

Origin of the Mutations in the *parkin* Gene in Europe: Exon Rearrangements Are Independent Recurrent Events, whereas Point Mutations May Result from Founder Effects

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A wide variety of mutations in the *parkin* gene, including exon deletions and duplications, as well as point mutations, result in autosomal recessive early-onset parkinsonism. Interestingly, several of these anomalies were found repeatedly in unrelated patients and may therefore result from recurrent, de novo mutational events or from founder effects. In the present study, haplotype analysis, using 10 microsatellite markers covering a 4.7-cM region known to contain the *parkin* gene, was performed in 48 families, mostly from European countries, with early-onset autosomal recessive parkinsonism. The patients carried 14 distinct mutations in the *parkin* gene, and each mutation was detected in more than one family. Our results support the hypothesis that exon rearrangements occurred independently, whereas some point mutations, found in families from different geographic origins, may have been transmitted by a common founder.

Introduction

Parkinson's disease (PD) is a frequent neurodegenerative disorder with a prevalence of ~2% in persons >65 years old (Elbaz et al. 1999). The main clinical features are rigidity, bradykinesia, and tremor, associated with a good response to levodopa. The disorder is caused by a massive loss of dopaminergic neurons in the pars compacta of the substantia nigra and is characterized by the presence of Lewy bodies (cytoplasmic eosinophilic hyaline inclusions) (Fearnley and Lees 1991). Genetic risk factors are probably involved in the pathogenesis of Par-

kinson's disease (de Silva et al. 2000), and several families with clearly established monogenic inheritance have been reported. The majority of such cases are caused by mutations in the *parkin* gene, which result in autosomal recessive early-onset parkinsonism (MIM 600116) (Hattori et al. 1998a, 1998b; Kitada et al. 1998; Leroy et al. 1998a; Lücking et al. 1998, 2000; Abbas et al. 1999). The number of patients with mutations in the α -synuclein (Polymeropoulos et al. 1997) or ubiquitin carboxy-terminal hydrolase (UCH)-L1 genes (Leroy et al. 1998b) is much smaller. The phenotype associated with *parkin* gene mutations is variable but is usually characterized by early-onset parkinsonism and slow disease progression (Ishikawa and Tsuji 1996; Lücking et al. 2000). Postmortem examinations reveal massive loss of dopaminergic neurons in the substantia nigra pars compacta and the absence of Lewy bodies, results suggesting that the pathologic process may differ from that of idiopathic Parkinson's disease (Takahashi et al. 1994; Mori et al. 1998; van de Warrenburg, in press). A wide variety of mutations in the *parkin* gene have been detected, including exon deletions and duplications, as well as point mutation (Hattori et al. 1998a, 1998b; Kitada et al.

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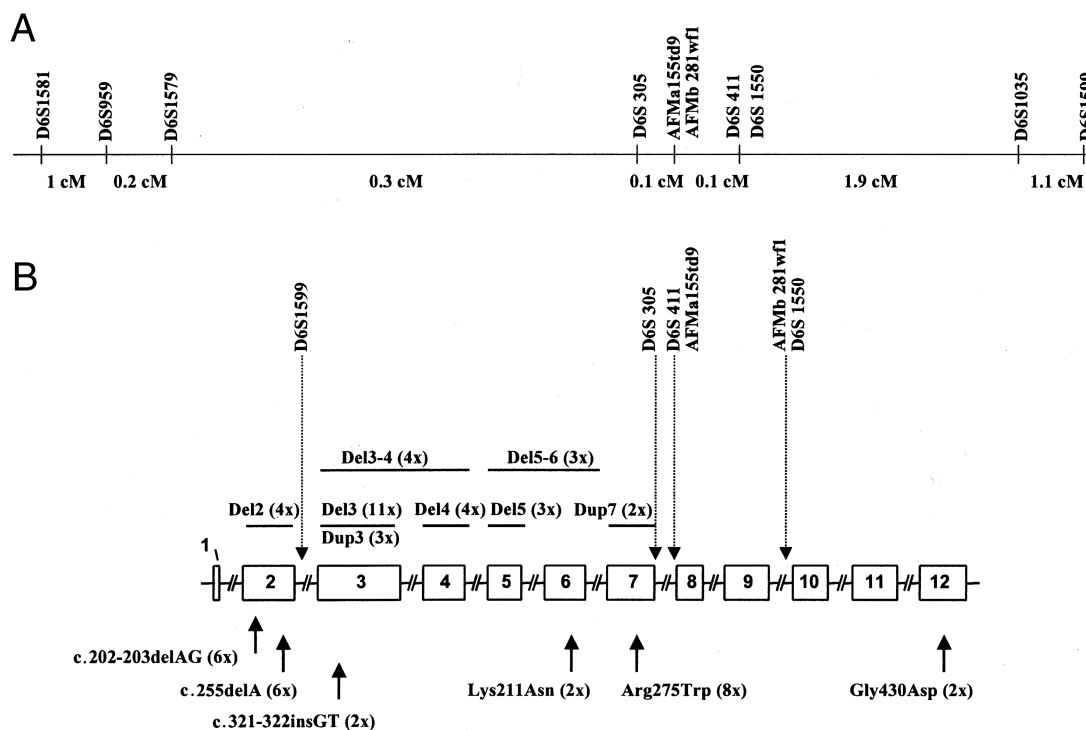


Figure 1 Recurrent mutations in the *parkin* gene and localization of the intragenic microsatellites, showing genetic map of the 10 microsatellites studied (A), exon rearrangements (B, top), and point mutations (B, bottom) that were detected more than once. Exon rearrangements are represented as bars corresponding to their size and position, and they are divided into deletions (del) and duplications (dup). The positions of point mutations are indicated by arrows. The ATG of the initiator methionine codon begins at nucleotide 102 of the published cDNA (Kitada et al. 1998). The number of index patients with the same mutation is indicated in parentheses. The positions of the microsatellites in the *parkin* gene are indicated by dotted arrows. Note the difference, arising from recent results of chromosome 6 sequencing, between the genetic map and physical map.

1998; Leroy et al. 1998a; Lücking et al. 1998, 2000; Abbas et al. 1999; Klein et al. 2000; Maruyama et al. 2000; Muñoz et al. 2000; Yamamura et al. 2000). The frequency of these mutations in Europe was estimated at 50% in families with early-onset parkinsonism that could have been autosomal recessive inheritance and at 18% in patients who had isolated parkinsonism with onset at age ≤ 45 years (Lücking et al. 2000).

Interestingly, several mutations were found repeatedly in index patients (Hattori et al. 1998a; Lücking et al. 1998, 2000; Abbas et al. 1999). In our series of patients, the deletion of exon 3 ($n = 11$), the Arg275Trp mutation ($n = 8$), the c.202-203 delAG mutation ($n = 6$), and the c.255delA mutation ($n = 6$) were found repeatedly. They may therefore result from recurrent, de novo mutational events or from founder effects. Divergent alleles of markers closely linked to the *parkin* locus would suggest independent de novo mutations, whereas conservation of alleles would support the hypothesis of a founder effect.

In the present study, haplotype analysis was performed with 10 microsatellite markers covering a 4.7-

cM region that contains the *parkin* gene, which is localized on chromosome 6q25.2-q27. The subjects were members of 48 families with early-onset autosomal recessive parkinsonism who carried 14 different mutations of the *parkin* gene found in more than one family. Our results support the hypothesis that exon rearrangements occurred independently, whereas there is evidence of founder effects in some families with point mutations.

Patients and Methods

Patients

Forty-eight families with early-onset parkinsonism caused by mutations in the *parkin* gene, including 69 patients and 49 unaffected relatives, were studied. All but four families (families TRUS and KUZ from Russia, families EGPD 25-95 and PW from Germany) have been described elsewhere (Lücking et al. 1998, 2000; Tassin et al. 1998; Abbas et al. 1999). The families were selected for *parkin* analysis according to the following criteria: (1) symptoms of parkinsonism (akinesia, rigidity, or

Table 1
Number and Origin of Index Patients with *parkin* Mutations Detected More than Once

MUTATION ^a	NO. OF INDEX PATIENTS ^b		NO. OF HAPLOTYPES	COUNTRY OF ORIGIN ^c (No.)
	Familial	Isolated		
Del2	4	0	3	Ir (3), F (1)
Del3	9	2	8	F (3), N(3), UK (1), Ir (2), Po (1), R (1)
Del3-4	3	1	2	Ir (2), F (1), G (1)
Del4	4	0	4	F (1), Ir (1), UK (1), G (1)
Del5	3	0	3	F (1), Ir (1), V (1)
Del5-6	0	3	2	L (1), Pa (1), UK (1)
Dup3	2	1	2	Ir (1), Al (1), S (1)
Dup7	2	0	1	Ar (1), N (1)
Lys211Asn	2	0	2	N (2)
c.321-322insGT	2	0	1	F (1), G (1)
Gly430Asp	1	1	1	UK (1), Ir (1)
c.255delA	4	2	5	F (4), S (1), Po (1)
Arg275Trp	4	4	6	F (2), It (2), Ir (1), UK (1), G (1), G/Ir (1)
c.202-203delAG	3	3	4	UK (2), F (1), N (1), It (1), R (1)

^a Del = deletion; Dup = duplication.

^b Because index patients were counted separately for the various repeated mutations, the total number (*n* = 60) is greater than the number of families studied (*n* = 48).

^c Al = Algeria; Ar = Argentina; F = France; G = Germany; Ir = Ireland; It = Italy; L = Lebanon; N = The Netherlands; Pa = Pakistan; Po = Portugal; R = Russia; UK = United Kingdom; V = Vietnam.

tremor), (2) marked improvement resulting from levodopa treatment, (3) age at onset ≤45 years for at least one affected sib, and (4) family history compatible with autosomal recessive inheritance (except family PW). In 13 of the 48 families, only one member was affected (referred to as isolated cases). These individuals were selected according to the same clinical criteria but had no family history of parkinsonism. They carried *parkin* gene mutations, on one or both alleles, that were detected at least twice in unrelated index patients (fig. 1B). The numbers and origins of the patients are summarized in table 1.

Genotyping

Blood samples were taken, after written informed consent was obtained, from 69 patients and 49 unaffected relatives, and genomic DNA was extracted using standard procedures. Genotyping was performed by PCR, using the primers specified in the Genome Database (GDB), with the following microsatellite DNA markers: D6S1581 (1 cM), D6S959 (0.2 cM), D6S1579 (0.3 cM), D6S305 (0.1 cM), AFMa155td9 (0 cM), AFMb281wf1 (0.1 cM), D6S411 (0 cM), D6S1550 (1.9 cM), D6S1035 (1.1 cM), and D6S1599 (fig. 1A) (genetic distances according to the Whitehead Institute for Biomedical Research). The physical positions of markers D6S411, AFMa155td9, AFMb281wf1, D6S1550, and D6S1599 differ partly from the genetic map and were determined with the use of clones from the Sanger Center (PAC 292F10 and RP1-45F6). Marker D6S305 was positioned, on the basis of PCR results, in patients with various exon deletions (Leroy et al. 1998b; Lücking et

al. 2000). Marker D6S1599 was amplified with the following primers: D6S1599 forward, 5'-GGG TGT GCT TGG ATT CCT TCA TG-3', and D6S1599 reverse, 5'-TAG CAT GTG GAC TGC ATA TCA AC-3'. The primers were labeled by fluorescence, and PCR products were analyzed on an ABI 377 automated sequencer with the GENESCAN 3.1 and GENOTYPER 1.1.1 software pro-

Table 2
Genotypes and Haplotypes of Index Patients with an Exon 2 Deletion in the *parkin* Gene

	IT31 (F)	IT22 (F)	F155 (F)	IT67 (F)
Origin	Italy	Italy	France	Italy
Consanguinity	No	No	No	No
Mutations	del2/del2-4	del2/del3	del2/del3	del2/ND
Marker:				
D6S1581	<u>7</u> 1	<u>8</u> 3	<u>7</u> 7	[3 7]
D6S959	<u>1</u> 1	<u>2</u> 2	<u>1</u> 2	<u>2</u> 2
D6S1579	<u>2</u> 5	<u>7</u> 2	<u>5</u> 5	<u>2</u> 2
<u>D6S1599</u>	<u>del/del</u>	<u>del/1</u>	<u>del/12</u>	[3 13]
D6S305	<u>7</u> 1	<u>3</u> 1	<u>9</u> 7	[1 3]
D6S411	<u>3</u> 4	<u>3</u> 3	<u>4</u> 3	<u>3</u> 3
AFMa155td9	<u>1</u> 3	<u>1</u> 1	<u>2</u> 2	[1 2]
AFMb281wf1	<u>3</u> 3	<u>3</u> 2	<u>3</u> 3	<u>3</u> 3
D6S1550	<u>2</u> 3	<u>4</u> 1	<u>4</u> 4	[2 3]
D6S1035	<u>2</u> 6	<u>5</u> 4	<u>4</u> 3	[4 5]

NOTE.—Reconstructed disease haplotypes bearing the analyzed mutation are underlined. The marker closest to the mutation is also underlined. When phase transmission was unknown, genotypes of the index patients are indicated in brackets. The haplotypes reconstructed in families with known consanguinity were considered as a single haplotype for statistical analysis. del = deletion; dup = duplication; F = familial parkinsonism; I = isolated parkinsonism; ND = not determined.

Table 3**Genotypes and Haplotypes of Index Patients with an Exon 3 Deletion in the *parkin* Gene**

	F141 (F)	F155 (F)	F431 (F)	F192 (F)	F195 (F)	UK57 (F)	F711 (F)	IT22 (F)	KUS (F)	JMP10 (I)	UK10551 (I)
Origin	France	France	France	N	N	N	Portugal	Italy	Russia	Italy	UK
Consanguinity	Yes	No	No	No	No	No	Yes	No	No	No	No
Mutations	Del3/ Del3	Del3/ Del2	Del3/ ND	Del3/ Dup7	Del3/ Lys211Asn	Del3/ Del4	Del3/ Del3	Del3/ Del2	Del3/ /Del7	Del3/ Del2-3	Del3/ Gly430Asp
Marker:											
D6S1581	3 4	7 7	3 7	1 8	8 3	7 7	7 7	3 8	[7 8]	[1 7]	3 3
D6S959	1 2	2 1	2 2	2 2	3 5	2 2	1 1	2 2	[1 2]	[2 5]	[2 3]
D6S1579	2 2	5 5	2 2	5 2	2 2	2 2	2 2	2 7	[2 5]	[2 6]	[2 5]
D6S1599	2 2	12/del	3 2	2 3	3 2	3 2	2 2	1/del	[4 12]	13/del	2 2
D6S305	1 8	7 9	1 4	7 6	7 3	3 7	3 3	1 3	3 3	1 1	[3 7]
D6S411	3 3	3 4	3 5	3 3	3 4	3 3	3 3	3 3	3 3	[2 5]	3 3
AFMa155td9	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1	[1 2]	[1 2]
AFMb281wf1	2 2	3 3	3 3	3 3	2 3	2 3	3 3	2 3	3 3	3 3	[2 3]
D6S1550	2 2	4 4	2 4	2 2	2 2	1 2	2 2	1 4	[2 4]	[3 4]	2 2
D6S1035	3 3	3 4	3 5	6 4	4 4	5 2	4 4	4 5	[3 5]	[3 5]	[2 4]

NOTE.—Data are as defined in Note to table 2. N = The Netherlands; UK = United Kingdom.

grams (all from Applied Biosystems). To ensure accurate sizing of the alleles, a control DNA sample of individual 1347.02, from the Centre d'Etude du Polymorphisme Humain, was tested for each marker, and allele numbers were assigned in increasing order from the smallest to the largest PCR product.

Haplotypes and Linkage Disequilibrium

Haplotypes were constructed manually to include a minimum number of recombinations. For each mutation, the disease-associated haplotypes (DHs) were compared among families, to detect (1) common *parkin* haplotypes that would indicate that the families were related and (2) a common ancestral DH. The haplotypes constructed in families with known consanguinity were considered as a single haplotype for statistical analysis.

The difference in allele distribution between normal and carrier chromosomes was evaluated by χ^2 and two-tailed Fisher's exact tests, with Yates correction when appropriate. The most frequent allele on disease-bearing chromosomes was defined as a single allele, and the others were pooled to form a second allele. The presence of linkage disequilibrium was tested by $D = \sqrt{(\chi^2/N)}$, where N is the total number of DHs and control chromosomes used. A P value $<.01$ was considered to be statistically significant. The proportion of carrier chromosomes bearing the original associated allele was calculated with the equation $\delta = (P_D - P_N)/(1 - P_N)$, where P_D and P_N are the frequencies of carrier and normal chromosomes, respectively. The control population comprised 140 chromosomes from normal, white subjects.

Results

The origins of the 69 patients and 49 relatives from 48 families with early-onset parkinsonism are shown in

table 1. DHs were constructed in the 38 families in which unaffected relatives were available (tables 2–4).

Exon Rearrangements

Genotypes or haplotypes of patients with deletion of exon 2, 3, or 4 are shown in tables 2, 3, and 4, respectively. Patients with exon 2 deletions did not share common haplotypes, and alleles at markers D6S1579 and D6S305, which flank the deletion, also differed (table 2). Although allele 3 at marker AFMb281wf1 was present on 100% of the disease-causing chromosomes, the association was not statistically significant, because this allele was present in 80% of the control population. Interestingly, marker D6S1599, located in intron 2, was deleted with exon 2 in 3 of 4 families (table 2). This observation indicated the existence of at least two distinct breakpoints and supported the hypothesis of independent mutational events. Haplotypes of the two patients with deletions of exons 3 and 4, only one of which was associated with a deletion of marker D6S1599, also differed (data not shown).

Four of the five haplotypes that segregated with exon 4 deletions were identical at markers AFMa155td9, AFMb281wf1, and D6S1550 (table 4). However, the association was not statistically significant, because these were the most frequent alleles in the control population (50%, 82%, and 46%, respectively). Furthermore, the haplotypes for the markers closest to the deletion (D6S1599 and D6S305) differed, suggesting independent recurrent mutations (table 4). However patient IT05, who was homozygous for an exon 4 deletion and who had no known consanguinity, was homozygous for markers D6S1599, D6S305, and D6S411, which flank the deletion. Although the patient's parents were not

Table 4
Genotypes and Haplotypes of Index Patients with an Exon 4 Deletion in the *parkin* Gene

	F29 (F)	DE25 (F)	UK57 (F)	IT05 (F)
Origin	France	Germany	United Kingdom	Italy
Consanguinity	No	No	No	No
Mutations	del4/ND	del4/ND	del4/del3	del4/del4
Marker:				
D6S1581	3 3	8 4	7 7	9 3
D6S959	2 5	6 1	2 2	5 1
D6S1579	2 3	2 3	2 2	2 5
D6S1599	10 3	4 1	2 3	3 3
D6S305	3 5	3 3	7 3	1 1
D6S411	3 3	3 3	3 3	4 4
AFMa155td9	2 1	2 2	2 1	3 2
AFMb281wf1	3 3	3 3	3 2	1 3
D6S1550	2 4	2 3	2 1	2 2
D6S1035	2 5	3 3	2 5	6 2

NOTE.—Data are as defined in Note to table 2.

known to be related, a common founder for the exon 4 deletion on both chromosomes may be suspected.

Eleven index patients had exon 3 deletions, the most frequent mutation in the *parkin* gene, but no significant association was observed between allele markers and the DHs (table 3). Allele 2 at marker D6S1599, the marker closest to the mutation, was present on 4 of 10 disease-bearing chromosomes but was also the most frequent allele in the control population (43%). Furthermore, patients F155 and JMP10 carried alleles 12 and 13, respectively, (whose sizes were very different from those of alleles 2 and 3), which probably arose from independent events. Patients F141 and F711 shared alleles 1-2-2 at markers D6S959, D6S1579, and D6S1599, but this association was not statistically significant, because these alleles were very frequent in the control population (32%, 50%, and 43%, respectively). This is also the case for patients F431 and UK57, who shared alleles 2-

2-3 for the same markers, and for patient F195, who had alleles 2-3 for markers D6S1579 and D6S1599. Alleles 2-2-3 were present in 57%, 50%, and 24% of the control population, respectively. Patients F155 and KUS shared alleles 2-5-12 at markers D6S959, D6S1579, and D6S1599; however, because the phase transmission for patient KUS was not known, we do not know whether these alleles were carried by the chromosome associated with the mutation. Because alleles 5 and 12 are rare in the control population (8% and 6%, respectively), these two patients may share a common haplotype, but no definite conclusion can be made.

No common haplotype, or even common marker alleles, were observed for the other exon deletions (exons 3 and 4, exons 5 and 6, exon 5) or duplications (exon 3 and exon 7) (data not shown).

Point Mutations

Genotypes or haplotypes of patients with the c.255delA, c.202-203delAG, and Arg275Trp mutations are shown in tables 5, 6, and 7, respectively. It is possible that F744 and S70 (alleles 3-2-2-7 at markers D6S1581, D6S959, D6S1579, and D6S1599) or S70 and UK17275 (alleles 2-7-3-3-1 at markers D6S1579, D6S1599, D6S305, D6S411, and AFMa155td9) share a common haplotype for the c.255delA mutation. No definite conclusion can be drawn, however, because the phase of transmission could not be determined for all markers or patients (table 5). However, genotype analysis revealed that, in most cases, the mutation segregated with allele 7 of marker D6S1599 (table 5), which is located 24 kb downstream of exon 2. This allele was not found in the 62 patients who did not carry the c.255delA mutation (not determined in one patient) (6/6 vs. 0/62; *P* < .0001).

Regarding allele frequencies (independent of the trans-

Table 5
Genotypes and Haplotypes of Index Patients with c.255delA Mutations in the *parkin* Gene

	F171 (F)	F744 (F)	F96 (F)	S70 (I)	UK17275 (F)	S74 (I)
Origin	France	France	France	Spain	Portugal	France
Consanguinity	No	No	No	No	Yes	No
Mutations	c.255delA/Dup6	c.255delA/Del5	c.255delA/c.255delA	c.255delA/c.255delA	c.255delA/c.255delA	c.255delA/Del3-4
Marker:						
D6S1581	3 8	3 3	3 3	[3 8]	[3 8]	[1 7]
D6S959	1 5	2 1	1 2	2 2	[1 6]	2 2
D6S1579	3 6	2 5	7 7	[2 3]	[2 3]	[2 5]
D6S1599	7 2	7 4	7 9	[7 9]	7 7	[2 7]
D6S305	8 9	6 3	7 1	[3 8]	[3 6]	3 3
D6S411	4 3	3 3	4 3	3 3	3 3	[3 4]
AFMa155td9	2 1	2 2	[1 2]	1 1	1 1	2 2
AFMb281wf1	3 3	2 3	3 3	[2 3]	3 3	2 2
D6S1550	4 2	2 4	3 2	[2 4]	[1 2]	[2 3]
D6S1035	5 4	5 2	3 2	[3 4]	[3 4]	[3 4]

NOTE.—Data are as defined in Note to table 2.

Table 6

Genotypes and Haplotypes of Index Patients with the c.202-203delAG Mutation in the *parkin* Gene

	IT20 (F)	UK86 (F)	NL24 (F)	TRUZ (F)	S749 (I)	UK4720 (I)
Origin	Italy	United Kingdom	Netherlands	Russia	France	United Kingdom
Consanguinity	No	No	No	No	Yes	No
Mutations	c.202-203delAG/ Lys161Asn	c.202-203delAG/ ND	c.202-203delAG/ Lys211Asn	c.202-203delAG/ ND	c.202-203delAG/ c.202-203delAG	c.202-203delAG/ c.202-203delAG
Marker:						
D6S1581	3 3	4 4	3 1	3 3	3 3	3 3
D6S959	[1 2]	6 1	2 5	[1 2]	[1 2]	[1 2]
D6S1579	[2 5]	3 4	2 2	[6 8]	4 4	3 3
D6S1599	[2 3]	3 2	2 3	[2 4]	2 2	2 2
D6S305	[3 9]	8 8	9 8	[5 6]	3 3	[1 4]
D6S411	3 3	3 1	3 3	3 3	[3 4]	3 3
AFMa155td9	2 2	[1 2]	2 1	2 2	[1 2]	[1 2]
AFMb281wf1	3 3	3 3	3 2	3 3	[2 3]	[2 3]
D6S1550	2 2	1 4	2 2	2 2	[2 4]	2 2
D6S1035	[3 6]	[3 5]	3 4	[2 3]	[4 5]	[2 5]

NOTE.—Data are as defined in Note to table 2.

mission phase), all index patients, whether homozygous or heterozygous for the c.202-203delAG mutation in exon 2, carried allele 2 at marker D6S1599 (table 6), but about half the patients with other mutations also carried this allele (6/6 vs. 29/62; $P > .01$). Furthermore, in family UK86, allele 2 did not segregate with the c.202-203delAG mutation (table 6). Patients IT20, NL24, and TRUZ shared alleles 3-2-3-2 at markers D6S411, AFMa155td9, AFMb281wf1, and D6S1550, but this association was not significant because of the high frequency of these alleles in the control population (59%, 50%, 82%, and 46%, respectively). Although it cannot be proved, IT20 and NL24 may share a common haplotype over the entire region.

At marker D6S305, in intron 7, all patients with the Arg275Trp mutation in exon 7 carried allele 4 (table 7). This allele was observed in three other patients who did not carry the Arg275Trp mutation (8/8, vs. 3/61; $P < .0001$). Furthermore, a common haplotype was observed between patients IT15 and IT63, who shared alleles 2-2-4-3 at markers D6S1579, D6S1599, D6S305, and D6S411; however, these alleles were the most frequent alleles in the control population (50%, 43%, and 59%, for D6S1579, D6S1599, and D6S411, respectively), except for allele 4 at marker D6S305, which was observed in only 9% in the control population. A common haplotype was also observed in UK2329 and S96 who shared alleles 3-1-2 at markers D6S411, AFMa155td9, and AFMb281wf1, with allele frequencies of 59%, 38%, and 9%, respectively. Patients IT15, IT63, S92, and UK4823 may share this haplotype, but the phase transmission was unknown for these markers.

For the other point mutations (Lys211Asn, c.321-322 insGT, and Gly430Asp), no common haplotypes or allelic associations were observed (data not shown).

Linkage Disequilibrium

We observed a strong linkage disequilibrium (LD) of 0.75 between the c.255delA mutation and allele 7 of marker D6S1599 (table 8). Allele 7 was present on five of seven independent chromosomes associated with the mutation, in patients from France, Portugal, and Spain (table 5, excluding patient S74, in whom the allele cannot be attributed to the mutation) but was absent from the control population ($P < .0001$) (table 8). However, patient F96 (from France) and patient S70 (from Spain), both of whom were homozygous for the c.255delA mutation, carry alleles 7 and 9 at marker D6S1599 (table 5). This observation could be explained by the existence of two distinct founder mutations or by a recombination between the mutation and marker D6S1599. Alternatively, a mutation in marker D6S1599 could have changed allele 7 to 9.

At locus D6S1599, allele 2 was present on 4 of 5 of the chromosomes associated with the c.202-203delAG mutation for whom the phase transmission was known. Because allele 2 of marker D6S1599 was also very frequent in the control population (43%), this association was not statistically significant ($P > .01$) (table 8).

At locus D6S305, (LD = 0.24) was observed between allele 4 and the Arg275Trp mutation (table 8). Allele 4 of marker D6S305 was present in five of five patients from France, Germany, and Italy on chromosomes carrying the mutation for which the phase transmission was known (table 7). This association was statistically significant since allele 4 was present in only 28% of the control chromosomes ($P < .003$). LD for marker AFMb281wf1 could not be tested, because the phase transmission was known in only three subjects. However, the frequency of allele 2 was much higher in the

Table 7**Genotypes and Haplotypes of Index Patients with the Arg275Trp Mutation**

	IT15 (F)	IT63 (F)	UK 2329 (I)	S92 (I)	S96 (I)	UK 4823 (I)	PW (F)	EGPD 25-95 (F)
Origin	Italy	Italy	Ireland	France	France	United Kingdom	Germany	Germany/Ireland
Consanguinity	No	No	No	No	No	No	No	No
Mutation	Arg275Trp/ ND	Arg275Trp/ ND	Arg275Trp/ Gly430	Arg275Trp/ del3-6	Arg275Trp/ ND	Arg275Trp/ del5-6	Arg275Trp/ 40pbdel3	Arg275Trp/ ND
Marker:								
D6S1581	4 8	7 3	3 8	3 3	3 3	3 3	3 3	7 3
D6S959	[1 2]	1 1	2 5	2 1	[1 2]	[1 2]	ND	1 2
D6S1579	2 5	2 7	2 5	2 2	[2 5]	3 3	2 2	ND
D6S1599	2 2	2 4	10 13	4 4	[3 4]	2 2	ND	3 2
D6S305	4 8	4 3	[4 3]	4 8	[4 5]	[4 1]	4 3	4 9
D6S411	3 3	3 3	3 3	3 3	3 3	3 3	2 1	[4 6]
AFMa155td9	[1 2]	[1 2]	1 1	[1 2]	1 1	[1 2]	3 2	1 1
AFMb281wf1	[2 3]	[2 3]	2 2	[2 3]	2 2	[2 3]	2 2	[2 3]
D6S1550	2 2	2 4	ND	2 2	2 2	ND	[3 4]	3 3
D6S1035	7 3	3 5	3 2	4 5	[2 4]	[2 5]	5 4	6 4

NOTE.—Data are as defined in Note to table 2.

eight patients with the Arg275Trp mutation compared with the control population (68% and 9%, respectively) ($P < .0001$). It is possible that allele 2 is carried by all the chromosomes carrying the Arg275Trp mutation.

Discussion

To discriminate between single founder effects and independent recurrent events, DHs in the *parkin* region were established for intragenic and tightly flanking markers in 48 families with repeatedly observed mutations in the *parkin* gene.

The disease-associated alleles and haplotypes in families with the same exonic rearrangement were discordant in most of the families, even for markers located close to the rearrangements. Furthermore, genotypes of marker D6S1599 revealed at least two distinct breakpoints for exon 2 deletions in four unrelated families. These results suggest that these recurrent mutations originated independently, although we cannot exclude a very ancient founder effect associated with recombinations or mutations involving tightly linked markers. The hypothesis of independent recurrent events was recently confirmed by molecular analysis of the breakpoints of the *parkin* gene in patients with some of the deletions analyzed here (Asakawa et al. 2000). These deletions were classified into 18 types. However, one type of exon 4 deletion was commonly found among six families. This may reflect the case of our patient IT05, who is homozygous, without known consanguinity, for an exon 4 deletion, as well as for three markers flanking the deletion. Thus, even if de novo mutations occur regularly, a given exon deletion that is transmitted to subsequent generations becomes a new founder for this deletion.

Unequal inter- or intrachromosomal crossovers that

result from the misalignment of two homologous flanking sequences may account for the existence of deletions and for duplications of the same regions of the *parkin* gene, as has been reported elsewhere in several genes, such as the steroid sulfatase gene (Yen et al. 1990) or the α -globin locus (Nicholls et al. 1987). Unequal crossovers, which result in duplications and deletions of a 1.5-Mb region of chromosome 17p11.2, give rise, respectively, to Charcot-Marie-Tooth Type 1A (CMT1A) (Lupski et al. 1991) and hereditary neuropathy with liability to pressure palsies (HNPP) (Chance et al. 1993). Excisions of an intrachromatid loop, resulting exclusively in deletions, also occur in this region of 17p11.2 (Kiyosawa et al. 1995; Lopes et al. 1998; Lopes et al. 1999). If both these mechanisms also cause exon rearrangements in the *parkin* gene, this would explain why deletions appear to be more frequent than duplications (29 vs. 5 in our series) in the *parkin* gene.

Transposable elements (TEs), such as Alu elements, are also involved in recombination. Almost 0.5% of known genetic disorders result from TE insertions or TE-mediated recombination (Deragon and Capy 2000; Deininger and Batzer 1999). TEs are relatively abundant in the genome (~1 Alu/6 kb). The density of Alu elements in the first 130 kb of intron 2 of the *parkin* gene, which is involved in the deletion of exons 2 and 3, reaches 1 Alu/2.5 kb. This may explain the frequency of deletions of exons 2, 3, and 3–4, duplications of exon 3, and triplication of exon 2.

Unlike the exon rearrangements, point mutations in the patients studied may be accounted for by a limited number of founders. Strong linkage disequilibrium was observed between the c.255delA mutation in exon 2 and allele 7 of marker D6S1599 in intron 2 (table 5) and between the Arg275Trp mutation in exon 7 and allele 4 of marker D6S305 in intron 7 (table 7). These

Table 8

Linkage Disequilibrium for Three-Point Mutations Found Two or More Times in the *parkin* Gene

Mutation	Locus	Allele	P _D ^a	P _N ^b	χ ²	P	P _{corrected} ^c	D ^d	δ ^d
c.255delA	D6S1599	7	5/7	0/124	73.65	<.0001	<.001	.75	.75
c.202-203delAG	D6S1599	2	4/5	53/124	1.40	>.01	>.1	ND	ND
Arg275Trp	D6S305	4	5/5	39/140	8.71	<.003	<.03	.24	1

^a The frequency of carrier chromosomes is calculated by $P_D = (n_{\text{patients}}/N)$, where N is the number of haplotypes in which the allele segregating with the mutation could be determined.

^b The frequency of control chromosomes is calculated by $P_N = (n_{\text{controls}}/N)$, where N is the number of control chromosomes used.

^c P value was corrected by a factor 10 (correction of Bonferroni), because the same control population was used to test allelic frequencies of the 10 microsatellites.

^d ND = not determined.

associations may reflect the transmission of a single ancestral mutation or may be caused by recurrent mutations on a predisposing haplotype, as shown in spinocerebellar ataxia 7 (Stevanin et al. 1999). Interestingly, alleles at markers D6S1579 and D6S305, which cover a 0.3-cM interval that includes D6S1599, were not conserved in patients with the c.255delA mutation in exon 2. These observations indicate that multiple recombinations and/or mutations have modified the alleles that segregate with the closely flanking markers, suggesting that this mutation can be attributed to very ancient founder effects. The geographic diversity of the patients carrying the same allele also supports this hypothesis: patients with the c.255delA mutation come from France, Spain, and Portugal (tables 5-7).

In contrast, the Arg275Trp mutation may be more recent, because a common ancestral haplotype between markers D6S1579 and D6S1550 is suspected. Patients IT15 and IT63 shared a haplotype between markers D6S1579 and D6S1550 (not proved for markers AFMa155td9 and AFMb281wf1, because the phase was not determined). Furthermore, patients UK2329 and S96 (who were from Ireland and France, respectively) and probably patients S92 and UK4823 (who were from France and the United Kingdom) also shared part of this haplotype between marker D6S411 and marker AFMb281wf1. Although these associations were not statistically significant for the majority of the markers, associations between the Arg275Trp mutation and the markers D6S305 and AFMb281wf1 were significant ($P < .001$). The ancestral founder haplotype could thus have been 2-2-4-3-1-2-2 for markers D6S1579 to D6S1550 before a recombination separated haplotype 2-2 (marker D6S1579 and D6S1599) from haplotype 4-3-1-2-2 (markers D6S305 to D6S1550). Because the suspected ancestral haplotype was observed in two Italian patients, the mutation may have arisen in Italy.

Allele 2 of marker D6S1599 was present in the six index patients with the c.202-203delAG mutation in

exon 2 and on four of the five chromosomes associated with the mutation. The association was not statistically significant, because allele 2 is also very frequent in the control population. Because of the absence of other known markers in the introns flanking this mutation, we cannot distinguish between a single founder effect on a chromosome carrying allele 2 or recurrent mutations on chromosomes that carry this allele by chance.

It is also difficult to draw conclusions concerning the three other point mutations analyzed in this study (Lys211Asn, c.321-322insGT and Gly430Asp). No allelic associations were observed. It is difficult, however, either to demonstrate or to exclude a founder effect because of (1) the small number of index patients, (2) the lack of polymorphic markers in flanking introns (except for mutation c.321-322insGT in exon 3), and (3) the absence of relatives who, if available, would enable us to determine the phase of transmission.

The release of sequence data for the *parkin* region of chromosome 6, which is expected in the near future, should allow us to clarify some of the patients in this study. The sequencing of a contig of BACs that contain the *parkin* gene is in progress, and some of the data are already available. It will therefore be possible to define new microsatellite markers in each intron of the *parkin* gene and to detect ancient founder effects. In addition, single-nucleotide polymorphism (SNP) databanks, which help in the reconstruction of haplotypes, are also being created. Only one SNP in the *parkin* gene, localized in intron 8, has been identified to date. The known SNPs in the coding region of the *parkin* gene (Abbas et al. 1999) were not informative for the present study.

In conclusion, a wide variety of mutations in the *parkin* gene have been found repeatedly in patients from many European populations. Interestingly, the majority of exon rearrangements seem to result from distinct mutational events, whereas at least two point mutations may have arisen from a small number of founders. Therefore, the mechanisms underlying these two groups

of mutations appear to be different. According to this hypothesis, the frequency of exon rearrangements would be expected, in the absence of selection, to increase with the passage of time because of new mutational events, whereas the frequency of point mutations would be expected to remain stable, because new mutations would be rare.

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Appendix

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Centre d'Etude du Polymorphisme Humain, <http://www.cephb.fr> (for the control DNA sample)
DNA Databank of Japan, <http://www.ddbj.nig.ac.jp> (for the

cDNA sequence of the *parkin* gene [accession number AB009973])

Genome Database, <http://www.gdb.org> (for primer sequences)
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for early-onset parkinsonism [MIM 600116])

Sanger Center, <http://www.sanger.ac.uk> (for the clone 292F10 and RP1-45F6 sequences [accession numbers 2760544 and 5924005, respectively])

Whitehead Institute for Biomedical Research, <http://www.genome.wi.mit.edu> (for integrated map of chromosome 6)

References

- Abbas N, Lücking CB, Ricard S, Durr A, Bonifati V, De Michele G, Bouley S, Vaughan JR, Gasser T, Marconi R, Broussolle E, Brefel-Courbon C, Harhangi BS, Oostra BA, Fabrizio E, Bohme GA, Pradier L, Wood NW, Filla A, Meco G, Deneffe P, Agid Y, Brice A (1999) A wide variety of mutations in the *parkin* gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. *Hum Mol Genet* 8:567-574
- Asakawa S, Hattori N, Shintani A, Sasaki T, Kawasaki K, Shimizu A, Kitada T, Matsumine H, Minoshima S, Shimizu Y, Mizuno Y, Shimizu N (2000) Molecular analysis of the deletion breakpoints of *parkin* gene. *Am J Hum Genet Suppl* 67:398
- Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Distchele CM, Bird TD (1993) DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell* 72:143-151
- de Silva HR, Khan NL, Wood NW (2000) The genetics of Parkinson's disease. *Curr Opin Genet Dev* 10:292-298
- Deininger PL, Batzer MA (1999) Alu repeats and human disease. *Mol Genet Metab* 67:183-193
- Deragon JM, Capy P (2000) Impact of transposable elements on the human genome. *Ann Med* 32:264-273
- Elbaz A, Grigoletto F, Baldereschi M, Breteler MM, Manubens-Bertran JM, Lopez-Pousa S, Dartigues JF, Alperovitch A, Tzourio C, Rocca WA (1999) Familial aggregation of Parkinson's disease: a population-based case-control study in Europe. *Europarkinson Study Group. Neurology* 52:1876-1882
- Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 114:2283-2301
- Hattori N, Kitada T, Matsumine H, Asakawa S, Yamamura Y, Yoshino H, Kobayashi T, Yokochi M, Wang M, Yoritaka A, Kondo T, Kuzuhara S, Nakamura S, Shimizu N, Mizuno Y (1998a) Molecular genetic analysis of a novel *parkin* gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the *parkin* gene in affected individuals. *Ann Neurol* 44:935-941
- Hattori N, Matsumine H, Asakawa S, Kitada T, Yoshino H, Elibol B, Brookes AJ, Yamamura Y, Kobayashi T, Wang M, Yoritaka A, Minoshima S, Shimizu N, Mizuno Y (1998b) Point mutations (Thr240Arg and Gln311Stop [correction of

- Thr240Arg and Ala311Stop]) in the parkin gene. *Biochem Biophys Res Commun* 249:754–758
- Ishikawa A, Tsuji S (1996) Clinical analysis of 17 patients in 12 Japanese families with autosomal-recessive type juvenile parkinsonism. *Neurology* 47:160–166
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392:605–608
- Kiyosawa H, Lensch MW, Chance PF (1995) Analysis of the CMT1A-REP repeat: mapping crossover breakpoints in CMT1A and HNPP. *Hum Mol Genet* 4:2327–2334
- Klein C, Pramstaller PP, Kis B, Page CC, Kann M, Leung J, Woodward H, Castellán CC, Scherer M, Vieregge P, Breakefield XO, Kramer PL, Ozelius LJ (2000) Parkin deletions in a family with adult-onset, tremor-dominant parkinsonism: expanding the phenotype. *Ann Neurol* 48:65–71
- Leroy E, Anastasopoulos D, Konitsiotis S, Lavedan C, Polymeropoulos MH (1998a) Deletions in the parkin gene and genetic heterogeneity in a Greek family with early onset Parkinson's disease. *Hum Genet* 103:424–427
- Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH (1998b) The ubiquitin pathway in Parkinson's disease. *Nature* 395:451–452
- Lopes J, Ravise N, Vandenberghe A, Palau F, Ionasescu V, Mayer M, Levy N, Wood N, Tachi N, Bouche P, Latour P, Ruberg M, Brice A, LeGuern E (1998) Fine mapping of de novo CMT1A and HNPP rearrangements within CMT1A-REPs evidences two distinct sex-dependent mechanisms and candidate sequences involved in recombination. *Hum Mol Genet* 7:141–148
- Lopes J, Tardieu S, Silander K, Blair I, Vandenberghe A, Palau F, Ruberg M, Brice A, LeGuern E (1999) Homologous DNA exchanges in humans can be explained by the yeast double-strand break repair model: a study of 17p11.2 rearrangements associated with CMT1A and HNPP. *Hum Mol Genet* 8:2285–2292
- Lücking CB, Abbas N, Durr A, Bonifati V, Bonnet AM, de Broucker T, De Michele G, Wood NW, Agid Y, Brice A (1998) Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. The European Consortium on Genetic Susceptibility in Parkinson's Disease and the French Parkinson's Disease Genetics Study Group. *Lancet* 352:1355–1356
- Lücking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Deneffe P, Wood NW, Agid Y, Brice A (2000) Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. *N Engl J Med* 342:1560–1567
- Lupski JR, de Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas O, Barker DF, Killian JM, Garcia CA (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* 66:219–232
- Maruyama M, Ikeuchi T, Saito M, Ishikawa A, Yuasa T, Tanaka H, Hayashi S, Wakabayashi K, Takahashi H, Tsuji S (2000) Novel mutations, pseudo-dominant inheritance, and possible familial effects in patients with autosomal recessive juvenile parkinsonism. *Ann Neurol* 48:245–250
- Mori H, Kondo T, Yokochi M, Matsumine H, Nakagawa-Hattori Y, Miyake T, Suda K, Mizuno Y (1998) Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. *Neurology* 51:890–892
- Muñoz E, Pastor P, Marti MJ, Oliva R, Tolosa E (2000) A new mutation in the parkin gene in a patient with atypical autosomal recessive juvenile parkinsonism. *Neurosci Lett* 289:66–68
- Nicholls RD, Fischel-Ghodsian N, Higgs DR (1987) Recombination at the human alpha-globin gene cluster: sequence features and topological constraints. *Cell* 49:369–378
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetrooulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045–2047
- Stevanin G, David G, Durr A, Giunti P, Benomar A, Abada-Bendib M, Lee MS, Agid Y, Brice A (1999) Multiple origins of the spinocerebellar ataxia 7 (SCA7) mutation revealed by linkage disequilibrium studies with closely flanking markers, including an intragenic polymorphism (G3145TG/A3145TG). *Eur J Hum Genet* 7:889–896
- Takahashi H, Ohama E, Suzuki S, Horikawa Y, Ishikawa A, Morita T, Tsuji S, Ikuta F (1994) Familial juvenile parkinsonism: clinical and pathologic study in a family. *Neurology* 44:437–441
- Tassin J, Durr A, de Broucker T, Abbas N, Bonifati V, De Michele G, Bonnet AM, Broussolle E, Pollak P, Vidailhet M, De Mari M, Marconi R, Medjbeur S, Filla A, Meco G, Agid Y, Brice A (1998) Chromosome 6-linked autosomal recessive early-onset Parkinsonism: linkage in European and Algerian families, extension of the clinical spectrum, and evidence of a small homozygous deletion in one family. The French Parkinson's Disease Genetics Study Group, and the European Consortium on Genetic Susceptibility in Parkinson's Disease. *Am J Hum Genet* 63:88–94
- Van de Warrenburg BPC, Lammens M, Lücking CB, Deneffe P, Wesseling P, Booij J, Praamstra P, Quinn N, Brice A, Horstink MWIM. Parkinsonism associated with *parkin* gene mutations: clinical and pathological abnormalities in a Dutch family. *Neurology* (in press)
- Yamamura Y, Hattori N, Matsumine H, Kuzuhara S, Mizuno Y (2000) Autosomal recessive early-onset parkinsonism with diurnal fluctuation: clinicopathologic characteristics and molecular genetic identification. *Brain Dev (Suppl)* 22:87–91
- Yen PH, Li XM, Tsai SP, Johnson C, Mohandas T, Shapiro LJ (1990) Frequent deletions of the human X chromosome distal short arm result from recombination between low copy repetitive elements. *Cell* 61:603–610