinergics. Our patients also had an extended length of follow-up, eliminating some of the atypical patients with DRD.

The *GCHI* gene appears to be highly mutable. To date, there are 85 different mutations reported in the database (www.bh4.org/biomdb.summary.html), although the *GCHI* gene is relatively small with six exons and an open reading frame of 750 bp. Interestingly, our ethnically diverse sample included four mutation carriers of Ashkenazi Jewish descent, all of whom carried a different mutation, excluding a founder effect. Overall, the rate of recurrent mutations is low, and only one mutation in the present study was identified in more than one individual. However, a common founder in these cases is un-

likely because of their different ethnic origin (Asian vs European). Fifteen of the 20 identified mutations represent novel mutations, further broadening the mutational spectrum of *GCHI*.

Individual K180 carried two sequence changes, one of which (68C>T) was also rarely found in controls.⁹ Interestingly, this change was previously also detected in a patient with DRD in combination with another mutation, suggesting that the 68C>T alteration represents either a rare polymorphism or is disease causing only in conjunction with another mutation.⁹

It remains unclear whether the mutation-negative cases may have *GCHI* mutations, unidentifiable by our comprehensive methods, for example, in regulatory or intronic regions. This idea is supported by the observation that there was no significant phenotypic difference between the mutation-positive and mutation-negative group. However, this conclusion is

limited by the relatively small sample size, especially in the mutation-negative group. Alternatively, they could have another etiology of DRD such as mutations in an additional autosomal dominant DRD gene like *DYT14*¹⁰ or atypical juvenile parkinsonism. It is unlikely that the three mutation-negative patients represent cases of tyrosine hydroxylase deficiency because their family history was suggestive of autosomal dominant disease. Further investigations to elucidate the cause of the disease in these families will be necessary.

Finally, our results confirm the value of the levodopa test in DRD even in the absence of any molecular genetic findings. If genetic testing is performed, gene dosage studies should be carried out, at least in cases with no detected sequence alterations.

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References

1. Furukawa Y. Update on dopa-responsive dystonia: locus heterogeneity and biochemical features. *Adv Neurol* 2004;94:127–138.
2. Bandmann O, Valente EM, Holmans P, et al. Dopa-responsive dystonia: a clinical and molecular genetic study. *Ann Neurol* 1998;44:649–656.
3. Steinberger D, Korinthenberg R, Topka H, Berghäuser M, Wedde R, Müller U. Dopa-responsive dystonia: mutation analysis of *GCHI* and analysis of therapeutic doses of L-dopa. *Neurology* 2000;55:1735–1737.
4. Nygaard TG, Wooten GF. Dopa-responsive dystonia: some pieces of the puzzle are still missing. *Neurology* 1998;50:853–855.
5. Furukawa Y, Guttman M, Sparagana SP, et al. Dopa-responsive dystonia due to a large deletion in the GTP cyclohydrolase I gene. *Ann Neurol* 2000;47:517–520.
6. Klein C, Hedrich K, Mohrmann K, et al. Exon deletions in the *GCHI* gene in two of four Turkish families with dopa-responsive dystonia. *Neurology* 2002;59:1783–1786.
7. Nygaard TG, Marsden CD, Duvosin RC. Dopa-responsive dystonia. *Adv Neurol* 1988;50:377–384.
8. Bressman SB, Raymond D, Wendt K, et al. Diagnostic criteria for dystonia in *DYT1* families. *Neurology* 2002;59:1780–1782.
9. Jarman PR, Bandmann O, Marsden CD, Wood NW. GTP cyclohydrolase I mutations in patients with dystonia responsive to anticholinergic drugs. *J Neurol Neurosurg Psychiatry* 1997;63:304–308.
10. Grotzsch H, Pizzolato GP, Ghika J, et al. Neuropathology of a case of dopa-responsive dystonia associated with a new genetic locus, *DYT14*. *Neurology* 2002;58:1839–1842.

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