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Association analysis of *EIF4G1* and Parkinson disease in Xinjiang Uygur and Han nationality

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Abstract

This study is to investigate whether the known mutations P.R1205H and P.A502V were pathogenic factors of Parkinson disease (PD) in Xinjiang Uygur and Han people.

A case–control study with polymerase chain reaction–restriction fragment length polymorphism method was performed on 150 cases of PD and 130 cases of age, sex, and national-matched healthy controls for rs200221361 polymorphism analysis and Sanger sequencing. Specific mutations were chosen for further sequencing in a case–control study.

The 3 variants located on the exon 10, and the rs200221361 was a nonsynonymous variant. The frequencies of rs200221361 genotype and allele between PD and control groups in Uygur and Han people showed no significant difference (for genotype, $\chi^2 = 0.91$, $P > .05$; for allele, $\chi^2 = 0.91$, $P > .05$). Statistical analysis showed that there were no differences in allele and genotype frequencies of rs200221361 genotype and allele between PD and control groups among the age, gender, or race ($P > .05$).

P.Ala502Val and P.Arg1205H may not be pathogenic mutations to PD in Xinjiang Uygur and Han people. The polymorphism of the rs200221361 may have no association with the occurrence of PD in Uygur and Han people of Xinjiang.

Abbreviations: PCR = polymerase chain reaction, PD = Parkinson disease.

Keywords: *EIF4G1*, Han nationality, Parkinson disease, polymorphism, Uygur nationality

1. Introduction

Parkinson disease (PD) is a common neurodegenerative disease, and its main clinical symptoms are resting tremor, bradykinesia, muscle rigidity, gait disturbance, and other motor symptoms. Patients with severe PD symptoms suffer from memory impairment and dementia. With the development of this disease, nonmotor symptoms will appear, such as autonomic dysfunction, cognitive disorders, depression, olfactory disorders, psychotic disorders, and sleep disorders.[1] One of the main pathological changes is selective degeneration of substantia nigra dopaminergic neurons, which could induce dopamine dysfunction. Another main change is the disorder of eosinophilic intracytoplasmic inclusion bodies (Lewy bodies). The incidence of PD among the seniors age above 65 is 1% to 2% and 4% among those ages above 85.[2] According to the epidemiological survey in China, the incidence of autonomic dysfunction is about 1.7% in males ages above 65 and 1.6% in females of the same age group.[3] The pathogenesis of PD is still unclear and the identified reasons may contain genetic factors, oxidative stress, toxicity of excitatory amino acids, mitochondrial defects, cell apoptosis, immune factors, and environmental toxins.[4,5] PD might be an autosomal dominant disorder, in which 5 pathogenic genes are found, namely, *SNCA*, *UCHL1*, *LRRK2*, *VPS35*, and *EIF4G1*.

eIF4G1 is a protein scaffold subunit of the translation initiation complex eIF4F, which binds the ribosomal 40S. A decrease in the levels of eIF4G1 protein in cells results in a reduction of overall protein synthesis linked to nutrient sensing.[6] Reported pathogenic eIF4G1 substitutions P.R1205H and P.A502V are shown to disrupt binding to eIF3E and eIF4E, respectively, and result in impaired nutrient sensing and mitochondrial dysfunction.[6,7] Interestingly, over-expression of eIF4G1 protein has been implicated in cell proliferation as observed in some malignant disorders, especially inflammatory breast cancer.[8] This evidence supports a role for *EIF4G1* mutations in cell survival and potentially the neuronal damage observed in PD.

According to our knowledge, the report on the polymorphism of *EIF4G1* gene is very limited among ethnic minorities with PD. One study performed in 2012 to analyze the *EIF4G1* in Han nationality and found the *EIF4G1* gene is not a pathogenic gene.[9] However, there was no relevant study performed on the association of *EIF4G1* gene and PD in Xinjiang Uygur nationality. In Xinjiang, there are many ethnic groups. For each nationality, the climatic conditions, geographical features, living habits, food culture, and predispositions for diseases are all different. Uighur nationality occupied 45.94% of the population in Xinjiang. They may have different genetic backgrounds compared with Han people. So they may have differences in etiology of disease and compared with Han people. And studies which was performed among cases age ≥ 55 years have shown that the prevalence of PD in Uighur was 1.1%, and the prevalence of PD in Han was 0.7% in the Kashi region of Xinjiang.[10] This

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article chose 4 different populations, including Uygur people from Xinjiang with PD, Han people with PD, and case–matched healthy controls. The aim of this study was to detect the correlation between polymorphism of *EIF4G1* gene and PD among patients from different ethnic groups and to determine whether *EIF4G1* gene was also a pathogenic gene among the Uygur people. We also analyzed the difference in allele and genotype frequency distribution of *EIF4G1* gene between Han people and Uygur people.

2. Materials and methods

2.1. General information

A total of 150 sporadic PD patients, either of Uygur ethnic minority or of Han nationality, treated at Department of Neurology, People's Hospital of Xinjiang Uygur Autonomous Region from March 2012 to July 2015, were included. PD patients from Uygur ethnic minority totaling 80 were ages 30 to 80 years (mean 63 ± 10.9 years, 42 males and 38 females); PD patients of Han nationality totaling 70 were ages 33 to 83 (mean 60 ± 10.2 years, 37 males and 33 females).

Healthy Uygur people and Han people who received physical examination in the same period at our hospital were included, totaling 130. These subjects had no past history or familial history of organic diseases of the brain, neurological lesions, extrapyramidal diseases, and mental diseases. Among 70 healthy Uygur subjects, 38 cases were males and 42 females, ages 40 to 80 years (mean 70 ± 6.5 years); of 60 Han subjects, 30 cases were males and 30 females, ages 33 to 85 years (mean 57.8 ± 10.2 years). The study was approved by the Ethical Review Committee of People's Hospital of Xinjiang Uygur Autonomous Region. Written informed consent was obtained from all cases.

Diagnosis was made by 2 experienced neurologists based on the criteria by Parkinson's Disease Society.[11] Those subjects who had a history of encephalitis, cerebrovascular diseases, poisoning using antipsychotics, familial PD, and Parkinsonism-Plus were excluded.

2.2. Experiment

The informed consent was obtained, and from each subject 3 mL peripheral venous blood was drawn into an ethylene diamine tetra-acetic acid-containing tube. DNA extraction was performed using whole-blood genomic DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instruction. DNA purity was determined as 1.7 to 1.9 and DNA concentration was $\geq 10\,\mu g/mL$. The primers of P.1205H and P.A502V were designed by Sangon Biotech Co, Ltd (Shanghai, China): F: 5′-TTCATACCTGTCCTGGTTGG-3′, R: 5′-CTTTCCCTCCATTCCTCTCC-3′, and F: 5′-CAAGGAGGT-GACAGCATCAA-3′, R: 5′-GCAGCCAAAGAACATTATTT CC-3′. The polymerase chain reaction (PCR) system consisted of the followings: 12.5 μL 2× *Taq*PCR master mix, 2 μL template, upstream and downstream primers 1 μL each, and 8.5 μL ddH_2O. PCR conditions: predenaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, 35 cycles, final extension at 72°C for 10 min. Sequencing of the primer and the amplified products was carried out by Sangon Biotech Co, Ltd. The target fragments were analyzed by agarose gel electrophoresis, identified by restriction enzyme digestion and then sequenced. Chromas 2.0 and Seqman softwares were used for the analysis of the sequencing results. The sequences were aligned against those from http://www.ncbi.nlm.nlh.gov, and known polymorphisms, mutations, and novel base changes were identified.

2.3. Statistical analysis

SPSS 17.0 software was used for statistical analysis. Genotype frequencies and allele frequencies were compared between different populations and different subgroups using the chi-squared test. Measurements were reported as mean ± standard deviation. Pair-wise comparison of measurements was done using t test. $P < .05$ indicated significant difference.

3. Results

3.1. Identification of PCR products

In order to identify the PCR products, the P.R1205H and P.A502V mutants of the *EIF4G1* gene were amplified and analyzed by electrophoresis. The product fragments of P.R1205H and P.A502V mutants had the length of 412 and 424 bp, respectively (Fig. 1, left panel). The products acquired were just the target fragments.

3.2. Detection of P.R1205H and A502V mutants

To detect the presence of P.R1205H and A502V mutants, the normal populations were tested. Meanwhile, mutation screening was also conducted for the healthy controls. The results indicated that the P.R1205H and A502V mutants were detected in 150 sporadic PD patients and in 130 gender-, age- and ethnic-matched normal controls by using Sanger sequencing (Fig. 1, right panel). No P.Arg1205H and P.Ala502Val mutations were discovered in PD patients and control cases.

3.3. Detection of rs200221361 polymorphism

To identify the rs200221361 polymorphism, Sanger sequencing was used to detect the alleles A and C in both PD patients and healthy controls, with 3 genotypes (AA, AC, and CC; Fig. 2). The number of 150 PD cases carrying AA/AC/CC genotypes was 148, 2, and 0, respectively; the number of 130 healthy controls carrying the 3 genotypes was 130, 0, and 0, respectively. The frequency of allele A and C in sporadic PD cases was 98.7% and 1.3%, respectively; among the healthy controls, the frequency was both 0. Using chi-squared test, the PD cases and the healthy controls did not show significant differences in genotype and allele frequencies of rs200221361 polymorphism ($P = 1.746$, $\chi^2 = 0.186$; $P = 3.457$, $\chi^2 = 0.063$).

3.4. Differences in genotype and allele frequency distributions

To analyze the relation between the rs200221361 polymorphism and PD, the difference in genotype and allele frequency distributions of rs200221361 polymorphism was tested among Uygur people and Han people. The results show that there was no relation between the rs200221361 polymorphism and PD (Table 1).

Based on age stratification, genotype and allele frequencies of rs200221361 polymorphism were compared between PD cases and healthy controls. Given the effect of aging on PD, PD cases were stratified by age. Cases with onset age below 50 were defined as early onset, and those with onset age above 50 were as

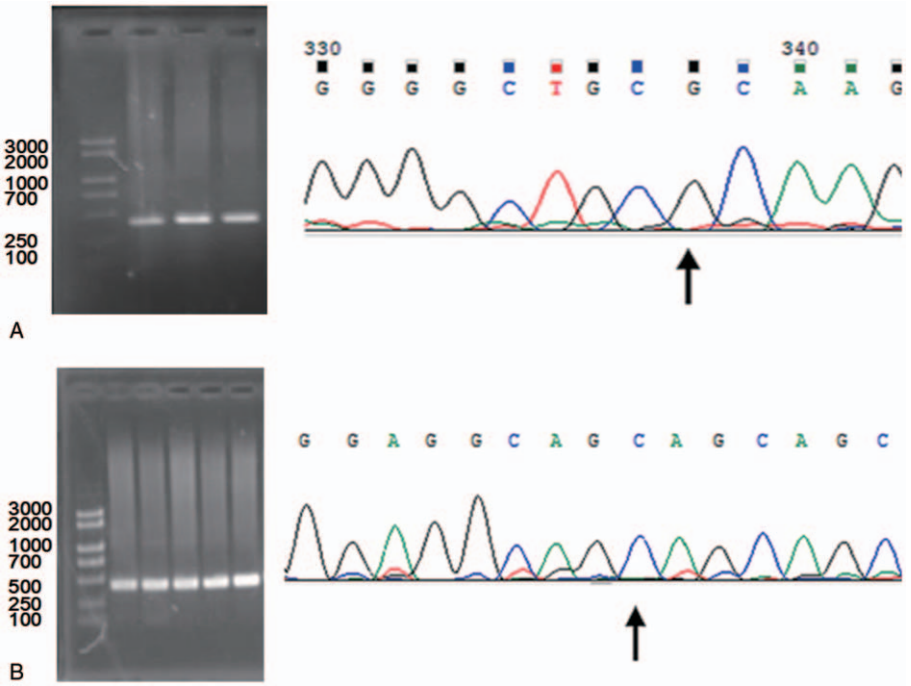

Figure 1. The P.R1205H mutants and P.A502V mutants amplification results. (A) The P.R1205H of the *EIF4G1* gene analyzed by electrophoresis (left panel) and GG genotype sequencing pattern unraveled by Sanger sequencing (right panel). (B) The P.A502V of the *EIF4G1* gene analyzed by electrophoresis (left panel) and CC genotype sequencing pattern illustrated by Sanger sequencing (right panel).

late onset. Then, the genotype and allele frequencies of rs200221361 polymorphism were compared between PD cases and healthy controls. Table 2 shows that no significant difference in genotype and allele frequencies was found among late-onset PD cases of Uygur ethnic minority or Han nationality as compared with the matched healthy controls ($\chi^2=0.104$, $P=2.638$; $\chi^2=0.106$, $P=2.619$).

Next, based on gender and ethnic stratification, genotype and allele frequencies of rs200221361 polymorphism were compared between subgroups of PD cases and healthy controls. Epidemiological survey has indicated differences in incidence of PD among males and females and also among different ethnic groups. Thus, the PD cases were stratified by gender and ethnic group, respectively. No significant differences in genotype and allele frequencies were found among PD cases of different gender and ethnic group as compared with the healthy controls ($P>.05$, Tables 3 and 4).

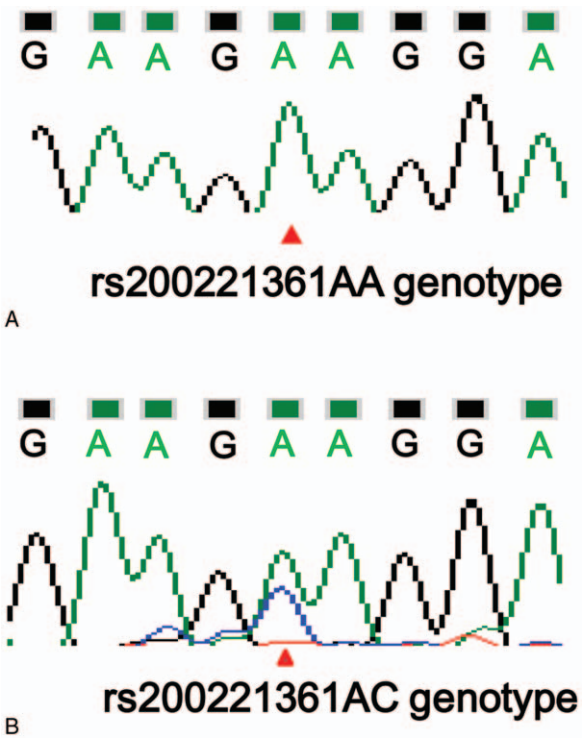

Figure 2. Alleles A (A) and C (B) were detected in both Parkinson disease patients and healthy controls, with 2 genotypes (AA, AC).

4. Discussion

P.Ala502Val and P.Arg1205H mutants of the *EIF4G1* gene play an important role in the pathogenesis of PD. Chartier-Harlin et al performed whole-genome analysis and cosegregation analysis for 1 late-onset case of inherited familial PD from France in 2011 and discovered missense mutation in the *EIF4G1* gene for the first time.[7] Besides the P.R1205H mutant, other mutations that were associated with PD were also found, including P.A502V, P. G686C, P.S1164R, and P.R1197W. The genetic sequencing was conducted among 251 patients with autosomal recessive PD and 235 normal controls. Lesage et al identified a novel candidate mutant P.E462dellnsGK in 2 sisters with PD and the known mutant P.G686C in 2 cases of sporadic PD.[12] Moreover, among the healthy controls, P.R1197W mutant was detected. The previous study claimed no healthy controls were found to be the carriers of P.A502V, P.G686C, P.S1164R, and P.R1197W, but these mutations were detected in 2 cases with Lewy body disorder by biopsies.[7] Thus, the mutations were considered as benign because they did not cause functional changes of the *EIF4G1* gene and hence PD. This raises doubt about the pathogenicity of

Table 1

rs200221361 Polymorphism allele and genotype frequency in PD group and control group.

		Genotype, n (%)				Allele, n (%)		
Group	**N**	**AA**	**AC**	**CC**	***P***	**A**	**C**	***P***
PD	150	148 (98.7)	2 (1.3)	0 (0)	1.746	298 (99.3)	2 (0.7)	3.457
Control	130	130 (100)	0 (0)	0 (0)		260	0	

PD = Parkinson disease.

Table 2

rs200221361 Polymorphism allele and genotype frequency of the people with different age in PD group and control group.

		Genotype, n (%)				Allele, n (%)		
Age	**N**	**AA**	**AC**	**CC**	***P***	**A**	**C**	***P***
≤50								
PD	60	58 (96.7)	2 (3.3)	0 (0)	2.638	118 (98.3)	2 (1.7)	2.619
Control	78	78 (100)	0 (0)	0 (0)		156	0	
≥50								
PD	90	90 (100)	0 (0)	0 (0)		180	0	
Control	52	52 (100)	0 (0)	0 (0)		104	0	

PD = Parkinson disease.

the above mutations, and the incomplete penetrance must be reassessed.

Emerging evidence has proved mutants affecting the *EIF4E* gene related to PD. Three nonsynonymous variants (p.T318I, p. V541G, and p.G698A) were identified among German 975 PD patients and 1014 normal controls.[13] The p.G698A variant was only detected in 1 PD patient, which was suspected of being pathogenic. In addition, Nuytemans et al performed case–control study using 213 PD patients and 272 controls, and found a novel mutation p.E525del that possibly affected the *EIF4E* gene.[14] This mutant was detected in 1 PD patient, but not in normal controls. Particularly, P.R1205H was found in 3 German normal controls, which was considered prone to PD after reaching a certain age.[13] Then, it was detected in familial PD and inherited diseases related to cosegregation. An 86-year-old carrier of the P. R1205H mutant showed no PD symptoms, thus P.R1205H mutation was considered a risk factor, which was agreed with a previous report.[7] It is suggested that P.R1205H mutant of the *EIF4G1* gene may be a rare pathogenic gene in familial PD. Tucci et al did not find the presence of P.Arg1205H and P.Ala502Val mutants in either the case group or the control group, but they found the mutants in population from NHLBI with frequency of 0.02%.[15] However, in 29 cases of familial PD from Han nationality, 503 cases of sporadic PD, and 508 normal controls,

Table 3

rs200221361 Polymorphism allele and genotype frequency of the people with different sex in PD group and control group.

		Genotype, n (%)				Allele, n (%)		
Gender	**N**	**AA**	**AC**	**CC**	***P***	**A**	**C**	***P***
Male								
PD	79	79	0	0		158	0	
Control	68	88	0	0		176	0	
Female								
PD	71	69 (97.2)	2 (2.8)	0 (0)	2.057	140 (98.6)	2 (1.4)	2.042
Control	72	72 (100)	0 (0)	0 (0)		144	0	

PD = Parkinson disease.

Table 4

rs200221361 Polymorphism allele and genotype frequency in Xinjiang Uygur and Han nationality.

		Genotype frequency				Allele frequency		
Group	**N**	**AA**	**AC**	**CC**	***P***	**A**	**C**	***P***
Uygur								
PD	80	80	0	0		160	0	
Control	70	90	0	0		180	0	
Han								
PD	70	68 (97.1)	2 (2.9)	0 (0)	1.741	138 (98.6)	2 (1.4)	1.703
Control	60	60 (100)	0 (0)	0 (0)		120	0	

PD = Parkinson disease.

P.Arg1205H mutant was absent.[9] Huttenlocher et al found only 1 heterozygous carrier of P.Arg1205H mutant among a total of 2146 PD patients and no mutations of P.Ala502Val were identified.[16] A large number of case–control studies have been carried out in China,[17] southwestern China,[18] India,[19] Japan,[20] South Africa,[21] and Italy.[22] Some healthy controls were also carriers of the P.R1205H mutant, which was then considered as benign. Furthermore, no P.Arg1205H and P. Ala502Val mutations were discovered and they were not associated with PD, as was also indicated by our study.

Similarly, the present study did not identify pathogenic mutations in the *EIF4G1*. GG and CC genotypes were identified for P.Arg1205H (rs112176450) and P.Ala502Val (rs111290936). We performed polymorphism analysis by using the PCR–restriction fragment length polymorphism technique among PD cases of Uygur ethnic minority, Han nationality, and the matched healthy controls. The 4 different populations did not show significant differences in allele and genotype frequencies of the rs200221361 polymorphism. No significant differences between PD patients of different ethnic group were found. The allele and genotype frequencies of the rs200221361 polymorphism did not show significant differences after subgroup analysis based on gender and age stratification either.

However, the sample size of this study was small and relevant research with a large sample should be performed in the future. In our study, the familial PD patients have been excluded and we also need study the association between these mutations and familial PD in Xinjiang Uygur and Han nationality.

To conclude, the polymorphism of the *EIF4G1* gene was not pathogenic in sporadic PD among Uygur people from Xinjiang and Han people.

Author contributions

Conceptualization: Hongyan Li.
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Funding acquisition: Hongyan Li.
Investigation: Yu Ma.
Methodology: Yu Ma.
Resources: Daxiong Zheng.
Software: Daxiong Zheng.
Supervision: Hongyan Li.
Writing – original draft: Yu Ma.
Writing – review & editing: Hongyan Li.

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