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Phactr2 and Parkinson's disease

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Abstract

Attempts at replicating the first genome-wide association study (GWAS) in Parkinson's disease (PD) have not successfully identified genetic risk factors. The present study reevaluates data from the GWAS and focuses on the SNP (rs11155313, located in the *Phactr2* gene) with the lowest *P*-value in the Tier 2 patient-control series of the first PD GWAS. We employed four case-control series to examine the nominated SNP rs11155313 and identified association in US (OR: 1.39, *P*=0.032), Canadian (OR: 1.41, *P*=0.014) and Irish (OR: 1.44, *P*=0.034) patient-control series, but not in the Norwegian series (OR: 1.15, *P*=0.27). When combining all four series the observed trend was statistically significant (OR: 1.30, *P*<0.001). This study shows reappraisal of publicly available results of GWAS may help nominate new risk factors for PD.

Keywords

Genome-wide association; Parkinson's disease; *Phactr2*

Introduction

Genome-wide association studies (GWAS) may hold the most promise in the mapping of causative/modifying genetic loci in complex disorders. The first GWAS in Parkinson's disease (PD) was reported using a two-tiered approach with an initial screen of ~200,000 SNPs in a cohort of 443 discordant sib-pairs (Tier 1) [14]. A matched unrelated patient-control series (n=664) was then used in the replication study (Tier 2) to assess the influence of SNPs identified in Tier 1 (*P*<0.01). Maraganore and colleagues reported the eleven most significant SNPs that generated the lowest overall *P*-values from the combined analysis of the two tiers. Independent

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studies by our group and others have not been able to replicate the association of the nominated SNPs [3,5,6,12,13,15], therefore reanalysis of the recently released raw data may be warranted.

Notably neither SNP with the most significant P -values in either Tier 1 or 2 were among those nominated. The SNP with the lowest P -value in Tier 1 (rs3746736; $P=1.3\times 10^{-5}$) is located in the Type II Cystatin gene cluster containing the *Cystatin C* gene (aka *Cystatin 3*). *Cystatin C* is located on Chromosome 20 (23.56-23.57Mb) and contains 3 exons (mRNA 775bp; 146 amino acids). Mutation of the *Cystatin C* gene is a known cause of amyloidopathy and a linkage peak of late-onset Alzheimer's disease is also located over this gene cluster [10,11]. Common variation has been associated with Alzheimer's disease and it is a top hit of meta-analysis on ALZforum (<http://www.alzforum.org/res/com/gen/alzgene/default.asp>)[2]. However SNP rs3746736 was also examined in the second GWAS in PD patients by Fung and colleagues [8] and showed no association with disease. The SNP with the lowest P -value in the study of Fung et al. (rs10501570; Chr11q14; $P=4.9\times 10^{-4}$) was also examined in Tier 1 of the Maraganore et al. study and demonstrated no significant association with PD ($P=0.53$).

The most significant SNP in Tier 2 of Maraganore et al., a matched patient-control series, is located in a gene *Phactr2* (KIAA0680; Chr6q24.2), encoding protein phosphatase 1 and actin regulator 2 (rs11155313; $P=1.5\times 10^{-5}$). This SNP was not included in the GWAS of Fung et al. and has never been replicated in a population-based patient-control series. The reason this SNP was not nominated in the original study is because it did not replicate in the combined analysis of both tiers. Herein we investigate the association of rs11155313 with PD in four Caucasian patient-control series from the US, Canada, Norway, and Ireland.

Subjects and Materials

The US series included 251 patients (51% male) with a mean age of 71 ± 10 years (range 36-87 years; age-at-onset 62 ± 12 years; range 23-85), the Norwegian series included 312 patients (59% male) with a mean age of 73 ± 11 years (range 47-99; age-at-onset 58 ± 11 years; range 25-88), and the Irish series included 186 patients (39% male) with a mean age of 61 ± 12 years (range 33-90; age-at-onset 50 ± 11 years; range 18-77). In the US, Norwegian and Irish series each patient was individually matched based on age (± 4 years), gender, and ethnicity to an unrelated control without evidence of neurological disease. The Canadian series (61% male) included 461 patients with a mean age of 73 ± 10 years (range 43-94; age-at-onset 62 ± 11 years; range 28-88) and 247 controls subjects (30% male) with a mean age of 67 ± 13 years (range 20-94). All patients were examined and observed longitudinally by a movement disorders neurologist and diagnosed with PD according to published criteria [9]. The ethical review board at each institution involved approved the study and all participants provided informed consent.

SNP genotyping was performed using TaqMan chemistry on an ABI7900 and analyzed with SDS 2.2.2 software. In the US, Norwegian and Irish series (individually matched for age and gender) where genotype data was only available for one sample of a matched pair, the other subject was retained in the analysis; however, this occurred in less than 5% of matched pairs. For the controls in each population, χ^2 tests of Hardy-Weinberg equilibrium (HWE) were examined. For the Irish, US, and Norwegian matched series, association between PD and rs11155313 was measured by odds ratios (OR's) and corresponding 95% confidence intervals (CI's) obtained from single variable conditional logistic regression under an additive model. For the Canadian and combined series, association between PD and rs11155313 was measured by OR's and 95% CI's obtained from logistic regression models adjusted for age, gender, and series under an additive model (combined series only).

Results and Discussion

Our analysis demonstrated an association between SNP rs1155313 and susceptibility to PD within the US (OR: 1.39, $P=0.032$), Canadian (OR: 1.41, $P=0.014$) and Irish (OR: 1.39, $P=0.034$) patient-control series (Table). However, this result was not replicated in the Norwegian patient-control series, although a trend in the same direction was observed (OR: 1.15, $P=0.27$). A combined analysis of the four series showed a significant association between rs1155313 and susceptibility to PD (OR: 1.30, $P<0.001$).

The original study of Maraganore et al. observed an increase in the minor allele frequency of rs1155313 in Tier 1 examining 443 discordant sib-pairs (32% in affected siblings versus 27% in unaffected subjects; OR 1.67; $P=2.17 \times 10^{-3}$). However, a highly significant decrease in the minor allele frequency of SNP rs1155313 was observed in Tier 2 in their sporadic PD patients (27% versus 37% in controls; OR 0.58; $P=1.54 \times 10^{-5}$). It was this opposite direction of effect that was the reason it was not nominated in the original study. These findings would suggest the positive association observed with SNP rs1155313 in Tier 1 and the present study should be treated with caution. In addition, the study by Fung et al. did not find an association between *Phactr2* genetic variability and risk of PD. However, they did not include rs1155313, nor any of the four other *Phactr2* SNPs in strong LD ($r^2 \geq 0.8$) with rs1155313.

The *phactr* family of proteins contains four members (*phactr* 1-4) which are abundantly expressed in the nervous system [1]. There is very little known about the function of these proteins but they are suggested to regulate protein phosphatase 1 and bind to cytoplasmic actin. The *phactr1* member has also been shown to be expressed at high levels in the cortex, hippocampus and striatum, with an increased presence at the synapse [1]. The role of kinase genes in PD is established (*PINK1* and *LRRK2*), but the dysregulation of phosphatase activity may have a similar effect.

Recent results in *LRRK2*-associated parkinsonism have identified common variants in PD. In the ethnic Chinese population of Taiwan, the risk factors *Lrrk2* G2385R and R1628P have been observed at 3-5% in the healthy control population while increasing to 6-10% of sporadic patients [4,7,16]. It is also worth noting that these functional variants on the whole appear absent in other populations, both *Lrrk2* G2385R and R1628P have not been observed in Caucasian populations.

Given the identification of genetic risk variants in PD, our study supports a reappraisal of publicly available GWAS' results in PD which may lead to a better understanding of genes implicated in PD risk. If this association is confirmed, further studies are needed to better characterize the role of *Phactr2* in PD.

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Genotype and allele frequencies for rs11155313

In the original GWAS rs11155313 minor allele frequency was increased in patients in Tier 1 (0.32 versus 0.27; OR 1.67; *P*-value 2.17 × 10⁻³) and decreased in patients in Tier 2 (0.27 versus 0.37; OR 0.58; *P*-value 1.54 × 10⁻⁵). Odds ratios in the present study correspond to an increase in one copy of the minor allele (G). Allele and genotype frequencies have been adjusted to account for individuals missing genotype data. For the matched US, Irish, and Norwegian series, estimated odds ratios and *P*-values result from single variable conditional logistic regression models. For the Canadian series, the estimated odds ratio and *P*-value results from a logistic regression model adjusted for age and gender.

Table

Series/rs11155313	AA	AG	GG	G (%)	OR (95% CI)	P
US						
Controls	118	103	17	137 (29)	1.39 (1.03 – 1.88)	0.032
Patients	103	111	28	167 (35)		
Canada						
Controls	122	95	14	123 (27)	1.41 (1.07-1.85)	0.014
Patients	201	193	50	293 (33)		
Norway						
Controls	136	125	27	179 (31)	1.15 (0.89 – 1.49)	0.270
Patients	125	131	35	201 (35)		
Ireland						
Controls	92	73	12	97 (27)	1.44 (1.03 – 2.02)	0.034
Patients	80	74	26	126 (35)		
Combined						
Controls	468	396	70	536 (27)	1.30 (1.13-1.48)	<0.001
Patients	509	509	139	787 (33)		