Original Article

Lack of Association between the *LRRK2* A419V Variant and Asian Parkinson's Disease

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Abstract

Introduction: The G2385R and R1628P *LRRK2* gene variants have been associated with an increased risk of Parkinson's disease (PD) in the Asian population. Recently, a new *LRRK2* gene variant, A419V, was reported to be a third risk variant for PD in Asian patients. Our objective was to investigate this finding in our cohort of Asian subjects. Materials and Methods: Eight hundred and twenty-eight subjects (404 PD patients, and 424 age and gender-matched control subjects without neurological disorders) were recruited. Genotyping was done by Taqman® allelic discrimination assay on an Applied Biosystems 7500 Fast Real-Time PCR machine. Results: The heterozygous A419V genotype was found in only 1 patient with PD, compared to 3 in the control group (0.4% vs 1.3%), giving an odds ratio of 0.35 (95% confidence interval (CI), 0.01 to 3.79; *P* = 0.624). Conclusion: A419V is not an important *LRRK2* risk variant in our Asian cohort of patients with PD. Our data are further supported by a literature review which showed that 4 out of 6 published studies reported a negative association of this variant in PD.

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Key words: Asian, A419V, Genetics, *LRRK2*, Parkinson's disease

Introduction

Recent studies have linked certain single nucleotide polymorphisms (SNPs) in the *LRRK2* gene with familial and sporadic Parkinson's disease (PD). Interestingly, there appear to be important population differences in the contribution of these SNPs to the risk of PD occurrence. The G2019S variant is very common amongst Ashkenazi Jews and African Arabs with PD,[1,2] but it is undetectable in Asian populations.[3,4] On the other hand, in Asians and in particular, the Han Chinese, the G2385R and R1628P variants have been consistently identified as important risk factors.[5-7]

Recently, Ross and colleagues[8] reported the *LRRK2* A419V variant (position c.1256 C>T, rs34594498) to be a third common risk variant in Asian patients with PD, with an odds ratio (OR) of 7.51 in the Taiwan Han Chinese, 2.21 (Koreans), and 1.26 (Japanese). In contrast, Di Fonzo and colleagues[9] found a low frequency (below 1%) of A419V in Han Chinese PD patients from Taiwan, with no significant difference between PD patients. Similarly, an Asian multicentre study reported this variant to be monomorphic in their cohort of PD patients.[7] More recently, Wu et al,[10] in 2012 and Wu-Chou et al,[11] in 2013 found that there was no association with A419V in Chinese PD patients in a multicentre study in Taiwan and Singapore. These discrepant findings prompted us to investigate if A419V is

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a risk variant in our cohort of Asian subjects, comprising individuals of Chinese, Malay and Indian ethnicity. Whilst A419V has been studied in Chinese individuals, much less is known in the Malay and Indian ethnic groups.

Materials and Methods

A total of 828 subjects attending 5 hospitals (404 PD, 424 controls) were recruited. PD patients were diagnosed based on the United Kingdom PD Brain Bank Criteria. Control subjects were age and gender-matched and did not suffer from any known neurological disorder. Three ethnic groups were selected: Chinese, Malays and Indians. Samples were only included if subjects reported a 3-generational pure lineage of a particular ethnic group, without racial admixture. Ethical approval was obtained and all participants provided written informed consent. The rs34594498 variant was genotyped using TaqMan® SNP genotyping assay (Applied Biosystems) on a 7500 Fast Real-Time PCR machine. Genotypes were confirmed by sequencing in a random subset of 20 individuals and the error rate was 0%. The allele and genotype frequencies in cases and controls were compared with chi square and Fisher's exact tests whenever appropriate. Statistical analyses were performed using a free and open source software (OpenEpi).

Results

Deoxyribonucleic acid (DNA) samples from a total of 828 subjects comprising 404 PD (223 Chinese, 122 Malays and 59 Indians) and 424 controls (236 Chinese, 110 Malays and 78 Indians) were analysed. The mean age, age at onset of PD, and age of controls was 64.5 ± 10.0 (27 to 92) years, 57.1 ± 11.8 (16 to 90) years and 58.6 ± 9.4 (29 to 85) years, comprising 56% and 48% men, respectively.

Results of the A419V variant genotyping are shown in Table 1. No subject was homozygous. In the Chinese cohort, 1 PD patient was positive for A419V, compared to 3 positive cases in the controls, giving an odds ratio (OR) of 0.35 (95% confidence interval (CI), 0.01 to 3.79; P = 0.624). The c.1256 C>T polymorphism was present at a minor allele frequency of 0.22% in the PD patients and 0.64% in the controls. The PD patient positive for A419V was diagnosed at 45 years and the median age of the 3 controls was 61 years. The PD patient had no family history of PD. Both the Malay and Indian cohorts were monomorphic. A combined analysis of the total cohort gave an OR of 0.35 (95% CI, 0.04 to 3.38; P = 0.625).

Table 1. Analysis of *LRRK2* A419V Genotype and Allele Frequencies

	PD (MAF)	Control (MAF)	OR (95% CI)
Chinese	n = 223	n = 236	
Wildtype Allele (C)	445 (0.998)	469 (0.994)	0.35 (0.01 to 3.79)
Variant Allele (T)	1 (0.002)	3 (0.006)	P = 0.624
Malay	n = 122	n = 110	
Wildtype Allele (C)	244 (1.0)	220 (1.0)	NA
Variant Allele (T)	0	0	
Indian	n = 59	n = 78	
Wildtype Allele (C)	118 (1.0)	156 (1.0)	NA
Variant Allele (T)	0	0	
Total	n = 404	n = 424	
Wildtype Allele (C)	807 (0.998)	845 (0.996)	0.35 (0.04 to 3.38)
Variant Allele (T)	1 (0.002)	3 (0.004)	P = 0.625

C: cytosine nucleotide; CI: confidence interval; OR: odds ratio; MAF: minor allele frequency; NA: not applicable; PD: Parkinson's disease; T: thymine nucleotide

Discussion

We focused on the A419V variant to determine whether it represents a risk factor in our Asian PD cohort, based on the recent findings reported by Ross et al.[8] We investigated the involvement of A419V in our larger cohort of Chinese patients, and conducted an exploratory study in our smaller Malay and Indian PD cohorts. We found that the c.1256 C>T polymorphism was present at a low frequency (0.86%) in the Chinese cohort. Only 1 Chinese PD patient was positive (minor allele frequency (MAF) 0.22%) compared to 3 Chinese controls (MAF 0.64%), yielding an OR of 0.35. This result contrasts with that recently reported by Ross and colleagues,[8] where an OR of 7.51 was found in their Taiwanese Chinese cohort, despite the 2 cohorts being of the same ethnic group (i.e. Han Chinese).

While we acknowledge that our sample size is relatively small, our finding of a low MAF is similar to that reported recently by Wu and colleagues[10] with over 3000 samples, suggesting that the MAF in our cohort is representative of the ethnic group as a whole (Table 2). A review of the available literature on A419V (Table 2) shows that the reported MAFs in the Han Chinese ethnic group ranges from 0.3% to 0.44% for controls and 0.42% to 1.9% for patients with PD.

We performed a meta-analysis of data from our study together with the published reports on A419V using only samples that are of known Chinese descent (this study, Li et al,[12] Wu et al,[10] Wu-Chou et al,[11] DiFonzo et al,[9] and Tan et al[7]). The analysis shows that the OR value is 1.49 (95% CI, 0.72 to 3.06), P = 0.197. Although this combined analysis suggests that the A419V variant tended towards being a risk factor, we would like to argue that the result is skewed

Table 2. Summary of Published Literature (July 2006 to February 2013) on *LRRK2* A419V

Ethnic Group	MAF, T Allele	Result	Age of Onset of PD	Reference
Han Chinese from Taiwan	PD: 0.8 Control:0.4 Cohort size: PD: 608 Control: 373	Not significantly different	54.9 ± 11.9 years	Di Fonzo et al,[9] 2006
Han Chinese from Taiwan, Singapore and China	Monomorphic Cohort size PD: 250 Control: 250	Monomorphic	Median age = 61.0 (29.0 to 88.0) years	Tan et al,[7] 2010
Han Chinese from Japan, Korea, Taiwan	Combined Asian series: 1.9 Cohort size Japan PD: 173 Control: 75 Korea PD: 844 Control: 587 Taiwan PD: 369 Control: 300	Significantly different in the combined Asian series Risk factor with an OR of 2.27 (1.35 to 3.83) Japan: OR 1.26 (0.38 to 4.22) Korea: OR 2.21 (1.2 to 4.06) Taiwan: OR 7.51 (0.95 to 59.6)	Combined Asian series: 54 ± 12 years	Ross et al,[8] 2011
Han Chinese from China	PD combined: 1.5 EOPD: 3.5 LOPD: 0.98 Control: 0.3 (combined <50 and >50 years) Cohort size PD: 729 Control: 585	Significantly different in the EOPD group OR 14.89; 95% CI, 1.94 to 114.20 Not significantly different in the LOPD group OR 2.37; 95% CI, 0.67 to 8.43 Combined: OR 4.14; 95% CI, 1.53 to 12.74	53.81 ± 11.04 years (this included a cohort of early onset PD cases)	Li et al,[12] 2012
Han Chinese from Taiwan, Singapore and China	PD: 0.42 Controls: 0.44 Cohort size PD: 1517 Control: 1487	Not significantly different Taiwan: OR 0.6777 (0.19 to 2.41) Singapore: OR 0.98 (0.24 to 3.93) China: OR 1.56 (0.37 to 6.5) Combined: 0.98 (0.45 to 2.18)	Taiwan : 63.06 ± 11.2 years Singapore: 64.6 ± 10 years China: 60.16 ± 11.0 years	Wu et al,[10] 2012
Han Chinese from Taiwan	Monomorphic Cohort size PD: 626 Control: 473	Monomorphic	63.2 ± 7.8 years	Wu-Chou et al,[11] 2013
Chinese	PD: 0.2 Control: 0.6 PD: 223 Control: 236	Not significantly different Chinese: OR 0.35 (0.01 to 3.79)	Chinese: 57.9 ± 12.0 years	Current study

CI: confidence interval; EOPD: early onset PD; LOPD: late onset PD; OR: odds ratio; MAF: minor allele frequency; PD: Parkinson's disease; T: thymine nucleotide

by the Li et al[12] data, which showed a stronger correlation with early onset PD. If we remove the data from the Li et al[12] paper, the combined analysis indicates an OR value of 1.05 (95% CI, 0.57 to 1.91) $P = 0.763$.

Out of 7 papers describing the A419V in PD in Chinese, 2 papers reported that the A419V was monomorphic in their cohort (Tan et al,[7] and Wu-Chou et al[11]). Meanwhile, 5 out of 6 papers (Di Fonzo et al,[9] Tan et al,[7] Wu et al,[10] Wu-Chou et al,[11] and this study) showed that the OR value was below 1.

In addition, out of 7 papers, only 2 suggest that A419V is a risk factor in PD (Ross et al,[8] and Li et al[12]). The combined number of Chinese samples from Ross et al[8] and Li et al[12] are 1098 for PD and 885 for controls. The combined number of Chinese samples from the other papers (Wu et al,[10] Wu-Chou et al,[11] DiFonzo et al,[9] Tan et al[7] and this study) are 3224 for PD and 2819 for controls. We would like to put forth the possibility of population stratification or possible gene-environment interactions that contribute to the findings in Ross et al[8] and Li et al[12], and that the data from these papers do not represent the true contribution of the A419V variant in Chinese PD patients. The combined analysis from the other papers together with this study on a much larger cohort size (3224 for PD and 2819 for controls) should be regarded as being more representative of the A419V variant in this ethnic group.

Therefore, our analysis strongly suggests the A419V variant is not a risk factor in PD in Chinese PD patients.

We found that the allele was monomorphic in the Malay and Indian ethnic groups, regardless of PD status. We recognise that the size of our cohort of Malay and Indian subjects is relatively small with 369 subjects in total. However, the potential contribution of *LRRK2* towards PD risk in these populations has received little attention, with only 1 report by Tan et al[7] analysing the *LRRK2* G2385R variant in a Malay and Indian cohort.[13] Similar to the Tan et al[13] study on G2385R, there were no A419V carriers in our Malay and Indian subjects. However as the minor allele frequency is below 1%, we acknowledge that further work with a larger sample size is needed to address this issue more closely.

Conclusion

In conclusion, based on our findings and the existing literature, we call for cautious interpretation of the A419V data. Independent replication studies in Taiwanese Chinese and other Asian populations will be required before concluding that A419V is a risk variant for PD amongst Asians.

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