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Replication of association between *ELAVL4* and Parkinson disease: the *Gene*PD study

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Abstract

Genetic variants in embryonic lethal, abnormal vision, Drosophila-like 4 (*ELAVL4*) have been reported to be associated with onset age of Parkinson disease (PD) or risk for PD affection in Caucasian populations. In the current study we genotyped three single nucleotide polymorphisms in *ELAVL4* in a Caucasian study sample consisting of 712 PD patients and 312 unrelated controls from the *Gene*PD study. The minor allele of rs967582 was associated with increased risk of PD (odds ratio = 1.46, nominal *P* value = 0.011) in the *Gene*PD population. The minor allele of rs967582 was also the risk allele for PD affection or earlier onset age in the previously studied populations. This

replication of association with rs967582 in a third cohort further implicates *ELAVL4* as a PD susceptibility gene.

Introduction

Parkinson disease (PD) is a complex neurodegenerative disorder with both environmental and genetic factors contributing to risk. Specific mutations in several genes have been identified which cause rare familial forms of PD including *SNCA* (Park1) (Polymeropoulos et al. 1997), *Parkin* (Park2) (Kitada et al. 1998), *PINK1* (Park6) (Valente et al. 2004), *DJ1* (Park7) (Bonifati et al. 2003), and *LRRK2* (Park8) (Paisan-Ruiz et al. 2004). The role that these genes play in idiopathic PD is an area of active investigation, with *LRRK2* being the most common.

Embryonic lethal, abnormal vision, Drosophila-like 4 (*ELAVL4*) (aliases HUD, PNEM) is located on chromosome 1p in a region [PARK10] linked to late onset PD in an Icelandic population (Hicks et al. 2002) and to age at onset of PD in a set of families ascertained from the US and Australia (Li et al. 2002). Follow-up studies in the US and Australian cohorts revealed evidence of association between single nucleotide polymorphism (SNP) markers in *ELAVL4* and age at onset of PD (Noureddine et al. 2005). Subsequent examination in distinct cohorts identified association between *ELAVL4* and risk for PD in an Irish case-control cohort, but not in Norwegian or US samples (Haugarvoll et al. 2007). Given Celtic origins in the Icelandic population this may suggest an Irish founder effect of the *ELAVL4* association (Haugarvoll et al. 2007).

ELAVL4 is a human homologue of the Drosophilia gene elav, which is implicated in neuronal differentiation and maintenance. The ELAVL proteins are mRNA binding proteins and all members of the family contain three RNA-recognition motifs. Among other targets ELAVL4 binds to tau (Aranda-Abreu et al. 1999), which has been previously implicated in PD (see Healy et al. (2004) for meta analysis).

We examined association between *ELAVL4* and the outcomes PD affection and onset age in the *Gene*PD cohort.

Subjects and methods

Subjects

PD cases from families with multiple affected individuals were ascertained from 30 clinical sites as part of the *Gene*PD Study. Neurologists from the participating sites examined and confirmed the diagnosis of each PD affected individual included in the study as previously described (Maher et al. 2002). Diagnostic criteria for PD were based on the United Kingdom PD Society Brain Bank Criteria (Gibb and Lees 1988) with slight modification. For example, multiple affected family members and head trauma were not considered exclusionary criteria. PD patients who were known to carry two parkin mutations (Sun et al. 2006) or a LRRK2 mutation were not included in the current analysis. The controls include Caucasian individuals recruited by the GenePD sites, which include some spouses, as well Caucasian controls recruited independently of the GenePD families. A set of 712 Caucasian PD patients from 404 families (55.5% male; mean onset age 60.8 ± 11.8) and 312 Caucasian unrelated controls (52.6% male; mean age at DNA collection 62.6 ± 11.0) were available for association testing. All participants signed informed consent forms and the study was approved by the Institutional Review Board at each of the participating clinical sites.

Genotype data

Tag SNPs with minor allele frequency >0.05 were selected to cover the region where previous association had been observed (Noureddine et al. 2005). Tag SNPs were replaced by previously published SNPs when there was substantial linkage disequilibrium between the Tag SNP and previously published SNP. Three SNPs in *ELAVL4* (see Table 1) were genotyped in all individuals using TaqMan technology on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA). Call rates were 0.95, 0.93, and 0.93 for rs967582, rs3902720, and rs12024093, respectively and all three SNPs were in Hardy–Weinberg equilibrium in controls subjects ($P > 0.05$).

ELAVL4 association analysis

Linear and logistic models were used to assess the association between the genotype data in the *ELAVL4* SNPs and age at onset of PD and PD affection status, respectively. For all analyses generalized estimating equations were employed to account for the non-independence of observations from within the same family. Analyses of age at onset included PD affected individuals only. Additive, dominant and recessive genetic models were considered. Analyses for PD affection and onset age were performed both with and without covariates in the model. Covariates included in the adjusted model for affection status were sex, age, and smoking defined as “ever smoked” (individuals who reported having, prior to the onset of PD symptoms, smoked regularly for at least 1 year) or “never smoked” (individuals who did not meet the ever smoked criteria). Covariate information was missing for some subjects and there were 662 PD and 285 control individuals included in the adjusted analysis for affection status. For the age at onset outcome, a single covariate indicating whether or not the subject was a heterozygote for a parkin mutation (Sun et al. 2006), was utilized and 676 PD subjects were included in the adjusted analysis.

Meta analysis of previously published results (Haugarvoll et al. 2007; Noureddine et al. 2005) and the current study examining SNP rs967582 and PD affection status was performed by combining *P* values as implemented in the program Metal (http://www.sph.umich.edu/csg/abecasis/metal/). Specifically, for each SNP a Z statistic was computed for each study based on the study specific *P* value and direction of the estimated effect. An overall Z statistic (and then corresponding *P* value) was computed as a weighted average of the study specific Z statistics, with the weights proportional to the square root of the number of individuals within each study. Weights were based on sample size defined as the number of subjects included in case control studies or the number of families used in the pedigree disequilibrium test. This method of meta analysis does not provide a summary effect measure but allows combination of association measures from different study designs such as the case-control (Haugarvoll et al. 2007) and family based designs (Noureddine et al. 2005) used in prior analysis of this region. No information about the direction of effect was provided for one population (Noureddine et al. 2005). Meta analysis was performed first assuming the direction of the effect was the same as the other studies and again, conservatively assuming the opposite direction from the other studies. Age at onset results were available for only two of the five populations and meta analysis was not performed for that outcome.

Results

Association results for the three *ELAVL4* SNPs are shown in Table 1. Significant association was observed with either a dominant ($P = 0.011$) or additive model ($P = 0.021$) for PD affection status and SNP rs967582. Significant association remained after covariate adjustment ($P = 0.029$ for additive model and $P = 0.020$ for dominant model). No association was observed for age at onset of PD.

Meta analysis across five populations indicated overall association between SNP rs967582 and risk of PD affection status. In a conservative analysis, which assumed that the observed direction of effect that was unreported in (Noureddine et al. 2005) was opposite that of the other studies the meta analysis *P* value equaled 0.0063. Figure 1 shows the odds ratio and 95% confidence interval for the four populations for which estimates are available.

Discussion

We examined association between risk of PD or age at onset of PD and SNPs in *ELAVL4* in the *Gene*PD study cohort. Significant association between the rs967582 SNP, and PD affection status was observed in this internationally recruited sample of familial PD cases, when examining nominal *P* values. A total of nine tests (three SNPs and three genetic models) were performed. Using a conservative Bonferroni correction the adjusted *P* value for rs967582 is 0.099 for the dominant model.

The minor allele of this SNP had previously been identified as associated with earlier age at onset of PD using family based association testing (Noureddine et al. 2005). In the current study, family relationships were accounted for analytically, and a population based association test was implemented. SNP rs967582 was recently associated with risk for PD in an Irish population but no significant association was observed for this SNP or others in *ELAVL4* in an independent US or Norwegian cohort (Haugarvoll et al. 2007). Although it was suggested that the pattern of significance was due to a Celtic founder effect, the ORs for rs967582 in the US and Norwegian samples are in the same direction as those seen for the Irish population (1.14 and 1.16, respectively) (Haugarvoll et al. 2007). The current study, which represents an international sample of familial PD cases, suggests that the Haugarvoll et al. (2007) study may have had insufficient power to detect small increased odds of PD associated with the minor allele in their non-Celtic samples. In the Haugarvoll et al. (2007) study, as with the current study, the C minor allele is the risk allele, and the two studies report similar allele frequencies and odds ratio estimates. Meta analysis provides evidence of association across five populations.

The current meta analysis included all published association analyses between PD and SNPs in *ELAVL4*. Publication bias may lead to the dissemination of positive association findings rather than negative. This is a limitation of meta analyses performed using published results.

Replication of results is an important, but not final, step in implicating a gene as a risk factor for a complex disease. The minor allele in SNP rs967582 has now been implicated in three Caucasian PD populations for *ELAVL4*. Association studies in additional populations as well as biological studies to clarify the role of this gene in PD risk are justified.

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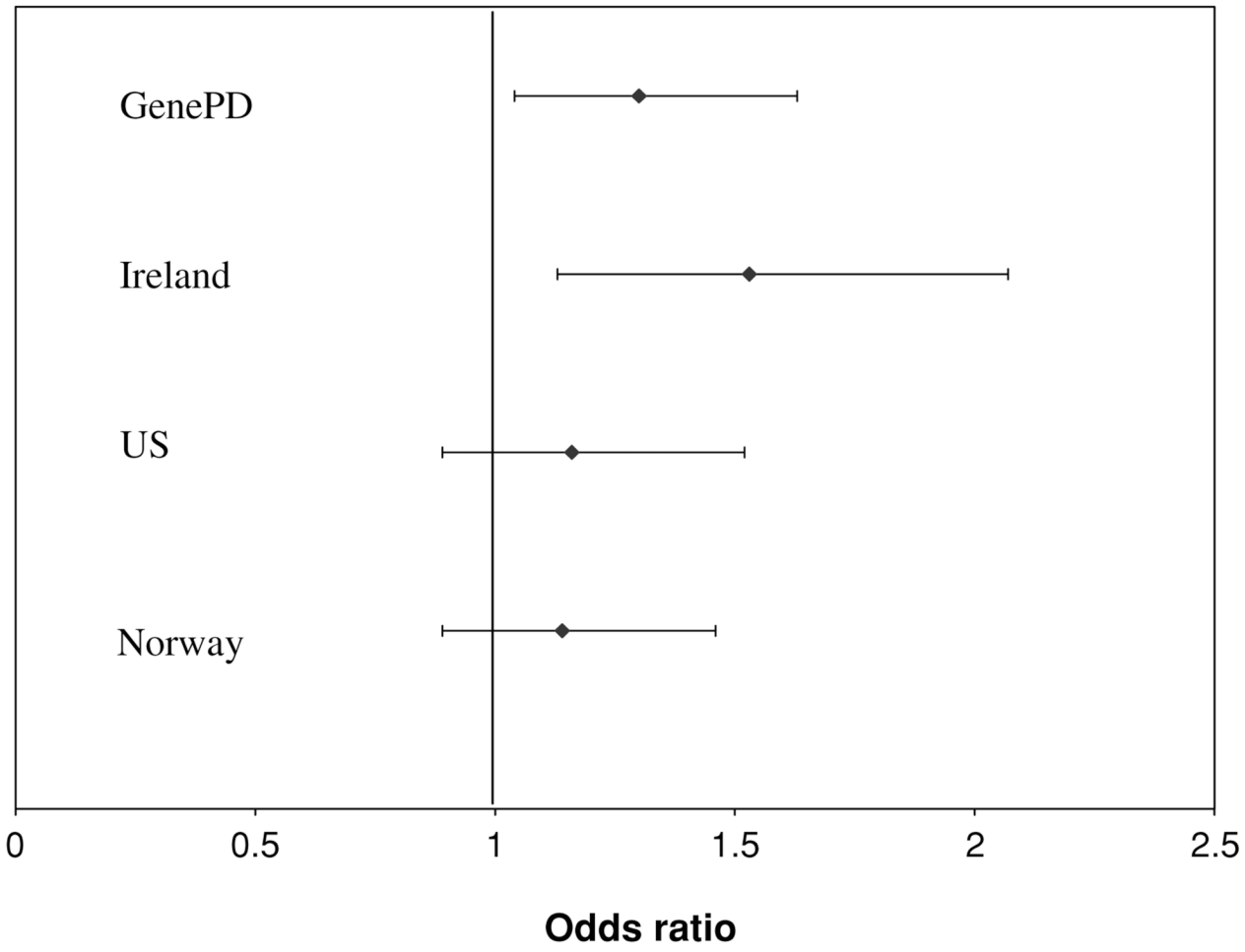

Fig. 1.

Odds ratio and 95% confidence interval for risk of PD affection status with increasing number of minor alleles of rs967582 from the current analysis (GenePD) and previously published populations (Haugarvoll et al. 2007). Another study in a US population found no association using a pedigree disequilbrium test. This study is not included in the figure as no effect measure was available (Noureddine et al. 2005)

Table 1

Association results [odds ratio (OR), nominal P values (*P*), and minor allele frequencies (MAF)] for PD affection status and age at onset for *ELAVL4* SNPs

SNP	bp location[a]	MAF cases (%)	MAF controls (%)	Affection						Age at onset					
				Additive		Dominant		Recessive		Additive		Dominant		Recessive	
				OR	*P*	OR	*P*	OR	*P*	Beta	*P*	Beta	*P*	Beta	*P*
rs967582	50294192	36.6	30.8	1.303	0.021	1.460	0.011	1.272	0.294	−0.208	0.764	−0.058	0.951	−0.644	0.616
rs3902720	50317357	31.7	30.0	1.080	0.559	1.137	0.454	1.016	0.955	−0.670	0.390	−0.667	0.499	−1.244	0.472
rs12024093	50340871	18.9	17.3	1.114	0.502	1.147	0.460	1.056	0.909	−1.212	0.223	−1.591	0.144	0.001	1.000

Results are from analyses with no covariate adjustment

[a]Chromosome 1 NCBI build 35; HapMap pairwise LD r^2 (rs967582, rs3902720) = 0.03, r^2 (rs967582, rs12024093) = 0.11, r^2 (rs3902720, rs12024093) = 0.55