How Much Phenotypic Variation Can Be Attributed to *parkin* Genotype?

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To establish phenotype–genotype correlations in early-onset parkinsonism, we have compared the phenotype of a large series of 146 patients with and 250 patients without *parkin* mutations. Although no single sign distinguished the groups, patients with mutations had significantly earlier and more symmetrical onset, dystonia more often at onset and hyperreflexia, slower progression of the disease, and a tendency toward a greater response to levodopa despite lower doses. After forward stepwise multiple logistic regression analysis, dystonia at onset and brisk reflexes were not longer significantly different but were correlated with age at onset rather than the presence of the *parkin* mutation. Age at onset in carriers of *parkin* mutations varied as did the rate of progression of the disease: the younger the age at onset the slower the evolution. The genotype influenced the phenotype: carriers of at least one missense mutation had a higher United Parkinson's Disease Rating Scale motor score than those carrying two truncating mutations. The localization of the mutations was also important because missense mutations in functional domains of *parkin* resulted in earlier onset. Patients with a single heterozygous mutation had significantly later and more asymmetrical onset and more frequent levodopa-induced fluctuations and dystonia than patients with two mutations.

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In the last few years, five genes and four loci have been identified in families with Parkinson's disease (PD). Mutations in the *alpha-synuclein* gene on chromosome 4q,[1] the *UCH-L1* gene on chromosome 4p,[2] and the *NR4A2* gene mapped to chromosome 2q22-23[3] are autosomal dominant, whereas mutations in the *parkin* gene on chromosome 6q[4] and the *DJ-1* gene on chromosome 1p[5] are autosomal recessive. Four other loci have been localized on chromosomes 2p13,[6] 4p14-16,[7] 1p35-36,[8] and 12p11-q13.[9] Other susceptibility factors, such as the Icelandic 1p32 locus,[10] the polymorphic variant S18Y of the UCH-L1 gene,[11] or the A0 allele of the tau gene,[12] have been reported. Whereas mutations in *alpha-synuclein* and *UCH-L1* have been found in only a few families,[1,2,13] mutations in the *parkin* gene have been described in many patients with autosomal recessive juvenile parkinsonism (ARJP).[4,14–16] Pathological studies have shown several differences be-

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tween ARJP with *parkin* mutations and idiopathic PD: in four cases there was an absence of Lewy bodies and a severe generalized loss of dopaminergic neurons from the substantia nigra pars compacta.[17–19] One of these four brains showed additional involvement of the substantia nigra pars reticulata,[19] the second showed additional neurofibrillary tangles and argyrophilic astrocytes in cerebral cortex and brainstem nuclei,[18] the third showed neuronal loss in parts of the spinocerebellar system,[17] and the fourth case, with a single heterozygous mutation, showed additional tau aggregates consistent with progressive supranuclear palsy.[20] Only one case, carrying compound heterozygous mutations of the *parkin* gene, has been described as having Lewy bodies.[21] The *parkin* gene, which contains 12 exons, spans more than 1.5Mb and encodes a protein of 465 amino acids with similarity to ubiquitin at the amino terminus (UBL domain) and a RING-in between ring (IBR)–RING motif at the carboxy terminus. Like many other proteins with a RING-finger domain, *parkin* has E3 ubiquitin ligase activity, linking *parkin*-associated PD to a defect in the ubiquitin-proteasome system.[22] Because mutations in *parkin* are associated with recessively inherited parkinsonism, they presumably result in reduction or loss of its E3 ligase activity.[22–25] This might interfere with the ubiquitin-mediated proteolysis of certain substrates, the accumulation of which might lead to neuronal death. Why dopaminergic neurons in the substantia nigra are particularly vulnerable to the loss of *parkin* function remains to be determined.

Many different *parkin* mutations have been found in familial as well as in sporadic cases of different origins. They are reported to be associated with phenotypes including typical early-onset parkinsonism, but also late-onset PD[14,26,27] and phenotypes resembling Dopa-responsive dystonia,[28] hemiparkinsonism-hemiatrophy,[29] forms with cerebellar ataxia,[17,30] pyramidal tract dysfunction,[30] or peripheral neuropathy.[31] However, no clinical correlations have been established so far between a given phenotype and specific mutations in the *parkin* gene. In addition, in some families the disease is associated with heterozygous *parkin* mutations that appear to be transmitted dominantly.[21,26] This suggests that carriers of some single *parkin* mutations might be at risk for developing PD.[32–34] This differs from the pseudodominant inheritance described in rare families in which all patients carry two mutations.[35–37]

The aim of our study was to compare large series of patients with and without *parkin* mutations and to assess the influence of the number and the nature of the mutations on the phenotype of the patients.

Patients and Methods

Patients

Five hundred patients with isolated or familial parkinsonism with ages at onset of up to 55 years (in at least one affected family member for the familial cases) were included as in our previous studies,[14,38,39] even though mutations in the *parkin* gene also have been reported in patients with onset up to 72 years of age.[27] The diagnostic criteria for PD in our study were at least two of the Parkinsonian triad of signs (bradykinesia, rigidity, rest tremor) and at least 30% improvement after L-dopa therapy in familial or isolated cases. Exclusion criteria were the existence of extensor plantar reflexes, ophthalmoplegia, early dementia, or early autonomic failure. A standardized form was used to assess the history of the disease and of the family, the clinical signs and the response to treatment. The response to treatment was calculated only in patients who had United Parkinson's Disease Rating Scale III assessment both "on" and "off" treatment. The percentage of improvement was obtained by comparing the "off" and "on" values. Quality control of clinical information was performed by one clinician.

One hundred and four patients were excluded because clinical information was incomplete. Among the remaining 396 patients, there were 210 cases from 76 families, including 207 compatible with autosomal recessive inheritance and 3 families with pseudodominant inheritance, two of which have been reported already.[36,37] The other 186 had no known family histories of PD in first or second-degree relatives, but in 15 cases there was consanguinity. The families were mostly of European descent (n = 329), including patients from France (n = 165), Italy (n = 73), the Netherlands (n = 26), United Kingdom (n = 26), Germany (n = 20), Portugal (n = 11), Spain (n = 4), and Eastern Europe (n = 4). There were also patients from North Africa (n = 27), South America (n = 15), and North America (n = 2), Asia (n = 9), Russia (n = 6), Middle East (n = 1) and Near East (n = 3), and Turkey (n = 4). Most families were white (91%).

Molecular Analysis

All index cases were screened for exon rearrangements in the *parkin* gene with a semiquantitative multiplex polymerase chain reaction assay, in which several exons of the *parkin* gene were coamplified together in the same reaction. To identify the mutations, we analyzed the polymerase chain reaction products on denaturing polyacrylamide gels on an ABI 377 automated sequencer with the GENESCAN 3.1 and GENOTYPER 1.1.1 softwares. The ratios between the heights of the peaks of each of the exons amplified in a given reaction were calculated and then compared with the ratios for nonrearranged exons in the control sample from a normal subject (for detailed description of this method, see Lücking and Brice[40]). The consequences of the exon rearrangements at the protein level (in-frame or frameshift) were deduced from published exon sequences (DNA Data Bank of Japan, accession no. AB009973). In the patients in whom the assay detected only one or no mutations, the entire coding sequence was analyzed by sequencing as previously described.[41]

To establish phenotype–genotype correlations, we considered all exon rearrangements, nonsense mutations, and small

insertions/deletions resulting in frameshifts to be truncating mutations. Although all of the truncating mutations do not have the same consequences on the protein, they all affect one of the functional domains of the *parkin* protein. All other mutations resulting in single amino acid changes were classified as missense mutations. In addition, patients were considered to carry a single *parkin* mutation only when one unambiguous *parkin* mutation was detected; this excludes all patients with rearrangements of consecutive exons where it cannot be concluded whether there are multiple exon rearrangements on a single allele or two different mutations, one on each allele. Patients with single heterozygous mutations were genotyped for the −258 parkin promoter single nucleotide polymorphism (SNP) according to West and colleagues.[42]

Statistical Analyses

Comparisons were first made between *parkin* carriers and patients without *parkin* mutations. Then, comparisons were made among subgroups of carriers of *parkin* mutations. Because age at onset younger than 40 years usually defines early-onset parkinsonism, we compared patients with age at onset younger than 40 years with those with age at onset 40 years or older. Patients with one or two missense mutations were compared with those carrying two truncating mutations. Patients who carried at least one missense mutation outside of the known functional domains were compared with those carrying two missense mutations within functional domains, such as the UBL and the RING-IBR-RING domains. Finally, patients with only one detected mutation in the *parkin* gene, as defined above, were compared with those with two known mutations. We used the χ^2 test or the Fisher's exact test when appropriate for comparison of proportions and analysis of variance for comparison of means. Forward stepwise multiple logistic regression analysis was performed to sort out the independent variables of items that defined significantly between patients with and without *parkin* mutations (Hoehn and Yahr "off" was not included in this analysis because the available data were unevenly distributed among groups and the sample size was too small in the last step of the analysis). No corrections were applied for multiple testing.

Results

Forty-five of 396 patients are reported for the first time including five isolated cases without *parkin* mutation and 40 cases with family histories including 18 carriers of *parkin* mutations. The remaining patients have been reported previously.[14,34,38,39] The proportion of patients with *parkin* mutations from families with autosomal recessive parkinsonism decreased significantly as a function of age at onset, ranging from 82% before age 20 years to 28% between 46 and 55 years (Fig, $p < 0.01$). This decrease already has been shown in isolated cases in which the difference was even greater.[14,38]

The clinical comparison between *parkin* carriers and noncarriers is shown in Table 1. The groups differed in four respects: (1) mean age at onset in patients with *parkin* mutation (31.4 ± 11.9 years) was earlier (38.1 ± 11.2 years, $p < 0.001$). The range was similar,

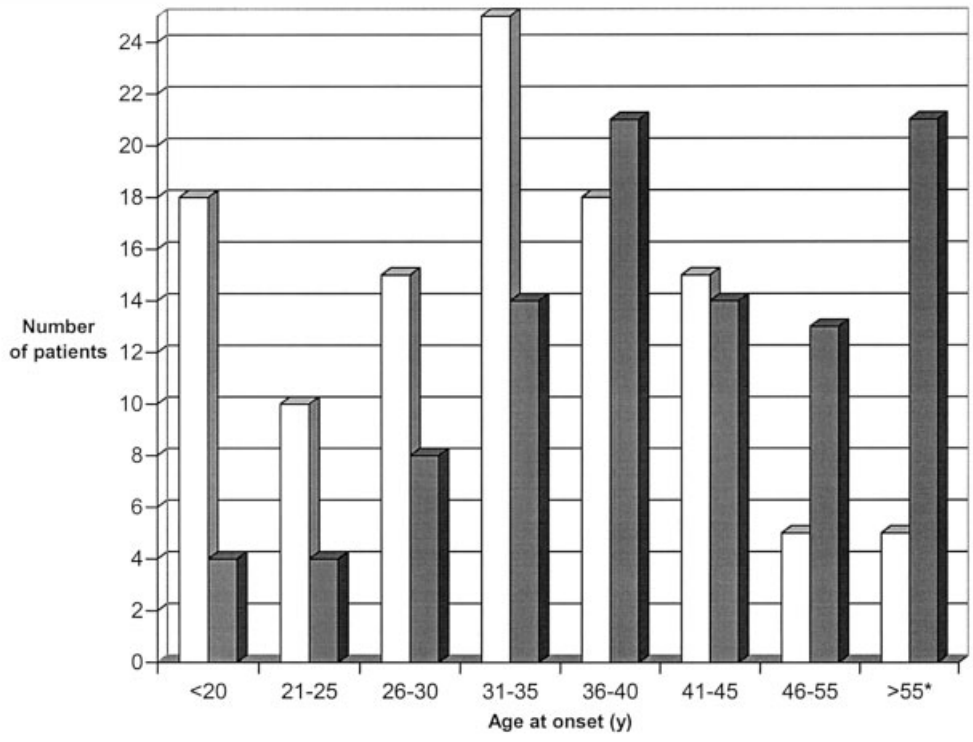

Fig. Frequency of familial parkin cases according to the age at onset. (unfilled bars) *patients with parkin mutations;* (filled bars) *patients without parkin mutations.* (asterisk) *Ages at onset: 56, 58, 68, 68, and 70 years.*

however, in both groups (7–70 vs 12—76 years); (2) dystonia and symmetry at onset and brisk reflexes at examination were more frequent in *parkin* carriers; (3) despite longer mean durations of disease (16.7 ± 10.4 vs 11.8 ± 9.2 years, $p < 0.001$) and L-dopa treatment (11.5 ± 8.3 vs 8.7 ± 7.7 years, $p < 0.005$), the daily dose of L-dopa was lower (497 ± 312 vs 610 ± 405mg, $p < 0.05$), and the percentage of improvement tended to be greater (62 ± 20 vs 55 ± 23%, not significant) in patients with *parkin* mutations; (4) the mean rate of progression, assessed by the Hoehn and Yahr score "off" (without treatment) adjusted for disease duration was lower in the *parkin* group (0.23 ± 0.27, n = 57 vs 0.40 ± 0.40, n = 87, $p = 0.01$) independent of age at onset. The gender of patients had no influence of the phenotype.

The regression analysis showed that age at onset ($p < 0.01$), disease duration ($p < 0.01$), asymmetry at onset ($p < 0.05$), and daily dose of L-dopa ($p < 0.05$) are significant independent variables. Interestingly, dystonia at onset as well as brisk reflexes were no longer independent after forward stepwise multiple logistic regression analysis. However, there was a significant correlation between age at onset and dystonia at onset ($p < 0.01$).

Age at onset was highly variable among *parkin* carriers, ranging from 7 to 70 years. We therefore analyzed the clinical features according to the age at onset (<40 vs ≥40 years). The rate of progression was greater in the group with age at onset after 39 (0.5 ± 0.62, n = 8 vs 019 ± 0.12, n = 49, $p < 0.01$). Presenting signs were also different because dystonia at onset was more frequent in the early-onset than in the late-onset group (30/95 vs 2/22, $p < 0.05$). There were no significant differences according to the age at onset for other clinical features.

To explain the differences in age at onset, we investigated the possible influence of the nature and the localization of the mutation in the *parkin* gene. We first compared patients with at least one missense with those with two truncating mutations (Table 2), reasoning that missense mutations usually have less functional consequences than truncating mutations in autosomal recessive disorders.[43] Patients carrying at least one missense mutation had higher United Parkinson's Disease Rating Scale motor score without treatment than patients carrying two truncating mutations (51.1 ± 20.7 vs 31.3 ± 16.5, $p < 0.01$) after similar disease durations. There were no other differences between the groups. This suggests that missense mutation carriers have a more severe disease than those carrying two truncating mutations.

We then compared phenotypes of patients with missense mutations according to their location with regard to functional domains (Table 3). Patients carrying two missense mutations within the functional domains (RING-IBR-RING and UBL domains) developed symptoms earlier than patients with one mutation outside the functional domains (29.9 ± 13.2 vs 39.5 ± 8.6 years, $p < 0.05$). The latter therefore were significantly older at examination (59.2 ± 9.7 vs 45.5 ± 13.1 years, $p < 0.05$).

Because several observations supported the hypothesis that the presence of a single *parkin* mutation might represent a risk factor for PD,[21,26,32–34] we compared patients with only a single *parkin* mutation to those carrying two mutations (Table 4). Patients with a single mutation showed symptoms later than those with

Table 1. Phenotype of 396 Patients with and without Parkin Mutations

Characteristic	*Parkin* (n = 146)	No. of Patients	Non-*Parkin* (n = 250)	No. of Patients
Gender ratio (M:F)	74:72	146	138:112	250
Age, yr (range)	48.6 ± 12.8 (16–89)	140	49.9 ± 12.6 (17–80)	250
Age at onset, yr[a] (range)	31.4 ± 11.9 (7–70)	146	38.1 ± 11.2 (12–76)	250
Disease duration, yr[a] (range)	16.7 ± 10.4 (1–58)	140	11.8 ± 9.2 (1–54)	250
Signs at onset				
Asymmetry[a]	87%	137	96%	231
Dystonia[a]	27%	117	10%	204
Clinical signs at examination				
Rigidity	92%	143	96%	233
Bradykinesia	94%	144	97%	231
Rest tremor	73%	141	74%	235
Hoehn & Yahr "off"[a] (range)	3.3 ± 1.1 (1.5–5)	57	2.9 ± 1.2 (1–5)	87
Treatment				
Daily dose of L-dopa, mg[a] (range)	497 ± 312 (62–1500)	118	610 ± 405 (50–3,000)	192
Duration of treatment, yr[a] (range)	11.5 ± 8.3 (0.2–40)	115	8.7 ± 7.7 (0.08–55)	182
Other signs				
Brisk reflexes[a]	33%	112	19%	190

[a]Two-tailed $p < 0.05$.

Table 2. Comparison of Parkin Patients with At Least One Missense Mutation and Parkin Patients with Two Truncating Mutations

Characteristic	At Least One Missense Mutation (n = 42)	No. of Patients	Two Truncating Mutations (n = 76)	No. of Patients
Gender ratio (M:F)	23:19	42	38:38	76
Age, yr (range)	49.8 ± 13.6 (16–75)	42	48.6 ± 12.9 (21–89)	70
Age at onset, yr (range)	32.9 ± 12.7 (9–68)	42	30.2 ± 10.0 (7–58)	76
Disease duration, yr (range)	16.9 ± 11.2 (1–42)	42	17.4 ± 10.8 (2–58)	70
Clinical signs at examination				
UPDRS III "off"[a]	51.1 ± 20.7 (25–87)	15	31.3 ± 16.5 (4–62)	23
Treatment				
Daily dose of L-dopa, mg (range)	538 ± 354 (150–1,400)	35	453 ± 286 (62–1,500)	60
Dyskinesia	76%	37	75%	65

[a]Two-tailed $p < 0.01$.

UPDRS = United Parkinson's Disease Rating Scale.

two mutations (37.3 ± 11.3 vs 30.5 ± 10.7 years, $p < 0.05$). L-Dopa–induced fluctuations and dystonia were more frequent in the latter (94% vs 69%, $p < 0.05$, and 70% vs 40%, $p < 0.05$, respectively) and clinical signs were more frequently asymmetrical at onset (100 vs 82%, $p < 0.05$). One of 34 relatives of patients with known *parkin* mutations carrying heterozygous R275W mutations had PD with onset at age 68 years. Among these relatives, there were 16 siblings with a mean age at examination of 41 ± 7.9 years (ranging from 28 to 50 years) and 18 parents with a mean age at examination of 71.1 ± 6.9 years (ranging from 63 to 84 years). None of these individuals were examined at an age younger than the age at onset of their affected relatives. Recently, a genetic association was described between idiopathic PD and the −258 T/G promoter SNP of the parkin gene.[34] The −258G allele, associated with reduced *parkin* expression in in vitro experiments, is also suspected to contribute to early-onset parkinsonism.[34] To determine whether the status of heterozygous *parkin* mutation carrier could be responsible for parkinsonism in conjunction with a *parkin* polymorphism, we genotyped the −258 T/G *parkin* promoter SNP by polymerase chain reaction restriction in the 23 patients with a single *parkin* mutation. The −258 G allele was observed in 45.6% of alleles inherited by heterozygous *parkin* carriers compared with 14% of Northern European controls ($p < 0.001$).[34]

Because some mutations were recurrent, we compared seven groups of patients with the same genotype. No specific clinical pattern could be associated with any of the genotypes. Mean disease durations were significantly different, but not ages at onset or at examination, which were highly variable (Table 5). Patients homozygous for the exon 3 deletion had ages at onset that differed by as much as 34 years. Patients carrying the R275W missense mutation tended to have an earlier age at onset than those from all other groups with two truncating mutations ($p = 0.09$). This suggests that the R275W mutation, located within the RING-IBR-RING domain, could have a more deleterious effect than truncating mutations. Unfortunately, the disease durations in patients with this mutation were very short, precluding the assessment of the severity of disease progression.

Atypical manifestations in *parkin* patients are re-

Table 3. Comparison of Parkin Patients with and without Mutations in Functional Domains (UBL and RING-IBR-RING)

Characteristic	Mutation in Functional Domains (n = 29)	No. of Patients	Mutation outside the Functional Domains (n = 13)	No. of Patients
Gender ratio (M:F)	17:12	29	6:7	13
Age, yr[a] (range)	45.5 ± 13.1 (16–70)	29	59.2 ± 9.7 (44–75)	13
Age at onset, yr[a] (range)	29.9 ± 13.2 (9–68)	29	39.5 ± 8.6 (18–55)	13
Disease duration, yr (range)	15.7 ± 10.5 (2–40)	29	19.7 ± 12.5 (1–42)	13
Clinical signs at examination				
Hoehn and Yahr "on" (range)	2.3 ± 0.7 (1–4)	24	2.6 ± 0.9 (1–4)	12
Treatment				
Daily dose of L-dopa, mg (range)	523 ± 309 (150–1,400)	25	577 ± 466 (150–1,400)	10

[a]Two-tailed $p < 0.05$.

UBL = ubiquitin at the amino terminus; IBR = in between ring.

Table 4. Comparison of Parkin Patients with a Single Mutation and Parkin Patients with Two Mutations

Characteristic	Single (n = 23)	No. of Patients	Two (n = 109)	No. of Patients
Gender ratio (M:F)	10:13	23	55:54	109
Age, yr	51.5 ± 9.2 (31–70)	23	48.3 ± 13.3 (16–89)	103
Age at onset, (yr)[a] (range)	37.3 ± 11.3 (18–68)	23	30.5 ± 10.3 (7–58)	109
Disease duration, yr (range)	14.2 ± 8.2 (2–37)	23	17.2 ± 10.9 (1–58)	103
Signs at onset				
Asymmetry[a]	100%	23	82%	100
Treatment				
Daily dose of L-dopa, mg (range)	461 ± 283 (125–1400)	22	476 ± 310 (62–1500)	84
Dyskinesia	82%	22	74%	92
Fluctuation[a]	94%	17	69%	83
Dystonia[a]	70%	17	40%	70

[a]Two-tailed $p < 0.05$.

ported in Table 6. In nine patients from different families, psychiatric disorders were evident and included psychosis, panic attacks, depression, disturbed sexual behavioral and obsessive-compulsive behaviors. Four patients had abnormal magnetic resonance imaging findings, but no evident pattern could be seen. A single patient had axonal polyneuropathy.

Discussion

We report the first evidence to our knowledge of phenotype–genotype correlations in patients with *parkin* mutations in a large cohort of 396 patients. Our previous studies had shown that *parkin* mutations accounted for 49% (36/73 families) of ARJP and 14% (20/146 patients) of isolated cases with an age at onset younger than 45 years.[14,38] The relative frequencies of *parkin* mutations in ARJP or isolated cases with age at onset at 55 years or younger found in this study are similar to those in our previous report: 61% (54/89 families) and 19% (35/186 patients), respectively. However, in familial cases with ages at onset 20 years or younger, greater than 80% of the patients have *parkin* mutations, whereas only 28% of those between the age of 46 and 55 are *parkin* carriers. This confirms that *parkin* mutations are much more frequent in early-onset patients.

There is no individual sign or symptom that distinguished between *parkin* carriers and noncarriers with early-onset parkinsonism. However, patients with *parkin* mutations tended to have earlier and more symmetrical onset, slower progression of the disease, and greater response to L-dopa despite lower doses. Nevertheless, our *parkin* carriers did not present all of the signs associated with ARJP previously reported: coarse tremor, foot dystonia, and diurnal fluctuations were absent.[44] They have, however, consistently slower disease progression and a marked response to L-dopa, but they do not have more complications from treatment, such as dyskinesias and fluctuations, than patients without *parkin* mutations, as reported in several previous studies.[44–46] The younger the age at onset, the more frequently dystonia is observed at onset. Thus, dystonia does not represent a specific sign of carriers of *parkin* but rather is associated with young onset parkinsonism regardless of the genetic origin. Age at onset among patients carrying *parkin* mutations varies (range, 7–70 years), as does the rate of progression of the disease: the younger the age at onset the slower the disease. In addition, atypical signs also were observed, such as psychiatric manifestations, cerebellar signs, neuropathy, and abnormal findings on brain magnetic resonance imaging, enlarging the spectrum of the disease (see Table 6). Behavioral disorders in *parkin* disease have been mentioned previously, but only in a few reports.[31,47,48] We did not find a homogenous psychiatric pattern in our *parkin* patients, and, because psychiatric manifestations are frequent in the general population and delirium and hypersexuality can be triggered by antiparkinsonism drugs, it cannot yet be concluded whether they are part of the phenotype or if they occurred by chance in several patients. Systematic psychiatric evaluation of *parkin* patients and their relatives will be required to resolve this question. Additional neurological manifestations, such as axonal neuropathy or cerebellar ataxia, were observed in several of our cases. Cerebellar signs,[17,30] pyramidal tract dysfunction[30] and peripheral neuropathy[31] have been reported previously, but not the presence of abnormalities on brain magnetic resonance imaging.

Mutations in the *parkin* gene are polymorphous and often private. To date, 79 different *parkin* mutations have been described: 45 point mutations including 20 truncating, 22 missense, and 3 splice mutations, as well as 34 exon rearrangements including 24 deletions and 10 multiplications.[4,14,15,21,25–27,32–35,37,41,47–58] The large spectrum of *parkin* gene defects, which differ in their predicted consequences on the function of the protein, raises the question of their role in the variabil-

ity of the phenotype. We indeed have found some differences. Despite the fact that the age at onset is similar in patients with at least one missense mutation and those with two truncating mutations, the phenotype is significantly more severe in the former. Although missense mutations usually have less functional consequences than truncating mutations in autosomal recessive disorders,[43] the consequences of several types of mutations is very difficult to predict (eg, missense mutations might have effects on splicing and therefore they can have more drastic consequences at the protein level).[59] Furthermore, the location of the mutation within the gene plays a role in the phenotype because mutations in functional domains resulted in onset of the disease approximately 9 years earlier than mutations in domains not known to be essential for *parkin* function. However, the influence of the nature and position of the mutation on the phenotype appears to be of limited importance, because no evident pattern emerged from comparison of patients with the same genotype. Furthermore, for most genotypes, there was great variability (up to 34 years) in age at onset despite the presence of the same mutations. The number of cases in each of the seven genotype groups was too low for a specific clinical pattern to emerge, but it appears that the mean age at onset of the patients who carry both the R275W mutation and a truncating mutation tended to be earlier than in those with two truncating mutations. This result suggests that the R275W mutation does not result in a simple loss of function as postulated for missense mutations. The R275W mutation might have dominant negative effect, which would account for the greater severity of the phenotype. However, this hypothesis is not supported by the fact that four heterozygous relatives (aged 28–70 years) are not affected by PD. Another explanation is that that R275W might confer a partial loss of function which, in conjunction with an additional, still unknown, predisposing factor is more deleterious than complete ablation of the gene. However, this discussion remains speculative, and the results must be confirmed by analyzing more patients.

Single mutations were detected in several patients[36,37] despite thorough molecular analyses of the gene including the promoter region.[34] Furthermore, families with apparently true autosomal dominant inheritance[21,26] or decreased striatal F-DOPA uptake in asymptomatic carriers of heterozygous *parkin* mutations[60] have been described. This prompted us to compare the phenotype in *parkin* carriers as a function of the number of mutations detected. Patients with a single *parkin* mutation were characterized by later and more asymmetrical onset and more frequent fluctuations and dystonias than patients with two characterized mutations. Their age at onset extends up to 68 years, 10 years later than in our series of patients with two *parkin* mutations. This suggests that the presence of a single *parkin* mutation might represent a risk factor for later onset PD. This result should be interpreted with caution, however, because the size and the complexity of the *parkin* gene may cause some mutations to escape detecting by our current screening techniques.[34]

However, note that among the 34 heterozygous carriers who were relatives of patients with two identified mutations only one was affected with onset at age 68 years. This may be coincidental or may suggest reduced penetrance or a slightly higher risk of developing PD in heterozygous carriers, even though the age at examination was earlier in siblings than parents, and they may develop symptoms in the future. In contrast, all their relatives with two mutations were affected. This observation indicates that being carrier of a single *parkin* mutation is not sufficient to produce the phenotype in all individuals and that other genetic or environmental factors are probably necessary in combination with the mutation. In this regard, note that the frequency of the −258 G allele of the *parkin* promoter SNP was significantly higher in our patients who carry a single heterozygous mutation than in European controls. These findings are consistent with a previous study[34] and suggest that the −258 G allele also may contribute to early-onset parkinsonism, particularly in individuals who also carry a heterozygous *parkin* mutation.

Table 5. Comparison of Parkin Patients with the Same Genotype

Characteristic	ex3del/ex3del (n = 8)	ex3-4del/ex3-4del (n = 4)	ex2del/ex3del (n = 6)
Gender ratio (M:F)	3:5	3:1	3:3
Age, yr (range)	46.9 ± 9.8 (35–59)	52.5 ± 6 (44–58)	49 ± 6.8 (42–59)
Age at onset, yr (range)	35.4 ± 10.6 (21–55)	37 ± 10.6 (23–47)	33.2 ± 10.4 (14–42)
Duration of disease, yr (range)[a]	11.5 ± 5.3 (4–17)	15.5 ± 6.2 (7–21)	15.8 ± 10.6 (3–33)

[a]Significant differences among the groups ($p < 0.05$).

ex = exon, del = deletion.

Table 6. Parkin Patients with Atypical or Additional Features

Patient	First Mutation	Second Mutation	Age at Onset (yr)	Age at Examination (yr)	Atypical Features
FPD-171-3	Exon 6 duplication	c255delA	26	29	Obsessive-compulsive behavior and depression
FPD-192-6	Exon 3 deletion	Exon 7 duplication	27	53	Psychosis
SPD-70	c255delA	c255delA	19	30	Delirium
SPD-92	Exon 3 to 6 deletion	R275W	16	46	Panic attacks
SPD-169	Exon 2 deletion	Exon 3 deletion	29	42	Verbal aggressiveness
BHAM-3	Exon 5 to 6 deletion	Exon 5 to 6 deletion	29	59	Hypersexuality, behavioral changes
BHAM-18	Exon 3, 4, 5 deletion[a]	?	18	53	Hypersexuality and depression
UK-4720	c202-203delAG	c202-203delAG	29	53	Psychosis
SAL-711-4	Exon 3 deletion	Exon 3 deletion	33	50	Depression, anxiety, hysterical episodes, and conversional symptoms. Cerebral MRI: T2 periventricular white matter hyperintensities
SAL-711-5	Exon 3 deletion	Exon 3 deletion	40	55	Cerebral MRI: important dilatation of the lateral ventricles and T2 hyperintensities in the frontal white matter
SAL-711-6	Exon 3 deletion	Exon 3 deletion	28	41	Axonal polyneuropathy
FPD 235-7	Exon 3 to 6 deletion	c255delA	27	55	Presenting sign: resting tremor in both legs that increased markedly when standing and was absent when walking
IT-48-97	Exon 3 to 4 deletion	Exon 3 to 4 deletion	43	58	Orthostatic tremor diagnosed before PD. Cerebral cortical atrophy at MRI, T2 hyperintensities in the upper pons and midbrain.
JMP-28	Exon 3 deletion	Exon 3 deletion	28	34	Cerebellar signs (nystagmus, slight left limb dysmetria). Cerebral MRI: bilateral sickle-shaped abnormal signals (decreased in T_1-, increased in T_2-weighted images) in the cerebellum.

[a]The phase of transmission was not known for this patient, and we could not determine if the deletion was present on one or both alleles.
MRI = magnetic resonance imaging; PD = Parkinson's disease.

Conclusion

There were significant clinical differences between patients with and without *parkin* mutations: *parkin* carriers tended to have earlier onset, more dystonia at onset, and brisk reflexes. They also took lower doses of L-dopa despite longer durations but did not have more

Table 5. (Continued)

c255delA/c255delA (n = 4)	c202-203delA/c202-203delA (n = 2)	ex3-4del/c255delA (n = 2)	ex3del/R275W (n = 3)
3:1	1:1	0:2	1:2
48.8 ± 16 (30–62)	48 ± 7.1 (43–53)	33 ± 17 (21–45)	25.3 ± 8.3 (16–32)
28.5 ± 13.1 (17–45)	26 ± 4.2 (23–29)	26.5 ± 17.7 (14–39)	21.3 ± 8.1 (12–27)
20.3 ± 7.9 (11–29)	22 ± 2.8 (20–24)	6.5 ± 0.7 (6–7)	4 ± 1 (3–5)

complications from treatment than noncarriers. There were no clinical signs that permit identification of a patient with *parkin* mutations, but *parkin* carriers represented a specific subgroup of early-onset parkinsonism. The phenotype differs with the genotype: patients with missense mutations had a more severe disease than those with truncating mutations, suggesting that missense mutations result in more than a loss of function. A dominant negative effect, as suggested for the R275W mutation, would produce a more severe clinical course. Not unexpectedly, missense mutations in functional domains resulted in an earlier onset than mutations in other regions of the protein. Finally, patients with two detected mutations had earlier onset than those with only one detected mutation, although the interpretation of this result is subject to caution. It is not yet clear whether the second mutation was not detected by the techniques used or whether the presence of a single mutation constitutes a real risk factor for PD.

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Appendix

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