

WE found a new mutation in the GTP cyclohydrolase gene involved in dopa-responsive dystonia. We sequenced the GTP cyclohydrolase gene in a family with four siblings affected by this disorder and identified an A–T mutation in exon 2, leading to a non conservative amino acid substitution at codon 135 of the protein (Ile135Lys), which may change the conformation of the binding site of this enzyme. The clinical evolution was heterogeneous among carriers of the same mutation, underlining the involvement of other determinants modulating the occurrence of the disease such as genetic or environmental susceptibility factors. *NeuroReport* 10:487–491 © 1999 Lippincott Williams & Wilkins.

A new GTP-cyclohydrolase I mutation in an unusual dopa-responsive dystonia, familial form

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Introduction

Dopa-responsive dystonia (DRD) is a rare disorder characterized by a fluctuating dystonia which develops during childhood with typical clinical features of lower limb dystonia, diurnal fluctuation and possible concomitant parkinsonism. However, it is now clear that later in life it may resemble idiopathic Parkinson's disease (PD) [1]. Indeed, these two diseases share some clinical features such as parkinsonism, focal dystonia, leading sometimes to confusion between both disorders. However, DRD can be distinguished by its sustained and dramatic responses to low doses of levodopa therapy and its different clinical evolution. In most cases, DRD is inherited as an autosomal dominant trait. A locus responsible for DRD was mapped to chromosome 14q21-22 and numerous heterozygous mutations in the GTP cyclohydrolase I gene were recently identified as a cause of DRD in sporadic and familial cases [2–15]. This gene contains 6 exons coding for the rate-limiting enzyme for the biosynthesis of tetrahydrobiopterin (BH4). BH4 is a necessary cofactor for hydroxylases of aromatic amino acids. DRD patients carrying a GTP-CH mutation have a partial de-

crease in enzyme activity and also in striatal biopterin and dopamine concentrations [16]. However, the GTP-CH gene mutations cause symptoms in some but not in all carriers and other factors may influence the susceptibility to develop DRD [2]. In this paper we describe a new mutation in the GTP-CH gene in a family in which four siblings, affected with DRD, presented a different clinical evolution between the men and women.

Subjects and Methods

Family: A detailed clinical description of the family was reported previously [17]. Two sisters and two brothers belonging to the same sibship developed fluctuating dystonia during childhood or adolescence (Fig. 1). Their parents were described as unaffected by relatives, but they had never been examined by a neurologist. The sisters were 7 (II.4) and 8 (II.3) years old when dystonia appeared. A simultaneous parkinsonism developed in II.4 whereas it occurred after the age of 54 years in II.3. Levodopa treatment proposed at age 51 (II.4) and 56 (II.3) was and is still effective for both of them. For the brothers, dystonia began at age 13 (II.1) and 15

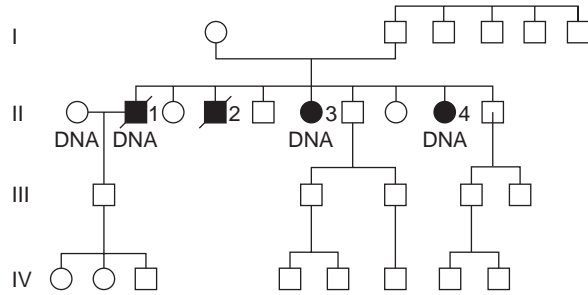


FIG. 1. Generations are indicated by Roman numerals. Shaded symbols represent individuals with DRD, clear symbols indicate individuals presently unaffected. Individuals marked DNA were sequenced.

(II.2). Parkinsonism (rest tremor) appeared at age 15 in case II.1. Treatment with levodopa was not proposed. However, dystonia and parkinsonism spontaneously disappeared at age 40 in individual II.1 and at age 44 in II.2. For 17 years the brothers were free of symptoms; parkinsonism then reappeared in both of them, but was dramatically improved by levodopa. Serum and urinary biopterin levels were lowered in the four patients and normal in an unaffected sister. According to Nygaard's criteria, the diagnosis of DRD was proposed in spite of the strange course in two steps for the two brothers. After 2 years (II.2) or 7 years (II.1) of treatment, their clinical evolution worsened with the appearance of levodopa-related motor complications such as the wearing-off phenomenon and dyskinesias. Cases II.1 and II.2 then presented similar characteristics to those of PD, not reported in DRD: predominant parkinsonism expression, initial and transient levodopa response, wearing off and dyskinesias.

Genetic analysis: Genomic DNA was extracted from EDTA-anticoagulated peripheral blood of the three patients available for analysis (II.1, II.3, II.4) and the spouse of II.1 and 92 unrelated control subjects (informed consent was obtained). All six exons and splicing junctions of the GTP-CH gene were amplified by PCR using intronic primers as described by Ichinose and co-workers [2]. Direct sequencing of the PCR products for the four members of the DRD family were performed with the 70770 Sequenase version 2.0 sequencing Kit (Amersham Life Science) using $\beta[^{35}\text{S}]\text{dATP}$. Reactions were run on a 6% acrylamide/8 M urea gel for 1–2 h at 60 W. The gels were then dried and exposed to X-ray film. Mutated sequences were confirmed on both strands.

Ninety-two healthy Caucasian samples were screened for the polymorphism found in exon 2 by allele-specific oligonucleotide (ASO). Two oligonucleotides, 5'-AAGGACA(T/A)AGACATG-3' were

5' end-labelled with DIG (Boehringer Mannheim). Allele-specific hybridization was obtained after washing for 3 min in a 0.1% SDS and $0.5\times$ SSC solution at 42°C for both the G and the T allele hybridization. Detection was realized as described by the supplier (Boehringer Mannheim).

Results

The three patients taking part in the analysis were heterozygous for an A–T substitution at a codon in the exon 2 of the GTP-CH gene (Fig. 2). This nucleotide alteration changed a isoleucine to a lysine at position 135 in the protein. No other nucleotide change was detected in the GTP-CH gene. None of the 92 controls nor the spouse of II.2 carried this polymorphism.

Discussion

We found a novel missense mutation in exon 2 (Ile135Lys) of the GTP-CH gene in a French family with DRD, which may modify the GTP-CH activity. The GTP-CH hydrolase is a homodecamer composed of 10 identical subunits. In DRD, this enzyme is composed of wild and mutant subunits. It has been suggested that the active site of this enzyme formed a narrow pocket at the interface of three monomers involving different parts of the molecule, one of them comprising the residues 131–139. Furthermore, the inner wall of the pocket is constituted of hydrophobic residues [18]. The substitution of the hydrophobic residue by the hydro-

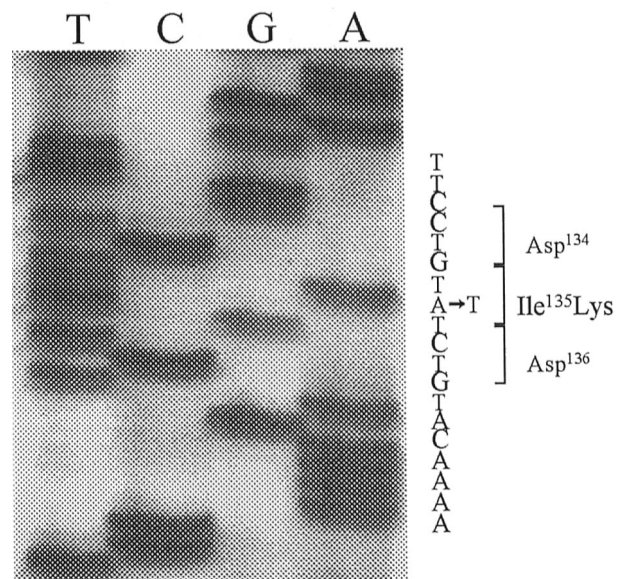


FIG. 2. Direct sequence analysis of the exon 2 of the GTP cyclohydrolase I gene in dopa-responsive dystonia patient 1 presenting the mutation (A–T) at codon 135.

Table 1. Characterization of the GTP-CH mutations in DRD.

Location in gene	Nucleotide aberration	Mutant type	Predicted effect of mutation	Phenotypes	References
5'-UTR	C ⁽⁻³²⁾ →T	Transcription/translation defect?	Decreased transcription/translation?	DRD	*Bandmann <i>et al.</i> , 1998
Exon 1	ATG ³ →ATC	Substitution	Met 1 Ile	DP/A	Tamaru <i>et al.</i> , 1997
Exon 1	ATG ³ GAG→ATGGGGAG	Insertion/frameshift	3 ins GG	DRD	*Ichinose <i>et al.</i> 1994
Exon 1	CC ⁶⁸ C→CTC	Substitution	Pro 23 Leu	D	De la Fuente-Fernandez, 1997; *Jarman <i>et al.</i> , 1997
Exon 1	GC ¹⁴⁹ G	Deletion/frameshift	149 del C	DRD	Furukawa <i>et al.</i> , 1998
Exon 1	G ¹⁸¹ AG→TAG	Substitution/truncation	Ser 60 Stop	aDRD/D	Nitsche <i>et al.</i> , 1998
Exon 1	G ¹⁶⁶ AG→TAG	Substitution/truncation	Glu 56 Stop	DRD	*Bandmann <i>et al.</i> , 1998
Exon 1	G ¹⁹³ AG→TAG	Substitution/truncation	Glu 65 Stop	D	*Furukawa <i>et al.</i> , 1996
Exon 1	CT ²¹² G	Deletion/frameshift	212 del T	DRD	Furukawa <i>et al.</i> , 1998
Exon 1	CT ²¹² G→CAG	Substitution	Leu 71 Gln	DRD	*Bandmann <i>et al.</i> , 1998
Exon 1	GC ²²¹ C→GTC	Substitution	Ala 74 Val	DRD	*Bandmann <i>et al.</i> , 1998
Exon 1	T ²²⁹ CCATCCGAGCTCGCTG	Deletion from Ser77 to Leu 82	229 del 18 bp	DRD	Furukawa <i>et al.</i> , 1998
Exon 1	CT ²³⁶ G→CCG	Substitution	Leu 79 Pro	A/DRD	*Ichinose <i>et al.</i> , 1995
Exon 1	GG ²⁴⁸ C→GCC	Substitution	Gly 83 Ala	ADRD	Bandmann <i>et al.</i> , 1998
Exon 1	C ²⁶² GG→TGG	Substitution	Arg 88 Trp	DRD/A	*Ichinose <i>et al.</i> , 1994
Exon 1	CG ²⁶³ G→CCG	Substitution	Arg 88 Pro	DRD	*Bandmann <i>et al.</i> , 1996
Exon 1	A ³⁰⁴ TG→AAG	Substitution	Met 102 Lys	DRD	Illarioshkin <i>et al.</i> , 1998
Exon 1	G ³⁰⁹ TT	Deletion/frameshift/truncation	309 del G	D/A	Steinberger <i>et al.</i> , 1998
Exon 1	GG ³²³ C→GAC	Substitution	Gly 108 Asp	HPA-DRD/A	Furukawa <i>et al.</i> , 1998
Exon 1	TC ³⁴¹ A→TAA	Substitution/truncation	Ser 114 Stop	D	*Furukawa <i>et al.</i> , 1996
Exon 1	TCA ³⁴² →TCT	Splicing defect?	Exon skipping?	DRD	*Bandmann <i>et al.</i> , 1998
Exon 1	G ³⁴³ AT→AAT	Substitution	Asp 115 Asn	D/A	*Jarman <i>et al.</i> , 1997
Exon 1	TC ³⁴¹ A→TAA	Substitution/truncation	Ser 144 Stop	DRD	Furukawa <i>et al.</i> , 1998
Exon 1	CA ³²⁹ GGAG→CAA GGA	Insertion/frameshift	329 ins A	DRD	Furukawa <i>et al.</i> , 1998
Intron 1	a ⁽⁻²⁾ g→gg	Splicing defect/frameshift/truncation	Exon 2 skipping	D/DP/A	Weber <i>et al.</i> , 1997; Steinberger <i>et al.</i> , 1998
Intron 1	a ³⁴⁴⁻² g→aa	Splicing defect	Exon 2 skipping	D	*Furukawa <i>et al.</i> , 1996; Furukawa <i>et al.</i> , 1998
Exon 2	CTA ³⁵¹	Deletion/frameshift	351 del A	HPA-DRD/DRD	Furukawa <i>et al.</i> , 1998
Exon 2	GA ⁴⁰¹ C→GTC	Substitution	Asp 134 Val	DRD/A	*Ichinose <i>et al.</i> , 1994
Exon 2	AT ⁴⁰⁴ A→AAA	Substitution	Ile 135 Lys		Family Te (present report)
Exon 2	TGT ⁴²³ →TGG	Substitution	Cys 141 Trp	DRD	Illarioshkin <i>et al.</i> , 1998
Exon 2	CA ⁴³¹ C→CCC	Substitution	His 144 Pro	DRD/A	*Hirano <i>et al.</i> 1996; Tamaru <i>et al.</i> , 1997
Intron 2	gt →ct	Splicing defect	Exon 2 skipping	DRD/A	Hirano <i>et al.</i> , 1995; Tamaru <i>et al.</i> , 1997
Intron 2	a ⁽⁻²⁾ g→gg	Splicing defect/frameshift/truncation	New splice acceptor site	D/P?	Weber <i>et al.</i> , 1997
Exon 3	CA ⁴⁵⁸ T→CCT	Substitution	His 153 Pro	ND	*Bandmann <i>et al.</i> , 1996
Intron 3	a ⁵¹⁰⁻² g→aa	Splicing defect	Exon 4 skipping	DRD	Furukawa <i>et al.</i> , 1998
Exon 4	A ⁵¹¹ TT GTA GAA ATC TAT	Deletion/frameshift	511 del 13 pb	ND	*Ichinose <i>et al.</i> , 1995
Exon 4	AG ⁵²⁷ T→ACT	Substitution	Ser 176 Thr	DRD	Illarioshkin <i>et al.</i> , 1998
Exon 4	GA ⁵³⁴ C→GCC	Substitution	Arg 178 Ser		Beyer <i>et al.</i> , 1997
Intron 5	gt →at	Splicing defect	Exon 5 skipping	DRD/A	Hirano <i>et al.</i> , 1998
Intron 5	gt →at	Splicing defect	Exon 6 skipping	DRD	*Bandmann <i>et al.</i> , 1998
Exon 5	C ⁵⁴⁴ AG→TAG	Substitution/truncation	Glu 182 Stop	D/DP/P	Steinberger <i>et al.</i> , 1998
Exon 5	AC ⁵⁵⁷ A→AAA	Substitution	Thr 186 Lys	DP	*Hirano <i>et al.</i> , 1997; Imaiso <i>et al.</i> , 1998
Exon 5	G ⁵⁷¹ TA→ATA	Substitution	Val 191 Ile	DRD	*Bandmann <i>et al.</i> , 1998
Exon 5	GG ⁶⁰² A→GAA	Substitution	Gly 201 Glu	DRD	*Ichinose <i>et al.</i> , 1994
Exon 5	G ⁶⁰⁷ GG→AGG	Substitution	Gly 203 Arg	ND	*Bandmann <i>et al.</i> , 1996

(Continued)

Table 1. (Continued).

Location in gene	Nucleotide aberration	Mutant type	Predicted effect of mutation	Phenotypes	References
Exon 6	A⁶³¹T	Deletion	631 del AT	ND	Furukawa et al., 1998
Exon 6	C⁶⁴⁶G → TGA	Substitution/truncation	Arg 216 Stop	ND	* Bandmann et al., 1996
Exon 6	AT⁶⁶²G → ACG	Substitution	Met 221 Thr	DP/A/HPA-DRD	Bezina et al., 1998; Furukawa et al., 1998
Exon 6	A⁶⁷⁰AA → TAA	Substitution/truncation	Lys 224 Stop	HPA-DRD/P/A	* Jarman et al., 1997
Exon 6	A⁶⁷¹A → AGA	Substitution	Lys 224 Arg	DRD	* Bandmann et al., 1996; Furukawa et al., 1998
Exon 6	TT⁷⁰¹C → TCC	Substitution	Phe 234 Ser	ND	* Bandmann et al., 1996
Exon 6	C⁷²¹GG → TGG	Substitution	Arg 224 Trp	ND	* Bandmann et al., 1998

Phenotypes: DRD, dopa-responsive dystonia; ND, not described; P, parkinsonism; D, dystonia; DP, dystonia and parkinsonism; A, asymptomatic; aDRD: atypical signs for diagnosis of DRD; HPA-DRD, intermediate phenotype between hyperphenylalaninemia and DRD. The references cited in [6] are identified by an *; the others correspond to [3–15].

philic one at position 135 may modify this binding site and may disrupt the enzyme activity. In this context, the low bipterin levels observed in these DRD cases suggested the perturbation of dopamine biosynthesis and would tend to confirm the involvement of the Ile135Lys mutation. Furthermore, the absence of this non conservative amino acid substitution in 92 healthy individuals led to the suggestion that this mutation is not a simple polymorph marker. To date in DRD, more than 30 mutations have been found scattered along the GTP-CH gene with quite a wide range of phenotypic expression [2–21] (Table 1). Most mutations described were in the coding region in particular in exon 1 and exon 6 often leading to amino acid substitution or stop codon. Some non-coding mutations in the splice site or in the 5'-end region have also been described [6]. However, no apparent correlation between the type or the location of these mutations and the phenotypes could be made with the available information.

The finding of a GTP-CH mutation in this DRD family raises new questions. Indeed, the two brothers presented peculiar features, including the disappearance of the dystonic symptoms for 17 years without treatment and then the reappearance of levodopa-responsive parkinsonism, and the occurrence of the wearing-off phenomenon and dyskinesias after 3 years of levodopa treatment.

The occurrence of both DRD and PD phenotypes in the same family may only be due to chance, since the prevalence of Parkinson's disease is relatively high. These PD symptoms in DRD may either be directly related to the specific Ile135Lys GTP-CH mutation or to other susceptibility factors. However, the lack of symptoms in some carriers of other GTP-CH mutations, the phenotype variation inside the same families and the spontaneous disappearance of clinical manifestation of the two brothers during 17 years suggest the involvement of other determinants modulating the occurrence of the disease such as environmental factors or gender effect [13,22,23,24].

Conclusion

The new mutation reported in this work reinforces the potential contribution of the GTP-CH gene to the development of DRD. However, the different clinical evolution observed in individuals presenting with the same mutation underlines the hypothesis that other genetic or environmental factors may modulate the occurrence and presentation of the disease. Therefore, both detailed clinical manifestations and genetic profiles are needed to try to bring out a genotype-phenotype correlation. This may allow to direct the search towards the other susceptibility factors involved in this disorder. Further-

more, the occurrence of both DRD and PD phenotypes in this family may also suggest that genetic susceptibility factors involved in DRD might also be candidate for PD.

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