Heterozygous *parkin* Point Mutations Are as Common in Control Subjects as in Parkinson's Patients

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Objective: Homozygous or compound heterozygous *parkin* mutations cause juvenile parkinsonism. Heterozygous *parkin* mutations are also found in patients with typical Parkinson's disease (PD), but it is unclear whether a single "mutation" in a patient is related to disease or is coincidental, because the mutation frequency in control subjects is unknown. We present a comprehensive sequence analysis of *parkin* in control subjects.
Methods: A total of 302 patients and 301 control subjects were sequenced, and findings were replicated in 1,260 additional patients and 1,657 control subjects.
Results: Thirty-four variants were detected, of which 21 were novel; 12 were polymorphisms and 22 were rare variants. Patients and control subjects did not differ in the frequency, type, or functional location of the variants. Even P437L, a common mutation thought to be pathogenic, was present in unaffected control subjects.
Interpretation: *parkin* point mutations are not exclusive to PD. The mere presence of a single point mutation in a patient, in the absence of a second mutation, should not be taken as a cause of disease unless corroborated by family data and functional studies. This study does not support the notion that heterozygous *parkin* sequence variants (mutations or polymorphisms) are risk factors for PD. Whether heterozygous dosage anomalies are associated with PD remains to be determined.

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Parkinson's disease (PD) is a severe and progressive neurodegenerative disorder characterized clinically by tremor at rest, bradykinesia, rigidity, and postural instability.[1] PD is the second most common neurodegenerative disorder after Alzheimer's disease. Several causative genes have been identified for PD,[2] including *parkin (PARK2)* on chromosome 6q25-27 (OMIM *602544).[3] *parkin* mutations, which can range from point mutations to large exon deletions and multiplications,[4] cause autosomal recessive juvenile parkinsonism (AR-JP).[3] Approximately 50% of AR-JP, defined as onset at or before age 30, is due to mutation of the *parkin* gene.[5] In AR-JP, mutations are either homozygous or heterozygous for two different mutations (compound heterozygous), suggesting that loss of Parkin protein function results in disease. The Parkin protein functions as an E3 ubiquitin-

protein ligase, in support of the theory that the ubiquitin-proteasome protein degradation pathway may be important in PD pathogenesis.[6]

Since its discovery as a cause of AR-JP, the *parkin* gene has been analyzed in patients with typical PD, and mutations have been found in virtually every disease subtype including late-onset, dominant, and sporadic PD.[4,5,7–23] In most of these cases, patients were heterozygous. The mutations were individually rare, and when screened in control subjects, they were not found. These observations led to the suggestion that the presence of a single *parkin* mutation may be sufficient to predispose to PD. However, most studies performed detailed analysis of *parkin* in patients and only screened the control subjects for the mutations found in patients. The reciprocal experiment (ie, analysis of *parkin* in con-

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Table 1. Subject Characteristics

Characteristics	Tier 1: Sequence Analysis		Tier 2: Replication Study	
	PD Patients	Control Subjects	PD Patients	Control Subjects
N	302	301	1,260	1,657
Mean age at blood draw ± SD (range), yr	67.8 ± 10.6 (36–87)	67.0 ± 11.7 (32–88)	67.9 ± 10.6 (30–93)	67.3 ± 20.3 (20–109)
Race (% white)	96.7	97.0	94.4	93.7
Sex (% male)	61.9	46.8	68.9	35.1
Mean age at onset ± SD (range), yr	56.7 ± 13 (7–82)	—	58.2 ± 11.9 (15–90)	—
Positive family history[a]	28.8%	—	22.4%	—

[a]Patients with a first- or second-degree relative with Parkinson's disease (PD).

SD = standard deviation.

trol subjects followed by mutation screening in patients) has not been performed. A comprehensive analysis of *parkin* in control subjects is necessary to establish whether the presence of single mutations in patients is truly related to disease or is coincidental. The purpose of this study was to analyze *parkin* sequence in patients and control subjects using identical comprehensive methodology, to determine whether *parkin* point mutations are found exclusively in patients, and to identify novel mutations that may be protective against PD and present only in control subjects.

Subjects and Methods

Study Participants

The study was originally designed as a two-tiered study intended to use the patient population from one movement disorder clinic in Portland, OR. The Oregon clinic patient population of 609 unrelated PD patients (who had already been enrolled in the study) was divided into 2 groups with equal proportions of men and women, familial and sporadic PD, and early- and late-onset PD. The first half received comprehensive sequence analysis (tier 1); the second half was intended for replication of significant findings by screening an independent sample (tier 2). However, the findings in tier 1 were smaller in magnitude than anticipated, and the original tier 2 sample size was insufficient for replication. We therefore expanded tier 2 to include patients from the NeuroGenetics Research Consortium (NGRC), hence increasing the tier 2 sample size from about 300 patients and 300 control subjects to 1,260 patients and 1,657 control subjects. The NGRC includes movement disorder clinics in academic institutions and their affiliated community-based clinics in Portland, OR; Seattle, WA; Albany, NY; and Atlanta, GA. This study used cases and control subjects from Oregon, Washington, and New York; Georgia has recently joined NGRC. NGRC clinics use standardized study protocols. Patients must have a diagnosis of PD by a movement disorder specialist according to the modified UK Parkinson's Disease Brain Bank criteria (family history is not used as an exclusion criterion).[1] Patients are enrolled sequentially; approximately 85% consent and participate. Control subjects, recruited at each NGRC site, consisted of spouses, friends, and coworkers of patients participating in

research; community healthy volunteers from religious and civil organizations; and employees of the institutions where research was being conducted. Community healthy volunteers were recruited via advertisement and outreach programs. The population represented diverse socioeconomic and education levels. A subset of 508 control subjects was examined by neurologists, determined to be free of neurological disorders, and entered into a longitudinal study of healthy brain aging. The remaining control subjects were screened using a standardized, self-administered questionnaire and personal interview at the time of informed consent and blood draw, and by self-report were free of neurodegenerative disease. All cases and control subjects were unrelated genetically as far as it could be determined. Subject characteristics are summarized in Table 1. Tier 1 included 302 PD patients, who represented half of the clinic population in Oregon, and 301 control subjects, balanced with patients for age, race/ethnicity, geography, and sex. The remainder of cases and control subjects were used in tier 2 for replication. Ethnicity and race categories were defined according to National Institutes of Health guidelines and presented to subjects for self-assignment. This study was approved by the institutional review boards of all participating institutions.

Procedures

Genomic DNA was extracted from peripheral blood using standard methods. For tier 1, all 12 exons of *parkin* and surrounding intronic sequence were polymerase chain reaction–amplified and sequenced on both strands using BigDye chemistry (Applied Biosystems, Foster City, CA) on an ABI 377 DNA Sequencer or an ABI 3100 Genetic Analyzer. Dosage analysis for detection of heterozygous copy number anomalies was performed on 39 early-onset PD cases[16] and on the 40 subjects who were found in tier 1 to have sequence variants. Among the 39 early-onset cases, 2 were compound heterozygous and 3 were heterozygous for deletions/duplications (reported previously).[16] For the 40 subjects identified in this study, *parkin* exon copy number was determined using semiquantitative polymerase chain reaction, similar to previously published protocols.[5] In brief, each exon was amplified simultaneously with an internal control of comparable size (from *APOE* or *MAPT*). Peak height and area was examined using GeneScan software (version 3.7) on an ABI 3100 Genetic Analyzer. Copy number

was estimated by comparing the ratio of the exon peak to the internal control peak. The results from the semi-quantitative method were, at times, inconsistent; therefore, we re-analyzed all 40 cases using the multiplex ligation-dependent probe amplification (MLPA) method. We used the SALSA MLPA P052 Parkinson 2 probemix kit (MRC-Holland) and GeneMarker Software (version 1.51, SoftGenetics, LLC), according to the manufacturer's instructions. This article addresses the prevalence of point mutations, which are seen frequently in late-onset PD and are suspected to be pathogenic or a risk factor for typical PD. Dosage analysis was performed to distinguish true heterozygotes from those who may have compound sequence/dosage mutations. For tier 2, the P437L mutation (c.1411 c>t) and IVS8+48 c>t (the only two variants whose frequencies in tier 1 were different in cases and control subjects) were genotyped in an additional 1,260 cases and 1,657 control subjects. P437L was genotyped using a restriction fragment length polymorphism (RFLP) assay and analyzed on an ABI 3100 Genetic Analyzer. In brief, a 354-base pair region surrounding c.1411 c>t was polymerase chain reaction–amplified and digested with *Pst*I, which specifically cleaves only the mutant allele. IVS8+48 c>t was genotyped using a custom TaqMan SNP Assay and analyzed on an ABI PRISM 7900HT sequence detection system (Applied Biosystems). Primer and probe sequences are available on request.

Statistical and Bioinformatics Analyses
Allele frequencies were estimated by allele counting. Standard statistical methods were used to test *parkin* allele frequencies in patients versus control subjects (χ^2 test and Fisher's exact test), to compute odds ratios and corresponding 95% confidence intervals (2 × 2 table), and to compare the mean age at onset of patients with mutations to the mean age of control subjects with mutations (*t* test). The program SIFT was used to predict the effect of each missense mutation on the Parkin protein.[24]

Results

Tier 1: Sequence Analysis in 302 Patients and 301 Control Subjects
We found 34 different *parkin* sequence variations in the 1,206 chromosomes analyzed. Twelve of the 34 variants were common polymorphisms (minor allele frequency ≥ 0.01) found in both patients and control subjects, and 22 were rare variants (minor allele frequency < 0.01), including 5 missense mutations. Twenty PD patients and 20 control subjects carried rare *parkin* variants (Table 2). Of the five missense mutations, only one (R402C) was found in patients only, two were found in controls only (T173M and V244I), and two were found in both patients and control subjects (A82E and P437L). Although unique to patients in this study, R402C has been previously reported to occur in unaffected control subjects.[15,16,22] One control subject carried two rare variants (Subject C3: P37P and IVS2+248 c>t), and one patient carried three rare variants (Subject P9: IVS7−60 g>c, IVS7−58 a>g,

IVS8+93 c>t), although we could not determine whether the variants were carried on the same or different chromosomes. With the possible exception of these two individuals, all carriers appeared to be heterozygous for the sequence variants. All 40 cases and control subjects were screened for copy number anomalies; none had a mutation. The average age at onset did not differ between patients who had a rare *parkin* variant (57.8 ± 16.4 years, range, 14−82 years and those who did not (56.9 ± 12.4, range, 18−81 years). The average age of control subjects with rare variants was significantly greater than the age at onset of patients with rare variants (68.1 ± 12.7 vs 57.8 ± 16.4 years, $p = 0.03$.

Of the 22 rare variants seen in this study, only 4 have been reported previously; 18 were novel (Table 3). Ten of the 22 variants were found in control subjects only, 8 were in patients only, and 4 were seen in both groups. None of the 8 variants that were unique to patients was in a functional Parkin domain, whereas 2 of the 4 found in both patients and control subjects (S255S and P437L) and 1 of the 10 found only in control subjects (V244I) were in the RING domains, which are thought to be important for Parkin substrate interactions (Fig). All five missense mutations (R402C, T173M, V244I, A82E, P437L) were in residues that are evolutionarily conserved in mouse and rat Parkin homologues. Four of the five missense mutations (R402C, T173M, A82E, P437L) result in altered amino acid charge or size, which could affect protein structure and function. The program SIFT predicted that the alterations that are caused by A82E, V244I, and P437L might be tolerated, whereas R402C and T173M would not be tolerated.

There was no significant difference between patients and control subjects in the frequency of any variant individually or combined (Table 4). The odds ratio for all rare variants combined was effectively one (odds ratio, 1.00; 95% confidence interval, 0.52–1.89). Considering only coding variants, the odds ratio increased to a statistically insignificant 1.47 (95% confidence interval, 0.67–3.23). However, further narrowing the coding variants to include only missense mutations did not increase the odds ratio; on the contrary, it reduced it to 1.38 (95% confidence interval, 0.55–3.49). The odds ratio for coding variants decreased from 1.47 to 1.11 when P437L was excluded. The only notable finding in tier 1 was that of P437L, whose allele frequency reached 1% in patients compared with 0.3% in control subjects. The odds ratio for P437L was 3.02, but it was not significant ($p = 0.16$) and was driven primarily by an insignificant increased frequency of the mutation in female patients. Although the tier 1 results were statistically insignificant, P437L was of interest because it is one of the most commonly observed rare *parkin* variants and is thought to be a mutation asso-

Table 2. Characteristics of Subjects with Rare parkin Sequence Variants

Subject No.	*parkin* Variant	Sex	Age, yr	FH[a]
Control Subjects				
C1	−3 g>a	F	66	—
C2	c.49 c>t	F	55	Negative
C3	P37P, IVS2+248 c>t	F	72	—
C4	A82E	F	69	Negative
C5	A82E	F	78	—
C6	A82E	F	65	Negative
C7	A82E	M	79	Negative
C8	IVS2+35 g>a	M	77	Negative
C9	IVS2+35 g>a	F	82	—
C10	IVS2+62 g>a	F	79	Negative
C11	IVS2+62 g>a	M	49	—
C12	IVS2+62 g>a	M	81	—
C13	T173M	F	60	—
C14	T177T	F	61	Negative
C15	V244I	M	32	—
C16	S255S	F	61	Negative
C17	IVS7−45 c>t	F	82	Negative
C18	IVS8+101 t>c	M	73	—
C19	P437L	M	73	Negative
C20	P437L	F	68	Negative
PD Patients				
P1	c.58 c>a	F	55	Negative
P2	A82E	F	61	MD (2)
P3	A82E	M	64	Negative
P4	IVS2+62 g>a	M	72	Negative
P5	L174L	F	51	Negative
P6	L174L	F	63	MD (1)
P7	L174L	F	72	PD (1)
P8	S255S	M	60	MD (1)
P9	IVS7−60 g>c, IVS7−58 a>g, IVS8+93 c>t	M	64	PD (2), MD (2)
P10	L307L	F	71	MD (4)
P11	IVS8+61 g>a	F	56	Negative
P12	R402C	F	39	MD (1)
P13	R402C	M	53	MD (1)
P14	R402C	M	60	PD (1)
P15	P437L	F	72	PD (1)
P16	P437L	M	75	Negative
P17	P437L	F	36	Negative
P18	P437L	F	82	PD (1)
P19	P437L	F	14	Negative
P20	P437L	M	36	PD (2), MD (2)

A total of 302 Parkinson's disease (PD) patients and 301 control subjects were sequenced for all 12 parkin exons and intron/exon boundaries. The table shows the subjects who were found to have a rare sequence variant (minor allele frequency < 0.01). Subject C18 was African American, Subject C15 was Native American, and the remaining subjects were white. Patients P12, P17, and P19 have been reported previously.[16]

[a]Family history (FH) of PD or other movement disorders (MD) was considered positive if one or more first- or second-degree relatives were affected. Negative indicates no PD or MD in first- or second-degree relatives. Dash indicates unknown family history. Number in parentheses is number of affected relatives.

ciated with PD.[9–12,14,16] P437L was therefore followed up in tier 2.

Table 5 shows the more common sequence variants (polymorphisms, minor allele frequency ≥ 0.01) that were detected in tier 1. Each polymorphism was detected in both cases and control subjects and in similar frequencies overall, and when analyzed stratified by age at onset, sex, or family history. No significance difference was detected, with the possible exception of a reduced

frequency of IVS8+48 c>t in patients (uncorrected $p = 0.02$). This polymorphism was genotyped in tier 2.

Tier 2: Replication
An independent sample, consisting of 1,260 patients and 1,657 control subjects, was tested for the P437L mutation and IVS8+48 c>t. The increased frequency of P437L in PD seen in tier 1 was not confirmed (see Table 4, Tier 2); the reduced frequency of IVS8+48

Table 3. Frequency of Rare parkin *Variants in Cases and Control Subjects*

	N Alleles/N Chromosomes (frequency)	
Variant	Parkinson's Disease Patients	Control Subjects
Tier 1: Sequence Analysis		
Found in control subjects only		
−3 g>a[a]	0/508 (0)	1/602 (0.002)
c.49 c>t[a]	0/508 (0)	1/602 (0.002)
P37P[a]	0/604 (0)	1/602 (0.002)
IVS2+35 g>a	0/180 (0)	2/236 (0.008)
IVS2+248 c>t[a]	0/180 (0)	1/236 (0.004)
T173M[a]	0/598 (0)	1/602 (0.002)
T177T[a]	0/604 (0)	1/602 (0.002)
V244I[a]	0/604 (0)	1/602 (0.002)
IVS7−45 c>t[a]	0/604 (0)	1/602 (0.002)
IVS8+101 t>c[a]	0/508 (0)	1/602 (0.002)
Found in patients only		
c.58 c>a[a]	1/508 (0.002)	0/602 (0)
L174L[a]	3/604 (0.005)	0/602 (0)
IVS7−60 g>c[a]	1/604 (0.002)	0/602 (0)
IVS7−58 a>g[a]	1/604 (0.002)	0/602 (0)
L307L[a]	1/604 (0.002)	0/602 (0)
IVS8+61 g>a[a]	1/604 (0.002)	0/602 (0)
IVS8+93 c>t[a]	1/508 (0.002)	0/602 (0)
R402C	3/604 (0.005)	0/602 (0)
Found in control subjects and patients		
IVS2+62 g>a[a]	1/180 (0.006)	3/236 (0.013)
A82E	2/604 (0.003)	4/602 (0.007)
S255S[a]	1/604 (0.002)	1/602 (0.002)
P437L	6/604 (0.010)	2/600 (0.003)
Tier 2: Replication Study		
P437L	8/2520 (0.003)	9/3094 (0.003)

[a]Novel mutations.

c>t in PD also was not confirmed (see Table 5, Tier 2). P437L allele frequency in tier 2 was 0.003 in patients and 0.003 in control subjects. Subanalysis by sex showed a nonsignificant elevated frequency of P437L in female PD patients. Overall, the average age of control subjects with P437L was greater than the average
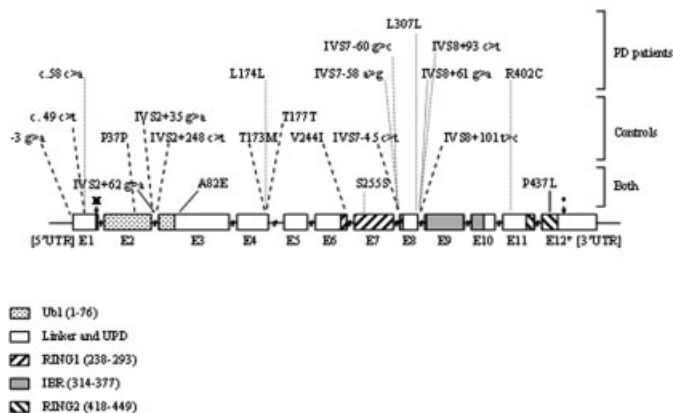

Fig. Nucleotides are numbered according to NCBI accession NT_007422, where the ATG initiation codon begins at nucleotide position c.102 (depicted above as M; *the termination codon is labeled with an* asterisk). parkin *contains large introns that are not drawn to scale (depicted as* double slash). *Listed below exon (E) structure (depicted as* rectangles) *are the Parkin protein domains (numbered as in Shimura and colleagues[6]): Ubl = ubiquitin-like domain, linker domain; UPD = unique parkin domain; RING1 = really interesting new gene domain 1; IBR = in-between RING domain; and RING2.* +Exon 12 is larger than ap-pears here. PD = Parkinson's disease; UTR = untranslated region.

Table 4. Carrier Frequency in Patients and Control Subjects, Odds Ratios, and 95% Confidence Intervals

| | Carrier Frequency | | |
	Patients (95% CI)	Control Subjects (95% CI)	OR (95% CI)
Variant			
Tier 1: Sequence Analysis			
All variants	0.07 (0.04–0.10)	0.07 (0.04–0.10)	1.00 (0.52–1.89)
Coding variants	0.05 (0.03–0.08)	0.04 (0.01–0.06)	1.47 (0.67–3.23)
Missense mutations	0.04 (0.01–0.06)	0.03 (0.01–0.05)	1.38 (0.55–3.49)
P437L	0.020 (0–0.04)	0.007 (0–0.02)	3.02 (0.60–15.09)
All coding variants except P437L	0.03 (0.01–0.05)	0.03 (0.01–0.05)	1.11 (0.44–2.77)
Tier 2: Replication Study			
P437L	0.006 (0.002–0.011)	0.006 (0.002–0.010)	1.09 (0.42–2.84)

None of the results was significant.

age at onset of patients with P437L (61.3 ± 20.4, range, 26–86 vs 53.9 ± 19.0, range, 14–82 years).

Discussion

This study has established that rare sequence variants in *parkin,* including mutations that alter amino acid sequence in functional protein domains, are not found exclusively in PD patients. In most prior studies, patients were subjected to thorough sequence analyses, but control subjects were screened only for mutations that had been detected in patients. Here, we demonstrate that when equally rigorous sequencing analyses are applied to patients and control subjects, rare variants are found in control subjects and patients with similar frequencies. Most of the rare variants that were found in patients were absent in control subjects, and most of the rare variants that were found in control subjects were absent in patients. Overall, there was no difference between patients and control subjects in the frequency (individually or combined), types (coding vs noncoding), or location (functional domains) of *parkin* sequence variations, nor was there a relation with the nature (charge or size) or evolutionary conservation of the affected amino acid residue.

There was no significant difference between patients and control subjects in the individual or overall frequency of sequence variants (including point mutations, rare variants, and polymorphisms). The only promising result from the sequence analysis was the slightly higher frequency of P437L in patients, primar-

Table 5. Frequency of parkin Polymorphisms in Cases and Control Subjects

| | N Alleles/N Chromosomes (frequency) | | |
Polymorphism	Parkinson's Disease Patients	Control Subjects	p^a
Tier 1: Sequence Analysis[a]			
IVS2+25 t>c	127/604 (0.210)	134/602 (0.223)	ns
IVS2−18 t>a	6/508 (0.012)	6/602 (0.010)	ns
IVS3−82 c>a[b]	1/74 (0.014)	2/90 (0.022)	ns
IVS3−20 c>t	50/602 (0.083)	47/602 (0.078)	ns
S167N	13/604 (0.022)	6/602 (0.010)	ns
IVS4−77 c>t[b]	34/508 (0.067)	45/602 (0.075)	ns
IVS7−68 g>c	210/556 (0.378)	213/592 (0.360)	ns
IVS7−35 a>g	255/604 (0.422)	256/602 (0.425)	ns
IVS8+48 c>t	123/602 (0.204)	158/600 (0.263)	0.02
V380L	98/604 (0.162)	94/602 (0.156)	ns
D394N	24/604 (0.040)	23/602 (0.038)	ns
3′untranslated region repeat[b]			
AC repeat[b]	8/508 (0.016)	9/602 (0.015)	ns
GC repeat[b]	8/508 (0.016)	10/602 (0.017)	ns
AC repeat[b]	0/508 (0.000)	3/602 (0.005)	ns
Tier 2: Replication Study			
IVS8+48 c>t	557/2492 (0.224)	715/3272 (0.219)	0.6

[a]Significance level was set at $p < 0.05$, uncorrected, two-tailed. Subanalysis by sex, age at onset (early-onset: ≤50 years; late-onset: >50 years), or family history (familial, sporadic) did not yield any significant differences.

[b]Novel polymorphisms.
ns = nonsignificant.

ily in women, which although insignificant, was noteworthy because P437L has been reported in PD before, has been shown to segregate with disease in some families, and has been implicated in predisposition to PD.[9–12,14,16] However, when followed up in a large sample size with sufficient analytic power (>90%), the putative association was not confirmed. The frequency of P437L in patients and control subjects was identical. It is possible that one or more of the other rare variants is associated with increased risk or protection against PD, and that given sufficiently large sample sizes, they may be detectable. However, even if such associations exist, the attributable risk would be negligible due to rarity of the variant. None of these variants could explain a significant portion of PD risk. Considering the relatively low public health impact, large association studies for extremely rare alleles are difficult to justify.

One of the aims of this study was to assess whether certain variants in *parkin* may be protective against PD. We anticipated that a comprehensive sequence analysis of control subjects would show such alleles. However, we found no variant that was significantly more frequent in control subjects than in patients. A third aim was to test the possible association of *parkin* polymorphisms with PD risk. Only one polymorphism showed a different frequency in cases versus control subjects in the initial sample of 603 subjects sequenced, but when tested in an independent sample of 2917 subjects, the association was not confirmed. In conclusion, we found no evidence for association of heterozygous *parkin* sequence variants, mutations or polymorphisms, with risk or protection against typical nonjuvenile PD.

There is no question that *parkin*-associated juvenile parkinsonism is recessive; that is, both alleles are mutant, but whether a single mutation (heterozygous) can cause or increase the risk for PD (ie, fully or partially dominant) remains an issue of debate. The question of mode of inheritance has implications for functional studies and creation of animal models because hypotheses are built on pathogenicity of haploinsufficiency. In the clinical setting, knowing the mode of inheritance is necessary for proper interpretation of diagnostic gene tests and family counseling. There have been many attempts to address these issues, which all converge on the question of mode of inheritance. Cosegregation of *parkin* mutations with disease has been convincing in families with AR-JP, in which mutations affecting both gene copies correlate tightly with juvenile-onset disease, but it is less convincing in kindreds with late-onset and dominant inheritance because some affected individuals lack mutations and some mutation carriers remain unaffected to advanced ages. On one hand, this suggests a single *parkin* mutation is sufficient to cause disease (dominant); on the other hand, cosegregation of mutations with disease is weak. In an earlier study, we reasoned that if *parkin* heterozygosity was associated with late-onset PD, and

homozygosity and compound heterozygosity were associated with early-onset parkinsonism, one would expect to see families that appear autosomal dominant but exhibit intergenerational differences in age at onset resembling anticipation. Although such families exist, they do not carry *parkin* mutations.[25] Chien and colleagues[26] recently reported a large pedigree with 225 individuals, 15 of whom had PD. They demonstrated complete segregation of a homozygous *parkin* point mutation with disease, and the absence of PD in heterozygous carriers, which supports a genuinely recessive mode of inheritance. They concluded that *parkin* haploinsufficiency is not a relevant risk factor for early- or late-onset PD. A population study by Lincoln and colleagues[19] also found that the frequency of *parkin* mutations, some of which were previously reported as pathogenic in the homozygous or compound heterozygous state, was similar in cases and control subjects. This study supports Chien and colleagues'[26] and Lincoln and colleagues'[19] findings and provides additional evidence that heterozygous *parkin* point mutations are not associated with PD. Positron emission tomography studies suggest *parkin* heterozygotes, although asymptomatic, may exhibit nigrostriatal dysfunction.[27] However, whether such modest alterations in function are tolerated or lead to clinical disease is unknown and again converges on the question of mode of inheritance and penetrance of clinical disease. In sum, the collective data suggest that heterozygosity for *parkin* point mutations may result in somewhat compromised protein function, but does not lead to clinical disease.

Clinical *parkin* gene testing has been available commercially for several years. The utility of this test has been controversial because the clinical interpretation of heterozygous findings, which are the most common outcome, have been unclear. Some of the variants reported here, P437L specifically, are probably pathogenic when they occur in the homozygous or compound heterozygous state (recessive). The unanswered question was whether they cause disease in the heterozygous state (dominant). Should a PD patient found to have a single mutation be assured that the cause of his or her disease is genetic and hereditary? Should *parkin* AR-JP patients be told that all of their children will be at increased risk because they are obligate heterozygotes? This finding that point mutations occur as frequently in control subjects as in patients argues against heterozygosity being associated with PD and supports a recessive mode of inheritance. The frequency of heterozygous carriers of rare sequence variants in control subjects is as would be expected for a recessive disorder. This study demonstrates that the mere presence of a point mutation in an individual should not be taken as the cause of disease unless a second mutation is also identified, nor should it be assumed to be a risk factor for PD unless case–control

studies establish an association. It is crucial that both sequencing and dosage analysis are performed for diagnostic gene testing and for genotype–phenotype correlation studies. In cases where an individual is found to be heterozygous, carrying only one point mutation, the appropriate clinical interpretation is "unknown significance," because although a causal relation should not be assumed, it cannot be ruled out either, because the individual might have an undetected second mutation embedded in the opposite chromosome.

Notably, this study addressed only the sequence variations in *parkin,* and not gene dosage alterations. Deletions and duplications that involve *parkin* exons are presumed to be pathogenic because they likely abolish Parkin function. However, it is not known whether a heterozygous gene dosage anomaly resulting in partial loss of function is sufficient to increase PD risk. In summary, this study rules out the notion that rare *parkin* sequence variants occur predominantly in PD patients. Whether heterozygous dosage anomalies are associated with PD remains to be determined.

References

1. Hughes A, Daniel S, Kilford L, Lees A. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181–184.
2. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of parkinson's disease. Annu Rev Neurosci 2005;28:57–87.
3. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–608.
4. Hedrich K, Eskelson C, Wilmot B, et al. Distribution, type and origin of *Parkin* mutations: review and case studies. Mov Disord 2004;19:1146–1157.
5. Lucking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. N Engl J Med 2000;342:1560–1567.
6. Shimura H, Hattori N, Kubo S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000;25:302–305.
7. Abbas N, Lucking C, Ricard S, et al. 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 1999;8:567–574.
8. Lucking C-B, Chesneau V, Lohmann E, et al. Coding polymorphisms in the Parkin gene and susceptibility to Parkinson disease. Arch Neurol 2003;60:1253–1256.
9. Hedrich K, Marder K, Harris J, et al. Evaluation of 50 probands with early-onset Parkinson's disease for *Parkin* mutations. Neurology 2002;58:1239–1246.
10. Rawal N, Periquet M, Lohmann E, et al. New parkin mutations and atypical phenotypes in families with autosomal recessive parkinsonism. Neurology 2003;60:1378–1381.
11. Nichols WC, Pankratz N, Uniacke SK, et al. Linkage stratification and mutation analysis at the parkin locus identifies mutation positive Parkinson's disease families. J Med Genet 2002;39:489–492.
12. Foroud T, Uniacke SK, Liu L, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. Neurology 2003;60:796–801.
13. Kann M, Jacobs H, Mohrmann K, et al. Role of Parkin mutations in 111 community-based patients with early-onset parkinsonism. Ann Neurol 2002;51:621–625.
14. Oliveira SA, Scott WK, Martin ER, et al. Parkin mutations and susceptibility alleles in late-onset Parkinson's disease. Ann Neurol 2003;53:624–629.
15. Bertoli-Avella AM, Giroud-Benitez JL, Akyol A, et al. Novel parkin mutations detected in patients with early-onset Parkinson's disease. Mov Disord 2005;20:424–431.
16. Poorkaj P, Nutt JG, James D, et al. parkin mutation analysis in clinic patients with early-onset Parkinson's disease. Am J Med Genet A 2004;129A:44–50.
17. Wiley J, Lynch T, Lincoln S, et al. Parkinson's disease in Ireland: clinical presentation and genetic heterogeneity in patients with parkin mutations. Mov Disord 2004;19:677–681.
18. Hedrich K, Kann M, Lanthaler AJ, et al. The importance of gene dosage studies: mutational analysis of the parkin gene in early-onset parkinsonism. Hum Mol Genet 2001;10:1649–1656.
19. Lincoln S, Maraganore D, Lesnick T, et al. Parkin variants in North American Parkinson's disease: cases and controls. Mov Disord 2003;18:1306–1311.
20. Periquet M, Latouche M, Lohmann E, et al. Parkin mutations are frequent in patients with isolated early-onset parkinsonism. Brain 2003;126:1271–1278.
21. West A, Periquet M, Lincoln S, et al. Complex relationship between Parkin mutations and Parkinson disease. Am J Med Genet 2002;114:584–591.
22. Schlitter AM, Kurz M, Larsen JP, et al. Parkin gene variations in late-onset Parkinson's disease: comparison between Norwegian and German cohorts. Acta Neurol Scand 2006;113:9–13.
23. Sinha R, Racette B, Perlmutter JS, Parsian A. Prevalence of parkin gene mutations and variations in idiopathic Parkinson's disease. Parkinsonism Relat Disord 2005;11:341–347.
24. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res 2001;11:863–874.
25. Poorkaj P, Moses L, Montimurro JS, et al. Parkin mutation dosage and the phenomenon of anticipation: a molecular genetic study of familial parkinsonism. BMC Neurol 2005;5:4.
26. Chien HF, Rohe CF, Costa MD, et al. Early-onset Parkinson's disease caused by a novel parkin mutation in a genetic isolate from north-eastern Brazil. Neurogenetics 2005:1–7.
27. Khan NL, Scherfler C, Graham E, et al. Dopaminergic dysfunction in unrelated, asymptomatic carriers of a single parkin mutation. Neurology 2005;64:134–136.