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A large-scale genetic association study to evaluate the contribution of *Omi/HtrA2* (PARK13) to Parkinson's disease

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Abstract

High-profile studies have provided conflicting results regarding the involvement of the *Omi/HtrA2* gene in Parkinson's disease (PD) susceptibility. Therefore, we performed a large-scale analysis of the association of common *Omi/HtrA2* variants in the Genetic Epidemiology of Parkinson's disease (GEO-PD) consortium.

GEO-PD sites provided clinical and genetic data including affection status, gender, ethnicity, age at study, age at examination (all subjects); age at onset and family history of PD (patients). Genotyping was performed for the five most informative SNPs spanning the *Omi/HtrA2* gene in approximately 2–3 kb intervals (rs10779958, rs2231250, rs72470544, rs1183739, rs2241028). Fixed as well as random effect models were used to provide summary risk estimates of *Omi/HtrA2* variants.

The 20 GEO-PD sites provided data for 6378 cases and 8880 controls. No overall significant associations for the five *Omi/HtrA2* SNPs and PD were observed using either fixed effect or random effect models. The summary odds ratios ranged between 0.98 and 1.08 and the estimates of between-study heterogeneity were not large (non-significant Q statistics for all 5 SNPs; I^2 estimates 0–28%). Trends for association were seen for participants of Scandinavian descent for rs2241028 (OR 1.41, $p = 0.04$) and for rs1183739 for age at examination (cut-off 65 years; OR 1.17, $p = 0.02$), but these would not be significant after adjusting for multiple comparisons and their Bayes factors were only modest.

This largest association study performed to define the role of any gene in the pathogenesis of Parkinson's disease revealed no overall strong association of *Omi/HtrA2* variants with PD in populations worldwide.

Keywords: Omi; HtrA2; Genetics; Parkinson's disease; PARK13

1. Introduction

The constant decline of genotyping costs and the development of robust analytical methods have contributed to the report of many susceptibility genes for complex diseases (Ioannidis et al., 2009). However, most of these genetic associations have not been replicated consistently across different populations (McCarthy et al., 2008). Indeed for Parkinson's disease (PD), as of April 10, 2009, at least 594 studies of 402 candidate genes and 1892 polymorphisms have yielded only 16 genes with nominally significant associations ($p < 0.05$) upon evaluation of the published data (http://www.pdgene.org) and even these associations are often of borderline significance and almost always correspond to small genetic effects. Furthermore, at least three published genome-wide association studies of PD have queried several hundred thousand SNPs without yielding solidly reproducible associations (Elbaz et al., 2006; Evangelou et al., 2009; Fung et al., 2006; Maraganore et al., 2005; Pankratz et al., 2009). The reasons for non-replication probably include overestimation of effect size and significance by initial studies, small sample sizes, and disease and population heterogeneities (Zondervan and Cardon, 2004, 2007). Given this setting, large multi-centred pooled analyses have gained increasing interest. Large consortia can overcome the above mentioned limitations of genetic association studies and publication biases that may limit the value of meta-analyses (Chanock et al., 2007). For example, a consortium analysis has validated that a REP-1 promoter variant in the *alpha-synuclein* gene (*SNCA*) confers susceptibility to PD in populations worldwide (Maraganore et al., 2006).

A number of genetic linkage studies in high-profile journals have supported the role of mitochondria in the pathogenesis of PD. Mutations in *Parkin* (*PARK2*), *DJ-1*, and *PTEN-induced kinase 1* (*PINK1*) cause autosomal recessive forms of PD, possibly via an impairment of mitochondrial function and/or dynamics (Bonifati et al., 2003; Kitada et al., 1998; Valente et al., 2004). Subsequently, point mutations in the *Omi/HtrA2* gene were reported in PD (Strauss et al., 2005). These mutations cause a loss of serine protease function of the human Omi/HtrA2 protein *in vitro*, and paralleled observations in rodents, where loss of Omi/HtrA2 function was critically related to neurodegeneration mimicking relevant clinical aspects of parkinsonism (Jones et al., 2003; Strauss et al., 2005). In this context, *in vitro* and

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in vivo studies revealed mitochondrial dysfunction in loss of function models of Omi/HtrA2 as a primary mechanism for neurodegeneration (Jones et al., 2003; Strauss et al., 2005).

Subsequently, Bogaerts and colleagues performed an *Omi/HtrA2* mutation analysis in a large Belgian cohort. They identified a novel amino acid exchange that was not observed in controls, leading to potential stabilization of the inactive Omi/HtrA2 protein (Bogaerts et al., 2008). Moreover, the authors identified nine patients carrying specific heterozygous mutations in the 5′ and 3′ regulatory region, suggesting that mutations in the *Omi/HtrA2* promoter affect the transcriptional activity of the gene (Bogaerts et al., 2008). However, a large sequence-based study on a North-American PD patient-control series detected the original pathogenic *Omi/HtrA2* variants in healthy control subjects (Simon-Sanchez and Singleton, 2008); whether this occurrence is due to reduced penetrance as observed for other PD mutations was unclear.

Therefore, to determine the relevance of *Omi/HtrA2* variation in susceptibility to PD, we conducted the largest study in terms of number of participants ever conducted in PD genetics for any gene. Our study is a large multi-centre study which includes over 15,000 subjects from 20 sites representing 14 countries and 4 continents.

2. Methods

2.1. Sampling

As of April 10, 2009 the Genetic Epidemiology of Parkinson's Disease (GEO-PD) consortium includes investigators from 35 sites representing 22 countries, and six continents. Together, we have pledged to share clinical and genetic data and DNA bio specimens for more than 20,000 PD cases and 20,000 controls, when considering the anticipated total recruitment of all sites by the end of 2010. All GEO-PD sites were invited to participate in this study. A total of 20 teams representing 14 countries and 4 continents agreed to participate and contributed clinical and genotypic data for a total of 15,258 individuals (6378 cases and 8880 controls). Of these 2372 individuals were part of a previous study (Ross et al., 2008).

2.2. Genotyping

A total of five SNPs were selected for genotyping: rs10779958, rs2231250, rs72470544, rs1183739 and rs2241028, listed in order from 5′ to 3′ end of the gene. It has been shown previously that tagSNPs selected from HapMap sufficiently capture the common genetic variation in different populations (de Bakker et al., 2005). Therefore, the HapMap was used to select tagSNPs with following criteria: (i) SNP with an r^2 threshold of 0.8, (ii) coding SNPs or (iii) SNPs in the 5′ and 3′UTR. Apart from that, the gene is enriched with rare variant(s) which were described previously (Strauss et al., 2005). Genotyping was done either by each site, a combination of sites, or by a commercial contract. The genotyping platforms employed include TaqMan® SNP Genotyping Assays (Applied Biosystems Inc.), MassARRAY™ Analyzer Compact (Sequenom) and Illumina GoldenGate (Illumina Inc.). Data from sites that showed missing rates >5% were excluded from further analyses. An exact test was used to test for Hardy Weinberg equilibrium (HWE) distortion in controls. Deviation from HWE was considered significant for $p < 0.05$. We observed deviations for different SNPs and different sites. To control for potential genotypic error, we asked sites that provided data for SNPs that were not in HWE to repeat the genotyping. Site-specific data for SNPs that still deviated significantly from HWE were removed from further analysis.

2.3. Statistical analysis

Effect estimates based on major versus minor allele contrast were computed. Results were then synthesised across different sites using both fixed effect and random effect models. Fixed effect models assume that the odds ratio is the same in all sites and that observed differences are due to chance alone. Random effect models allow that the odds ratios might be different due to heterogeneity across different sites. Random effect calculations take into account the estimated between-study heterogeneity. Heterogeneity between sites was tested using the χ^2-based Q statistic and was also assessed with the I^2 metric, which ranges from 0% to 100% I^2 is estimated by the ratio $(Q–df)/Q$, where Q is χ^2 based statistic and df is degree of freedom. Typically estimates of $I^2 < 25\%$ are considered to reflect little or no heterogeneity, 25–50% moderate heterogeneity, 50–75% large heterogeneity and >75% very large heterogeneity. It should be acknowledged that I^2 can have large uncertainty in its estimation (Ioannidis et al., 2007), especially for variants with low minor allele frequency. Thus it represents a tentative working interpretation for heterogeneity.

The main analyses considered all sites and populations regardless of ancestry. In secondary analyses, we examined separately populations of Caucasian versus Asian descent due to relevant differences in genotypic distribution; and further evaluated within Caucasians specifically in populations of Scandinavian descent. Omi/HtrA2 is located on chromosome 2p close to the PARK3 locus that was previously linked to families with Northern European descent (OMIM %602404). We also evaluated subgroup effects for PD with age at examination (cut-off 65 years).

Given that 5 SNPs were tested, nominal statistical significance was claimed for a threshold of $p < 0.01$ (i.e. 0.05/5). Power calculations showed that with 6000 cases and 9000 controls, our study would have at least 86% power to detect

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Table 1
Description of datasets contributed by each study site.

Site	Country	Total sample (*N*)	Case	Control	Male (%)	Mean age of onset	Mean age at study	Familial PD (*n*)	Diagnostic criteria
Annesi	Italy	368	184	184	176 (47.8)	60.09	64.77	NA	UKPDBB
Brice	France	545	293	252	323 (59.2)	47.53	57.79	NA	UKPDBB
Elbaz	France	710	209	501	415 (58.4)	63.49	66.93	45	Bower
Ferrarese	Italy	182	87	95	108 (59.3)	61.17	64.48	15	Gelb
Lynch	Ireland	369	184	185	145 (39.2)	49.55	63.34	21	UKPDBB
Opala	Poland	223	101	122	145 (65.0)	63.68	72.41	13	UKPDBB
Farrer/Wszolek	US	536	312	224	308 (57.4)	63.85	73.67	102	UKPDBB
Klein	Germany	458	226	193	227 (49.5)	48.51	61.28	NA	UKPDBB
Maraganore	US	2155	1076	1079	1299 (60.2)	61.64	66.87	847	Bower
Mellick	Australia	547	273	274	270 (49.3)	62.0	67.95	107	Bower
Aasly	Norway	1349	391	958	677 (50.1)	58.03	72.65	97	UKPDBB
Valente	Italy	288	192	96	143 (49.6)	58.30	67.87	2	UKPDBB
Wirdefeldt	Sweden	273	95	178	142 (52)	65.88	75.20	69	Gelb
Hadjigeorgiou	Greece	600	300	300	343 (57.1)	64.36	69.64	NA	Bower
V. Broeckhoven	Belgium	539	266	273	310 (57.5)	59.80	65.14	38	Pals/Engelborghs
Sharma/Gasser	Germany	1700	678	1020	977 (57.4)	55.33	54.94	NA	UKPDBB
Toda	Japan	3509	988	2521	NA	NA	NA	NA	UKPDBB
Hattori	Japan	373	240	133	NA	37.76	47.46	NA	UKPDBB
Tan	Singapore	368	179	189	173 (47.0)	61.44	66.52	NA	UKPDBB
Lin	Taiwan	200	100	100	102 (51)	59.29	64.59	6	UKPDBB
Total		**15298**	**6378**	**8879**		**59.72**	**65.66**		

UKPDBB, United Kingdom Parkinson's Disease Brain Bank; NA, not available.

an allele-based odds ratio of 1.15 for minor allele frequencies of 10% or higher for alpha = 0.01. Power would be only 20% for a minor allele frequency of 2% and odds ratio of 1.15, but it would be 78% for the same minor allele frequency of 2% and odds ratio of 1.3. Furthermore, for associations with uncorrected $p < 0.05$, we also expressed the strength of the putative associations by calculating the respective Bayes factor, assuming that average genetic effects for PD susceptibility with common variants may reflect odds ratios of 1.30 and using a lump-and-smear prior, as described by Ioannidis (Ioannidis, 2008).

Meta-analyses were performed using Review Manager 4.2.7 and STATA 10.0 (Stata Corp., College Station, TX, USA). Reporting of methods and results follows the STREGA guidance (Little et al., 2009).

Table 2
Allele frequencies of the five SNP (rs10779958, rs2231250, rs1183739, rs2241028 and rs72470544) in cases and controls for each site.

Sites	Cases (major /minor allele)					Controls (major /minor allele)				
	rs10779958	rs2231250	rs1183739	rs2241028	rs72470544	rs10779958	rs2231250	rs1183739	rs2241028	rs72470544
Annesi	306/56	301/55	327/35	348/14	NA	293/67	292/68	318/44	352/14	NA
Brice	501/85	499/87	NA	551/33	569/15	434/70	434/70	NA	479/25	499/5
V. Broekhoven	428/94	423/93	446/78	491/39	511/15	448/80	450/86	453/91	515/31	533/13
Elbaz	357/57	357/57	354/62	399/17	409/7	839/145	839/145	855/133	936/62	966/20
Ferrarese	145/27	144/28	NA	165/7	171/1	154/34	153/35	NA	179/9	185/3
Lynch	316/52	317/53	280/74	347/25	350/18	306/64	304/64	306/52	340/30	353/15
Opala	176/26	176/26	165/27	192/10	190/8	216/28	216/28	188/44	234/12	231/11
Farrer/Wszolek	524/100	514/98	431/87	585/39	586/40	371/77	369/75	363/69	421/25	429/19
Klein	381/71	384/68	377/75	432/20	592/8	379/85	380/84	386/78	425/39	598/2
Maraganore	1845/307	1846/306	1814/338	2024/128	NA	1853/305	1853/305	1819/339	2023/135	NA
Mellick	444/94	442/94	458/82	503/37	NA	441/85	445/85	450/82	502/34	NA
Aasly	667/113	663/107	629/115	738/38	716/28	1614/302	1608/300	1495/293	1774/128	1786/110
Valente	300/62	300/62	320/42	344/18	350/12	124/30	124/30	135/19	142/12	147/7
Larrisa	470/114	472/114	512/74	NA	592/8	500/90	508/92	512/84	NA	598/2
Wirdefeldt	159/27	162/26	150/38	183/7	151/5	303/53	301/51	287/55	335/21	301/7
Sharma/Gasser	1114/210	1144/212	1176/182	1282/76	1315/45	1731/299	1727/295	1694/330	1912/116	1953/69
Toda	1684/5903	NA	NA	1527/5489	NA	292/1115	NA	NA	407/1487	NA
Hattori	400/618	NA	NA	NA	NA	80/128	NA	NA	NA	NA
Tan	289/611	NA	NA	319/653	NA	69/127	NA	NA	39/85	NA
Lin	165/343	165/343	NA	169/334	NA	29/47	29/47	NA	25/56	NA

NA: not applicable either due to significant HWE deviations or genotype failure that led to the exclusion of these data from further analyses.

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3. Results

3.1. Database

Twenty sites contributed 6378 cases and 8880 controls combined (Table 1). Most of the cases were of Caucasian ancestry (71.5%), but some were of Asian ancestry (28.5%). No subjects of African ancestry were included in the study, since a Nigerian site of GEO-PD could not contribute data and a South African site only recently joined the GEO-PD consortium. To maintain homogeneity in the Caucasian sample, 0.2% of those subjects who reported a mixed ancestry were removed from further analysis. The proportion of men and women ranged from 42% to 58% across different participating sites (Table 1). The median age at onset of PD in our studied population was 61 years and the median age at study in the overall sample was 67 years.

The distribution of allele frequencies for each SNP and at each participating site is shown in Table 2. The genotypes were in HWE in controls for most of sites and for most of the SNPs, however, data from a total of 5 sites had to be excluded either due to HWE deviation or failure to genotype SNP rs72470544 (two sites showed HWE distortion and genotyping was not successful for remaining three sites) and 4 sites were excluded similarly for rs1183739 (two sites showed HWE distortion and genotyping was not successful for the remaining two sites).

3.2. Overall results

The overall results did not reveal nominally significant associations for any of the SNPs with PD, either for random effect or fixed effect models (Table 3). The odds ratios ranged from 0.98 to 1.02 (Fig. 1) and no p-value was less than 0.07, even without adjustment for 5 comparisons. We observed no substantial heterogeneity for three SNPs: rs2241028, rs2231250 and rs10779958 (I^2 estimates ranged from 0% to 8%), while for rs1183739 and rs72470544 the I^2 estimates were larger (23% and 28%, respectively), but not significant. The Q test was non-statistically significant for all 5 SNPs.

3.3. Subgroup analyses

Subgroup analyses on Caucasian versus Asian ancestry samples did not reveal any subgroup differences that would be beyond chance (data not shown). We observed a non-significant trend for association with SNP rs2241028 in our Caucasian samples ($p = 0.07$ uncorrected for multiple comparisons). When restricting the analysis to the Scandinavian sites, including Sweden and Norway, the odds ratio was 1.44 (95% CI, 1.02–2.02, $p = 0.04$ uncorrected for multiple comparisons). Furthermore, we identified a subgroup of 238 subjects (143 cases and 95 controls) of only Swedish or Norwegian descent from our North-American site (Mayo Clinic /Maraganore). Incorporating these data also

Table 3
Summary effect estimates and confidence interval for Omi/HtrA2 gene.

SNP	Overall				Scandinavian			Age at examination*		
	Sites	RE OR (95% CI)	FE OR (95% CI)	Het. P (I^2)	RE OR (95% CI)	FE OR (95% CI)	Het. P (I^2)	RE OR (95% CI)	FE OR (95% CI)	Het. P (I^2)
rs72470544	13	0.98(0.77–1.26)	1.02(0.84–1.22)	0.17(28%)	1.27(0.63–2.56)	1.45(0.97–2.15)	0.20(39%)	0.96(0.72–1.27)	0.96(0.72–1.27)	0.35(9%)
rs10779958	20	0.99 (0.93–1.06)	0.99(0.93–1.06)	0.41(3%)	1.02(0.82–1.27)	1.03(0.85–1.25)	0.33(9%)	1.03(0.91–1.16)	1.03(0.92–1.17)	0.56(0%)
rs2231250	17	1.01(0.94–1.09)	1.02(0.94–1.09)	0.49(0%)	1.03(0.79–1.34)	1.07(0.87–1.30)	0.27(24%)	1.02(0.91–1.16)	1.03(0.91–1.16)	0.56(0%)
rs1183739	14	1.03(0.93–1.13)	1.04(0.96–1.12)	0.20(23%)	1.01(0.83–1.23)	1.01(0.83–1.23)	0.39(0%)	**1.17(1.03–1.33)**	**1.17(1.03–1.33)**	0.76(0%)
rs2241028	18	1.07(0.99–1.17)	1.08(0.99–1.17)	0.58(0%)	**1.41(1.02–1.94)**	**1.41(1.02–1.94)**	0.90(0%)	1.05(0.76–1.44)	1.01(0.81–1.26)	0.18(36%)

FE, fixed effects; RE, random effects; CI, confidence interval; Het., heterogeneity (Q statistic); (*) age at examination <65 years; bold: $p < 0.05$.

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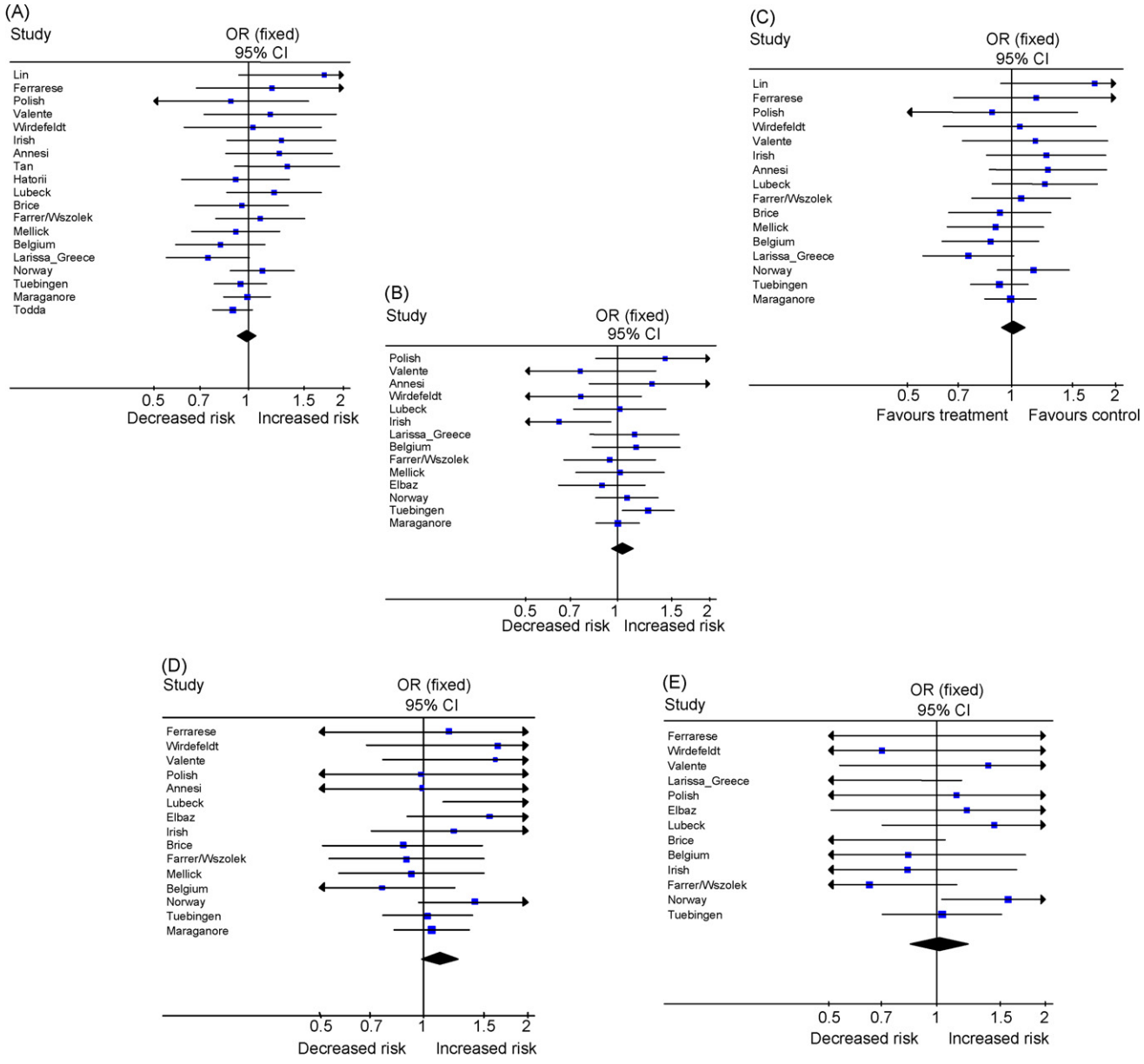

Fig. 1. A panel showing the forest plot for each SNP. The summary effect estimate is indicated by the diamond (A) Forest plot for SNPs rs10779958, (B) rs1183739, (C) rs2231250, (D) rs2241028 and (E) rs72470544.

into the analysis of Scandinavian descent population lead to marginal change in odds ratio from 1.44 to 1.41 (95% CI, 1.02–1.94, $p = 0.04$ uncorrected for multiple comparisons). Another SNP (rs72470544) also had a trend for association by fixed effects only in Scandinavian populations, but this was not formally significant (odds ratio 1.45, $p = 0.07$ uncorrected). These effects were not different beyond chance compared with the effects in non-Scandinavian ancestry participants.

Finally, we observed a nominally significant association of SNP rs1183739 with age at examination (cut-off 65 years) (odds ratio 1.17 95% CI 1.03–1.33; $p = 0.02$), but this was also not significant when corrected for multiple comparisons and the difference against non-young age at examination (odds ratio 0.97 95% CI 0.87–1.07; $p = 0.52$) was not beyond chance.

For both the rs2241028 and rs1183739 subgroup effects in Scandinavian descent populations and young onset PD, the estimated Bayes factors were 0.4, suggesting that the nominally significant results increased 2.5-fold (1/0.4) the odds that the association was true compared with before running the study.

4. Discussion

Our study revealed no evidence for an overall association of common variants in the *Omi/HtrA2* gene with PD

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across populations worldwide. Our generally "negative" findings employing a candidate gene approach are also consistent with the findings of three published genome-wide association studies of PD, none of which highlighted *Omi/HtrA2* as a susceptibility gene in PD (Fung et al., 2006; Maraganore et al., 2005; Pankratz et al., 2009).

Our subgroup analyses should be interpreted with caution, however, we cannot fully exclude the possibility that Omi/HtrA2 may confer susceptibility to PD in persons of Scandinavian descent. As shown in a large study on genetic variation among Europeans the geographic distribution of a given sample needs to be considered during the evaluation of genetic association studies (Novembre et al., 2008). In this context the observed trend for marker rs2241028 as a susceptibility factor for PD in Caucasians was more pronounced in the Scandinavian population and was also seen when we included participants of Scandinavian descent from the USA. The same tentative level of support is offered by the data for the borderline association of rs1183739 in the subgroup of PD patients with an earlier age at examination. The results on both of these subgroup effects increase the odds of these associations being true only by about 2.5-fold compared with before running the study, therefore they are still very likely to reflect false-positive results in subgroup analyses, but they should not be entirely dismissed and it would be interesting to have some additional studies in Scandinavian populations and young-age PD respectively.

Our genetic study shows, that as for other established PD genes, i.e. Parkin (PARK2) and PINK1 (PARK6), also for Omi/HtrA2 no clear genetic association of sequence variations with PD was observed among different populations. By contrast *in vitro* and *in vivo* studies strongly implicate loss of Omi/HtrA2 protein in disrupted mitochondrial homeostasis and subsequent cell death; these studies have included a knockout mouse model that recapitulated a range of relevant clinical aspects of PD (Jones et al., 2003; Strauss et al., 2005). Moreover, recent pathoanatomical studies indicate that Omi/HtrA2 represents a consistent pathological marker for neurodegeneration in different alpha-synucleinopathies (Kawamoto et al., 2008). Loss of Omi/HtrA2 function may contribute to a broader spectrum of neurodegeneration, as decreased levels of Omi/HtrA2 were demonstrated in brains of Huntington's disease patients (Inagaki et al., 2008). Therefore, while our genetic association studies provide no consistent support for an association of *Omi/HtrA2* and PD, functional studies suggest that further study of this gene in the context of neurodegenerative disorders is justified. We cannot exclude the possibility that other neurodegenerative diseases besides PD may be influenced by Omi/HtrA2 variations. Moreover, we should acknowledge that here we have examined SNPs that have allele frequencies of 2% or higher. We cannot exclude the possibility that rare mutations with frequency of 1% or less may associate with PD.

Acknowledging these caveats, our study is the largest in sample size conducted on PD genetics for any gene to date and the results are strong enough to suggest that these variants are unlikely to be a clinically important determinant of PD risk. For the most uncommon variants among those examined (those with minor allele frequency in the range of 2%), we cannot exclude subtle associations, but power was adequate to exclude odds ratios of 1.30 or higher even for these relatively uncommon variants. Our approach demonstrates the utility of large-scale consortia in clarifying controversial associations that have been hotly debated in the literature.

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