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Levodopa-responsive dystonia
GTP cyclohydrolase I or parkin mutations?

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Summary
Autosomal dominant DOPA-responsive dystonia (DRD) is usually caused by mutation in the gene encoding guanosine triphosphate-cyclohydrolase I (GTPCH I). We studied 22 families with a phenotype of levodopa-responsive dystonia by sequencing the six coding exons, the 5′-untranslated region and the exon–intron boundaries of the *GTPCH I* gene. Eleven heterozygous mutations were identified, including five missense mutations, one splice site mutation, two small deletions and two nonsense mutations, in 12 families that included 27 patients and 13 asymptomatic carriers. Six mutations were new and five had already been reported. Four of the mutations caused truncation of the GTPCH I protein. One family carried a base-pair change in the 5′-untranslated region, not detected in controls, that could be responsible for the phenotype. Three of the remaining 10 families had deletions in the parkin gene on chromosome 6, underlining how difficult it is to distinguish, in some cases, between DRD and parkin mutations. No mutations were identified in seven families. The clinical spectrum extended from the classical DRD phenotype to parkinsonism with levodopa-induced dyskinesias, and included spastic paraplegia as well as the absence of dystonia.

Keywords: DOPA-responsive dystonia, *GTPCH I* gene, sequence analysis, mutations, phenotype–genotype correlation

Abbreviations: bp = base pair(s); DRD = DOPA-responsive dystonia; GTPCH 1 = guanosine triphosphate cyclohydrolase I; PCR = polymerase chain reaction

Introduction
DOPA-responsive dystonia (DRD) is a disorder characterized by childhood or adolescent onset of dystonia sometimes associated with parkinsonism. Treatment of symptoms is based on the marked and sustained response to low doses of levodopa (Segawa *et al*., 1976). An autosomal recessive form of DRD has been described in a few families with homozygous mutations in the gene encoding tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis

(Knappskog *et al.*, 1995; Ludecke *et al*., 1995, 1996; van den Heuvel *et al.*, 1998). Autosomal dominant DRD, mapped to chromosome 14 (Nygaard *et al.*, 1993), has been shown to be caused by mutations in the gene for guanosine triphosphate cyclohydrolase I (GTPCH I) (Ichinose *et al.*, 1994). GTPCH I is the initial and rate-limiting enzyme in the production of tetrahydrobiopterin, which serves as the cofactor for tyrosine hydroxylase.

Several different mutations in the *GTPCH I* gene have been reported throughout the world (Ichinose *et al.*, 1994; Blau *et al.*, 1995; Hirano *et al.*, 1995; Bandmann *et al.*, 1996, 1998; Furukawa *et al.*, 1996; Beyer *et al.*, 1997; Illarioshkin *et al.*, 1998) and several studies have demonstrated a dominant negative effect of mutant GTPCH I on the normal enzyme (Hirano *et al.*, 1996, 1998; Hirano and Ueno, 1999). In most families, DRD is an autosomal dominant disorder with reduced penetrance (~30%) (Nygaard *et al.*, 1990). Female carriers are more often affected than males (3–4 : 1) (Nygaard, 1995). Homozygous mutations in the *GTPCH I* gene have also been described; they result in a more severe phenotype, resembling atypical phenylketonuria, that is not completely responsive to levodopa (Blau *et al.*, 1995; Furukawa *et al.*, 1998; Hirano and Ueno, 1999).

Recently, mutations in the parkin gene were found in families with autosomal recessive juvenile parkinsonism (Hattori *et al.*, 1998; Kitada *et al.*, 1998; Lucking *et al.*, 1998; Abbas *et al.*, 1999). The phenotype is highly variable, but dystonia with an excellent response to levodopa can be the major clinical sign. However, levodopa-induced dyskinesias are usually precocious and severe in patients with parkin gene mutations as opposed to those with DRD. We have sequenced the *GTPCH I* gene in 22 families with DRD, and have analysed the parkin gene in a subset of them in order to establish genotype–phenotype correlations.

Patients and methods

Families and patients

Twenty-two families with dystonia responsive to levodopa in at least one member were selected for molecular analysis. Twenty index cases had the classical DRD phenotype of early-onset dystonia with a complete response to low doses of levodopa. Two additional families were included after the descendants were found to have classical DRD: one with spastic paraplegia responsive to levodopa and one with Parkinson's disease, including levodopa-induced dyskinesias in the index cases. Eight cases had no family history and 14 had at least one affected first-degree relative. Blood samples were taken with informed consent from 33 patients and 40 clinically unaffected first-degree relatives. All families were of French origin except one from Algeria. Sixty-four Caucasian subjects with no neurological disorders were included as controls.

Molecular analysis of the GTP cyclohydrolase I gene

PCR amplification and sequence analysis

All exons and exon–intron junctions of the *GTPCH I* gene from 22 index patients were amplified from genomic DNA by the polymerase chain reaction (PCR) as described by Ichinose and collaborators (Ichinose *et al.*, 1994), except for exon 3, for which a new reverse primer was designed in the intronic sequence (5′-GATTCTCAGCAGATGAGGG-3′). We also amplified exon 6 of the alternatively spliced type 2 *GTPCH I* (Togari *et al.*, 1992) with primers 5′-GTGTGATCCATGTAGATGC-3′ and 5′-CGTTGGAC-ACAGCTCATAATG-3′. The 5′-untranslated region was amplified as described by Bandmann and collaborators (Bandmann *et al.*, 1998).

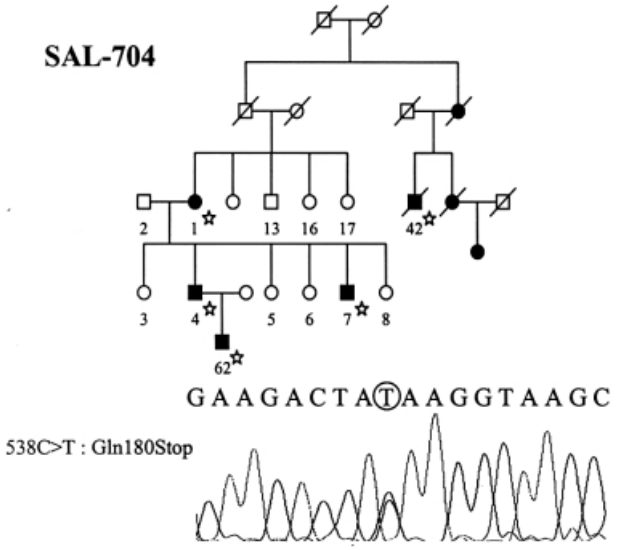
The same primers were used for sequencing the PCR products on both strands using the Big Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit (ABI Prism), according to the manufacturer's recommendations, on an ABI 377 automated sequencer. Sequences were analysed with the Sequence Analysis 3.0 (ABI Prism) software. When a mutation was identified in an index case, co-segregation with the disease was established in the patient's family. To confirm the presence of the mutation in relatives or to verify its absence in the control population, PCR amplification of genomic DNA was followed by sequence analysis and/or digestion with the appropriate restriction endonuclease followed by agarose gel electrophoresis, and/or polyacrylamide gel electrophoresis. In family SAL-444, mRNA extraction from leucocytes and RT–PCR (reverse transcription–PCR) amplification of the GTPCH I transcript was also used. Nucleotide positions were determined according to the cDNA sequence published by Togari and collaborators (Togari *et al.*, 1992) (Fig. 1).

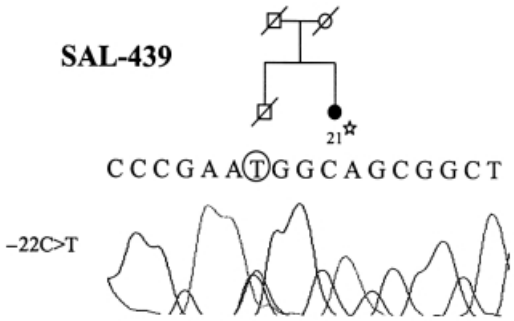
Restriction fragment assay

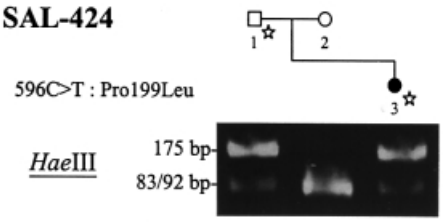
Twenty-five microlitres of the PCR product were digested with the appropriate restriction enzyme according to the manufacturer's recommendations and run on a 2% agarose gel.

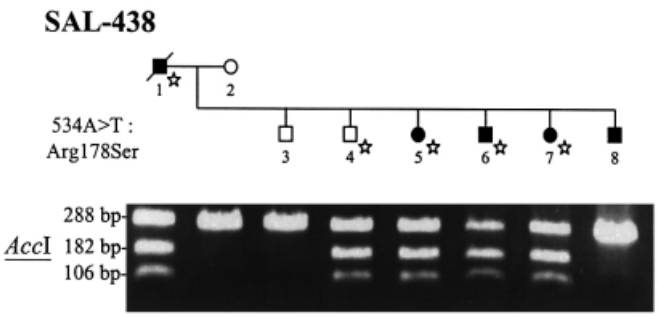
Polyacrylamide gel electrophoresis

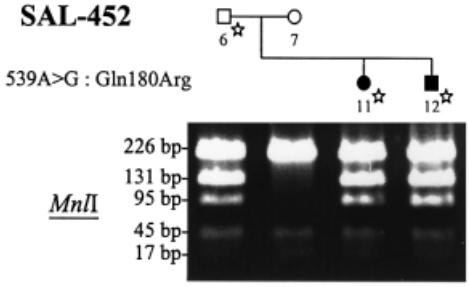
Two 5′-fluorescent (Fam) forward primers (Ichinose *et al.*, 1994) were used to amplify exon 1 and 6 in the two families with a small deletion. The presence of the 261–266delGCGGCA variant in exon 1, resulting in a shorter product [496 versus 502 base pairs (bp)], and the 631–632delAT variant in exon 6, also resulting in a shorter product (233 versus 235 bp), were established by fragment size measurement using an ABI 377 automated sequencer with Genescan 3.1 and Genotyper 1.1.1 software (ABI Prism).

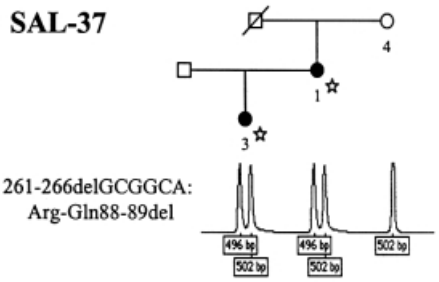

SAL-704
2
1
13
16
17
42
3
4
5
6
7
8
62
G A A G A C T A T A A G G T A A G C
538C>T : Gln180Stop

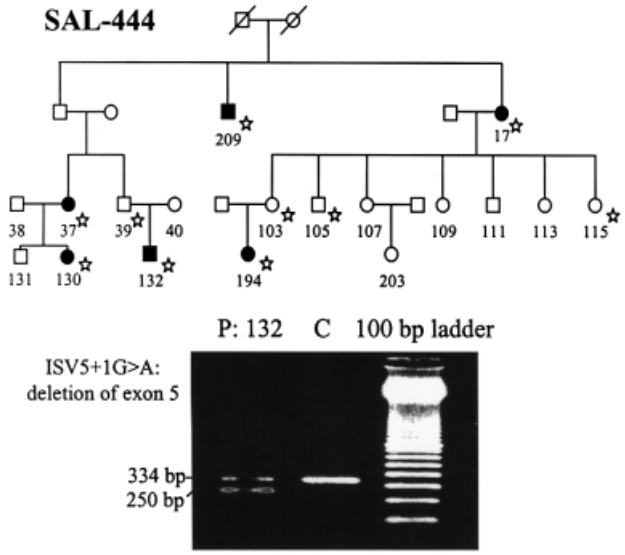

SAL-439
21
C C C G A A T G G C A G C G G C T
-22C>T

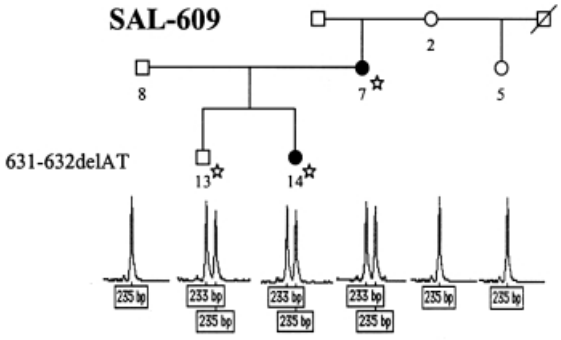

SAL-424
1
2
3
596C>T : Pro199Leu
HaeIII
175 bp
83/92 bp


SAL-438
1
2
3
4
5
6
7
8
534A>T :
Arg178Ser
288 bp
AccI
182 bp
106 bp


SAL-452
6
7
11
12
539A>G : Gln180Arg
226 bp
131 bp
95 bp
MnlI
45 bp
17 bp


SAL-37
4
1
3
261-266delGCGGCA:
Arg-Gln88-89del
496 bp
502 bp
496 bp
502 bp
502 bp


SAL-444
209
17
38
37
39
40
103
105
107
109
111
113
115
131
130
132
194
203
P: 132
C
100 bp ladder
ISV5+1G>A:
deletion of exon 5
334 bp
250 bp


SAL-609
2
8
7
5
631-632delAT
13
14
235 bp
233 bp
235 bp
233 bp
235 bp
233 bp
235 bp
235 bp

Extraction of mRNA and RT–PCR

Messenger RNA was extracted from ~10^7 lymphocytes of the index patient with the splice site mutation using the QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech, Uppsala, Sweden) and was resuspended in 50 µl water after precipitation. Random hexamer primed cDNAs were synthesized from 8 µl of mRNA in a final volume of 15 µl using the First-Strand cDNA Synthesis Kit (Pharmacia Biotech). To confirm the splicing of exon 5, we amplified 5 µl of the cDNA reaction using a forward primer corresponding to a sequence in exon 3 (5′-TTCCT-ACAAGCAAGTCC-3′) and a reverse primer corresponding to a sequence in exon 6 (5′-AATGCTACTGGCAGT-ACGATCGG-3′). PCRs were performed in a final volume of 25 µl containing 0.5 µM of each primer and 1.25 units of *Taq* DNA polymerase (Perkin Elmer, Foster City, Calif., USA). An initial denaturation for 3′ at 94°C was followed by 35 cycles of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, with a final extension of 5 min at 72°C. Exon 5 skipping, which resulted in a shorter product (250 versus 334 bp), was established by migration on a 2% agarose gel.

Molecular analysis of the parkin gene

The entire coding sequence of the parkin gene was tested as described (Abbas *et al.*, 1999). In addition, semiquantitative PCR experiments were used to detect heterozygous deletions of exons 2–12 (Lücking *et al.*, 2000).

Statistical analysis

Means were compared by the use of non-parametric tests and frequencies with the χ^2 test, with the Yates correction when appropriate.

Results

Molecular analysis

The entire coding sequence, the exon–intron boundaries, the 5′-untranslated region and the alternatively spliced form of the *GTPCH I* gene were analysed in 22 index patients with DRD. Eleven different sequence variations were detected in the *GTPCH I* gene in eleven index patients from France and one from Algeria. Five patients were isolated cases and seven had a family history of DRD. Six had newly identified mutations, and five had been described previously (Table 1 and Fig. 1).

Five non-conservative missense mutations were detected. The three new missense mutations (Fig. 1) were found in some of the relatives but not on chromosomes from 128 control subjects.

Two small deletions were identified and verified by polyacrylamide gel electrophoresis (Fig. 1). One, 261-266delGCGGCA(Arg-Gln88-89del), was new, and resulted in the deletion of two amino acids. Two nonsense mutations (Table 1), one of which was found in two families and one new 538C→T(Gln180Stop) mutation (Fig. 1), caused truncation of the GTPCH I protein.

A splice site mutation (IVS5+1G→A), described previously (Hirano *et al.*, 1998), abolished the highly conserved consensus sequence GT, which is the splice donor site of intron 5. RT–PCR amplification from exon 3 to exon 6 of the mRNA extracted from lymphocytes showed that all of exon 5 was skipped. Both the normal 334 bp fragment and a smaller fragment of 250 bp, which does not contain the 84 bp corresponding to exon 5, were detected in the DRD patient, whereas the control subject presented only the normal 334 bp band (Fig. 1). This mutation not only deletes exon 5 but also alters the reading frame and creates a premature stop codon (TAA) at position 215.

Sequence analysis of the 5′-untranslated region of the 11 index cases, in whom we had previously failed to find a mutation in the coding region or the splice sites, revealed a C→T base change at position –22 in the only patient of one family, but not in 64 controls. When more than one affected subject was available in the family, the specific mutation was present in the affected relative, demonstrating segregation with the disease. Because of reduced penetrance, it was sometimes also present in unaffected relatives in eight families. However, patient SAL-438-8, who was considered to be affected but only had brisk reflexes in all the limbs and pain during walking with no spasticity, did not carry the Arg178Ser mutation detected in his relatives.

No mutations in the *GTPCH I* gene were found in the 10 remaining index cases, seven of which were familial and three were isolated patients. However, in three of these families, in which the pattern of transmission was compatible with autosomal recessive inheritance, and where the patients had levodopa-induced dyskinesias, deletions were detected in the parkin gene. Homozygous deletion of exons 8 and 9 was found in the three patients of family FR-001 (Lucking *et al.*, 1998). Heterozygous deletion of exons 2 and 3 was

Fig. 1 Mutations in the *GTPCH I* gene in eight families with DRD. Simplified pedigrees are shown, with filled squares (men) and circles (women) representing affected individuals and barred symbols indicating deceased individuals. Only numbered individuals were examined and analysed. The stars indicate the presence of the mutation. For sequence electrophoretograms in families SAL-704 and SAL-439, the mutated base is circled. The agarose gels represent PCR products after digestion with the appropriate restriction enzyme in families SAL-452, SAL-424 and SAL-438. Size is indicated in bp. Allele sizes in bp are indicated below the polyacrylamide gel electrophoresis profiles for families SAL-609 and SAL-37. Exons 3–6 were amplified by RT–PCR and electrophoresed on agarose gel (P = patient; C = control) in family SAL-444.

Table 1 *Mutations in the GTPCH I gene on chromosome 14 detected in this study*

Family	Location	Nucleotide change (restriction enzyme)	Amino acid change	Previously described
SAL-439	5′-UTR	–22C→T		
MON-132	1	248G→C	Gly83Ala	Bandmann *et al*., 1998
SAL-37	1	261–266delGCGGCA	Arg-Gln88–89del	
SAL-438	4	534A→T (*Acc*I)	Arg178Ser	
SAL-704	4	538C→T	Gln180Stop	
SAL-452	4	539A→G (*Mnl*I)	Gln180Arg	
SAL-424	5	596C→T (*Hae*III)	Pro199Leu	
CLE-150	5	602G→A	Gly201Gln	Ichinose *et al*., 1994
SAL-444	Intron 5	IVS5+1G→A	Exon 5 skipping (Stop215)	Hirano *et al*., 1998
SAL-609	6	631–632delAT	Met211Val(Stop249)	Bandmann *et al*., 1998
SAL-426	6	645C→T	Arg216Stop	Bandmann *et al*., 1996
SAL-445	6	645C→T	Arg216Stop	Bandmann *et al*., 1996

observed in the single patient of family SAL-436. The three patients in family SAL-431 carried a heterozygous deletion of exon 3 but no point mutation was detected on the other allele after the entire coding region had been sequenced. However, considering that the parkin mutation is rare, the probability that the three patients of family SAL-431 were all heterozygous carriers by chance is low.

Clinical features (Table 2)

In the 12 families with a mutation in the *GTPCH I* gene, there were 27 patients (11 men, 16 women) and 13 asymptomatic carriers (10 men, 3 women), and the female : male ratios in patients and asymptomatic carriers were significantly different ($P < 0.05$). The difference between the numbers of asymptomatic male and female carriers was also statistically significant ($P < 0.05$). Age at examination was 36 ± 19 years and age at onset 16 ± 17 years. Onset occurred earlier in women (14 ± 15 years) than in men (21 ± 20 years). Two of the carriers had cramps with increased reflexes in the lower limbs but without functional impairment. In most patients, the initial symptom was a gait disorder (11 out of 23; 48%) or abnormal foot posture (seven out of 23; 30%). In two patients, tremor was the first sign. Difficulty in writing and torticollis were each observed in one patient. Reflexes in the lower limb were normal in 61% (14 out of 23), increased in 39% (nine out of 23), and there was extensor plantar reflex in 26% (six out of 23). The location of the dystonia, the presence of pyramidal signs and the degree of levodopa response (Table 2) were not affected by protein truncation ($n = 18$) or missense mutation ($n = 8$). In five patients, dystonia was not prominent; idiopathic Parkinson's disease had been diagnosed in three and spastic paraparesis in two.

Case reports

The following two case reports illustrate some of the diagnostic problems.

Family SAL-609

The 42-year-old mother (SAL-609-7) and her 12-year-old daughter (SAL-609-14), who had gait disorders, were diagnosed as having spastic paraplegia. The mother had suffered from spastic gait since the age of 12 years. On examination, the gait was broad, reflexes were increased in the lower limbs and the plantar reflex flexor, and *pes cavus* and scoliosis were mild. Postural tremor was more pronounced in the upper limbs and predominant in the right hand, EMG was normal, but auditory brainstem-evoked potentials suggested abnormal brainstem conduction. Tremor recordings were typical of essential tremor and possibly rest tremor. At age 48 years the patient could not walk without a cane and rest tremor worsened, leading to the introduction of levodopa at age 49 years. The treatment caused spectacular remission. The gait became normal after 3 days, but tremor recordings were unchanged. It was noticed that the daughter had frequent falls and a gait disorder at age 8 years, and she was confined to a wheelchair at age 13 years. Reflexes were normal, with decreased ankle jerks, the plantar reflex was extensor, and mild postural tremor in the arms was associated with a dystonic writing posture. After introduction of 125 mg levodopa daily at age 19 years, gait and writer's cramp were alleviated. After 6 months of levodopa treatment (150 mg/day), both patients developed typical dyskinesias in the face and choreic movements in the upper limbs, but neither complained about the involuntary movements.

Family SAL-704

Index patient SAL-704-1 was followed for 9 years at the Salpêtrière Hospital in Paris with the diagnosis of familial Parkinson's disease. Autosomal dominant heredity was suspected because a paternal aunt and two of her children had levodopa-responsive parkinsonism, which was confirmed in her cousin, SAL-704-42. Patient SAL-704-1, when examined at age 76 years after 19 years of evolution, showed a symmetrical akinetic–rigid syndrome, with a Unified

Table 2 *Clinical features of 27 patients from 12 families with DRD according to the mutation in the GTPCH I gene on chromosome 14*

Patient	Sex	Age (years)	Age at onset (years)	Location of dystonia	Additional features	LL reflexes	Plantar reflex	Response to low dose of levodopa (%)
Truncating mutation								
SAL-37-1	F	46	7	LL	Painful dystonia	N	F	100
SAL-37-3	F	14	7	LL	Painful dystonia	N	F	100
SAL-426-15	M	25	9	LL	Some UL dystonia	N	F	90
SAL-426-17	F	24	7	Generalized	LL spasticity	N	ND	100
SAL-444-17	F	57	10	Generalized	Writer's cramp	N	F	50
SAL-444-37	F	45	42	LL	None	ND	ND	NT
SAL-444-130	F	21	18	UL	None	ND	ND	NT
SAL-444-132	M	15	11	Generalized	Scoliosis, mental retardation	N	F	90
SAL-444-194	F	20	10	Generalized	None	Increased	E	90
SAL-444-209	M	68	52	None	Tremor, axial rigidity, amimia	N	F	NT
SAL-445-4	F	10	5	Torticollis, writer's cramp	None	Increased	F	80
SAL-609-7	F	47	12	None	LL spasticity, postural and rest tremor	Brisk	F	90*
SAL-609-14	F	12	8	Writer's cramp	LL spasticity, scoliosis	Brisk	E	90*
SAL-704-1	F	76	57	None	Parkinsonian syndrome, major instability, dementia	Brisk	EF	100*
SAL-704-4	M	39	10	LL	Facial grimacing, writer's cramp	N	F	90
SAL-704-7	M	37	13	LL, writer's cramp	None	N	F	90
SAL-704-42	M	66	55	None	Parkinsonian syndrome	N	F	ND
SAL-704-62	M	15	10	LL	Intermittent	N	F	NT
$n = 18$	11F/7M	35 ± 21	19 ± 18			5I/11N	13F/3E	
Missense mutation								
SAL-424-3	M	21	9	LL	None	ND	ND	100
SAL-438-1	M	57		LL	LL spasticity	Increased	E	NT
SAL-438-5	F	27	10	Generalized	None	Increased	F	100
SAL-438-6	M	25		None	Exercise-induced LL stiffness	Increased	F	0
SAL-452-11	F	10	5	Generalized	LL spasticity	Brisk	E	90
SAL-452-12	M	8		LL	None	N	F	>30
MON-132-3	F	14	8	LL	None	ND	ND	100
CLE-150-5	F	54	7	Generalized	Bradykinesia and tremor	N	F	80
$n = 8$	4F/4M	27 ± 19	8 ± 2			4I/2N	4F/2E	
Mutation in 5′-untranslated region								
SAL-439-21	F	49	5	LL	None	N	EF	90

F = female; M = male; LL = lower limb; UL = upper limb; F = flexor; E = extensor; N = normal; I = increased; ND = not determined; NT = not treated. *Levodopa-induced dyskinesias.

Parkinson's Disease Rating Score of 52 without treatment and 37 with small doses of levodopa. Diphasic dyskinesias were prominent on the left side, and monophasic dyskinesia was present in the neck and upper limb. Dementia was evident, with frontal behaviour and a Mini-Mental score of 13/30. At age 80 years she was confined to a wheelchair, with a fixed flexor posture of the upper limbs and severe dystonic hand deformations. A brain scan revealed enlarged lateral ventricles and sequelae of stroke. The diagnosis of DRD was proposed only when her two sons, with typical DRD, were examined.

The clinical features of the patients with deletions in the parkin gene are presented in Table 3. The mean age at onset was 17 ± 9 years, which is very similar to that of patients with *GTPCH I* gene mutations. The phenotype in the three families included dystonia at onset in five out of seven patients and progressive appearance of parkinsonian signs in all patients.

Table 3 *Clinical features of seven patients from three families with mutations in the parkin gene*

Patient	Sex	Age (years)	Age at onset (years)	Location of dystonia	Additional features	LL reflexes	Plantar reflex	Response to low dose of levodopa (%)
FR-001-11	F	34	14	LL	Bradykinesia, rigidity	Brisk	F	> 30
FR-001-14	F	35	18	Generalized	Parkinsonian syndrome, painful dystonia	Brisk	F	80*
FR-001-16	M	26	7	Generalized	Parkinsonian syndrome, painful dystonia	N	F	90*
SAL-431-9	F	42	25	None	Bradykinesia	ND	ND	90*
SAL-431-11	F	38	31	Writer's cramp	Bradykinesia, rigidity, painful dystonia	ND	ND	>30*
SAL-431-16	F	35	8	LL, blepharospasm, writer's cramp	Bradykinesia, rigidity	Brisk	F	100*
SAL-436-8	F	35	13	Generalized	Bradykinesia, rigidity, orthostatic hypotension	Brisk	F	70*
$n = 7$	1M/6F	35 ± 5	17 ± 9			4I/1N	5F	

F = female; M = male; LL = lower limb; I = increased; F = flexor; N = normal; ND = not determined. *Levodopa-induced dyskinesias.

Levodopa-induced dyskinesias were severe in six out of seven patients.

Discussion

We have reported the screening of 22 families with DOPA-responsive dystonia for mutations in the coding exons, the 5′-untranslated region and intron–exon junctions of the *GTPCH I* gene. Eleven of the 22 families carried 10 mutations in the coding sequence, which segregated with the disease in all seven kindreds in which at least two affected patients were available. One family had a base change in the 5′-untranslated region. Only five of these mutations have been described previously (Ichinose *et al.*, 1994; Bandmann *et al.*, 1996, 1998; Hirano *et al.*, 1998), underlining the large amount of allelic heterogeneity at the *GTPCH I* locus, which complicates molecular diagnosis. Five families carried a single heterozygous missense mutation in the coding region. These mutations were non-conservative, and affected amino acid residues that are highly conserved among species. They are probably causative mutations as they were not present on 128 control chromosomes. A new 6 bp deletion, affecting two highly conserved amino acids, was found in a single kindred. Four mutations, in five families, altered the reading frame and introduced a premature stop codon in the *GTPCH I* gene. These were a 2 bp deletion, an exon-5 splicing defect and two nonsense mutations.

A common haplotype for four microsatellite markers in the *GTPCH I* region (*D14S978*, *D14S1057*, *D14S285*, *D14S63*) segregated with the base change, an Arg216Stop nonsense mutation (data not shown), in two families that live in the same region of France, suggesting that they descend from a single founder. This mutation has also been reported in a British family (Bandmann *et al.*, 1996), but the haplotype segregating with the mutation is unknown.

The absence of a mutation in either the coding region or splice sites has been observed in several families with typical DRD (Bandmann *et al.*, 1996; Ichinose *et al.*, 1994). Recently, Bandmann and collaborators identified the first mutation in the 5′-untranslated region of the *GTPCH I* gene. This prompted us to extend our sequence analysis to the promoter region in 11 mutation-negative families. We found a heterozygous base-pair change at position –22 that was different from the change reported by Bandmann and collaborators (Bandmann *et al.*, 1998) in a sporadic DRD patient with a typical phenotype. Since it has not been described previously and it was not found in 128 control chromosomes, it cannot represent a frequent polymorphism. It may interfere with GTPCH I transcription and/or translation.

Mutations in the tyrosine hydroxylase gene also cause DRD but with autosomal recessive inheritance (Knappskog, 1995; Ludecke *et al.*, 1995, 1996; van den Heuvel *et al.*, 1998). They cannot, therefore, account for the four families without base changes in the *GTPCH I* gene, in which transmission of the disease was clearly autosomal dominant. Since most of the *GTPCH I* gene, including the exon-6 alternatively spliced form, has been sequenced we cannot exclude the existence of another causative gene in DRD. The enzymes involved in the synthesis of tetrahydrobiopterin or dopamine might be good candidates.

The clinical features of the mutation carriers are highly variable both within and between families. As previously reported (Nygaard *et al.*, 1990; Ichinose *et al.*, 1994), there is an excess of women among patients with DRD, and a tendency for earlier onset in women than in men. Conversely, there is a significant excess of men among the asymptomatic carriers. In addition, penetrance is clearly age-dependent in both sexes, which renders genetic counselling difficult. Another problem is illustrated by patient SAL-438-8, who had brisk reflexes and pain in the legs when walking, as did several of his affected relatives, some of whom also had spastic gait, but he did not carry the mutation characteristic of the family. Since the absence of the Arg178Ser mutation was verified in an

Table 4 *Atypical presentations in patients with mutations in the GTPCH I gene*

Atypical presentation	Amino acid change	Reference
Very early onset (feeding problems in the 1st week of life)	Gly83Ala	Bandmann *et al*., 1998
Relapsing–remitting course of dystonic features	Lys224Stop	Bandmann *et al*., 1996
Inversed diurnal fluctuations (improvement during the day)	Leu71Glu	Bandmann *et al*., 1998
Guitarist's cramp	Arg216Stop	Bandmann *et al*., 1998
Oromandibular dystonia	Ala196Ser	Steinberger *et al*., 1999
Severe motor delay, lack of speech development	351delA/Met221Thr	Furukawa *et al*., 1998
Paroxymal dystonia, oculogyric crises	Cys108Asp/Lys224Arg	Furukawa *et al*., 1998
Levodopa-induced dyskinesias	Gln180Stop Met211Val (Stop249)	Present study

independent blood sample, this patient is considered to have a phenocopy. There were no correlations between the type of mutation and age at onset or clinical presentation. Dystonia is the most frequent sign in cases with early onset, but its topography might be misleading. Late-onset cases often present with parkinsonism, as did three of our patients, but one of our late-onset cases had dystonia.

Symptoms may also be atypical. In family SAL-609, both affected members had early-onset spasticity in the lower limbs, but the mother also presented postural tremor. They were therefore diagnosed as having a complex form of spastic paraplegia before they were treated with levodopa. Atypical features have also been reported in patients with characterized mutations (Table 4), especially in compound heterozygotes. The presence of levodopa-induced dyskinesias, observed in three of the 27 patients in this series who carried a truncating mutation, has not been reported previously, although levodopa-induced chorea was described in a woman with athetoid cerebral palsy of unknown origin, which responded dramatically to the treatment (Fletcher *et al*., 1993).

The absence of levodopa-induced dyskinesias is considered to distinguish DRD from autosomal recessive juvenile parkinsonism caused by parkin mutations, as the other presentations show considerable overlap. This difference has been explained by the absence of dopaminergic cell loss in the substantia nigra in DRD (Rajput *et al.*, 1994), where the mutations are thought to cause only dysfunction of the neurons, as opposed to parkin mutations, which result in marked dopaminergic cell loss in the substantia nigra (Mori *et al.*, 1998). However, the observation of levodopa-induced dyskinesias in some of the patients with *GTPCH I* mutations suggests that the nigrostriatal denervation in DRD might affect both pre- and post-synaptic element. Moreover, this study included three families in which the index cases presented with dystonic features at onset and progressive parkinsonism but had a marked response to low doses of levodopa. They were found to have deletions in the parkin gene, which illustrates how difficult it is to distinguish between DRD and parkin mutations. However, although most parkin mutation patients had onset with dystonia, they all subsequently presented parkinsonian signs and all but one had levodopa-induced dyskinesias. Therefore, the presence of parkinsonism, especially in patients with young onset, and of severe levodopa-induced dyskinesias is suggestive of parkin mutations.

Our study also shows that the response of DRD to levodopa is not consistently good. Patient SAL-444-17, who had generalized dystonia, had only a 50% improvement on levodopa treatment, whereas the symptoms of his affected relatives were relieved almost completely. Patient SAL-438-6 had only exercise-induced stiffness, but this was not at all responsive to levodopa. It was reported that some patients with dystonia who respond to anticholinergic drugs also carry *GTPCH I* mutations (Jarman *et al.*, 1997). This was the case with patient SAL-37-3, who was initially treated successfully with trihexyphenidyl before the introduction of levodopa. These cases illustrate the importance of a levodopa trial in all patients with dystonia and in cases of spastic paraplegia with severe gait abnormalities and discrete pyramidal signs.

In conclusion, we have identified 11 different mutations in the *GTPCH I* gene, six of which are new, and have confirmed that the mutations can occur in many regions of this gene, including potential regulatory sequences. Our analysis showed that mutations of this gene are responsible for the majority of DRD cases, especially if autosomal dominant inheritance is present. Most of the mutations occurred in a single patient or family, and this, unfortunately, will not facilitate molecular analysis for genetic counselling. Finally, the clinical spectrum associated with these mutations is particularly large and now includes levodopa-induced dyskinesias, introducing the additional diagnostic problem of confusion with patients carrying mutations in the parkin gene. In these cases, only molecular analysis is decisive.

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