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Short Communication

Human leukocyte antigen variation and Parkinson's disease

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Short Title: HLA association in PD

Abstract

A role for the immune system in the pathogenesis of Parkinson's Disease (PD) has previously been suggested. A recent genome-wide association (GWA) study identified an association between one single nucleotide polymorphism (SNP) in the human leucocyte antigen (*HLA*) region (*HLA-DRA* rs3129882) and PD in a population of American patients with European ancestry. In that study, the minor rs3129882 allele (G) was associated with an increased risk of PD under an additive model. Due to the increased likelihood of obtaining false positive results in GWA studies compared to studies conducted based on a hypothesis-driven approach, repeated validation of findings from GWA studies are necessary. Herein, we evaluated the association between rs3129882 and PD in three different Caucasian patient-control series (combined 1,313 patients and 1,305 controls) from the US, Ireland, and Poland. We observed no association (OR: 0.96, P=0.50) between rs3129882 and PD when analyzing our data under an additive or dominant model. In contrast, when examined under a recessive model, the GG genotype was observed to be protective in the Irish (OR: 0.55, *P*=0.008), Polish (OR: 0.67, *P*=0.040) and combined (OR: 0.75, *P*=0.006) patient-control series. In view of these diverging results, the exact role of genetic variation at the HLA region and susceptibility to PD remains to be resolved.

Introduction

Familial forms of parkinsonism have provided insight into the underlying genetic determinants of disease. However, for the vast majority of patients with sporadic Parkinson's disease (PD), limited genetic risk factors have been identified. It was hoped that genome-wide association (GWA) studies would help resolve the role of common genetic variation in PD risk, however results to date have been restricted to only a few candidate genes including α-synuclein (*SNCA*) and microtubule-associated protein tau (*MAPT*) [1]. In the most recent GWA study performed on a US population (2,000 patients and 1,986 controls), Hamza *et al.* confirmed the *SNCA* and *MAPT* loci, and nominated a novel association within the human leukocyte antigen (HLA) locus on chromosome 6, with an association peak at the *HLA-DRA* single nucleotide polymorphism (SNP) rs3129882 [2]. The minor allele (G) was associated with an increased risk of PD under an additive model [2]. This was subsequently confirmed in two separate series (OR: 1.21, P=0.01 and OR: 1.17, P=0.06), where the effect sizes were similar [2], although smaller as would be expected after the initial data-mining approach of the GWA study. The association of PD risk with a genetic polymorphism in a gene involved in immunosurveillance was considered biologically reasonable, as an involvement of the immune system in PD pathogenesis has long been discussed [3]. Herein we attempt to replicate the previously reported association of rs3129882 with PD in patient-control series from the US, Ireland, and Poland.

Methods

The combined patient-control series contained 1,313 patients with clinical diagnoses of PD and 1,305 unaffected and unrelated control subjects. Key demographic and clinical data are summarized in Table 1. The *HLA-DRA* SNP rs3129882 was genotyped with an ABI on-demand Taqman assay and analyzed with SDS 2.2.2 software. All samples were genotyped at one study centre (Mayo Clinic). The rate of genotype calls was ≥95% in each population. The association between rs3129882 and PD was evaluated using a logistic regression model adjusted for age, gender, and series (combined series only), where ORs and 95% confidence intervals (CIs) were estimated. We examined association under an additive model (effect of an additional minor allele) to correspond with the model used by Hamza *et al.* [2], and also under a dominant (G/G or G/A vs. A/A) and a recessive model (G/G vs. A/A or G/A). *P*-values ≤ 0.05 were considered statistically significant. The ethical review boards at each institution approved the study, and all participants provided written informed consent.

Results

We examined the association of rs3129882 within our individual and combined patient-control series (Table 2). There was no evidence of deviation from Hardy-Weinberg equilibrium in the genotype frequencies in the controls of each separate series ($P>0.05$ after Bonferroni correction [3 tests], Table 2). Hamza and colleagues proposed (G) as a risk allele under an additive model, however under this model in our combined series we observed no association (OR: 0.96, P=0.50). Results were similar under a dominant model (data not shown). When examined under a recessive model, the GG genotype was

observed to be protective in the Irish (OR: 0.55, *P*=0.008), Polish (OR: 0.67, *P*=0.040) and combined (OR: 0.75, *P*=0.006) patient-control series (Table 2).

Discussion

The role of common genetic variation in susceptibility to PD remains to be resolved. The most recent GWA study nominated the HLA region as a risk factor for PD [2]. Interestingly, the highly polymorphic HLA complex has been associated with PD risk in two previous independent studies [4, 5]. Our results show a significant association at this locus with PD susceptibility although it is not the same effect observed by Hamza *et al.* [2]. In the study by Hamza *et al.*, the "G" allele was associated with an increased risk of PD under an additive model. Though we cannot rule out the possibility that the "G" allele is associated with increased risk of PD under an additive model, based on the 95% confidence intervals for odds ratio estimates we can conclude that any such association is unlikely to have an odds ratio of greater than 1.08 in our Caucasian populations. Interestingly, our findings under a recessive model are counterintuitive given those of Hamza *et al.* In our data, subjects with both copies of the "G" allele, the risk allele as proposed by Hamza *et al.*, were actually at a decreased risk of PD, with an odds ratio of 0.75 in the combined Caucasian series. This finding needs to be validated in future studies, and overall our study indicates that the association between rs3129882 and PD remains unclear and requires further study.

Differences in the substructure of the Caucasian populations studied may be one possible explanation for these conflicting results. Hamza *et al.* have investigated the ethnic and

genetic background of the individuals in their study and predicted that the association of HLA with PD may differ in populations with varying ethnic or geographical origin [2]. The association was strongest in the European populations compared to the overall US series. Given the complexity of the genetic architecture, gender- and ethnic specificity in allele frequencies, and the known high levels of polymorphism at the HLA complex, much larger meta-analytical approaches may be necessary to resolve the role of this locus in PD [2, 6].

The potential role of the immune response in both the risk and pathogenesis of PD has long been a focus of research [3]. The HLA complex plays a pivotal role in immunosurveillance within the body and is associated with a number of autoimmune disorders. It is an attractive hypothesis that variation within those genes that regulate the immune response may be important in determining risk of PD [7]. In fact, HLA-DR positive microglial cells contribute to the neuroinflammation in the substantia nigra of brains from PD patients [8]. While there are observations that individuals taking high amounts of anti-inflammatory medications had a lower likelihood to develop PD [9], metaanalyses of several similar studies showed diverging results [10]. Furthermore, the components of the immune system are regulated by a highly complex network of cytokines and receptors. The present associations of *HLA-DRA* SNP probably represent only one contribution among many others, although the different *HLA* alleles are not entirely independent of each other. Variation in cytokine genes has been proposed to increase the risk of PD, particularly variants in the tumor necrosis factor alpha gene which is located in the Class III region of the HLA complex [11] . It is likely that rs3129882 is acting as a marker in linkage disequilibrium with other *HLA* alleles, and a

more detailed look at HLA-A, -B, -DR and Class III genes is now warranted. Interestingly, after our initial submission of this manuscript, a meta-analysis of datasets from five PD genome wide association studies reported the association of additional HLA-DRA SNPs with PD [12].

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Figure Captions

Table 1. Demographic and Clinical Data of the three PD Case-Control Series

The sample mean ± SD (minimum – maximum) is given for age and age at onset. Information regarding age at onset was unavailable for some individuals in the Ireland (109 patients), Poland (5 patients) and the MCJ series (13 patients).

Table 2. Genotype and allele frequencies for *HLA-DRA* rs3129882

Odds ratios (ORs) and *P*-values result from logistic regression models adjusted for age, gender, and series (combined series only). ORs correspond to an additional "G" allele (additive model), and to presence of the "G/G" genotype (recessive model). All statistical analyses were performed using the SAS software package (SAS Institute; Cary, North Carolina).

Table 1

Series	**PD cases**	**Unrelated controls**
Irish	n=354	n=360
Age	68 ±10 (38-94)	73 ±22 (24-104)
Gender		
Male	198 (56%)	131 (36%)
Female	156 (44%)	229 (64%)
Age at onset	52 ±10 (18-77)	N/A
Family History		
Positive	16 (5%)	
Negative	147 (42%)	360 (100%)
Unknown	191 (54%)	
Polish	n=343	n=312
Age	69 ±11 (40-93)	65 ±16 (24-99)
Gender		
Male	211 (62%)	161 (52%)
Female	132 (38%)	151 (48%)
Age at onset	56 ±12 (23-81)	N/A
Family History		
Positive	40 (12%)	
Negative	303 (88%)	312 (100%)
US	n=616	n=633
Age	72 ±11 (31-99)	70 ±13 (21-94)
Gender		
Male	390 (63%)	267 (42%)
Female	226 (37%)	366 (58%)
Age at onset	63 ±12 (25-94)	N/A
Family History		
Positive	209 (34%)	
Negative	360 (58%)	633 (100%)
Unknown	9 (8%)	
Combined Caucasian series	**n=1313**	**n=1305**
Age	70 ±11 (31-99)	69 ±17 (21-104)
Gender		
Male	799 (61%)	559 (43%)
Female	514 (39%)	746 (57%)
Age at onset	59 ±13 (18-94)	N/A

Table 2

			Genotype counts (%)			*Allele frequency (%)*		*Additive model*		*Recessive model*	
Series		***N***	***A/A***	***A/G***	***G/G***	***A***	***G***	***OR (95% CI)***	***P-value***	***OR (95% CI)***	***P-value***
Irish											
	Patients	354	132 (37%)	184 (52%)	38 (11%)	448 (63%)	260 (37%)	0.86	*0.19*	0.55	*0.008*
	Controls	360	136 (38%)	162 (45%)	62 (17%)	434 (60%)	286 (40%)	(0.69 – 1.08)		(0.35 – 0.86)	
Polish											
	Patients	343	87 (25%)	196 (57%)	60 (17%)	370 (54%)	316 (46%)	0.99	*0.95*	0.67	*0.040*
	Controls	312	96 (31%)	136 (44%)	80 (26%)	328 (53%)	296 (47%)	(0.79 – 1.24)		(0.45 – 0.98)	
US											
	Patients	616	213 (35%)	299 (49%)	104 (17%)	725 (59%)	507 (41%)	1.02	*0.78*	0.98	*0.89*
	Controls	633	228 (36%)	295 (47%)	110 (17%)	751 (59%)	515 (41%)	(0.87 – 1.20)		(0.72 – 1.32)	
Combined											
	Patients	1313	432 (33%)	679 (52%)	202 (15%)	1543 (59%)	1083 (41%)	0.96	0.50	0.75	0.006
	Controls	1305	460 (35%)	593 (45%)	252 (19%)	1513 (58%)	1097 (42%)	(0.86 – 1.08)		(0.61 – 0.92)	