ASSOCIATION BETWEEN EARLY-ONSET PARKINSON'S DISEASE AND MUTATIONS IN THE *PARKIN* GENE

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ABSTRACT

Background Mutations in the *parkin* gene have recently been identified in patients with early-onset Parkinson's disease, but the frequency of the mutations and the associated phenotype have not been assessed in a large series of patients.

Methods We studied 73 families in which at least one of the affected family members was affected at or before the age of 45 years and had parents who were not affected, as well as 100 patients with isolated Parkinson's disease that began at or before the age of 45 years. All subjects were screened for mutations in the *parkin* gene with use of a semiquantitative polymerase-chain-reaction assay that simultaneously amplified several exons. We sequenced the coding exons in a subgroup of patients. We also compared the clinical features of patients with *parkin* mutations and those without mutations.

Results Among the families with early-onset Parkinson's disease, 36 (49 percent) had *parkin* mutations. The age at onset ranged from 7 to 58 years. Among the patients with isolated Parkinson's disease, mutations were detected in 10 of 13 patients (77 percent) with an age at onset of 20 years or younger, but in only 2 of 64 patients (3 percent) with an age at onset of more than 30 years. The mean (±SD) age at onset in the patients with *parkin* mutations was younger than that in those without mutations (32±11 vs. 42± 11 years, $P<0.001$), and they were more likely to have symmetric involvement and dystonia at onset, to have hyperreflexia at onset or later, to have a good response to levodopa therapy, and to have levodopa-induced dyskinesias during treatment. Nineteen different rearrangements of exons (deletions and multiplications) and 16 different point mutations were detected.

Conclusions Mutations in the *parkin* gene are a major cause of early-onset autosomal recessive familial Parkinson's disease and isolated juvenile-onset Parkinson's disease (at or before the age of 20 years). Accurate diagnosis of these cases cannot be based only on the clinical manifestations of the disease. (N Engl J Med 2000;342:1560-7.)

PARKINSON'S disease is one of the most frequent neurodegenerative disorders, with a prevalence of 1 to 2 percent among persons older than 65 years of age.[1] It is characterized by resting tremor, rigidity, and bradykinesia, all of which respond well to treatment with levodopa. The pathological hallmarks are the presence of Lewy bodies (cytoplasmic eosinophilic hyaline inclusions) and massive loss of dopaminergic neurons in the pars compacta of the substantia nigra.[2] The cause of the disease is still unknown, but the existence of genetic susceptibility factors is strongly suspected.[3,4] Two genes (α-synuclein[5] and ubiquitin carboxy-terminal hydrolase L1 [*UCH-L1*][6]) and two gene loci (on chromosomes 2p13 and 4p14–16.3, respectively[7,8]) have been implicated in the pathogenesis of autosomal dominant Parkinson's disease, but they seem to account for the cases in only a few families. In contrast, mutations in the gene designated *parkin* have recently been identified in several families with autosomal recessive early-onset parkinsonism.[9-14] However, the frequency of mutations in this gene in familial and isolated cases of early-onset parkinsonism has not yet been assessed in large series of patients.

The phenotype associated with mutations in the *parkin* gene has not been clearly established, making the selection of patients for genetic testing difficult. In Japanese patients with *parkin* mutations, the disease is characterized by an early onset, dystonia at

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onset, hyperreflexia, early complications resulting from levodopa treatment, and slow progression.[9-11] In contrast, the clinical characteristics of some European and North African patients with *parkin* mutations were indistinguishable from those of patients with idiopathic Parkinson's disease, with an age at onset of up to 58 years.[13,14] The number of families analyzed so far is small, however, and the correlations between genotype and phenotype are uncertain. Brain tissue from the patients studied did not contain Lewy bodies, suggesting that the pathologic process might differ from that of idiopathic Parkinson's disease.[15,16]

We performed a clinical and molecular study of 73 families with early-onset autosomal recessive Parkinson's disease, including 152 affected family members, and 100 patients with early-onset isolated Parkinson's disease. They were screened for mutations in the *parkin* gene by a semiquantitative polymerase-chain-reaction (PCR) assay designed to detect exon rearrangements (deletions and multiplications) and by genomic sequencing. Correlations between genotype and phenotype were assessed both in patients with *parkin* mutations and in those without such mutations.

METHODS

Patients and Families

We studied 73 families (152 patients with Parkinson's disease and 53 unaffected relatives) that met the following criteria: symptoms of parkinsonism in affected family members that were reduced by at least 30 percent by treatment with levodopa (the response could not be assessed in 3 untreated patients); a mode of inheritance compatible with autosomal recessive transmission (affected siblings without affected parents); an age at onset of 45 years or younger in at least one of the affected siblings; and the absence of extensor plantar reflexes, ophthalmoplegia, early dementia, or early autonomic failure in the family members we examined. Twenty of the families originated from Italy, 14 from France, 12 from Great Britain, 10 from the Netherlands, 9 from Germany, 2 from Portugal, and 1 each from Spain, Algeria, Morocco, Argentina, India, and Vietnam. Eight of the families were consanguineous, and 12 have been described previously.[13,14] In addition, we studied 100 patients with isolated Parkinson's disease with an age at onset of 45 years or younger, most of whom were European, and 8 of whom were from consanguineous marriages. These 100 patients were selected according to the same clinical criteria used for the patients with familial disease but who had no family history of Parkinson's disease. Eight of these 100 patients had never received treatment.

The enrollment of the subjects was random. For each subject, we obtained clinical information from the subject or the subject's records and peripheral blood for DNA analysis. DNA was extracted from peripheral-blood leukocytes according to standard procedures. The study was approved by ethics committees in the countries of all the participating investigators, and written informed consent was obtained from all the study subjects.

Molecular Analysis

Screening for mutations in the *parkin* gene was performed in all index patients (except those known to be homozygous or to have compound heterozygosity for such mutations[13,14]) with the use of a semiquantitative PCR assay for the detection of rearrangements of *parkin* exons. Exons 2 through 12 were amplified simultaneously in three groups by PCR (multiplex PCR): group 1 consisted of exons 4, 7, 8, and 11; group 2 consisted of exons 5, 6, 8, and 10; and group 3 consisted of exons 2, 3, 9, and 12 and an external control, C328, a 328-bp sequence of the transthyretin gene on chromosome 18. The primers used were the same as those described by Kitada et al.[9] except for the primer for exon 3, for which exonic primers were used: 5′AATTGTGACCTGGATCAGC3′ (Ex3iFor) as the forward primer and 5′CTGGACTTCCAGCTGGTGGTGAG3′ (Ex3iRev) as the reverse primer. The C328 forward primer was 5′ACGTTCCTGATAATGGGATC3′ (TTRForHex), and the reverse primer was 5′CCTCTCTCTACCAAGTGAGG3′ (TTR328Rev). All forward primers were fluorescently labeled with HEX-phosphoramitide. The PCR products (2.5 μl) were analyzed by 5 percent denaturing polyacrylamide-gel electrophoresis with an automated sequencer (model ABI 377) and GeneScan version 3.1 and Genotyper version 1.1.1 software (all from Applied Biosystems). All reactions were performed at least twice. The DNA from a patient known to have a heterozygous deletion of exons 8 and 9 was always processed in parallel as an internal control.

We calculated the ratios of all the peak heights in a given reaction and then compared the ratios with the ratios measured in a specimen from a normal subject. This comparison yielded the following rules: values of 0.6 or less were interpreted as indicating a heterozygous deletion of an exon; values of 0.8 to 1.2 were interpreted as normal; values of 1.3 to 1.7 were interpreted as indicating a heterozygous duplication of an exon; values of 1.8 to 2.3 were interpreted as indicating a homozygous duplication or heterozygous triplication of an exon; and values of more than 2.6 were interpreted as indicating a homozygous triplication of an exon. An exon rearrangement was confirmed only if all the ratios concerning this exon were abnormal. The consequence of the rearrangements at the protein level (a frame shift vs. an in-frame rearrangement) was deduced from the exon sequences published by Kitada et al.[9] (DNA Data Bank of Japan accession number AB009973).

Each PCR reaction involved, in a total volume of 25 μl, 40 ng of DNA, with 3 mM magnesium chloride, 0.2 mM of each deoxynucleoside triphosphate, and 1 U of *Taq* polymerase. Denaturation for 5 minutes at 95°C was followed by 23 cycles consisting of 30 seconds of denaturation at 95°C, 45 seconds of annealing at 53°C, and 2.5 minutes of extension at 68°C, with a final period of extension at 68°C for 5 minutes. Primer concentrations were chosen to yield — within the exponential phase of the PCR (data not shown) — similar peak heights in each multiplex reaction and ranged from 0.4 to 1.9 μM. Deletions and insertions of bases were deduced from the size of the PCR products.

In the case of 54 index patients with familial disease and 91 index patients with isolated disease, exon rearrangements or deletions or insertions of bases were not found on both chromosomes and thus did not account for the phenotype. Therefore, the entire coding region of the *parkin* gene (including exon–intron boundaries) was sequenced as described previously[14] in 53 index patients with familial disease and 50 patients with isolated disease.

Whether the *parkin* variants we identified cosegregated with the disease was assessed by genotyping of all available members, affected or not, of the respective families. In addition, 114 chromosomes from mostly European, unrelated controls who had no movement disorders were analyzed for the point mutations and the rearrangements of the exons represented in multiplex group 3. The techniques used were restriction assays, polyacrylamide-gel electrophoresis, and the semiquantitative PCR assay. For two point mutations — the substitution of A for G at nucleotide 939 of complementary DNA (cDNA) and the substitution of T for C at nucleotide 1101 of cDNA — mismatched reverse primers were used in order to create a restriction site that depended on the point mutation being looked for: 5′GGCAGGGAGTAGCCAAGTTGAGGAT3′ for digestion with *Alw*I and 5′AGCCCCGCTCCACAGCCAGCGC3′ for digestion with *Bst*UI (in each primer, the underlined nucleotide differs from the wild-type sequence).

Statistical Analysis

Means (±SD) were compared with the nonparametric Mann–Whitney U test. Frequencies were compared with the chi-square test, with Yates' correction when appropriate.

RESULTS

Frequency of Mutations in the *parkin* Gene

Twenty-five families (56 patients) with autosomal recessive Parkinson's disease had homozygous or compound heterozygous mutations on each allele of the *parkin* gene (Table 1). In addition, 11 families (27 patients) with a mutation in one allele were considered to have *parkin*-related disease, on the basis of the assumption that the second mutation was not detected by the methods used in this study. Thus, mutations in the *parkin* gene were detected in 36 of 73 families (49 percent), including 12 previously described families.[13,14] Among the 100 patients with isolated Parkinson's disease, 18 (18 percent) had *parkin* mutations. The frequency of mutations among consanguineous patients with isolated Parkinson's disease, a pattern that is suggestive of autosomal recessive inheritance, was similar to that among consanguineous patients with familial disease (50 percent vs. 62 percent). The frequency of mutations in the patients with isolated Parkinson's disease decreased significantly with increasing age at onset: mutations were detected in 10 of 13 patients (77 percent) with an age at onset of disease of 20 years or younger, but only in 2 of 64 patients (3 percent) with an age at onset of 31 to 45 years (Table 2). Sequencing of the *parkin* gene in 22 of the 64 patients with isolated Parkinson's disease who were older than 30 years at the onset of symptoms revealed a point mutation in only 1 patient. In 14 families in which the affected family members had *parkin* mutations on both chromosomes, none of 28 unaffected siblings had two *parkin* mutations, indicating the high penetrance of the mutations.

Clinical Studies

The 36 families with Parkinson's disease and *parkin* mutations and the 18 patients with isolated Parkinson's disease and *parkin* mutations came from a variety of regions: France (in 15 cases), Italy (in 13), Great Britain (in 7), the Netherlands (in 3), Spain (in 3), Germany (in 3), Portugal (in 2), Algeria (in 2) and Lebanon, India, Pakistan, Vietnam, Japan, and Argentina (in 1 case each).

As a group, the 100 patients with *parkin* mutations had a mean (±SD) age at onset of 32±11 years (range, 7 to 58); the age at onset was not known for 1 patient (Table 3). Among the patients with an age at onset of 45 years or younger, the onset of the disease was earlier in the 18 patients with isolated Parkinson's disease and *parkin* mutations than in the 75 patients with familial Parkinson's disease and mutations (mean age, 21±9 vs. 32±9 years; median, 20 vs. 33 years; $P<0.001$). This difference was not due to selection bias, because the mean ages at onset were similar in the two groups when all initially included patients with an age at onset of 45 years or younger were compared, whether or not they had *parkin* mutations (age at onset in 118 patients with familial disease, 34±9 years; in 100 patients with isolated disease, 32±9 years). The mean age at onset was significantly younger in the patients with *parkin* mutations than in those without mutations, both in the total sample (Table 3) and in the group with familial cases alone (34±10 years for 82 patients with familial disease and mutations and 43±12 years for 65 patients with familial disease but without mutations; $P<0.001$).

The initial manifestations of the disease in most patients with *parkin* mutations were tremor (65 per-

TABLE 1. FREQUENCY OF MUTATIONS IN THE *PARKIN* GENE IN 73 FAMILIES WITH AUTOSOMAL RECESSIVE EARLY-ONSET PARKINSON'S DISEASE AND 100 PATIENTS WITH ISOLATED EARLY-ONSET PARKINSON'S DISEASE.*

NO. OF MUTATIONS	FAMILIES WITH AUTOSOMAL RECESSIVE DISEASE (N=73)		PATIENTS WITH ISOLATED CASES (N=100)	
	NO. OF FAMILIES	NO. OF CONSANGUINEOUS FAMILIES	NO. OF PATIENTS	NO. OF CONSANGUINEOUS PATIENTS
Two	25	5	14	4
One	11†	0	4	0
None identified	37	3	82‡	4
Total no. with mutations (%)	36 (49)	5 (62)	18 (18)	4 (50)

*Early onset was defined as an onset at or before 45 years of age (in at least one of the affected siblings in affected families).

†The *parkin* gene was not sequenced in one family.

‡The *parkin* gene was not sequenced in 41 patients in whom the onset of disease was after 30 years of age.

TABLE 2. FREQUENCY OF MUTATIONS IN THE *PARKIN* GENE IN 100 PATIENTS WITH ISOLATED EARLY-ONSET PARKINSON'S DISEASE, ACCORDING TO THE AGE AT ONSET.

AGE AT ONSET	PATIENTS WITH HOMOZYGOUS OR HETEROZYGOUS MUTATIONS	CONSANGUINEOUS PATIENTS WITH HOMOZYGOUS OR HETEROZYGOUS MUTATIONS
	no. of patients/total no. (%)	
≤20 yr	10/13 (77)*	2/3 (67)
21–30 yr	6/23 (26)†	2/2 (100)
31–40 yr	1/49 (2)‡	0/2
41–45 yr	1/15 (7)§	0/1
Total no. of patients (%)	18/100 (18)	4/8 (50)

*P=0.003 for the comparison with patients with an age at onset of 21 to 30 years.

†P=0.005 for the comparison with patients with an age at onset of 31 to 40 years.

‡The *parkin* gene was not sequenced in 35 patients.

§The *parkin* gene was not sequenced in six patients.

TABLE 3. CHARACTERISTICS OF PATIENTS WITH PARKINSON'S DISEASE ACCORDING TO THE PRESENCE OR ABSENCE OF MUTATIONS IN THE *PARKIN* GENE.*

CHARACTERISTIC	PATIENTS WITH *PARKIN* MUTATIONS (N=101)	PATIENTS WITHOUT *PARKIN* MUTATIONS (N=85)	P VALUE
Sex (F/M)	49/52	31/54	
Age at onset (yr)	32±11	42±11	<0.001
Duration of disease (yr)	17±11	13±11	0.002
Clinical signs at onset (%)			
Micrography	30	47	0.02
Bradykinesia	63	65	
Tremor	65	75	
Dystonia	42	22	0.02
Asymmetric signs	89	98	0.02
Clinical signs at examination			
Bradykinesia (%)	95	98	
Rigidity (%)	92	99	
Resting tremor (%)	74	80	
Postural tremor (%)	54	47	
Urinary urgency (%)	11	25	0.01
Hyperreflexia (%)	44	21	0.04
No progression or slow rate of progression (%)	88	72	
Motor scale of UPDRS score†			
Without treatment	41±22	43±16	
During treatment	23±18	26±15	
Hoen–Yahr assessment without treatment‡			
Mean stage	3.2±1.0	3.1±0.8	
Interval from onset to stage 2 (yr)	11±9	5±3	
Interval from onset to stage 3 (yr)	19±10	17±8	
Interval from onset to stage 4 (yr)	26±8	33±2	
Interval from onset to stage 5 (yr)	40±19	44	
Mini–Mental State Examination score§	29±3	28±2	
Clinical signs during treatment			
Percent improvement with levodopa	72±20	64±17	0.03
Daily dose of levodopa (mg)	500±340	600±400	
Duration of levodopa treatment			
Months	123±102	111±99	
Years	10	9	
Levodopa-induced dyskinesia			
Percentage of patients	77	63	0.04
Months of treatment	64±65	60±55	
Levodopa-induced fluctuations in symptoms			
Percentage of patients	79	65	
Months of treatment	64±61	61±54	
Dystonia			
Percentage of patients	58	45	
Months of treatment	65±72	54±40	

*Plus–minus values are means ±SD. Among the patients with *parkin* mutations, 83 had familial disease and 18 had isolated disease. Among the patients without *parkin* mutations, 57 had familial disease and 28 had isolated disease.

†The motor scale of the Unified Parkinson's Disease Rating Scale (UPDRS)[17] assesses 14 motor functions of patients with Parkinson's disease. Some of the functions were tested separately for each side of the body, the arms and legs, the face, and the trunk, resulting in 27 subtests. Each subtest was scored on a scale from 0 (no impairment) to 4 (severe impairment), resulting in a total score ranging from 0 to 108.

‡The Hoehn and Yahr stages are used to describe the degree of functional disability of patients with Parkinson's disease. Stage 1 indicates mild unilateral symptoms, stage 2 bilateral or axial symptoms, stage 3 impairment of postural reflexes, stage 4 strongly disabling disease (the patient is able to stand and walk unassisted but is markedly incapacitated), and stage 5 severely disabling disease (the patient cannot stand or walk without assistance and is therefore confined to a wheelchair or bed).[18]

§The Mini–Mental State examination assesses orientation; short-term memory; attention span; and naming, copying, reading, writing, spatial, and constructive capacities with respect to 30 tasks, all scored as either 1 (succeeded) or 0 (failed). The maximal score is 30; dementia was considered to be present if the score was below 24.

cent) and bradykinesia (63 percent) (Table 3). The patients with *parkin* mutations had significantly higher frequencies of dystonia and symmetric symptoms at onset and of hyperreflexia at onset or later, as well as a better response to levodopa despite having had the disease for a longer period (Table 3) than those with no *parkin* mutations. Dystonia began in the lower limbs in 28 of 31 patients with mutations, but 2 patients first had torticollis and 1 had right-arm dystonia. Dyskinesia as a result of levodopa treatment was significantly more common in patients with mutations than in those with no mutations, but such dyskinesia occurred in both groups, on average, after nearly 5 years of treatment (range, 1 month to 20 years). There were no significant differences between the 24 patients with at least one missense mutation and the 52 patients with two truncating mutations; the 25 patients with single heterozygous truncating mutations were not assigned to either group, since the nature of the suspected second mutation was unknown.

Nineteen different homozygous and heterozygous exon rearrangements were found in 35 index patients, including 4 from previously described families with homozygous deletions of exons (Table 4 and Fig. 1A).[13,14] In addition to identifying the suspected deletions of an exon, our approach provided evidence of four duplications of an exon and one triplication of an exon. The results were highly reproducible and confirmed by cosegregation analysis. Rearrangements of exons 2, 3, 9, and 12 were not found in the controls.

Sixteen different exonic point mutations were found in 28 index patients, including 8 from previously described families[14] (Table 4 and Fig. 1B). In addition, an intronic deletion of 5 bp (IVS8 −21 to −17del) was detected. All point mutations cosegregated with the disease, and none were found in any control. The amino acids modified by mutations were conserved in the *parkin* orthologues in rats[20] and mice (Gene Bank accession numbers AF210434 and AB019558, respectively). However, in two patients from one family, the homozygous point mutation Arg334Cys was associated with the homozygous intronic 5-bp deletion and the heterozygous Asp280Asn mutation, so that the pathogenicity of the latter two mutations cannot be ascertained.

Many of the exon rearrangements were found repeatedly among the index patients, particularly deletions of exon 3 (in 10 patients), exon 2 (in 4), exon 4 (in 4), and exons 3 and 4 (in 4) (Fig. 1A). Six point mutations were found in more than one index patient: the deletion of A at nucleotide 255 of cDNA (in six index patients), the deletion of A and G at nucleotide 202 to 203 of cDNA (in five), Arg275Trp (in five), the insertion of G and T between nucleotide 321 and nucleotide 322 of cDNA (in two), Lys211Asn (in two), and Gly430Asp (in two) (Fig. 1).

TABLE 4. FREQUENCY OF HOMOZYGOUS, COMPOUND HETEROZYGOUS, AND SINGLE HETEROZYGOUS MUTATIONS AMONG 45 INDEX PATIENTS WITH FAMILIAL OR ISOLATED PARKINSON'S DISEASE, ACCORDING TO THE TYPE OF MUTATION.

TYPE OF MUTATION	EXON REARRANGEMENT	POINT MUTATION	EXON REARRANGEMENT PLUS A POINT MUTATION*	TOTAL NO. (%)
	no. of index patients (no. of consanguineous index patients)			
Homozygous	9 (6)	10 (3)	NA	19 (35)
Compound heterozygous	8	3	9	20 (37)
Single heterozygous	9†	6	NA	15 (28)

*NA denotes not applicable.

†The *parkin* gene was not sequenced in one index patient.

DISCUSSION

We detected mutations in the *parkin* gene in almost half the families with autosomal recessive Parkinson's disease in which at least one affected member was 45 years of age or younger at the onset of symptoms. The frequency of such mutations was lower in a group of patients with isolated early-onset Parkinson's disease.

On average, patients with *parkin* mutations began to have symptoms in their early 30s, but the age at onset ranged widely, from 7 to 58 years. The fact that the onset occurred at an earlier age in patients with isolated Parkinson's disease and *parkin* mutations than in those with familial Parkinson's disease and *parkin* mutations suggests that among patients who are older than 30 years at the onset of isolated Parkinson's disease, the disease is mainly due to causes other than *parkin* mutations.

Can patients with *parkin* mutations be distinguished clinically from patients with early-onset Parkinson's disease from other causes? As a group, those with *parkin* mutations had an earlier onset of disease, were more likely to have dystonia and symmetric signs at onset, as well as hyperreflexia at onset or later, and were more likely to have a better response to levodopa, but were also more likely to have dyskinesia during treatment, than were patients without *parkin* mutations. These signs were less frequent, however, than in previous reports[11,21] and could not be used specifically to identify patients with mutations. Furthermore, the clinical manifestations of the *parkin* mutations were independent of the age at onset.

In addition, patients with late-onset disease who have mutations can be difficult to distinguish from those with idiopathic Parkinson's disease. In general, however, the disease progressed slowly in the patients with mutations. Despite having had symptoms for

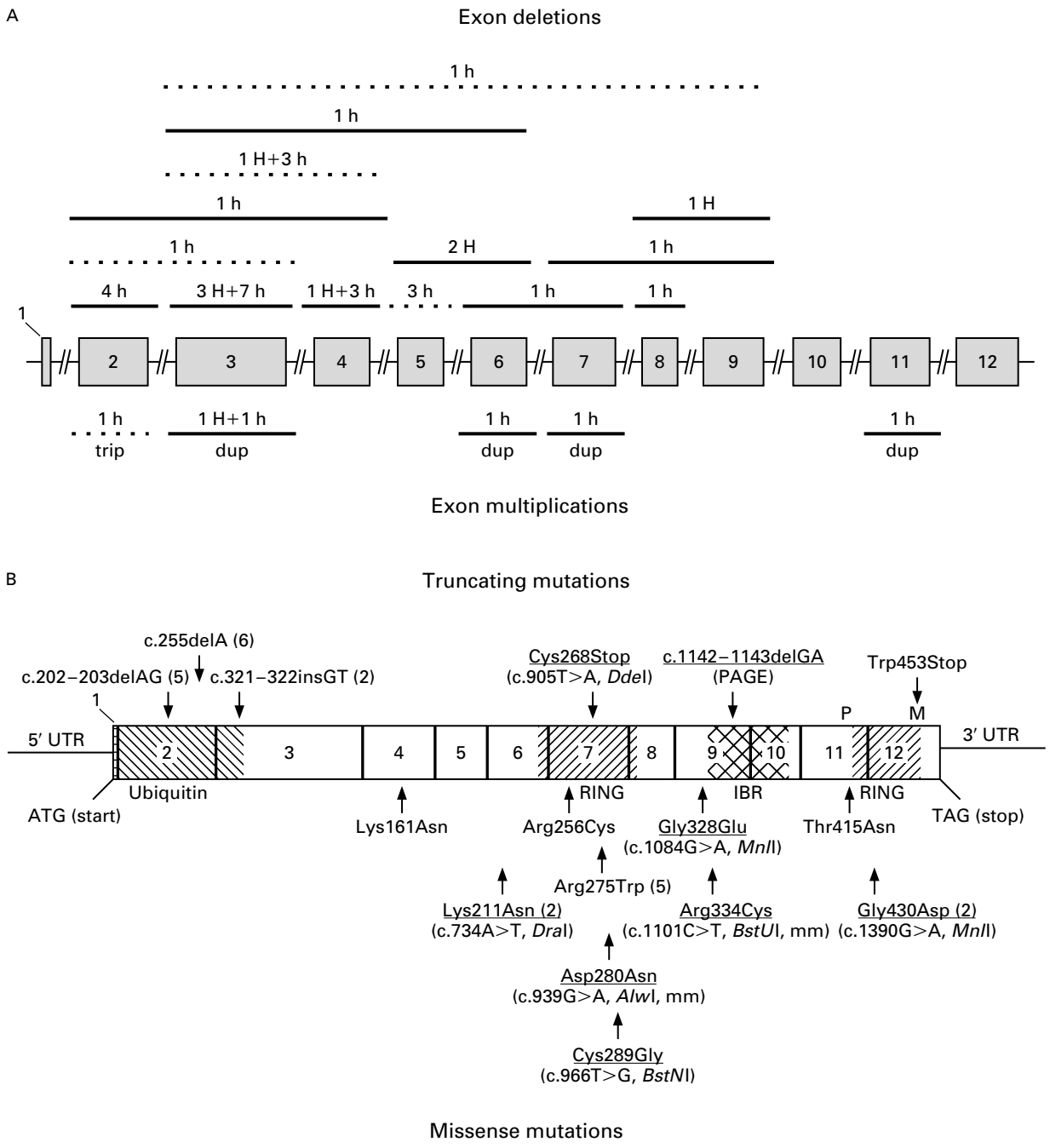

Figure 1. Mutations in the *parkin* Gene.

Panel A shows the exon rearrangements identified. Deletions are indicated above the sequence, and duplications (dup) and triplications (trip) are indicated below the sequence. Their deduced effect on the protein is represented by a dotted line for in-frame rearrangements and by a solid line for frame-shift rearrangements. The number of index patients with the rearrangement and the type of mutation — heterozygous (h) or homozygous (H) — are indicated above each mutation. Panel B shows the point mutations resulting in truncation of the sequence of 12 exons or in a missense mutation. The hatched regions indicate the ubiquitin-like domain and the RING–IBR–RING finger motif.[19] The 6 truncating mutations are indicated above the sequence, and the 10 missense mutations are indicated below the sequence. For mutations identified in more than one index patient, the number of index patients with the mutation is given in parentheses. The nucleotide change and the restriction enzymes used to screen family members and unrelated control subjects without movement disorders are given in parentheses below the mutation (mm denotes the mismatch primer used for the PCR, and PAGE polyacrylamide-gel electrophoresis). Mutations that were not based on published sequences[14] are underlined. Nucleotides prefaced by a small c indicate the numbers in the complementary DNA sequences described by Kitada et al.[9] The ATG of the initiator methionine codon begins at nucleotide 102. The putative site of phosphorylation (P) and an *N*-myristoylation site (M) affected by the Thr415Asn and Trp453Stop mutations, respectively, are indicated. UTR denotes untranslated region.

many years, the majority of patients with *parkin* mutations had good responses to low doses of levodopa. Although levodopa-induced dyskinesia was reported to develop early,[9,11,21] the mean delay in our patients was about 5 years, with a maximum of 20 years. This time frame was similar to that for the patients without *parkin* mutations.

Finally, dementia was rare among the patients with mutations. This might be explained by a less widespread neuronal loss in patients with mutations, in whom the substantia nigra and, to a lesser extent, the locus caeruleus are selectively affected, as compared with patients with idiopathic Parkinson's disease.[15,16] However, the low frequency of dementia in the patients with mutations could also be due to a younger mean age at examination or to the exclusion of patients who had dementia early in the course of the disease.

There were no clinical differences between patients with missense mutations and those with truncating mutations. This finding was surprising, since missense mutations might be expected to interfere less with the function of the *parkin* protein than truncating mutations and therefore to result in a milder phenotype. We therefore assume that the 10 conserved amino acids that were affected by the missense mutations are of crucial importance for the function of the protein or that their modification results in decreased protein synthesis or more rapid degradation.[22] In addition, the wide range of clinical signs, even within single families with mutations (e.g., variation of up to 20 years in the age at onset) suggests that additional factors contribute to the phenotype.

The chief histopathological differences between patients with *parkin* mutations and those with idiopathic Parkinson's disease that have been detected so far are the absence of Lewy bodies and the restriction of neuronal cell loss to the substantia nigra and the locus caeruleus in the patients with *parkin* mutations.[16] Thus, *parkin* gene mutations are responsible for the death of selective cells, the mechanism of which might differ from that in idiopathic Parkinson's disease.

The PCR-based technique that we used revealed numerous rearrangements of exons, including those identified in eight families in which no mutations were found by direct sequencing.[14] In combination with genomic sequencing, this technique greatly improves the sensitivity of the molecular diagnosis in patients with *parkin* gene mutations. The various combinations of exon deletions, the exon multiplications, and the newly identified point mutations increase the already wide variety of disease-related mutations identified in the *parkin* gene. The position of the mutations indicates functionally important protein regions such as the RING–IBR–RING domain, as does conservation of the corresponding amino acids in mice and rats.[20]

The presence of both deletions and multiplications of some exons (e.g., exon 2 and 3) suggests that a mechanism such as unequal recombination might be involved. The observation that 13 of the mutations were found repeatedly in as many as 10 families raises the possibility of a founder effect. However, many of the mutations were found in families from different European countries, suggesting that these alterations are recurrent. The point mutations that accounted for the disease in approximately 40 percent of our patients seem to be less frequent among Japanese patients.[11] Finally, the identification of 15 index patients with single heterozygous mutations indicates that other mutations remain to be discovered, perhaps in noncoding regions of the *parkin* gene.

In conclusion, mutations of the *parkin* gene are frequent among patients with autosomal recessive Parkinson's disease. Although dystonia at the onset of disease, hyperreflexia, and a slow rate of disease progression are characteristic features of patients with *parkin* mutations, there are no specific clinical signs that distinguish these patients from patients with other causes of Parkinson's disease. The wide spectrum of mutations in the *parkin* gene renders molecular diagnosis difficult, but the relatively simple semiquantitative PCR method that we used detected approximately 70 percent of the mutations found in this series of patients.

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APPENDIX

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