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Genetic variation of *Omi/HtrA2* and Parkinson's disease

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Abstract

Variants in the *Omi/HtrA2* gene have been nominated as a cause of Parkinson's disease. This sequencing study of *Omi/HtrA2* in 95 probands with apparent autosomal dominant inheritance of Parkinson's disease did not identify any pathogenic mutations. In addition, there was no association between common variations in the *Omi/HtrA2* gene and susceptibility to Parkinson's disease in any of our four patient-control series (n=2373). Taken together our results do not support a role for *Omi/HtrA2* variants in the pathogenesis of Parkinson's disease.

Keywords

PARK13; PD; HtrA2; mitochondria; neurodegeneration

Introduction

Mutations in the *Omi/HtrA2* gene were recently designated a novel autosomal dominant Parkinson's disease (PD) locus (*PARK13*) [1]. A heterozygous (p.G399S; ss102660599) substitution resulting in a loss of protein function was identified in four sporadic PD patients indicating reduced penetrance. A second substitution (p.A141S; ss102660598) was associated with PD susceptibility [1].

Omi/HtrA2 knockout and mutant mice present a neurodegenerative phenotype with parkinsonian symptoms or with motor neuron degeneration respectively [2,3]. The HtrA2

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protein is a serine protease which is targeted to the mitochondria, plays a role in apoptosis, and the mutants are reported to increase *in vitro* cell-death during stress conditions [1]. Evidence is also reported that the HtrA2 protein is found in Lewy bodies [1]. The confirmation for the role of *Omi/HtrA2* variants in PD will further support the role of mitochondrial dysfunction in disease and provide a novel target for functional studies, and the development of translational therapeutics [4]. Herein we investigate the frequency of *Omi/HtrA2* mutations in autosomal dominant parkinsonism and the associated risk of common variants with sporadic PD.

Patients and methods

DNA sequencing of familial PD probands

To estimate the frequency of *Omi/HtrA2* mutations in autosomal dominant parkinsonism and identify novel variants, we have sequenced 95 affected probands with a family history of parkinsonism. The average age of onset was 54±11 years (range 30–77 years, 59 men/36 women). Families were referred from movement disorders clinics throughout Europe, North America, North Africa, and Asia (90% Caucasians and 10% Asians). For each kindred, DNA was available from at least two affected family members (74% of the families had 2–5 known affected members, 16% had 6–10, and 10% had more than 10 affected members). Parkinsonism in each pedigree was consistent with an autosomal dominant pattern of inheritance and known mutations in *SNCA* and *LRRK2* were excluded. Each patient was assessed by neurologists specialized in movement disorders. The examination included a full medical history, including family history, and a standard neurological assessment. The Folstein Mini-Mental State Examination (MMSE) was used to assess cognitive function. A clinical diagnosis of PD required the presence of at least two of three cardinal signs (resting tremor, bradykinesia, and rigidity), improvement from adequate dopaminergic therapy, and the absence of atypical features or other causes of parkinsonism [5]. Appropriate institutional review and informed consent was obtained for clinicogenetic research.

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. Primer pairs for all *Omi/HtrA2* exons (NM_012347) were designed with Gene Runner version 3.05 software, and were used to amplify all 8 exons by polymerase chain reaction (PCR) (primer sequences available on request). PCR products were purified from unincorporated nucleotides using a Millipore PCR purification plate. A total volume of 6 μl, containing 20–50 ng of the clean product and 1.6 pM of one of the primers (forward or reverse), was used for sequencing. Electropherograms were analyzed with SeqScape v2.1.1 (ABI, Applied Biosystems, Foster City, CA, USA). If a putative pathogenic variant was observed segregation analysis was performed in the family by testing all available DNA from affected and unaffected family members.

Genetic Association study with PD

Four independent Caucasian PD patient-controls series from the US, Ireland, Norway and Poland were examined. The demographics for each series are displayed in Table 1. All patients were examined and observed longitudinally by a movement disorders neurologist with a PD diagnosis based on published criteria [5]. In the Irish and US series each patient was individually matched based on age (+/- 4 years), gender, and ethnicity to an unrelated control without evidence of neurological disease. The Norwegian and Polish controls are matched only for ethnicity. The ethical review boards at each institution involved approved the study, and all participants provided informed consent.

From our sequencing analysis and reported SNP frequencies we selected four variants (rs10779958, rs1183739, rs2231250 and rs2241028) with a minor allele frequency >5% and the two previously reported mutations (A141S and G399S) to examine in our patient-control

series [7]. Genotyping was performed on a Sequenom MassArray iPLEX platform (San Diego, CA); all primer sequences are available on request. Numerical variables were summarized with the sample mean, standard deviation (SD), and range. For the US and Irish matched series, associations between PD and *Omi/HtrA2* SNPs were measured by odds ratio (OR) and corresponding 95% confidence interval (CI) obtained from single variable conditional logistic regression models. For the Norwegian and Polish series, associations with PD were measured by OR and 95% CI obtained from logistic regression models adjusted for age, sex, and series (combined series only). Haplotype analysis was performed using S-Plus score tests for association [6], with adjustments made for age and gender; *P*-values were obtained from the asymptotic distribution of the score statistic. In PD cases, linear regression models adjusted for sex were used to examine associations between age-at-onset and *Omi/HtrA2* SNPs. Additive models were considered in all single marker analysis. Genotype and allele frequencies were in Hardy-Weinberg equilibrium for all SNPs and inter-marker linkage disequilibrium in study controls was measured by pairwise r-squared values (Figure 1). For each family of statistical tests, a Bonferroni adjustment for multiple testing was made in order to control the family-wise error rate at 5%; *P*-values ≤ 0.01 are considered statistically significant after this adjustment.

Results

Sequencing of *Omi/HtrA2* gene in 95 PD probands revealed a number of known SNPs and non-coding variants, however no putatively pathogenic substitutions were observed. A number of rare non-coding variants were observed including two intronic and four in the 5' and 3' UTRs of *Omi/HtrA2* (Figure 1). These variants were not observed to segregate with disease in the families although one (5'UTR c.-598 G>A) has been demonstrated to influence gene expression *in vitro* and others are predicted in *silico* to functionally effect the *Omi/HtrA2* locus (Figure 2).

The sequencing of probands did not identify the previously reported pathogenic substitution p.G399S. However this putative pathogenic variant was observed in our patient-control series, this mutation was identified in twelve (<1%) control individuals (1 US, 2 Polish, and 9 Norwegian) and four (<1%) PD patients (3 US and 1 Norwegian). These findings refute the proposed role of ss102660599 (p.G399S) as a pathogenic mutation.

An association study was performed on four independent PD patient-control series examining the frequency of five SNPs in the *Omi/HtrA2* gene (rs10779958, rs1183739, rs2231250, rs2241028 and ss102660598 (c.G421T; p.A141S)). There was a trend toward an association between PD susceptibility and rs1183739 in the Irish series (OR: 1.70, P=0.020), but this trend was not observed in any of the other series and was not statistically significant after adjusting for multiple testing. There was no evidence of any other associations between the five SNPs considered and susceptibility to PD (Table 2). There was no significant association with AAO or between haplotypes and disease (data not shown).

Discussion

Genetic forms of parkinsonism have had a major influence on the field, however the nomination of each new loci and pathogenic mutation must be greeted with caution. The first step for all putative pathogenic mutations or genetic associations must be independent confirmation. Mutation of *Omi/HtrA2* was nominated as a novel cause of sporadic PD and a non-synonymous SNP was associated with increased risk of disease. The pathogenicity of *Omi/HtrA2* was supported by the recent identification of a mutation (p.R404W) in one sporadic PD patient [7] and a functional link between the regulation of HtrA2 activity and Pink1, a known cause of recessive forms of PD [8].

The present study set out to investigate the frequency of *Omi/HtrA2* mutations in autosomal dominant forms of PD and the association of common variants with sporadic PD. No pathogenic substitutions were observed within our familial probands. A number of rare non-coding variants were observed including SNP ss99308628 (c.-598 G>A) in the 5'UTR which has previously been predicted to alter stress-related transcription factor binding sites and shown to affect transcription *in vitro* [7].

We observed no significant associations in our PD patient-control series, including the nominated risk factor p.A141S. In addition the previously reported pathogenic mutation p.G399S was observed in both patients and control individuals at a similar frequency (<1%). These findings support the report of Simon-Sanchez and Singleton (2008) who performed a comprehensive sequencing study in 644 PD patients and 828 control samples from the Neurogenetics repository housed at the Coriell Institute for Research and did not identify any pathogenic variants [9]. Taken together these studies do not support a role for the *Omi/HtrA2* gene in PD.

The designation of the *PARK13* locus for *Omi/HtrA2* variants warrants reconsideration. Over the coming years the number of putatively pathogenic genes and variants will increase. This is typified by the recent nomination of *GIGYF2* variants accounting for the *PARK11* locus [10] and also the high number of putatively pathogenic *LRRK2* mutations [11]. Co-segregation in large kindreds or independent consistent replication of significant association with disease susceptibility remain the gold-standards for confirmation of disease loci.

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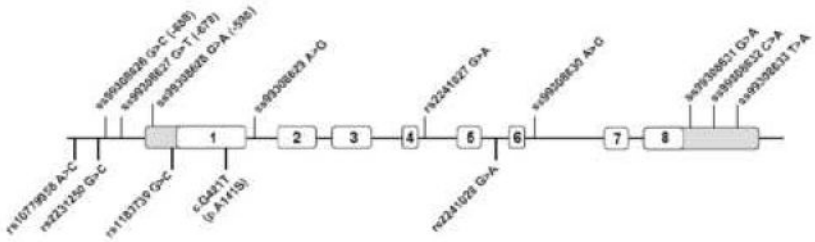
(a)



(b)

Marker	*rs2231250*	*rs1183739*	*G421T*	*rs2241028*
rs10779958	1.000	0.041	0.130	0.280
rs2231250	---	0.040	0.130	0.290
rs1183739	---	---	0.009	0.011
ss102660598 (G421T)	---	---	---	0.510
rs2241028	---	---	---	---

Figure 1. DNA variants identified through sequencing of *Omi/HtrA2* gene and linkage disequilibrium (r^2) between association markers

Ideogram of the *Omi/HtrA2* gene highlighting the variants identified through sequencing of the 95 probands above the gene. Below the diagram are the five variants used to assess the association of common *Omi/HtrA2* gene variation with PD risk. These variants were chosen based on minor allele frequency (>5%) and traverse the gene (5' region to intron5) (b) A presentation of the linkage disequilibrium (r^2) measure between the five common variants in our US control sample chosen to assess association with disease (all four control samples displayed similar r^2 values between the SNPs examined).

(a)

SNP	Genomic Position[a]	Coding DNA Position[b]	Location	*In silico* predicted effect[c]
ss99308626	g.74609955	c.-688 G>C	5'-region	-NF1 (1/0.85) -AP2 (0.83/0.92) -CP2F (1/0.97)
ss99308627	g.74609965	c.-678 G>T	5'-region	-CP2F (1/0.97)
ss99308628	g.74610045	c.-598 G>A	5' UTR	+CREB/ATF (0.75/0.84)
ss99308629	g.74611513	c.712-34 A>G	IVS2	No effect
ss99308630	g.74612991	c.1116-264 G>A	IVS6	No effect
ss99308631	g.74613625	c.+4 G>A	3' UTR	No effect
ss99308632	g.74615005	c.+384 C>A	3' UTR	Alters hsa-miR-589 target sequence
ss99308633	g.74614064	c.+443 T>A	3' UTR	No effect

(b)

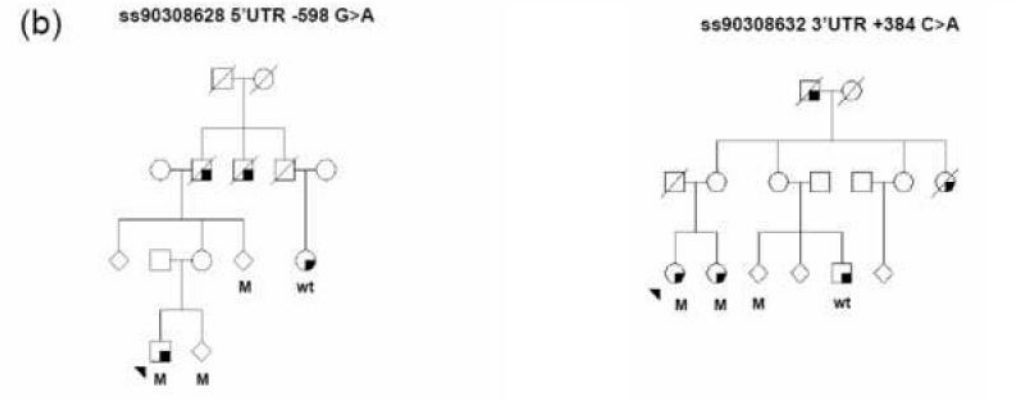


Figure 2. Rare non-coding variants observed in sequencing of *Omi/HtrA2*

The rare variants observed through sequencing in 95 probands with apparent autosomal dominant inheritance of PD. (a)[a] The genomic positions of the of each SNP are generated from the UCSC Genome Browser dbSNP build based on the Human Mar. 2006 Human Genome Assembly. [b] Coding DNA position relative to reference sequence NM_013247 and beginning at the ATG codon (IVS=intronic variable sequence and UTR=untranslated region). [c] *In silico* prediction of transcription factor binding site was performed using MatInspector analysis (Core/Matrix similarity), splicing Alternative Splice Site Predictor (ASSP), and microRNA (miRNA) binding sites by complementarity to the miRNA sequences listed in miRBase. No segregation within our families were observed for these variants, (b) represents the two US pedigrees harboring the UTR variants predicted to alter the functionality of the *Omi/HtrA2* locus. Squares represent males, circles represent females and to protect confidentiality some individuals gender is masked using a diamond. A diagonal line through the icon shows an individual is deceased. The proband is arrowed and a shaded lower right-hand quadrant represents an individual affected by PD. M= mutation carrier and wt= wild-type non-carrier of the mutant allele.

Table 1

PD patient-control series demographics

The sample mean ± SD (minimum, maximum) is given for age and age at onset. Total sample sizes given for each series do not account for genotyping failure, which occurred in <5% of samples.

Series	PD patients	Controls
US	*n=224*	*n=224*
Age	73 ± 10 (38 – 90)	73 ± 10 (38 – 90)
Age at onset	62 ± 12 (26 – 82)	N/A
Familial PD (%)	155 (37)	N/A
Gender (Male)	115 (51%)	115 (51%)
Ireland	*n=174*	*n=174*
Age	61 ± 12 (33 – 90)	61 ± 12 (33 – 90)
Age at onset	49 ± 11 (18 – 77)	N/A
Familial PD (%)	28 (16)	N/A
Gender (Male)	68 (39%)	68 (39%)
Norway	*n=391*	*n=958*
Age	72 ± 11 (30 – 99)	73 ± 11 (43 – 106)
Age at onset	59 ± 11 (25 – 88)	N/A
Familial PD (%)	120 (23)	N/A
Gender (Male)	325 (60%)	538 (47%)
Poland	*n=101*	*n=123*
Age	75 ± 7 (56 – 86)	70 ± 11 (34 – 89)
Age at onset	64 ± 7 (38 – 76)	N/A
Familial PD (%)	13 (13)	N/A
Gender (Male)	40 (40%)	48 (39%)

Table 2

Association results for variants in *Omi/HtrA2* gene and PD

For the matched US and Irish series, estimated odds ratios and *P*-values result from single variable conditional logistic regression models. For the Norwegian and Polish series, estimated odds ratios and *P*-values result from logistic regression models adjusted for age and gender. Estimated odds ratios correspond to an increase of one minor allele. *P*-values ≤ 0.01 are considered statistically significant after a Bonferroni adjustment for multiple testing.

Series/ SNP	US		Ireland		Norway		Poland	
	Patient n (%)	Control n (%)	Patient n (%)	Control n (%)	Patient n (%)	Control n (%)	Patient n (%)	Control n (%)
rs10779958								
AA	162	150	137	126	283	672	78	96
AC	58	69	42	54	101	270	20	24
CC	6	4	5	5	6	16	3	2
A	364	353	316	306	667	1614	176	216
C	64 (15)	75 (18)	52 (14)	64 (17)	113 (14)	302 (16)	26 (13)	28 (11)
OR (95% CI)	0.84 (0.59, 1.19)		0.80 (0.55, 1.17)		0.93 (0.73, 1.19)		1.29 (0.72, 2.32)	
P-value	0.33		0.25		0.56		0.39	
rs2231250								
GG	159	150	137	125	284	671	78	96
GC	56	67	43	54	95	266	20	24
CC	6	4	5	5	6	17	3	2
G	356	351	317	304	663	1608	176	216
C	62 (15)	73 (17)	53 (14)	64 (17)	107 (14)	300 (16)	26 (13)	28 (11)
OR (95% CI)	0.82 (0.57, 1.17)		0.82 (0.56, 1.19)		0.88 (0.69, 1.13)		1.29 (0.72, 2.32)	
P-value	0.28		0.29		0.32		0.39	
rs1183739								
GG	124	153	109	132	266	625	70	75
GC	56	56	62	42	97	245	25	38
CC	5	6	6	5	9	24	1	3
G	287	346	280	306	629	1233	165	188
C	63 (18)	66 (16)	74 (21)	52 (15)	115 (15)	293 (19)	27 (14)	44 (19)
OR (95% CI)	1.14 (0.76, 1.71)		1.70 (1.09, 2.65)		0.95 (0.75, 1.20)		0.68 (0.39, 1.20)	
P-value	0.54		0.02		0.65		0.18	
ss102660598 (G421T)								
GG	199	204	166	169	344	842	91	110
GT	25	19	18	15	28	102	8	11
TT	1	0	0	0	0	4	0	0
G	402	409	350	353	716	1786	190	231
T	24 (6)	19 (4)	18 (5)	15 (4)	28 (4)	110 (6)	8 (4)	11 (5)
OR (95% CI)	1.31 (0.69, 2.52)		1.20 (0.61, 2.38)		0.67 (0.43, 1.02)		1.00 (0.37, 2.72)	
P-value	0.41		0.6		0.06		0.99	
rs2241028								
GG	200	198	161	155	351	827	91	111
GA	23	23	25	30	36	120	10	12
AA	1	1	0	0	1	4	0	0
G	403	402	347	340	738	1774	192	234
A	21 (5)	24 (6)	25 (7)	30 (8)	38 (5)	128 (7)	10 (5)	12 (5)
OR (95% CI)	0.83 (0.46, 1.51)		0.83 (0.48, 1.42)		0.74 (0.50, 1.07)		1.15 (0.46, 2.90)	
P-value	0.55		0.49		0.11		0.76	