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Parkin and *PINK1* mutations in early-onset Parkinson's disease: comprehensive screening in publicly available cases and control

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Abstract

Background—Mutations in *parkin* and *PTEN-induced protein kinase (PINK1)* represent the two most common causes of autosomal recessive parkinsonism. The possibility that heterozygous mutations in these genes also predispose to disease or lower the age of disease onset has been suggested, but currently there is insufficient data to verify this hypothesis conclusively.

Objective—To study the frequency and spectrum of *parkin* and *PINK1* gene mutations and to investigate the role of heterozygous mutations as a risk factor for early-onset Parkinson's disease (PD).

Methods—All exons and exon-intron boundaries of *PINK1* and *parkin were* sequenced in 250 patients with early-onset PD and 276 normal controls. Gene dosage measurements were also performed, using high-density single-nucleotide polymorphism arrays.

Results—In total 41 variants were found, of which 8 have not been previously described (*parkin:* p.A38VfsX6, P.C166Y, P.Q171X, p.D243N, p.M458L; *PINK1:* p.P52L, P.T420T, P.A427E). 1.60% of patients were homozygous or compound heterozygous for pathogenic mutations. Heterozygosity for pathogenic *parkin* or *PINK1* mutations was over-represented in patients compared with healthy controls (4.00% vs. 1.81%) but the difference was not significant (p = 0.13). The mean age at disease onset was significantly lower in patients with homozygous or compound heterozygous mutations than in patients with heterozygous mutations (mean difference 11 years, 95% Cl 1.4 to 20.6, p = 0.03). There was no significant difference in the mean age at disease onset in heterozygous patients compared with patients without a mutation in *parkin* or *PINK1* (mean difference 2 years, 95% Cl −3.7 to 7.0, p = 0.54).

Conclusions—Our data support a trend towards a higher frequency of heterozygosity for pathogenic *parkin* or *PINK1* mutations in patients compared with normal controls, but this effect was small and did not reach significance in our cohort of 250 cases and 276 controls.

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Competing interests: None.

Parkinson's disease (PD) is a common neurodegenerative disorder characterised by bradykinesia, rigor, tremor and postural instability. Several lines of evidence suggest that genetic susceptibility plays an important role in the pathogenesis of PD.[1] Mutations in *parkin* and *PTEN-induced putative kinase 4 (PINK1)* have been found to cause early-onset PD (disease onset before the age of 50 years) with autosomal recessive inheritance.[23] *Parkin*, encoding the E3 ubiquitin ligase parkin, is one of the largest genes in the human genome, spanning approximately 1.4 Mb. It is located on chromosome 6q25.2-q27 in a highly unstable genomic region.[4] Point mutations and exon rearrangements in *parkin* are the most common genetic causes of early-onset PD; approximately 18% of sporadic and 50% of all familial early-onset cases have been attributed to *parkin* mutations.[5] *PINK1*, located on chromosome 1p36, has been implicated in PD based on linkage studies in families with autosomal recessive early-onset parkinsonism.[2] In contrast to *parkin*, *PINK1* is a much smaller gene, containing only eight exons that span about 18 kb. Homozygous or compound heterozygous loss of function mutations in *PINK1* have been found to lead to mitochondrial dysfunction.[67]

There is growing evidence that heterozygosity for *parkin* or *PINK1* mutations might act as a susceptibility factor for PD.[8–10] There is an increased frequency of heterozygous mutations in patients with PD compared with healthy controls, and cross-sectional imaging studies have shown subclinical dopaminergic dysfunction in heterozygous *parkin* or *PINK1* mutation carriers.[91112] In addition, an inverse relationship has been suggested between the number of pathogenic mutations and the age at disease onset.[813] However, the literature is not consistent, as some studies report a similar frequency of heterozygous mutations in cases and in controls.[14–16] Additional screening studies are therefore required to investigate the relevance of heterozygous pathogenic mutations to the pathogenesis of PD.

We undertook a comprehensive screening study of a large, publicly available cohort of patients with early-onset PD (defined as age at disease onset 50 years) and normal controls, to determine the frequencies and spectrum of mutations in *parkin* and *PINK1* and to investigate the role of heterozygous mutations as a risk factor for PD. We identified several novel mutations of which some are likely to be pathogenic, and found a trend towards a higher frequency of heterozygosity for pathogenic *parkin* or *PINK1* mutations in patients compared with normal controls.

METHODS

All participants gave written informed consent to participate in genetics research and the research protocol was approved by the institutional review board.

Subjects

We screened 250 early-onset patients with PD (mean age at disease onset 41 years, range 7–50) and 276 neurologically normal controls (mean age at sampling 39 years, range 15–53) for mutations in *parkin* and *PINK1*. These samples, precompiled in six plates (NDPT14, NDPT15, NDPT16, NDPT19, NDPT20 and NDPT21), are publicly available at the NINDS Neurogenetics Repository at the Coriell Institute for Medical Research, New Jersey, USA

(http://ccr.coriell.org/ninds). Clinical characteristics of cases and controls are summarised in table 1.

Sequencing

All exons and exon-intron boundaries of *parkin* (reference sequence: NM_004562.1) and *PINK1* (reference sequence: NM_0032409.2) were amplified by PCR, in a reaction mix of 15 ng of genomic DNA, 10 nmol/μl forward primer, 10 nmol/μl reverse primer and 12 μl of a master mix (FastStart PCR Master; Roche, Indiana, USA). For exon 1 in *parkin*, 1 μl of 5% dimethylsulphoxide (American Bioanalytical, Massachusetts, USA) was added to the PCR reaction mix and PCR amplification for exon 1 in *PINK1* was achieved by adding 12.5 nmol/μl 7-deaza-GTP (New England Biolabs, Massachusetts, USA) to the mix (primer sequences and fragment lengths are shown in supplementary table 1 online). For the PCR reaction, we used a 60 touch down 50 thermo-cycling program. PCR cycling conditions were as follows: initial denaturation step of 4 minutes at 94°C; 8 cycles of 30 seconds at 94°C, 30 seconds at 60°C and 30 seconds at 72°C; 20 cycles of 30 seconds at 94°C, 30 seconds at 60°C, decreasing by 0.5°C after each cycle, 30 seconds at 72°C; 16 cycles of 30 seconds at 94°C, 20 seconds at 50°C and 30 seconds at 72°C; and a final extension step of 5 min at 72°C.

After PCR product purification (AMPure; Agencourt Bioscience Corporation, Massachusetts, USA), we performed direct dye terminator sequencing (BigDye v3.1; Applied Biosystems, California, USA). The resulting reactions were cleaned with CleanSEQ (Agencourt Bioscience Corporation, Massachusetts, USA), processed on an automated analyser (3730 ×l DNA Analyzer; Applied Biosystems) and analysed with Sequencher V. 4.1.4 (Gene Codes Corp., Michigan, USA).

For sample ND00548 from plate NDPT14, chromatography analysis showed a complex mutation in exon 2 of parkin. We therefore performed allele-specific sequencing in this sample. Exon 2 was amplified by PCR and then cloned (pCR8/GW/TOPO TA Cloning Kit; Invitrogen, Carlsbad, California, USA), in accordance with the manufacturer's instructions. Transformed colonies were cultured overnight in LB medium containing 100 μg/ml spectinomycin and followed by isolation of plasmid DNA using PureLink Quick Plasmid Miniprep Kit (Invitrogen). DNA from 12 colonies was sequenced bidirectionally using direct sequencing as described above.

Gene dosage measurements

In all samples we performed genome-wide single-nucleotide polymorphism (SNP) genotyping using SNP chips (Infinium HumanHap 550K V.3; Illumina Inc., California, USA) in accordance with the manufacturer's protocol. Raw genotypes were determined using the genotyping module of BeadStudio V.3.1.12 (Illumina Inc.). Gene dosage, inferred from signal intensity data and genotype calls at the *parkin* and *PINK1* loci, was visualised using Genome Viewer V.3.1.4 within BeadStudio as previously described.[17] A heterozygous duplication was called if the log R ratio (a metric for gene dosage) was increased by 50%, whereas a 50% decrease in the log R ratio in combination with absence of heterozygous SNP calls was considered a heterozygous deletion.

In samples with an exon rearrangement involving the coding sequence of *parkin*, we performed quantitative PCR (qPCR) assays (TaqMan; Applied Biosystems) to confirm each rearrangement. *β-globin* was used as an endogenous reference. Each reaction contained two fluorescent-label TaqMan MGB probes (Applied Biosystems), one VIC-labelled probe that specifically bound to the respective *parkin* exon sequence and one 6-FAM-labelled probe that was specific for the *β-globin* reference (primer and probe sequences are shown in supplementary tables 2 and 3 online). The qPCR reaction mix consisted of 25 ng genomic DNA, 10 μl of 2×TaqMan Universal PCR Master Mix (Applied Biosystems), 72 nmol/μl primers, 10 nmol/μl of the β-globin probe and 10 nmol/μl test probe. PCR cycling conditions were: 95°C for 10 minutes, 95°C for 15 seconds and 60°C for 1 minute (40 cycles). Each sample was replicated six times and for each exon, two control samples were measured. The dosage of each exon relative to *β-globin* and normalised to control DNA was determined using the $2^{-\Delta\Delta CT}$ method.

Bioinformatic analyses

For multiple sequence alignments, human *PINK1* and *parkin* reference sequences were aligned with paralogues and orthologues from the NCBI conserved domain database using ClustalW2. First, the serine/threonine protein kinase catalytic domain (c109925) was identified in the *PINK1* sequence by querying NCBI conserved domain database and then sequence-to-structure alignments were applied to the kinase domain of *PINK1* to map the kinase functional subdomains to the *PINK1* kinase region. Similar approaches were applied on the RING-finger domains of *parkin.*

Statistical analyses

For sequence analysis, we defined any alteration from the reference sequence as a variant or a mutation. Changes in exon copy number were referred to as exon rearrangements. Polymorphisms were defined as variants that occurred at a minor allele frequency of at least 1% in the control population. To ensure accuracy of the statistical analyses, we only called a mutation pathogenic if one of the following criteria applied: stop mutation, frameshift mutation, exon rearrangement or a missense mutation that has been reported to be pathogenic (details are presented in table 2).

Fisher's exact tests on allelic association and tests for assessing departures from Hardy–Weinberg equilibrium were performed using PLINK (version 1.04; http://pngu.mgh.harvard.edu/~pur-cell/plink/).[18] Statistical significance for differences in age at onset was tested using *t* tests after Kolmogorov–Smirnov tests showed a normal distribution of the data (SPSS V.12.0.1). For the age at onset analysis, $p < 0.05$ was considered significant.

RESULTS

We found 41 sequence variants in a cohort of 250 patients with early-onset PD and 276 normal controls (fig 1, tables 3,4). No significant departures from Hardy–Weinberg equilibrium were found for any of these variants and none of the identified variants achieved

significance for allelic association after Bonferroni correction for multiple testing (adjusted p <0.0012).

Parkin analysis

The following variants were seen in *parkin:* 4 frameshift mutations, 13 missense mutations, one stop mutation, 5 intronic variants and 2 silent mutations (table 3). Five of these variants have not been previously described (p.A38VfsX6, p.C166Y, p.Q171X, p.D243N, p.M458L; chromatograms of all novel variants are shown in supplementary figs 1 and 2 online). In addition, we identified four patients (1.6%) and two controls (0.7%) with a heterozygous exon deletion, and four cases (1.6%) and one control (0.4%) with a heterozygous exon duplication (table 2, supplementary figures 4–6 online). Nine patients (3.6%) and five controls (1.8%) were heterozygous for pathogenic *parkin* mutations, three patients (1.2%) were compound heterozygotes as they carried two different pathogenic mutations, and one patient (0.4%) was homozygous for a pathogenic mutation.

PINK1 analysis

We found the following variants for *PINK:* nine missense mutations, five silent mutations and two intronic variants (table 4). Of these, three mutations have not been previously reported (p.P52L, p.T420T, p.A427G; chromatograms of all novel variants are shown in supplementary fig 3 online). None of the studied subjects had a *PINK1* gene duplication or deletion. One patient (0.4%) was heterozygous for a pathogenic mutation in *PINK1*, whereas none of the controls carried a heterozygous mutation. No subject had pathogenic *PINK1* mutations.

Combined analysis

In all, 14 patients (5.6%) and 5 controls (1.8%) had 1 pathogenic mutations in *parkin* or *PINK1*, and this difference was significant (p = 0.02). Of the 14 patients with 1 pathogenic mutation, 8 (57.1%) had a family history of parkinsonism, compared with 87 of 250 (34.8%) patients without a pathogenic mutation (missing family history data: 3 patients without a pathogenic mutation (1.2%)). Of 5 controls with a pathogenic mutation, 2 (40.0%) had a positive family history for PD, compared with only 7 of 271 (2.6%) controls without a pathogenic mutation.

Bioinformatic analysis of sequence conservation

To determine the extent of sequence conservation at newly discovered mutated sites, we aligned the human *PINK1* and *parkin* sequences with human domain paralogues and with orthologues from various species. Based on this analysis, at least three variants were probably pathogenic: p.A38VfsX6 and p.Q171X lead to an early termination of the coding sequence, and p.A427E is located in close proximity to the highly conserved serine-threonine kinase activation loop, and it is likely that mutations at this site will interfere with substrate binding to this crucial domain (fig 2).

DISCUSSION

We report the results of a comprehensive screening study for *parkin* and *PINK1* mutations in 250 patients with early-onset PD and 276 normal controls. We identified 41 sequence variants: 8 novel and 33 previously described. Conservation across various species strongly suggests that at least three of the novel mutations are likely to be pathogenic (p.A38VfsX6, p.A171X, p.A427E). In addition to sequence variants, we also found eight cases and three controls with a heterozygous exon rearrangement (table 2).

Overall, patients with pathogenic changes in *parkin* or *PINK1* accounted for 5.6% of all cases in this study. This frequency is lower than previous reports have suggested.[5–19] Possible explanations for this discrepancy could be a population-specific variability in allele frequencies, different case selection criteria, different age at onset distributions in the studied cohorts, and different criteria for determining which variant is considered pathogenic.

Increasing evidence indicates a role of heterozygous pathogenic mutations as a susceptibility factor for PD. Positron emission tomography imaging studies have reported a subclinical dopaminergic dysfunction in heterozygous *parkin* or *PINK1* mutation carriers.[11–12,20] This observation is supported by transcranial ultrasound studies that reported hyperechogenicity of the substantia nigra, which was shown to depend on mutation status.[11,21–22] Furthermore, subclinical sensory abnormalities have been described in patients with heterozygous *PINK1* mutations.[23] The frequency of heterozygous pathogenic mutations has been reported to be significantly increased in patients with parkinsonism compared with normal controls.[9] However, several studies have provided contradictory data.[14–16] The lack of cohesion between these studies may have arisen due to the differences in how variants were defined as pathogenic, which determined if they were included in the statistical analysis. For our study, we used conservative criteria, and only included mutations in the analysis that were frameshift or stop mutations, exon rearrangements or mutations that have been reported as probably pathogenic (table 2). Based on these strict criteria, we found an increased frequency of patients with a heterozygous pathogenic mutation compared with controls (4.0% vs. 1.8%), but the difference was not significant ($p = 0.13$). None of the patients was heterozygous for pathogenic mutations in both genes. Therefore, tests for possible additive effects were not feasible.

Age at onset analysis found a significantly lower mean age at disease onset in patients who were homozygous or compound heterozygous for pathogenic *parkin* mutations (mean (SD) 32 (7) years) than in patients with a heterozygous pathogenic mutation (43 (8) years) (mean difference 11 years, 95% CI 1.4 to 20.6, $p = 0.03$). In contrast to previous reports that have shown a significant decrease in age at onset in heterozygous carriers compared with patients without a heterozygous mutation,[8–24] our study did not reveal a significant difference (43 (8) years vs. 41 (9) years; mean difference 2 years, 95% CI −3.7 to 7.0, $p = 0.54$).

A possible limitation of this study is the moderate resolution of genome-wide SNP chips for the identification of exon rearrangements. On average, the Illumina 550K chip contains about 0.35 SNPs per kb at the *parkin* locus and 0.39 SNPs per kb at the *PINK1* locus. Small

duplications or deletions could therefore have been missed, and our finding of 11 exon rearrangements in the entire series could be an underestimation.

In conclusion, we present the results of a comprehensive analysis of variation in the parkinsonism genes *parkin* and *PINK1*. Our data found that there was a trend towards a higher frequency of heterozygosity for pathogenic *parkin* or *PINK1* mutations in patients compared with controls.

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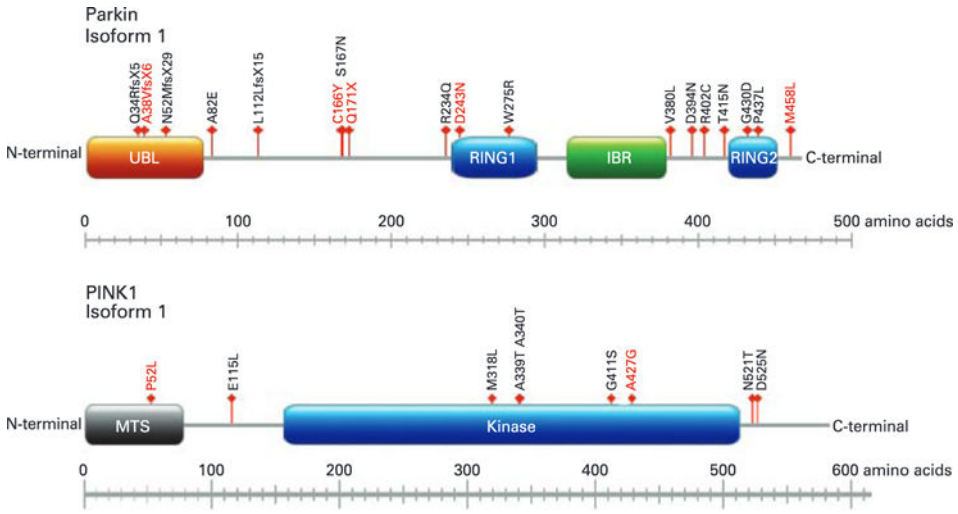

Figure 1.

Missense, nonsense and frameshift mutations observed in this study. Arrows indicate the position of each respective mutation relative to the protein domains. Novel mutations are indicated by red font, whereas previously described mutations are black. UBL, ubiquitin-like domain; RING1, RING finger motif 1; IBR, in-between-RING domain; RING2, ring finger motif 2; MTS, mitochondrion transit sequence.

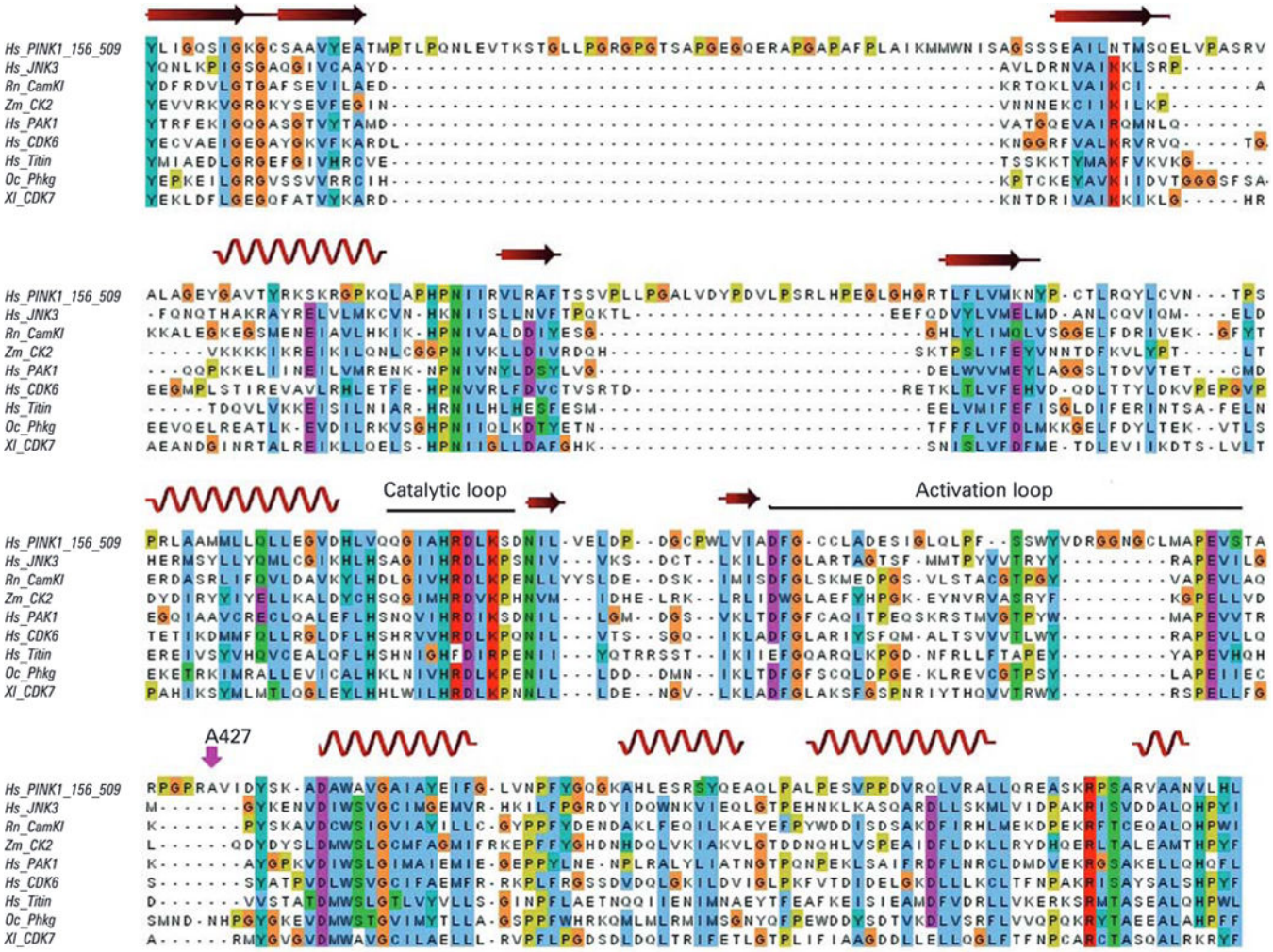

Figure 2.

Alignment of *PINK1* kinase domain with conserved serine-threonine protein kinases. Sequence alignment between the *PINK1* kinase domain and several serine-threonine protein kinase members in the NCBI conserved domain database. The red swirl ribbons and arrowed ribbons stand for the predicted conserved α-helix and β-strand in three dimensional structures, respectively. The mutation p.Ala427Glu lies close to the highly conserved APE triple that is the C-terminal anchor of the activation loop. It is likely that mutations in or close to this crucial domain interfere with substrate recognition.

Table 1

Clinical description of cases and controls

Characteristics	Patients	Controls
Subjects, n	250	276
Female/Male	97/153	199/77
Mean age at sampling, years	54	39
Mean age at onset (range), years	41 (7 to 50)	MA
Ethnicity		
Caucasian: Hispanic	7	3
Caucasian: not Hispanic	156	273
Caucasian: not specified	87	0
Positive family history*	95	44
No information on family history	3	0

NA, not applicable.

*Positive family history for one or more of: parkinsonism, tremor, dementia, restless legs syndrome, dystonia.

Table 2

Subjects with pathogenic mutations included in statistical analysis

Sample ID	Mutation(s)*		Age at onset, years	Family history
Pathogenic *parkin* mutations in cases				
ND00136	Heterozygous for p.Q171X	Heterozygous for p.R275W	38	Present
ND00153	Heterozygous deletion of exons 5–6		29	Present
ND00187†	Heterozygous duplication of exons 5–9		38	Present
ND00429†	Heterozygous duplication of exons 5–9		41	Present
ND00548	Heterozygous for p.A38VfsX6	Heterozygous for p.Q34RfsX5	36	Absent
ND01119	Heterozygous deletion exon 2		50	Absent
ND01122	Heterozygous for p.R275W		47	Absent
ND02639	Homozygous for p.L112LfsX15		30	Absent
ND02798	Heterozygous duplication of exon 6		32	Present
ND04581	Heterozygous deletion exon 2	Heterozygous for p.Q34RfsX5	22	Present
ND05921	Heterozygous deletion exon 4		49	Present
ND06330	Heterozygous duplication exons 2–3		50	Absent
ND06635	Heterozygous for p.N52MfsX29		42	Present
Pathogenic *PINK1* mutations in cases				
ND08471	Heterozygous for p.M318L		47	Absent
Pathogenic *parkin* mutations in controls				
ND03967	Heterozygous deletion exon 3		NA	Present
ND04990	Heterozygous duplication exon 2		NA	Absent
ND08538	Heterozygous deletion exons 2–4		NA	Absent
ND09912	Heterozygous for p.R275W		NA	Absent
ND10272	Heterozygous for p.T415N		NA	Present

NA, not applicable.

*Only frameshift mutations, stop mutations, exon rearrangements that span the coding sequence and missense mutations that have been reported to be pathogenic are listed.

†ND00187 and ND00429 are siblings.

Table 3

Variants in *parkin* in 250 PD cases and 276 controls

Nucleotide	Location/rs number	Amino acid change	Mutation type	Genotypes in cases ($A_1A_1/A_1A_2/A_2A_2$)	Genotypes in controls ($A_1A_1/A_1A_2/A_2A_2$)	MAF cases/controls	Ref.
Novel mutations that are likely to be pathogenic							
c.[214delC+216GR T]	Exon 2	p.Ala38ValfsX6	Frameshift	0/1/249	0/0/276	0.002/0.000	–
c.612CR T	Exon 4	p.Gln171X	Stop	0/1/249	0/0/276	0.002/0.000	–
Novel mutations of unknown significance							
C.598GR A	Exon 4	p.Cys166Tyr	Missense	0/1/249	0/0/276	0.002/0.000	–
C.828GR A	Exon 6	p.Asp243Asn	Missense	0/0/250	0/1/275	0.000/0.002	–
C.1473AR C	Exon 12	p.Met458Leu	Missense	0/1/249	0/0/276	0.002/0.000	–
Previously described mutations that are likely to be pathogenic							
c.202−203delAG	Exon 2	p.Gln34ArgfsX5	Frameshift	0/2/248	0/0/276	0.004/0.000	25
c.256delA	Exon 2	p.Asn52MetfsX29	Frameshift	0/1/249	0/0/276	0.002/0.000	25
c.437−477del	Exon 3	p.Leu112LeufsX15	Frameshift	1/0/249	0/0/276	0.004/0.000	25
c.924CR T	Exon 7/rs34424986	p.Arg275Trp	Missense	0/2/248	0/1/275	0.004/0.002	2527
c.1345CR A	Exon 11	p.Thr415Asn	Missense	0/0/250	0/1/275	0.000/0.002	25
Previously described mutations with unknown significance							
c.802GR A	Exon 6	p.Arg234Gln	Missense	0/1/249	0/0/276	0.002/0.000	28
c.1305CR T	Exon 11	p.Arg402Cys	Missense	0/0/250	0/1/275	0.000/0.002	29
c.1390GR A	Exon 12	p.Gly430Asp	Missense	0/1/249	0/0/276	0.002/0.000	5
c.1444CR T	Exon 12	p.Pro437Leu	Missense	0/3/247	0/3/273	0.006/0.005	24
Previously described mutations that are likely to be non-pathogenic							
c.273−18A	Intron 2	–	Intronic	0/4/246	0/2/274	0.008/0.004	16
c.34BCR A	Exon 3	p.Ala82Glu	Missense	0/0/250	0/2/274	0.000/0.004	3031
c.623CR T	Exon 4	p.Leu174Leu	Silent	0/1/249	0/2/274	0.002/0.004	15
c.884TR C	Exon 7/rs9456711	p.Leu261Leu	Silent	0/2/248	0/0/276	0.004/0.000	2832
Previously described polymorphisms							
c.272+25C	Intron 2/rs2075923	–	Intronic	13/83/154	10/95/171	0.218/0.208	25
c.514−20T	Intron 3/rs4709583	–	Intronic	1/35/214	1/42/233	0.074/0.080	25
c.601GR A	Exon 4/rs1801474	p.Ser167Asn	Missense	0/7/243	0/9/267	0.014/0.016	33

Nucleotide	Location/rs number	Amino acid change	Mutation type	Genotypes in cases ($A_1A_1/A_1A_2/A_2A_2$)	Genotypes in controls ($A_1A_1/A_1A_2/A_2A_2$)	MAF cases/controls	Ref.
c.973−35G	Intron 7/rs3765474	–	Intronic	52/126/72	52/138/86	0.460/0.438	25
c.1034+48T	Intron 8/rs10945756	–	Intronic	16/87/147	17/98/161	0.238/0.239	16
c.1239GR C	Exon 10/rs1801582	p.Val380Leu	Missense	6/78/166	5/89/182	0.180/0.179	25
c.1281GR A	Exon 11/rs1801334	p.Asp394Asn	Missense	0/16/234	0/14/262	0.032/0.025	25

MAF, minor allele frequency.

Genotype distribution of the major allele (A_2) and minor allele (A_1) is described for each variant.

Table 4

Variants in *PINK1* in 250 and 276 controls

Nucleotide	Location/rs number	Amino acid change	Mutation type	Genotypes in cases $A_1A_1/A_1A_2/A_2A_2$)	Genotypes in controls ($A_1A_1/A_1A_2/A_2A_2$)	MAF cases/controls	Ref.
Novel mutation that is likely to be pathogenic							
c.1374CR A	Exon 7	p.Ala427Glu	Missense	0/2/248	0/0/276	0.004/0.000	–
Novel mutation of unknown significance							
c.249CR T	Exon 1	p.Pro52Leu	Missense	0/1/249	0/0/276	0.002/0.000	–
Novel mutation that is likely to be non-pathogenic							
c.1354GR A	Exon 7	p.Thr420Thr	Silent	0/1/249	0/0/276	0.002/0.000	–
Previously described mutation that is likely to be pathogenic							
c.1046AR T	Exon 4	p.Met318Leu	Missense	0/1/249	0/0/276	0.002/0.000	34,35
Previously described mutations with unknown significance							
c.1109GR A	Exon 5/rs55831733	p.Ala339Thr	Missense	0/2/248	0/0/276	0.004/0.000	34
c.1325GR A	Exon 6/rs45478900	p.Gly411Ser	Missense	0/0/250	0/1/275	0.000/0.002	36
c.1667GRA	Exon 8	p.Asp525Asn	Missense	0/1/249	0/0/276	0.002/0.000	37
c.1822AR G	Exon 8	p.Ser576Ser	Silent	0/1/249	0/0/276	0.002/0.000	34
Previously described mutations that are likely to be non-pathogenic							
c.1030GR A	Exon 4/rs56200357	p.Arg312Arg	Silent	0/1/249	0/0/276	0.002/0.000	2
c.1267TR C	Exon 6/rs45499398	p.Asp391Asp	Silent	0/1/249	0/0/276	0.002/0.000	34,38
Previously described polymorphisms							
c.283CR T	Exon 1	p.Leu63Leu	Silent	9/81/160	5/78/193	0.198/0.159	34
c.438AR T	Exon 1	p.Gln115Leu	Missense	0/17/233	0/32/244	0.034/0.058	18
c.482−7A	Intron 1/rs2298298	–	Intronic	2/48/200	3/63/210	0.104/0.125	34
c.1054−5G	Intron 4/rs3131713	–	Intronic	2/51/197	2/63/211	0.110/0.121	34
c.111 MR A	Exon 5/rs3738136	p.Ala340Thr	Missense	1/33/216	0/18/258	0.070/0.033	34
c.1656AR C	Exon 8/rs1043424	p.Asn521Thr	Missense	15/110/125	21/110/145	0.280/0.275	34

Genotype distribution of the major allele (A_2) and minor allele (A_1) is described for each variant.

MAF, minor allele frequency.