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Sequencing analysis of *OMI/HTRA2* shows previously reported pathogenic mutations in neurologically normal controls

Javier Simón-Sánchez[1,2] and Andrew B. Singleton[1,3,*]

[1]Molecular Genetics Unit, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA, [2]Unidad de Genética Molecular, Departamento de Genómica y Proteómica, Instituto de Biomedicina de Valencia-CSIC 46010, Valencia, Spain and [3]Department of Public Health Sciences, Center for Public Health Genomics, University of Virginia, Charlottesville, VA 22908, USA

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A novel heterozygous non-synonymous mutation and a novel polymorphism in *OMI/HTRA2 locus* have been associated with Parkinson's disease (PD) in a German population. In an attempt to replicate these results in an independent population, we analyzed the entire coding region of *OMI/HTRA2* in a series of 644 North American PD cases with both young- and late-onset disease and in 828 North American neurologically normal controls. Our results show that neither of the variants previously related to PD were associated with PD in our cohort and that the risk variants were present in neurologically normal controls.

INTRODUCTION

Whether genetic factors are related to the appearance of Parkinson's disease (PD) has been an area of much discussion. An important step forward in favor of a genetic contribution to the etiology of PD was taken with the identification of several families in which PD was inherited on an autosomal dominant manner (1–7). Further genetic analysis identified mutations in *SNCA* as causative of disease in the largest of these families (8,9). After this finding, different approaches have been performed to identify *loci* related to the appearance of PD and mutations in five genes have been unequivocally linked to PD, these include two autosomal dominant *loci* [*SNCA*, *PARK1*, OMIM #168601 (8) and *PARK4*, OMIM #605543 (10); and *LRRK2*, *PARK8* OMIM #607060 (11,12)) and three autosomal recessive *loci* (*PRKN*, *PARK2* OMIM #602544 (13); *PINK1*, *PARK6* OMIM #605909 (14); *DJ-1*, *PARK7* OMIM #606324 (15)]. Although these cases represent only 5% of total PD cases, they are of considerable interest because understanding the molecular etiology associated with these *loci* may help in understanding the molecular basis of sporadic PD.

In addition to these five genes, genome-wide linkage analysis and candidate gene analysis have suggested an association of other *loci* with sporadic PD. One such gene is *OMI/HTRA2* (OMIM #606441), a gene encoding a 50 kDa nuclear-encoded serine-protease with proapoptotic activity and a mitochondrial targeting sequence at its N-terminal region. This gene was first linked to neurodegeneration when Gray *et al*. (16) showed that Omi/HtrA2 interacts with presenilin-1, previously related to young-onset inherited forms of Alzheimer's disease (17). Further support for a role of Omi/HtrA2 in neurodegeneration came when Park *et al*. (18) demonstrated that Amyloid-β, a key factor implicated in the pathogenesis of Alzheimer's disease, is directly and efficiently cleaved by the Omi/HtrA2 serine-protease activity both *in vitro* and *in vivo*. In addition, Jones *et al*. (19) found a missense mutation (p.S276C) in the protease domain of Omi/HtrA2 of the mouse mutant mnd2 (motor neuron degeneration 2). The loss of neurons in the striatum resulting in a neurodegenerative disorder with parkinsonian features was described in *OMI/HTRA* knockout mice (20); these findings lead Strauss *et al*. (21) to look for mutations in *OMI/HTRA2* in a large sample of 518 German PD patients. In this study, they reported that mutations in *OMI/HTRA2* are associated with PD in German population by identifying two novel missense substitutions in PD patients and demonstrating a functional consequence of these variants *in vitro*. The first (p.G399S) was identified in four apparently sporadic PD cases and not observed in 740 chromosomes of healthy control individuals. All carriers shared the same haplotype based on six neighboring single nucleotide

*To whom correspondence should be addressed at: Laboratory of Neurogenetics, NIA IRP NIH, Room 1A1014, Building 35, 35 Lincoln drive, Bethesda, MD 20892, USA. Tel: +1 3014516079; Fax: +1 3014515466; Email: singleta@mail.nih.gov

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Table 1. Minor allele frequencies (MAF), Fisher's exact test *P*-values, odds ratios (OR) and 95% confidence intervals (CI 95%) for variants p.G399S and p.A141S after analysis with Plink v0.99s

Variant	Samples included in analysis	Minor allele (cases–controls)	MAF (cases–controls), %	Fisher's *P*-value	OR	CI 95%
p.G399S	PD: all, $n = 644$; Controls: all, $n = 828$	A (5–6)	0.388–0.363	1	1.069	0.3256–3.511
p.G399S	PD: age at onset ≤ 40, $n = 106$; Controls: age at sampling ≤ 40, $n = 141$	A (2–1)	0.944–0.354	0.5794	2.676	0.2411–29.71
p.G399S	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	A (3–5)	0.278–0.365	1	0.7633	0.182–3.201
p.A141S	PD: all, $n = 644$; Controls: all, $n = 828$	T (28–46)	2.215–2.991	0.2365	0.7348	0.4566–1.182
p.A141S	PD: age at onset ≤ 40, $n = 106$; Controls: age at sampling ≤ 40, $n = 141$	T (7–9)	3.398–3.214	1	1.059	0.3879–2.892
p.A141S	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	T (21–37)	1.985–2.941	0.1815	0.6683	0.3887–1.149

These values were calculated in our entire population (644 PD cases and 828 nerulogically normal controls), only taking into account YOPD and late-onset PD cases independently along with corresponding age-matched control cohorts.

polymorphisms (SNPs). Secondly, they identified a novel polymorphism p.A141S significantly overrepresented in their PD cohort compared with 370 healthy individuals ($\chi^2 = 4.25$, $P = 0.039$, OR = 2.15, CI = 1.02–4.52).

They also detected Omi/Htra2 in Lewy bodies in brains of idiopathic PD patients.

In order to confirm the involvement of *OMI/HTRA2* genetic variants in the development of PD, we sequenced the entire coding region of this gene in a large series of 644 PD patients and 828 neurologically normal controls.

RESULTS

After analyzing the entire protein coding region of *OMI/HTRA2* (RefSeq NM_013247) in a total of 644 PD patients and 828 neurologically normal controls, we identified the c.1195G>A substitution in exon 7 (leading to p.G399S), previously identified as a mutation associated with PD by Strauss *et al*. (21), in a total of five PD patients (0.77%) and six neurologically normal control individuals (0.72%). The PD patients carrying this substitution were *ND00428*, *ND00154*, *ND01247*, *ND09816* and *ND00148*.

Both *ND00428* and *ND00154* were diagnosed with YOPD with ages at onset of 38 and 30, respectively. *ND01247*, *ND09816* and *ND00148* were all diagnosed with late-onset PD with ages at onset of 56, 65 and 77, respectively.

The control samples carrying p.G399S were *ND01699*, *ND05065*, *ND10345*, *ND03376*, *ND10860* and *ND08025*. These samples were derived from panels *NDPT002*, *NDPT006*, *NDPT019*, *NDPT020*, *NDPT0 21* and *NDPT023* with ages at sampling of 85, 70, 25, 42, 46 and 62 years, respectively. Fisher's exact association test showed no association of p.G399S and PD (Table 1).

Fisher's exact association test was performed considering either YOPD or late-onset PD samples, along with their corresponding age-matched controls. Using the threshold of appearance of PD at or before 40 years, 106 samples were considered as YOPD and 538 as late-onset PD. Using this same criterion for the age at sampling in the control population, 141 and 687 samples were used as age-matched controls for YOPD and late-onset PD, respectively. No association was seen after these analyses (Table 1).

Table 1 also shows the frequency of genotypes of the variant c.421G>T in exon 1 of *OMI/HTRA2* (leading to p.A141S). The T allele of this variant has previously been associated with PD (21); however, we failed to find such an association in our data set performing Fisher's exact association test considering all samples and only YOPD and late-onset PD cases independently, along with their corresponding age, neurologically normal controls (Table 1). Only *ND4314* and *ND04275* (two neurologically normal control individuals of 49 and 55 years, respectively) were homozygous for this change.

We also failed to find the synonymous p.F149F variant, previously identified by Strauss *et al*., in our population.

In addition to these variants, we identified eight novel coding variants, both synonymous (p.V109V, p.L118L, p.R209R and p.L367L) and non-synonymous (p.W12C, p.P128L, p.F172V and p.A227S), in both cases and control samples. None of these seem to be causative of disease in our population (Table 2).

Eight SNPs have been described within the *OMI/HTRA2* coding region, two being exonic and six residing within introns. Of these, only rs2231249, rs2231248, rs11538692, rs2241027 and rs2241028 were within our sequencing boundaries and, thus, were genotyped. Variants rs2231248 (exon 1), rs2241027 (intron 4) and rs2241028 (intron 5) had at least one sample with a variant allele; however, none of these SNPs showed association with PD using Fisher's exact association test (Table 2). In addition, none of the variants tested showed association with disease when comparing young-onset cases with controls with age at collection >40 years (data not shown).

Table 2. Minor allele frequencies, χ^2 values, *P*-values, OR, CI 95% and Fisher's exact test *P*-values for all new variants found in our data set, as well as three SNPs within *OMI/HTRA2*

Variant	Samples included in analysis	Minor allele (cases–controls)	MAF (cases–controls)	Fisher's *P*-value	OR	CI 95%
p.V109V	PD: all, $n = 644$; Controls: all, $n = 828$	A (2–0)	0.158%–0%	–	–	–
p.V109V	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	A (1–0)	0.4854%–0%	–	–	–
p.V109V	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	A (1–0)	0.0945%–0%	–	–	–
p.L118L	PD: all, $n = 644$; Controls: all, $n = 828$	G (0–2)	0%–0.130%	–	–	–
p.L118L	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	G (0–2)	0–0.714%	–	–	–
p.L118L	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (0–0)	0%–0%	–	–	–
p.R209R	PD: all, $n = 644$; Controls: all, $n = 828$	G (2–0)	0.156%–0%	–	–	–
p.R209R	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	G (0–0)	0%–0%	–	–	–
p.R209R	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (2–0)	0.187%–0%	–	–	–
p.L367L	PD: all, $n = 644$; Controls: all, $n = 828$	C (0–1)	0%–0.063%	–	–	–
p.L367L	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	C (0–0)	0%–0%	–	–	–
p.L367L	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	C (0–1)	0%–0.077%	–	–	–
p.W12C	PD: all, $n = 644$; Controls: all, $n = 828$	G (0–1)	0%–0.064%	–	–	–
p.W12C	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	G (0–0)	0%–0%	–	–	–
p.W12C	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (0–1)	0%–0.078%	–	–	–
p.P128L	PD: all, $n = 644$; Controls: all, $n = 828$	C (0–1)	0%–0.065%	–	–	–
p.P128L	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	C (0–1)	0%–0.357%	–	–	–
p.P128L	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	C (0–0)	0%–0%	–	–	–
p.F172V	PD: all, $n = 644$; Controls: all, $n = 828$	G (1–0)	0.0782%–0%	–	–	–
p.F172V	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	G (1–0)	0.4762%–0%	–	–	–
p.F172V	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (0–0)	0%–0%	–	–	–
p.A227S	PD: all, $n = 644$; Controls: all, $n = 828$	T (1–0)	0.078%–0%	–	–	–
p.A227S	PD: age at onset $\leq$40, $n = 106$; Controls: age at sampling $\leq$40, $n = 141$	T (0–0)	0%–0%	–	–	–
p.A227S	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	T (1–0)	0.093%–0%	–	–	–
rs2231248	PD: all, $n = 644$; Controls: all, $n = 828$	G (1–0)	0.079%–0%	–	–	–

Continued

Table 2. Continued

Variant	Samples included in analysis	Minor allele (cases–controls)	MAF (cases–controls)	Fisher's *P*-value	OR	CI 95%
rs2231248	PD: age at onset ≤40, $n = 106$; Controls: age at sampling ≤40, $n = 141$	G (0–0)	0%–0%	–	–	–
rs2231248	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (1–0)	0.094%–0%	–	–	–
rs2241027	PD: all, $n = 644$; Controls: all, $n = 828$	G (6–8)	0.465%–0.493%	1	0.9442	0.3268–2.728
rs2241027	PD: age at onset ≤40, $n = 106$; Controls: age at sampling ≤40, $n = 141$	G (3–1)	1.415%–0.354%	0.3187	4.033	0.4166–39.05
rs2241027	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (3–7)	0.278%–0.522%	0.5269	0.5324	0.1374–20.64
rs2241028	PD: all, $n = 644$; Controls: all, $n = 828$	G (60–85)	4.73%–5.400%	0.4412	0.8701	0.6199–1.221
rs2241028	PD: age at onset ≤40, $n = 106$; Controls: age at sampling ≤40, $n = 141$	G (13–20)	6.311%–7.140%	0.8556	0.8756	0.4251–1.804
rs2241028	PD: age at onset >40, $n = 538$; Controls: age at sampling >40, $n = 687$	G (47–65)	4.420%–5.020%	0.5596	08755	0.5961–1.286

These values were calculated in both the entire group (644 PD cases and 828 nerulogically normal controls) and only testing YOPD and late-onset PD cases independently with corresponding age-matched control groups.

DISCUSSION

Here we present an extensive analysis of *OMI/HTRA2 locus* in a large cohort of both young- and late-onset PD cases and age-matched neurologically normal controls.

The p.G399S variant, previously described as a PD-causative mutation in a German study (21), is not associated with the disease in our population and was found at the same frequency in controls. A rare polymorphism previously described to be associated with PD (21) was not associated with disease in our cohort after Fisher's exact test of association ($P = 0.2265$, OR = 0.7348, CI = 0.4566–1.182).

We also performed single marker association between three SNPs (exon 1 and introns 4 and 5) and disease. The results derived from this analysis are consistent with a lack of contribution of *OMI/HTRA2 locus* to the risk of sporadic PD.

Interestingly, we have identified eight novel variants in our population in both cases and controls, four of which are non-synonymous changes: p.W12C, p.P128L, p.F172V and p.A227S. Variants p.W12C and p.P128L were detected in two different control samples, p.F172V and p.A227S in two different PD cases. Although it cannot be ruled out, it seems unlikely that these variants are related to disease in these patients and are likely rare benign alterations.

It is worth noting that while the p.G399S change was identified in six controls, half of these were relatively young (age 25, 42, 46, 62, 70 and 85 years); however, we do not feel that it is reasonable to suppose that these patients could be control patients who would convert to disease at a later date; first, the average age that patients who carried this alteration in our case cohort was 53.2 years (range 30–77 years) and thus we would have expected some of these controls to convert to PD cases already; secondly, a pathogenic change should be enriched in a case cohort even when compared with a random population cohort and this is particularly true in a disease which is relatively rare in occurrence.

OMI/HTRA2 was included in the original *PARK3 locus*, spanning 10.3 cM from marker D2S134 to D2S286 (22). Refinement of this *locus* 3 years later to 2.5 Mb by West *et al*. (23) excluded *OMI/HTRA2*. However, since several independent linkage and association studies suggest the existence of a disease-modulating gene outside this refined *PARK3* region (24), Strauss *et al*. unsuccessfully looked for mutations in the coding region of this gene in families B, C, D and K that defined *PARK3 locus*. Since no mutations were found in these *PARK3*-linked families, *OMI/HTRA2* was later designated as *PARK13* (OMIM #610297). Along with *PARK3*, this would be the second *locus* identified in German population (three out of the six families used to define *PARK3* had a German background) and not replicated elsewhere.

While we cannot rule out a small genetic risk at this locus, particularly one present only in a subset of patients such as those with young-onset disease, the present study did have sufficient power to identify an effect of the magnitude and type originally described; thus, the weight of evidence presented here suggests that the variability at *OMI/HTRA2* does not contribute to risk for PD and shows the necessity of replicating association studies in populations with a different ethnic background.

MATERIAL AND METHODS

Subjects

All samples were taken directly from pre-compiled panels from the National Institute of Neurological Disorders and Stroke (NINDS) funded Neurogenetics repository hosted by

Table 3. Distribution and mean age at sampling/onset per decade of all samples included in this study

Age range	Neurologically normal controls			PD cases		
	Number of samples	Mean	Standard deviation	Number of samples	Mean	Standard deviation
0–9	–	–	–	2	7	0
10–20	5	18.6	2.07	7	14.71	3.59
21–30	51	26.25	2.33	17	27.29	2.88
31–40	86	36.02	2.84	80	36.66	2.86
41–50	103	46.07	2.92	144	46.36	2.93
51–60	159	56.58	2.7	134	56.83	3.10
61–70	205	65.49	2.94	177	65.35	2.89
71–80	162	74.81	2.83	73	75.12	2.63
81–90	52	84.17	2.17	10	83.3	1.76
91–100	5	92.4	1.94	–	–	–

the Coriell Institute for research (NJ, USA). All participants provided written informed consent.

Neurologically normal Caucasian control subjects were derived from nine panels of DNA: *NDPT002*, *NDPT006*, *NDPT009*, *NDPT019*, *NDPT020*, *NDPT021*, *NDPT022, NDPT023* and *NDPT024*. These contain DNA from 828 unrelated individuals from North America, 340 males and 488 females. Each panel contains 5 μg of DNA from 92 unrelated Caucasian individuals without history of Alzheimer's disease, amyotrophic lateral sclerosis, ataxia, autism, bipolar disorder, brain aneurism, dementia, dystonia or PD. None had any first-degree relative with a known primary neurological disorder and the mean age of participants was 74 (range, 55–88), 67 (range, 55–84), 68 (range, 55–84), 28 (range, 15–35), 40(range, 36–48), 49 (range, 46–53), 67 (range, 56–91), 68 (range, 56–94) and 67 (range, 56–95) for panels *NDPT002*, *NDPT006*, *NDPT009*, *NDPT019*, *NDPT020*, *NDPT021*, *NDPT022*, *NDPT023* and *NDPT024*, respectively. The mean age at onset of all controls was 58 years. Distribution of age per decades and mean age at sampling for each group is displayed in Table 3.

Parkinson's disease cases were taken from seven panels of DNA: *NDPT001*, *NDPT005*, *NDPT014*, *NDPT015*, *NDPT016*, *NDPT017* and *NDPT018*.

These panels contain 5 μg of DNA from 644 unrelated Caucasian individuals from North America with PD, including 363 males and 281 females. The mean age at onset was 70 (range, 55–70), 67 (range, 55–81), 32 (range, 7–40), 43 (range, 40–47), 49 (range, 47–52), 66 (range, 56–84) and 66 (range, 56–87), respectively.

Here, we have defined young-onset PD (YOPD) as onset of a parkinsonian syndrome at or before age 40 (≤40); only those samples from *NDPT014* and 14 samples from *NDPT015* were considered as YOPD. These samples (a total of 106 including 63 males and 43 females) had a mean age at onset of 31 (range 7–40), defined as when symptoms were first noted, including at least one of the following: resting tremor, rigidity, bradykinesia, gait disorder and postural instability.

The remaining samples comprise 538 cases, including 302 males and 236 females with late-onset PD. The mean age at onset is 59 years (range, 41–87) and they all show at least one of the main clinical signs of PD such as resting tremor, rigidity, bradykinesia, gait disorder and postural instability at the disease onset.

All subjects (from both young- and late-onset panels) were questioned regarding family history of parkinsonism, dementia, tremor, gait disorders and other neurological dysfunction. Subjects both with and without a reported family history of PD were included. None were included who had three or more relatives with parkinsonism, nor with clear Mendelian inheritance of PD. The mean age at onset of all PD cases was 55 years. Distribution of age per decades and mean age at onset for each group is displayed in Table 3.

A more detailed description of both case and control samples can be found at http://ccr.coriell.org/Sections/Collections/NINDS/DNAPanels.aspx?PgId=195&coll=ND.

Sequencing analysis

Screening of *OMI/HTRA2* (RefSeq NM_013247) was carried out using genomic DNA from 644 PD patients and 828 neurologically normal controls. Polymerase Chain reaction (PCR) amplification was performed in a final volume of 15 μl containing 10 ng of genomic DNA, 10 pmol of forward and reverse primers (sequence available upon request) and 10 μl FastStart PCR Master mix (Roche). Primers for amplification were designed using the online tool ExonPrimer (http://ihg.gsf.de/ihg/ExonPrimer.html). Each purified product was sequenced using forward or reverse primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry as per manufacturer's instructions (Applied Biosystems, Foster city, CA, USA). The resulting reactions were purified and resolved on an ABI3730XL genetic analyzer (Applied Biosystems) and analyzed with Sequencher software v4.1.4 (Gene Codes Corporation, VA, USA). All changes that deviated from the wild-type sequence were verified by PCR amplification using a fresh DNA aliquot and sequencing in both forward and reverse directions.

Statistical analysis

Power calculations were performed using the program PS v2.1.30 (25). For variant p.A141S, based on the minor allele frequency (0.02991) we obtained after sequencing 828 control samples, our series possesses sufficient power (98.5%) to detect a difference in allele frequency ($P = 0.05$) with an effect of OR = 2.15. This is the OR that Strauss *et al*. (21) obtained in their study for this same variant.

For p.G399S based on a minor allele frequency of 0.0036 (according to our control population), our series posses enough power (80%) to detect a difference in allele frequency ($P = 0.05$) with an effect of OR = 3.59.

Plink v0.99s (http://pngu.mgh.harvard.edu/~purcell/plink/) (26) was used to perform Fisher's exact tests of association between all identified variants and PD.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Waters, C.H. and Miller, C.A. (1994) Autosomal dominant Lewy body parkinsonism in a four-generation family. *Ann. Neurol.*, **35**, 59–64.
2. Payami, H., Bernard, S., Larsen, K., Kaye, J. and Nutt, J. (1995) Genetic anticipation in Parkinson's disease. *Neurology*, **45**, 135–138.
3. Plante-Bordeneuve, V., Taussig, D., Thomas, F., Ziegler, M. and Said, G. (1995) A clinical and genetic study of familial cases of Parkinson's disease. *J. Neurol. Sci.*, **133**, 164–172.
4. Wszolek, Z.K., Pfeiffer, B., Fulgham, J.R., Parisi, J.E., Thompson, B.M., Uitti, R.J., Calne, D.B. and Pfeiffer, R.F. (1995) Western Nebraska family (family D) with autosomal dominant parkinsonism. *Neurology*, **45**, 502–505.
5. Golbe, L.I., Di Iorio, G., Sanges, G., Lazzarini, A.M., La Sala, S., Bonavita, V. and Duvoisin, R.C. (1996) Clinical genetic analysis of Parkinson's disease in the Contursi kindred. *Ann. Neurol.*, **40**, 767–775.
6. Maraganore, D.M., Schaid, D.J., Rocca, W.A. and Harding, A.E. (1996) Anticipation in familial Parkinson's disease: a reanalysis of 13 United Kingdom kindreds. *Neurology*, **47**, 1512–1517.
7. Morrison, P.J., Godwin-Austen, R.B. and Raeburn, J.A. (1996) Familial autosomal dominant dopa responsive Parkinson's disease in three living generations showing extreme anticipation and childhood onset. *J. Med. Genet.*, **33**, 504–506.
8. Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R. *et al.* (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*, **276**, 2045–2047.
9. Polymeropoulos, M.H., Higgins, J.J., Golbe, L.I., Johnson, W.G., Ide, S.E., Di Iorio, G., Sanges, G., Stenroos, E.S., Pho, L.T., Schaffer, A.A. *et al.* (1996) Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. *Science*, **274**, 1197–1199.
10. Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R. *et al.* (2003) α-Synuclein locus triplication causes Parkinson's disease. *Science*, **302**, 841.
11. Paisan-Ruiz, C., Jain, S., Evans, E.W., Gilks, W.P., Simon, J., van der Brug, M., Lopez de Munain, A., Aparicio, S., Gil, A.M., Khan, N. *et al.* (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron*, **44**, 595–600.
12. Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R.J., Calne, D.B. *et al.* (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*, **44**, 601–607.
13. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, **392**, 605–608.
14. Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G. *et al.* (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science*, **304**, 1158–1160.
15. Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joosse, M. *et al.* (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science*, **299**, 256–259.
16. Gray, C.W., Ward, R.V., Karran, E., Turconi, S., Rowles, A., Viglienghi, D., Southan, C., Barton, A., Fantom, K.G., West, A. *et al.* (2000) Characterization of human HtrA2, a novel serine protease involved in the mammalian cellular stress response. *Eur. J. Biochem.*, **267**, 5699–5710.
17. Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K. *et al.* (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, **375**, 754–760.
18. Park, H.J., Seong, Y.M., Choi, J.Y., Kang, S. and Rhim, H. (2004) Alzheimer's disease-associated amyloid beta interacts with the human serine protease HtrA2/Omi. *Neurosci. Lett.*, **357**, 63–67.
19. Jones, J.M., Datta, P., Srinivasula, S.M., Ji, W., Gupta, S., Zhang, Z., Davies, E., Hajnoczky, G., Saunders, T.L., Van Keuren, M.L. *et al.* (2003) Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. *Nature*, **425**, 721–727.
20. Martins, L.M., Morrison, A., Klupsch, K., Fedele, V., Moisoi, N., Teismann, P., Abuin, A., Grau, E., Geppert, M., Livi, G.P. *et al.* (2004) Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol. Cell Biol.*, **24**, 9848–9862.
21. Strauss, K.M., Martins, L.M., Plun-Favreau, H., Marx, F.P., Kautzmann, S., Berg, D., Gasser, T., Wszolek, Z., Muller, T., Bornemann, A. *et al.* (2005) Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum. Mol. Genet.*, **14**, 2099–2111.
22. Gasser, T., Muller-Myhsok, B., Wszolek, Z.K., Oehlmann, R., Calne, D.B., Bonifati, V., Bereznai, B., Fabrizio, E., Vieregge, P. and Horstmann, R.D. (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat. Genet.*, **18**, 262–265.
23. West, A.B., Zimprich, A., Lockhart, P.J., Farrer, M., Singleton, A., Holtom, B., Lincoln, S., Hofer, A., Hill, L., Muller-Myhsok, B. *et al.* (2001) Refinement of the PARK3 locus on chromosome 2p13 and the analysis of 14 candidate genes. *Eur. J. Hum. Genet.*, **9**, 659–666.
24. Pankratz, N., Uniacke, S.K., Halter, C.A., Rudolph, A., Shults, C.W., Conneally, P.M., Foroud, T. and Nichols, W.C. (2004) Genes influencing Parkinson disease onset: replication of PARK3 and identification of novel loci. *Neurology*, **62**, 1616–1618.
25. Dupont, W.D. and Plummer, W.D Jr (1990) Power and sample size calculations. A review and computer program. *Control Clin. Trials*, **11**, 116–128.
26. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.*, **81**, 559–575.