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The *UCHL1* S18Y polymorphism and Parkinson's disease in a Japanese population

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Abstract

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Appendix. Supplementary data: Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.parkreldis.2011.01.019.

UCHL1 plays an important role in the ubiquitin-proteasome system and is a biologically plausible candidate gene for Parkinson's disease (PD). However, results from genetic association studies of the *UCHL1* S18Y polymorphism have been equivocal. Meta-analyses indicate that the polymorphism's risk effect might be restricted to Asian populations and early-onset disease. To further explore the role of *UCHL1* in PD, we genotyped S18Y in 605 PD patients and 1620 controls of Japanese ancestry. We did not find evidence of an association in the overall sample (SY vs. SS: adjusted OR = 1.11, $P = 0.37$; YY vs. SS: adjusted OR = 1.01, $P = 0.94$). In the early-onset stratum, however, we observed a trend toward a reduction in risk for those with the Y allele (SY vs. SS, adjusted OR, 0.75; 95% CI, 0.47–1.20; YY vs. SS, OR, 0.64; 95% CI, 0.36–1.14; trend test, $P = 0.12$). These results indicate that, if involved in PD, the S18Y variant is not a major determinant of risk and its effect might be restricted to early-onset disease.

Keywords

Parkinson's disease; *UCHL1*; Association study

1. Introduction

Parkinson's disease (PD) is complex and multifactorial, likely involving a combination of environmental exposures, polygenic inheritance, and gene-environment interactions. Ubiquitin-C-terminal hydrolase L1 (*UCHL1*) is a compelling candidate gene for PD on biological grounds because the protein it encodes plays a pivotal role in the ubiquitin-proteasome system (UPS), displays neuron-specific expression, and is found in Lewy bodies, the neuropathologic hallmark of PD. However, genetic association studies have yielded equivocal results.

S18Y is the most studied *UCHL1* variant, and there is evidence to suggest that it is a functional polymorphism. The *UCHL1* enzyme has multiple functions, including dimerization-dependent E3 ubiquitin ligase activity and, *in vitro*, this ligase activity promotes α -synuclein aggregation [1]. The 18Y variant protein is resistant to dimerization, reducing its function as an E3 ligase and resulting in lower levels of α -synuclein aggregation in comparison to the 18S variant. This decrease in ligase function displays a dose effect, with the largest reduction observed in 18Y homozygotes.

Some case-control association studies have reported that the 18Y allele protects against PD in a dose-dependent manner, which fits well with the observed effects of this variant on *UCHL1* enzyme function. However, this finding has not been consistently replicated, and even meta-analyses have yielded conflicting results [2–4]. The most promising evidence in favor of association has come from studies in Asian populations and of early-onset PD [3,4].

Because the association between *UCHL1* S18Y and PD is disputed and might be restricted to Asian populations, we sought to further assess this association in a large Japanese case-control sample.

2. Materials and methods

2.1. Sample description

DNA was collected from 605 unrelated patients with PD in central Japan through several neurology clinics. All patients met Calne criteria for clinically definite PD as determined by a neurologist. Thirty-seven (6.1%) of these patients reported a family history of PD in one or more first-degree or second-degree relatives. Control subjects came from two sources. The first control group included 314 unrelated persons of Japanese ancestry residing in the same

communities as the PD patients. All reported no history of PD or related disorders. The second control group consisted of 1306 individuals who were part of a community-based aging study of Japanese-Americans in King County, Washington, USA (The Kame Project) between 1991 and 2002 [5]. All reported no history of PD and that both of their parents were of Japanese origin. The institutional review boards at each participating site approved the study, and all subjects gave written informed consent.

2.2. Genotyping

The *UCHLI* S18Y variant (rs5030732) was genotyped by TaqMan assay on an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, California, USA) using primers 5'-CCTGGCCGCCTTGCTCT (forward) and 5'-CCCAGCACGTCCACGAA (reverse), and probes VIC-CAGCCGGGACAGCA and FAM-CCAGCCGGTACAGCA. Positive (previously confirmed by sequencing) and negative controls were included in all genotyping runs.

2.3. Data analysis

Two cases under 21 at PD diagnosis were excluded from the analyses. Fisher's exact test was used to evaluate sex differences between the case and control populations. Hardy-Weinberg equilibrium (HWE) for the S18Y genotypes was also assessed using an exact test. Logistic regression specifying a co-dominant model was used to test the S18Y-PD association. Homozygotes for the S allele were specified as the reference group. A trend test was performed to assess the impact of each additional copy of the Y allele on the odds of PD. We also performed age-stratified analyses, defining early-onset cases as those with an age at onset of 50 years or younger ($n = 99$) and late-onset cases as those with an age at onset over 50 years ($n = 506$). Analyses utilizing all cases adjusted for both age and sex, while age stratified analyses adjusted for sex only. Stata v 9.2 was used for all statistical analyses.

3. Results

The characteristics of the study participants are presented in Table 1. Sex ratios were approximately equal in the case and control populations (Table 1), and the S18Y genotypes were in HWE in the full sample and all sub-samples (Supplementary Table).

As shown in Table 2, there was no evidence for an association between S18Y genotype and PD in the overall sample. Because controls came from two separate sources (Japanese-Americans and individuals of Japanese ancestry living in Japan), the analyses were performed again using the individual control sources to determine if there was a bias in the overall result due to the controls' country of residence. There did not appear to be significant differences in the results obtained when using each of the two control samples separately (Table 2). Neither the early- nor late-onset stratified analyses identified a significant association between genotype and PD; however, in the early-onset stratum, there was a non-significant trend toward a reduced risk of PD with each additional Y allele (Table 2).

4. Discussion

This is the largest *UCHLI* S18Y PD association study reported in a Japanese sample to date [6,7] and the second largest reported in an Asian sample [8]. We did not find evidence of an association between the *UCHLI* S18Y polymorphism and PD in the overall sample (SY vs. SS, adjusted odds ratio [OR], 1.11; 95% confidence interval (CI), 0.88–1.41; YY vs. SS, OR, 1.01; 95% CI, 0.77–1.33). However, we observed a trend toward a reduction in risk for those with the Y allele in the early-onset stratum (SY vs. SS, adjusted OR, 0.75; 95% CI,

0.47–1.20; YY vs. SS, OR, 0.64; 95% CI, 0.36–1.14; trend test, $P = 0.12$), consistent with the results of several previous studies.

Three meta-analyses of *UCHL1* S18Y have been published to date [2–4]. The first [3], published in 2004, supported the inverse association between 18Y and PD in a decreasing linear trend in a sample of combined Asian and White participants (SY vs. SS, OR, 0.92; 95% CI, 0.79–1.07; YY vs. SS, OR, 0.71; 95% CI, 0.55–0.91; $P = 0.03$). A significant inverse association was also observed in the early-onset (SY vs. SS, OR, 0.80; 95% CI, 0.65–0.99; YY vs. SS, OR, 0.49; 95% CI, 0.35–0.69; $P < 0.001$) and Asian-only subgroups (SY vs. SS, OR, 0.76; 95% CI, 0.57–1.01; YY vs. SS, OR, 0.67; 95% CI, 0.47–0.94; $P = 0.05$). A meta-analysis published two years later [2] examined the effect of S18Y in Whites only and did not find a significant association (SY + YY vs. SS, OR, 0.96; 95% CI, 0.86–1.08; $P = 0.49$). However the analysis did not examine the effect of the variant in early-onset patients. The most recent meta-analysis [4] added two studies in Asian and three in White populations. Consistent with the results of the first meta-analysis, a significant association was observed between S18Y and PD in both Asian (under a recessive model) (YY vs. SS + SY, OR, 0.79; 95% CI, 0.67–0.94; $P = 0.01$) and White (under a dominant model) (SY + YY vs. SS, OR, 0.89; 95% CI, 0.81–0.98; $P = 0.02$) populations.

There are several limitations to this work that are related primarily to the choice of the control group. First, although all controls denied a history of PD, they were not examined by a neurologist, leaving the potential for the misclassification of true cases as controls. Any bias due to control misclassification is likely minimal, however, due to the low baseline prevalence of PD. Secondly, control subjects came from two different locations. The controls recruited in Japan from the same communities as the cases are an ideal comparison group; however, only 314 were collected in this fashion. Another 1306 controls came from a US-based study of Japanese–Americans. This could have introduced bias, as these populations have different environmental exposures. Additionally, although only controls with both parents of Japanese origin were included in the analyses, if Japanese individuals who move to the United States are genetically different from those who do not, population stratification could have also biased our results. However, an analysis stratified by control recruitment site did not indicate that this was the case (Table 2). Finally, the age distribution of the control group limited our ability to make age-specific comparisons. Because the mean age of cases diagnosed with early-onset PD was 53 and only 1.5% of the controls were 53 or younger at time of blood draw, we used the full control sample as the comparison group for all analyses. While the results for early-onset cases should be interpreted with some caution, it is unlikely that the effects observed in this group are due completely to uncontrolled confounding due to age. For example, although the incidence of PD increases with age, the *UCHL1* S18Y polymorphism is not known to be associated with longevity, and in fact, the S18Y variant was shown to be in HWE in the control sample. Thus, we would expect a similar genotypic distribution in younger controls and similar estimates of effect.

There are a number of possible reasons that we did not find an association between the S18Y variant and PD in the overall sample. Although ours is the largest Japanese case-control sample in which S18Y has been studied to date, we still had limited power to detect associations of small effect. For example, there was only 35% power to detect an OR of 0.90 under a log-additive model of inheritance with a false-positive fraction of 5%. Additionally, the effect of S18Y might be modified by environmental factors which were not assessed in our sample. For example, Kyratzi et al. [9] recently showed that the S18Y variant confers an antioxidant function to neuronal cells. As pesticides can induce oxidative stress in neurons, this variant might be protective only in exposed individuals. Only one association study has assessed this potential interaction; pesticide exposure was not found to have a modifying effect on the association between the S18Y variant and PD [10]. It is also possible that

UCHL1 is involved in PD, but S18Y is not the true risk variant. The majority of studies that investigated the role of *UCHL1* in PD have focused on S18Y. However, one study which looked at the effect of additional variants in *UCHL1* using a tagSNP approach found no evidence for association in analyses of individual markers or haplotypes [2]. Recent genome-wide association studies also did not report an association between PD and markers in *UCHL1* [11,12], though S18Y was not directly assayed in any of these studies. Lastly, despite its biological plausibility, variation in *UCHL1* might not modify risk for PD.

Our findings indicate that, if involved in PD, the S18Y variant is not a major determinant of risk and its effect might be restricted to early-onset disease. Future studies with larger sample sizes and meta-analyses of early-onset subgroups are needed to better examine the effect of this genetic variant on PD. Studies that examine interacting variables, such as pesticide exposure, might also be merited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Study population characteristics.

	Cases		Controls			
	All		Early onset	Late onset	All	Japanese-Americans
<i>N</i>	605		99	506	1620	1306
Age at enrollment Mean (SD)	67.1 (10.3)		52.9 (9.2)	70.0 (7.9)	70.3 (6.9)	70.6 (5.3)
Age at onset Mean (SD)	61.0 (10.8)		44.1 (5.5)	64.6 (7.8)	NA	NA
Female <i>N</i> (%)	347 (57.4)		58 (58.6)	289 (57.1)	887 (54.8)	687 (52.6)

NA, not applicable.

Table 2

Odds of PD by *UCHL1* S18Y genotype.

	SY vs. SS			YY vs. SS			Trend test	
	OR	95% CI	P-value	OR	95% CI	P-value	χ^2	P-value
All Subjects	1.11	0.88–1.41	0.37	1.01	0.77–1.33	0.94	0.38	0.54
All cases, controls from Japan	1.15	0.82–1.62	0.41	1.17	0.78–1.74	0.46	0.19	0.66
All cases, Japanese–American controls	1.13	0.88–1.44	0.35	1.00	0.75–1.33	1.00	0.16	0.69
Early onset cases, all controls	0.75	0.47–1.20	0.23	0.64	0.36–1.14	0.13	2.43	0.12
Late onset cases, all controls	1.15	0.90–1.48	0.10	1.06	0.79–1.41	0.70	0.14	0.71