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Risk of Parkinson's Disease in Carriers of *Parkin* mutations: Estimation Using the Kin-Cohort Method

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Abstract

Objective—To estimate the risk of Parkinson's disease in individuals with mutations in the *Parkin* gene.

Design—We assessed point mutations and exon deletions and duplications in the *Parkin* gene in 247 PD probands with age at onset ≤50 and 104 control probands enrolled in the Genetic Epidemiology of PD study. For each first-degree relative, a consensus diagnosis of PD was established. The probability that each relative carried a mutation was estimated from the proband's *Parkin* carrier status using Mendelian principles and the relationship of the relative to the proband .

Results—*Parkin* mutations were identified in 25 PD probands (10.1%), 72% of whom were heterozygotes. One *Parkin* homozygote reported 2 siblings with PD. The cumulative incidence of PD to age 65 in carrier relatives (age-specific penetrance) was estimated to be 7.0% (95% CI: 0.4-71.9%) compared to 1.7% (95% CI: 0.8-3.4%) in non-carrier relatives of cases (p=0.59) and 1.1% (95% CI: 0.3-3.4%), in relatives of controls (compared to non-carriers p=0.52).

Conclusions—The cumulative risk of PD to age 65 in a non-carrier relative of a case with AAO ≤50 is not significantly greater than the general population risk among controls. Age specific penetrance among *Parkin* carriers, in particular heterozygotes, deserves further study.

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Keywords

Parkin; Mutations; Parkinson's disease; Kin-cohort study; Early onset

Introduction

Mutations in the *Parkin* gene (PARK2)[1, 2] are associated primarily with early-onset Parkinson's disease (EOPD), defined as age at onset (AAO) ranging from ≤45 to ≤55[3-7], but have also been described in PD cases with an AAO over 70 years [7-10]. In PD cases with AAO ≤ 45 with a mode of inheritance consistent with autosomal recessive transmission, the frequency of *Parkin* mutations may be as high as 49%[3] while in cases without a family history of PD the range is 15-18%.[4, 6] AAO is inversely correlated with the frequency of *Parkin* mutations in both familial[3] and sporadic[6] cases.

Several studies have compared the AAO of PD in heterozygous, compound heterozygous, and homozygous *Parkin* mutation carriers [3-10] and found that heterozygous cases, both familial and sporadic, have older AAO. Heterozygous *Parkin* mutation carriers are more frequently reported among sporadic than familial cases[7].

Information on the risk of PD in individuals who carry *Parkin* mutations in either the homozygous, compound heterozygous, or heterozygous state (or penetrance) is essential for genetic counseling. The penetrance of *Parkin* mutations has only been reported for isolated families[7]. Most of the previous study designs sampled PD cases based on family history of PD, which would bias penetrance estimates upwards[11, 12]. To obtain an unbiased estimate of risk, a population-based random sample would be desirable, but *Parkin* mutations are so rare in the population that such a sample would have to be extremely large to obtain sufficient precision in penetrance estimates.

To obtain unbiased estimates of the risk of PD in *Parkin* carriers despite the low population frequency of *Parkin* mutations [13], we used a kin-cohort study design[11, 12] applied to participants in the Genetic Epidemiology of Parkinson's Disease (GEPD) study[14]. The kin-cohort design is highly efficient for estimating penetrance, because the relatives' mutation status is not required for the analyses, thus reducing costs for genetic analysis[15].

Methods

Subjects

Details of the GEPD study have been previously described [14, 16, 17]. Cases were ascertained based on AAO of motor signs ≤50 (EOPD) or >50 (LOPD). In this study we included all 247 PD cases with AAO ≤50. All cases were recruited from the Center for Parkinson's Disease and Other Movement Disorders at Columbia University (CPD), and EOPD cases were oversampled[14]. One hundred and five controls were randomly selected from 412 controls in the GEPD study for complete sequencing of the *Parkin* gene.[18] The majority of the controls were recruited by random digit dialing, with frequency matching by age, gender, ethnicity and area code/exchange. An additional sample of 40 controls of Hispanic descent, 11 African American (AA) controls and 170 Caucasian controls participating in GEPD were used to examine Parkin variants that have not been previously described. All PD cases and control probands were seen in-person and underwent an identical evaluation[14] that included a medical history, Unified Parkinson's Disease Rating Scale (UPDRS)[19] and videotape assessment.

Diagnosis of PD in Relatives

Information on the family history of PD in first-degree relatives was obtained by administering a reliable, validated interview to each case, control, and first-degree relative. For relatives who were deceased or otherwise unavailable for interview, the history was obtained by interviewing the most knowledgeable informant[16]. An algorithm was created to generate a final diagnosis for PD in each first-degree relative based on the family history interview and the direct interview with the relative. For relatives diagnosed with PD, a level of certainty was assigned as definite, probable, possible, uncertain, and unlikely. For first-degree relatives who met criteria for uncertain, possible, probable or definite PD, we tried to obtain additional information in the form of an examination, medical records, or an independent interview by a neurologist. A best estimate diagnosis of PD was assigned for each relative [14]. Family history information including age at onset of PD was available for 1330 relatives of 224 PD cases and 638 relatives of 103 controls included in the penetrance analysis. The Institutional Review Board at the College of Physicians and Surgeons, Columbia University approved this study.

Criteria for Inclusion of Mutation Carriers—All sequence variants identified in cases and controls were genotyped in ethnically matched controls. Sequence variants observed in controls but at a frequency of ≤1% were classified as rare variants. The remaining variants were classified as mutations and included in the analysis based on three criteria: 1) The mutation is absent in controls 2) The mutation is recurrent , has been reported in PD cases (unrelated) in more than one study, or the mutation changes an amino acid that is evolutionarily conserved and which is predicted to effect protein function 3) The mutation is located in the coding region and predicted to change the amino acid sequence.

Molecular Genetic Analysis of Probands

Mutation screening was performed in 247 cases and 105 controls to detect point mutations and exon deletions and duplications in the *Parkin* gene. In a previous study, we sequenced all *Parkin* exons and screened for exon deletions and duplications by semi-quantitative multiplex PCR in 101 cases and 105 controls[18]. One control was subsequently found to have a putative splice variant IVS6-14 C>G, that has not been reported in cases or controls. We consider this a rare variant and not a mutation and have excluded it from the analysis. In this study we report data on an additional 146 PD cases from the GEPD study who were screened for *Parkin* mutations by denaturing high performance liquid chromatography (DHPLC) (WAVE Transgenomic), which has 100% sensitivity and specificity. Primers and DHPLC conditions used for analysis of the *Parkin* gene have been described previously[20]. Amplicons were either directly sequenced (n=126) or analyzed using a *Parkin* genotyping array (n=20)[21] in DNA samples with abnormal elution profiles. The genotyping array has excellent sensitivity and specificity for detection of sequence variants when compared to the gold standard of sequencing[21]. The primers used for PCR amplification of *Parkin* exons 1-12 and intron-exon boundaries and sequencing have been described previously[22]. Cycle sequencing was performed on the purified PCR product as per the manufacturer's instructions (BigDye, Applied Biosystems). Products were analyzed on an ABI3700 genetic analyzer. Chromatograms were viewed using Sequencher (Genecodes) and sequence variants determined. All sequence variants identified in cases and controls were confirmed by analysis in a separate PCR followed by bi-directional sequencing. To identify genomic deletions and exon rearrangements in *Parkin*, semi-quantitative multiplex PCR was performed as previously described[18].

In addition to screening for mutations in *Parkin*, we genotyped five LRRK2 mutations (G2019S, L1114L, I1122V, R1441C and Y1699C) in 247 cases and 104 controls. Results of

the analysis of LRRK2 in all participants in the GEPD study, including both early and late onset PD cases, and all controls have been reported.[23]

Statistical methods

Demographic and clinical characteristics were compared between cases and controls and between mutation carriers and non-carriers. Fisher's exact test for categorical characteristics and Student's t-test for continuous characteristics were used to assess statistical significance. The penetrance of *Parkin* mutations was estimated using the kin-cohort[11] method. In this method, the genotypes of the relatives are first estimated using Mendelian principles and the relationship of the relatives to the proband. Then the observed disease occurrence in the relatives is evaluated in relation to these estimated genotypes. This method assumes that, although the PD case probands were sampled through their AAO, thereby increasing the proportion of carriers among cases and their first-degree relatives, the relatives of these PD cases are representative of randomly chosen individuals with certain genotypes. Hence familial influences on the relatives' PD risk other than the *Parkin* genotype are assumed to be negligible.

First, we computed the probability that each relative carries a mutation. Second, we used the kin-cohort method to estimate genotype-specific disease rates, using the consensus diagnoses of PD in the first-degree relatives[16, 17]. Third, we used Kaplan-Meier survival analysis to compute the cumulative risk of PD in the first-degree relatives of control probands. Kaplan-Meier analysis cannot be directly applied to estimate genotype-specific cumulative incidence in the relatives of cases because the carrier status in these relatives is unknown; however the kin-cohort method allows calculation of cumulative incidence through a method similar to Kaplan–Meier analysis. Confidence intervals for the penetrance and the cumulative incidence were computed using log-log transformation to ensure the lower limits of the confidence intervals were positive [24].

Results

Demographic and Clinical Characteristics of the Case and Control Probands

Demographic and clinical characteristics of the cases and controls are shown in Table 1. The mean AAO of the 247 cases was 41.8 years (SD: 6.8), mean disease duration was 10.7 (SD: 7.6) years, and the total motor score (UPDRS Part III) was 20.3 (SD: 12.8). Nineteen (8.5%) of the 224 cases on whom family history information was available had a diagnosis of PD in a first-degree relative.

Frequency of Parkin Mutations in Cases and Controls

Twenty-five (10.1%) of the 247 cases had a *Parkin* mutation; five (20%) were homozygous, two (8%) were compound heterozygotes and eighteen (72%) were heterozygous (Table 2). Twenty two of the mutations have been previously described in other studies [2, 21, 22] Three new mutations were identified that have not been previously published and were not detected in any of our control samples (Iso298Leu, Asp18Asn, and Pro153Arg). Eleven different point mutations (c.81G>T, Gly319Gly, Arg42Pro, Arg275Trp, Met192Leu, Cys253Tyr, Asp280Asn, Iso298Leu, Arg366Gln, Asp18Asn, Pro153Arg) and four different exon rearrangements were identified (Exon 5 del, Exon 3-4del, Exon 3 40bp del and Exon 2 del) (Table 2). Point mutations included nine missense mutations (Arg42Pro, Arg275Trp, Met192Leu, Cys253Tyr, Asp280Asn, Iso298Leu, Arg366Gln, Asp18Asn, Pro153Arg), one synonymous substitution (Gly319Gly), and a non-coding 5'UTR mutation (c.81G>T). Exon deletions were found in four different exons (exons 2, 3, 4 and 5). 40 percent (10/25) of the variants identified in cases were found in exons encoding functional domains including the ubiquitin domain (Exon 2) and RING1 domain (Exon 7). Six cases carried the Exon 3 40bp

deletion, five cases carried Arg275Trp and two carried Arg42Pro. We previously reported that the synonymous substitution, Leu261Leu, is a variant rather than a disease associated mutation and thus have not included carriers of Leu261Leu in our estimates[23]. Among the 25 carriers, three (12%) had AAO ≤20 (2/3 heterozygotes); four (16%) had AAO 21-30 (3/4 heterozygotes); six (24%) had AAO 31-40 (5/6 heterozgygotes); and twelve (48%) had AAO 41-50 (8/12 heterozgyotes). Among all 247 EOPD case probands, carriers represented 75% (3/4) of those with AAO≤20, 36% (4/11) of those with AAO 21-30, 8% (6/73) of those with AAO 31-40, and 8% (12/159) of those with AAO 41-50.

Clinical Characteristics of Case Probands With and Without Parkin Mutations

Demographic and clinical features of the 25 mutation carriers and 222 non-carriers are shown in Table 3. The AAO of PD was significantly younger in carriers (36.5 ± 10.3) than in non-carriers (42.4 ± 6.1) (p=0.01), but did not differ between heterozygotes compared to compound heterozygotes and homozygotes combined. Other comparisons of clinical features were not significant.

The clinical characteristics of first-degree relatives stratified by the probands' mutation status are presented in Table 4. Information on the family history of PD in first-degree relatives was available for 23/25 carriers and 201/218 non-carriers. One of 23 carrier probands (4.4%) had a family history of PD. This proband is homozygous for an 40 bp deletion in exon 3 and had two affected siblings (AAO 26, 30).

Penetrance Estimates of Parkin Mutations

The probability of a relative being a carrier, whether or not he/she was actually diagnosed with PD, stratified by the proband's carrier status is presented in Table 5. In the calculation, the population frequency of *Parkin* mutations, p, was assumed to be 0.03% {Lucking, 2000 #1168; Oliveira, 2003 #2;. We have run a sensitivity analysis by taking p to be 2.8% (the upper limit of the 95% exact confidence interval of the mutation frequency estimated from the controls) and the results did not change, suggesting that the estimates are robust to misspecification to the population frequency of *Parkin* mutations.

The expected genotype distribution in the relatives and the prevalence of a history of PD in relatives predicted to be carriers or noncarriers are shown in Table 6. To obtain these prevalence estimates, we first computed the probability that each of the relatives was a carrier based on the observed carrier status in the probands (see Table 1) and then combined the information on the predicted genotypes in the relatives with the observed PD diagnoses in the relatives. There were 93 relatives expected to be carriers (homozygotes or heterozygotes), among whom 2 had PD (Table 6). Therefore, the prevalence of a history of PD in carrier relatives was estimated at 2/93 [2.2% (CI: 0.3%, 7.6%)]. Among relatives expected to be noncarriers (N=1237), 19 had PD (Table 6); thus the prevalence of a history of PD in noncarrier relatives was estimated as 1.7% (0.9-2.4%). These two prevalence estimates did not differ significantly.

The cumulative incidence of PD in 1330 relatives of case probands (without regard to carrier status) was estimated to be 1.7% (95% CI: 0.9-3.3%) to age 65, and 5.9% (95% CI: 3.7-9.3%) to age 80. Estimates of the cumulative risk of PD in *Parkin* carriers and non-carriers and the cumulative risk of PD in 647 relatives of controls are presented in table 7. Among relatives expected to be carriers, the cumulative incidence of PD was 7.0% to age 65 and remained so up to age 80. Among relatives expected to be noncarriers, the cumulative incidence was 1.7% to age 65 and 6.1% to age 80. The ratio of the cumulative incidence to age 80 for carriers versus non-carriers was 1.1 (95% CI: 0.07-18.8). Estimates of risk in carriers and noncarriers did not differ significantly with our sample size. The cumulative

incidence in relatives of controls was similar to that in relatives expected to be noncarriers (1.1% to age 65 and 4.1% to age 80).

We have reported that LRRK2 mutations may be responsible for familial aggregation in both EOPD and LOPD in the GEPD study{Clark, 2006 #12300}. To separate out the effect of the LRRK2 G2019S mutation (the only observed LRRK2 mutation in the cases), we repeated our analyses using 214 case probands who did not carry a LRRK2 mutation. However, we can not we can not exclude the possibility that they carry 'other' mutations in the LRRK2 gene as we did not completely sequence the LRRK2 gene but only genotyped 5 previously reported LRRK2 mutations. There were 1274 relatives included in the analysis. Since these relatives were family members of probands who did not carry any of the five previously identified LRRK2 mutations, it is unlikely they carry any of these mutations. The cumulative incidence to age 80 for relatives expected to be non-carriers of either LRRK2 mutations or *Parkin* mutations was 5.9%, compared to 6.1% for relatives expected to be noncarriers of *Parkin* mutations. The cumulative incidence of PD to age 80 in non-*Parkin*, non-LRRK2 carrier relatives was not significantly different from controls.

Discussion

Using the kin-cohort method, we have shown that the cumulative risk to age 65 in a relative of an EOPD case who is not estimated to carry a *Parkin* mutation is not significantly greater than the general population risk among controls. We estimated a cumulative risk of PD in carriers of Parkin mutations (age-specific penetrance) of 7.0% (95% CI: 0.4-71.9%) up to age 80. We were unable to examine risk of PD among heterozygotes separately. *Parkin* heterozgyosity may not be sufficient for the development of PD. A recent paper suggested that *Parkin* variants were equally common in cases and controls in predominantly non-coding regions[25].

The sampling scheme of our study, in which probands were ascertained without regard to their family history of PD, differs markedly from that in other studies that included only families with multiple affected individuals. As noted in the context of estimating the penetrance of mutations in BRCA1 and BRCA2, penetrance estimates are inflated when based on samples selected though family history [12, 15], and hence we believe our estimates are more representative of those in the general population than are those derived from familial samples. While the confidence intervals are extremely wide, the observed low frequency of PD in first-degree relatives of *Parkin* mutation carriers, 72% of whom are heterozygotes, may be a reflection of the low penetrance in carriers.

The frequency of *Parkin* mutations was 10.1% (95% CI: 8.0-16.4%) in 247 cases with AAO ≤ 50, which is within the range of other series with primarily sporadic cases (15-18%)[6], or population-based series (9%)[26]. The mean AAO of our case sample was 41.6 years, and 48% of the cases had AAO between 40 and 50. The frequency of *Parkin* mutations has been reported to decrease with increasing age-at-onset[3, 6]. While the age-specific frequencies of carriers are similar to other reported series[6], the high percentage of older cases in this study may explain why we report a frequency at the lower end of the spectrum.

Limitations

There are three important limitations in this study. Most importantly, the number of relatives of probands who are estimated to carry *Parkin* mutations is limited, which results in cumulative risk estimates with wide confidence intervals. We have limited our study to only those who participated in the GEPD study for whom we have accurate information on vital status, a necessary criterion for the kin-cohort method. In addition, only 105 controls were completely sequenced for the *Parkin* gene, which may explain our inability to detect

differences between relatives of PD probands and relatives of control probands. The sample size required for a typical kin-cohort study is usually large, due to the low population prevalence of the mutation being studied. Second, the diagnosis in the relatives was a best estimate diagnosis. We have previously reported high sensitivity (95.5%) and specificity (96.2%) of our family history questionnaire based on examination of 141 relatives. Third, we did not have actual genotype data on relatives. We used the kin-cohort method to estimate penetrance in the absence of these data.

Some relatives may have been too young to manifest PD. Our sampling plan was to include all probands with AAO ≤50 regardless of their family history of PD and AAO in the relatives. The relatives of these probands are likely to be younger than the relatives of randomly selected PD patients. In a study of affected sibling pairs, the mean AAO of heterozygous *Parkin* carriers was reported to be 49.6.[10] Forty-nine percent of the relatives in our sample were younger than 49.6 (Table 4). Our estimates of the prevalence of a history of PD in the relatives do not account for this young age distribution, and thus underestimate prevalence of PD according to *Parkin* genotype in the general population. We accounted for the younger age distribution by computing age-specific cumulative incidence of PD according to genotype.

One assumption required for the kin-cohort analyses to be valid is that risk of PD in relatives within the same family is independent, given the proband's *Parkin* genotype. The presence of other genetic and environmental risk factors that aggregate in the families may violate this assumption and bias the penetrance estimation[15, 27]. Due to the possibility of additional unspecified familial risk factors in the relatives of early-onset PD probands, penetrance estimates obtained from our sample can be applied to the population of relatives of the early-onset PD probands but not to the entire population.

We are currently recruiting a larger multi-center sample of early-onset cases and examining and obtaining DNA in relatives of carrier probands. We hope to use this new sample to refine our estimates of penetrance in heterozygous, homozygous, and compound heterozygous carriers.

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Table 1

Demographic Characteristics of Case and Control Probands

	Total (n=351)	Cases (n=247)	Controls (n=104)	Significance Cases vs. Controls
% male (n)	59.7 (209)	61.1 (151)	55.8 (58)	0.40
Age (years) (sd)	54.9 (10.2)	52.5 (9.0)	60.8 (10.5)	<0.001
% White (n)	84.3 (296)	81.0 (200)	92.3 (96)	
% African-American (n)	2.3 (8)	2.0 (5)	2.9 (3)	
% Hispanic (n)	8.0 (28)	10.1 (25)	2.9 (3)	
% Other (n)	5.4 (19)	6.9 (17)	1.9 (2)	0.02
Years Education (sd)	15.5 (3.2)	15.5 (3.3)	15.5 (2.9)	0.97
% with *Parkin* variants (n)	7.1 (25)	10.1 (25)	0.0 (0)	<0.001
% with family history of PD* (n)	7.7 (25)	8.5 (19/224)	5.8 (6/103)	0.50

* Family history was available for 328 probands (224 cases and 103 controls)

Table 2

Parkin Mutations identified in cases

Patient	Exon	Mutation	Zygosity	AAO (years)	Ethnicity
1	2	Arg42Pro	Het	44	White/non Hispanic
2	2	Arg42Pro	Het	32	Hispanic
3	2	Exon 2 deletion	Hom	42	White/non Hispanic
4	2	Asp18Asn	Het	32	Hispanic
5	3	Exon 3 40bp deletion	Hom	38	White/non Hispanic
6	3,7	Exon 3 40bp deletion + Arg275Trp	Comp. Het	19	White/non Hispanic
7	3	Exon 3 40bp deletion	Het	29	White/non Hispanic
8	3	Exon 3 40bp deletion	Hom	47	White/non Hispanic
9	3	Exon 3 40bp deletion	Hom	45	White/non Hispanic
10	3	Exon 3 40bp deletion	Hom	27	White/non Hispanic
11	3,4	Exon 3-4 deletion	Het	16	Hispanic
12	4	Pro153Arg	Het	42	Hispanic
13	5	Exon 5 deletion	Het	48	White/non Hispanic
14	5	Exon 5 deletion	Het	50	White/non Hispanic
15	5	Met192Leu	Het	36	Hispanic
16	5	Met192Leu.	Het	42	Hispanic
17	7	Cys253Tyr +Asp280Asn	Comp. Het	47	White/non Hispanic
18	5’UTR	c.81G>T	Het	28	Hispanic
19	7	Arg275Trp	Het	47	White/non Hispanic
20	7	Arg275Trp	Het	24	Other
21	7	Arg275Trp	Het	35	White/non Hispanic
22	7	Arg275Trp	Het	42	Hispanic
23	8	Iso298Leu[1]	Het	37	White/non Hispanic
24	9	Gly319Gly	Het	47	Hispanic
25	10	Arg366Gln	Het	17	White/non Hispanic

Table 3

Characteristics of Case Probands With and Without *Parkin* Mutations

	Mutation (n=25)	No mutation (n=222)	*p* value
Current age (sd)	47.2 (9.9)	53.1 (8.7)	0.008
Age onset (sd)	36.5 (10.3)	42.4 (6.1)	0.01
% male (n)	40.0 (10)	63.5 (141)	0.03
% Ethnicity: White (n)	64.0 (16)	82.3 (184)	
African-American (n)	0.0 (0)	2.3 (5)	
Hispanic (n)	28.0 (7)	8.1 (18)	0.03
Other (n)	8.0 (2)	6.8 (15)	
Years education (sd)	14.1 (3.8)	15.6 (3.3)	0.06
Duration PD (yrs)(sd)	10.6 (7.5)	10.7 (7.7)	0.96
Total motor score (UPDRS III)(sd)	22.1 (15.8)	20.1 (12.2)	0.57
Total modified mini mental state (mMMS) (sd)	52.3 (5.3)	53.7 (4.6)	0.24
% Family history PD in first-degree relative*	4.4 (1/23)	8.9 (18/201)	0.72
% Bradykinesia	88.0 (22)	82.9 (184)	0.78
% Rest tremor	80.0 (20)	78.2 (172/220)	1.00
% Rigidity	96.0 (24)	98.7 (219)	0.35
% Postural instability	44.0 (11)	45.0 (99/220)	1.00
% Asymmetry	96.0 (24)	88.5 (192/217)	0.49
% on L dopa	70.8 (17/24)	70.2 (153/218)	1.00
% Improve L dopa	95.0 (19/20)	97.4 (150/154)	0.46
%On-off	51.7 (15)	53.6 (113/211)	1.00
Hoehn and Yahr (sd)	2.3 (0.9)	2.1 (0.9)	0.32
% First symptom†: rest tremor (n)	44.0 (11)	48.9 (108)	0.68
% First symptom: gait (n)	0.0	4.5 (10)	0.60
% First symptom: rigidity (n)	8.0 (2)	9.5 (21)	1.00
% First symptom: bradykinesia (n)	4.0 (1)	4.1 (9)	1.00
% First symptom: motor (n)	4.0 (1)	1.8 (4)	0.42
% First symptom: poor balance (n)	4.0 (1)	0.5 (1)	0.19
% First symptom: pain (n)	8.0 (2)	1.8 (4)	0.12

*Family history was available for 224 case probands.

†First symptom information was available for 221 out of 222 non-carriers.

Table 4

Characteristics of first-degree relatives of case probands stratified by probands' carrier status

	Total Relatives of Case Probands (n=1330)	Relatives of Homozyogous/ Compound Heterozygous Carriers (n=42)	*Relatives of Heterozygous carriers* (n=110)	*Relatives of Non-carriers* (n=1178)	*Relatives of Control Probands* (n=638)	*Significance across probands' genotypes*
% male (n)	47.7 (634)	52.4 (22)	44.6 (49)	47.8 (563)	50.9 (325)	0.09
Age (years) (sd)	49.7 (22.7)	47.6 (22.2)	45.4 (19.7)	50.2 (22.9)	53.9 (20.2)	0.08
Parent (years, sd)	73.0 (14.0)	72.8 (9.1)	65.8 (13.7)	73.6 (13.9)	72.6 (13.9)	0.01
Siblings (years, sd)	49.6 (11.6)	46.7 (8.8)	45.3 (12.1)	50.2 (11.6)	55.1 (14.4)	0.01
Offspring (years, sd)	24.9 (11.5)	19.3 (8.8)	22.8 (9.0)	25.2 (11.7)	33.6 (11.1)	0.14
Ethnicity (%, n)						
White	74.4 (989)	100 (42)	42.7 (47)	76.4 (900)	91.5 (584)	
African-American	2.9 (39)	0	0	3.3 (39)	1.7 (11)	<0.001
Hispanic	13.9 (185)	0	47.3 (52)	11.3 (133)	3.6 (23)	
Other	8.8 (117)	0	9.4 (11)	9.0 (106)	3.1 (20)	
Years Education (sd)	12.6 (4.9)	13.4 (4.4)	11.7(4.9)	12.7 (4.9)	13.3 (4.1)	0.11
Relationship %,(n)						
Parents	32.4 (431)	30.9 (13)	28.2 (31)	32.9 (387)	31.5 (201)	
Siblings	37.4 (497)	42.9 (18)	46.4 (51)	36.3 (428)	36.7 (234)	0.29
Offspring	30.2 (402)	26.2 (11)	25.5 (28)	30.8 (363)	31.8 (203)	
Number Affected (%, n)						
All relatives	1.6 (21)	4.8 (2)	0	1.6 (19)	0.9 (6)	0.09
AAO years for affecteds mean, (sd)	61.3 (16.1)	27.0 (2.8)	--	64.9 (11.9)	66.5 (12.8)	<0.001
Parents	3.5 (15)	0	0	3.9 (15)	3.0 (6)	0.51
AAO years for affecteds mean, (sd)	69.1 (7.3)	--	--	69.5 (7.7)	66.5 (12.8)	0.60
Siblings % (n)	1.2 (6)	7.7 (2)	0	1.0 (4)	0	0.07
AAO years for affecteds mean, (sd)	41.7 (15.3)	27.0 (2.8)	--	49.0 (13.2)	--	0.09
Offspring	0	0	0	0	0	

Table 5

Probability of a relative being a carrier stratified by the proband's mutation status*

Relatives of homozygous or compound heterozygous probands			
	Probability of the relative being a homozygous carrier	**Probability of the relative being a heterozygous carrier**	**Probability of the relative being a non-carrier**
Parent	*P*	1- *p*	0
Sibling	¼*(*p*^{2}-2 *p*+1)	1/2*(- *p*^{2}+1)	1/4*(1-*p*)^2
Offspring	*P*	1- *p*	0

Relatives of heterozygous probands			
	Probability of the relative being a homozygous carrier	**Probability of the relative being a heterozygous carrier**	**Probability of the relative being a non-carrier**
Parent	l/2**p*	1/2	1/2*(1-*p*)
Sibling	1/4*(*p*^{2} +*p*)	1/2*(- *p*^{2}+ *p*+1)	1/4*(*p*^{2}-3*p* +2)
Offspring	1/2**p*	1/2	1/2*(1-*p*)

Relatives of non-carrier probands			
	Probability of the relative being a homozygous carrier	**Probability of the relative being a heterozygous carrier**	**Probability of the relative being a non-carrier**
Parent	0	*p*	1-*p*
Sibling	1/4**p*^{2}	1/2(-*p*^{2}+2*p*)	1/4*(*p*^{2}-4p+4)
Offspring	0	*p*	1-*p*

* *p* is the population frequency of the mutation.

Table 6

Estimated genotype distribution and prevalence of a history of PD in relatives of cases

	Carriers (Homozygotes and Heterozygotes)		Non-carriers		Total
	PD	Non-PD	PD	Non-PD	
Parent	0	29	15	387	431
Siblings	2	38	4	453	497
Offspring	0	25	0	377	402
Total	2	91	19	1218	1330
Prevalence (exact 95% CI)	2.2% (0.3%, 7.6%)		1.7% (0.9%, 2.4%)		

Table7

Estimated cumulative incidence of PD in *Parkin* carrier relatives, non-carrier relatives, and relatives of controls

Age	*Parkin* Carrier (Homozygotes and heterozygotes) Relatives (95% CI)	Non-carrier Relatives (95% CI)	Relatives of Controls (95% CI)
25	3.7% (1.1, 84.0%)	0	0
35	7.0% (0.4,71.9%)	0	0
45	7.0% (0.4,71.9%)	0.2% (0.1, 1.0%)	0.2% (0.03-1.7%)
55	7.0% (0.4,71.9%)	0.4 (0.1, 1.4%)	0.2% (0.03, 1.7%)
60	7.0% (0.4,71.9%)	1.1 (0.5, 2.5%)	0.6% (0.1, 2.5%)
65	7.0% (0.4,71.9%)	1.7% (0.8, 3.4%)	1.1% (0.3, 3.4%)
70	7.0% (0.4,71.9%)	3.3% (1.9, 5.7%)	1.1% (0.3, 3.4%)
75	7.0% (0.4,71.9%)	4.6% (2.7, 7.5%)	2.8% (1.1, 7.2%)
80	7.0% (0.4,71.9%)	6.1% (3.8, 9.8%)	4.1% (1.7, 9.8%)