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Analysis of Thr12Met and Ala1144Thr Mutations of the *ATP13A2* Gene in Parkinson's Disease Patients in Xinjiang Uygur and Han Ethnic Groups

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Statistical Analysis C
Data Interpretation D
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Background: It has been reported that the *ATP13A2* gene is one of the most susceptible pathogenic genes of Parkinson's disease (PD). PARK9 mutations are found in early-onset PD and familial PD patients. Uygur and Han PD patients in the Xinjiang area were recruited as research subjects to study the differences in the Thr12Met and Ala1144Thr *loci* mutations of the *ATP13A2* gene in these PD populations. This study explored the mutations at the Thr12Met and Ala1144Thr gene *loci* of the *ATP13A2* gene in Parkinson's disease patients in the Uygur and Han populations in the Xinjiang province.

Material/Methods: The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to analyze the Thr12Met and Ala1144Thr mutations of the *ATP13A2* gene in a case-control study of 200 age- and sex- matched Uygur and Han PD patients.

Results: Of the 200 PD patients were studied, 2 from the Han group had a Thr12Met mutation, but Ala1144Thr mutations were not found. Among the Uygur PD patients, no Thr12Met or Ala1144Thr mutations were found.

Conclusions: Thr12Met and Ala1144Thr mutations of the *ATP13A2* gene are rare in the Uygur PD patients in Xinjiang. Overall, the mutation rates of Thr12Met and Ala1144Thr in the Uygur and Han PD patients in the Xinjiang region are low.

MeSH Keywords: **Mutation • Nerve Degeneration • Parkinson Disease**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/892821>



Background

Parkinson's disease (PD) is a progressive degenerative disease of the nervous system in middle-aged and older people. The causes of PD are not yet entirely understood. Studies have shown that aging, genetic, and environmental factors may be related to the onset of the disease [1]. Cases are mainly sporadic since known PD mutations occur only in about 5–10% of patients [2]. Presently, at least 18 PD-related *loci* and 15 PD-related genes have been identified [3–5]. The *ATP13A2* gene, also known as *PARK9*, is one area of genetic research focus in PD pathogenesis [6–8]. Cloning of *ATP13A2* gene has found a compound heterozygous mutations, 3 homozygous mutant, and 2 heterozygous mutations forms. The homozygous mutation and compound heterozygous mutations of the gene were similar, with typical clinical manifestations in PD patients. The *ATP13A2* gene contains 29 coding exons; encoding 1 contains 5 groups of P type ATPase 1180 amino acids, containing 10 transmembrane regions. Research has demonstrated that wild-type *ATP13A2* protein mRNA is expressed mainly in the brain, especially in the striatum, and expression is up-regulated in the brain in sporadic late-onset Parkinson's patients. It has been reported that the *ATP13A2* gene is one of the most susceptible pathogenic genes of PD. *PARK9* mutations are found in early-onset PD (EOPD) (younger than 50 years old) and familial PD patients [9]. Uygur and Han PD patients in the Xinjiang area were recruited as research subjects to study the differences in the Thr12Met and Ala1144Thr *loci* mutations of the *ATP13A2* gene in these PD populations.

Material and Methods

Ethics statement

The research protocol was approved by the ethics committee of First Affiliated Hospital of Xin Jiang Medical University (Protocol NO: 20130216-12). All the patients provided appropriate informed consent.

General information

From September 2012 to September 2013, 200 cases of primary PD patients from Uygur and Han ethnic groups treated in the First Affiliated Hospital of Xinjiang Medical University were selected. All subjects had lived in the Xinjiang area for more than 2 generations, without marriages to other ethnic groups. The matched groups were selected based on sex, age, living environment, etc. A total of 200 PD patients were selected (110 male, 90 female); 100 patients were Uygur and the others were Han.

The onset age range was 31–84 years, with an average of 58.45 ± 11.74 years. The age of 50 was used to separate the

early-onset from the late-onset PD subjects. In the early-onset group, there were 64 cases (42 Uygur, 22 Han; 28 male, 36 female). The onset age range was 31–50 years, with an average of 46.17 ± 3.52 years. In the late-onset group, there were 136 cases (58 Uygur, 78 Han; 82 male, 54 female). The onset age range was 51–84 years, with an average of 66.01 ± 8.04 years. The United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria for Idiopathic Parkinson Disease was used as the screening criteria. Neurology experts reviewed the cases with difficulties in diagnosis, and cranial MRI or CT scans were used when necessary to rule out secondary PD. There were no statistically significant differences in age or sex between the Uygur and Han groups.

Experimental method

After obtaining informed consent from patients, 2 mL of peripheral blood was drawn and placed in anticoagulant tubes containing 800 μ L of ethylene glycol tetra acetic acid. A genomic DNA extraction kit from Tiangen Biochemistry Co., Ltd. (Beijing) was used to isolate genomic DNA. Standard extracted DNA samples had a purity of 1.7–1.9 and concentrations ≥ 10 ng/ μ L. The samples were stored at -20°C .

The primers were synthesized by the Shanghai Biological Engineering Co. The primer sequences used to amplify the Thr12Met site were: PF: 5'-CAGGCTGATGTTTATTGTTTAA-3' (f), and PR: 5'-CTCTCACCTCTTGTCTCTTATT-3' (r). The primer sequences used to amplify the Ala1144Thr site were: PF: 5'-GCAGGGAGTTCAGTGTCTG-3' (f), and PR: 5'-GGTCTGGTCACCC TCAACTTC-3' (r). The polymerase chain reaction (PCR) mix contained 10 μ L 2 \times Qat PCR Master Mix, 0.5 μ L forward and reverse primers (10 μ mol/mL), 2 μ L DNA sample (100 ng/mL), and deionized water added to bring the final volume to 20 μ L. The PCR reaction conditions for the Thr12Met mutation were as follows: the initial denaturation was at 94°C for 3 minutes, followed by 25 cycles of reactions: denaturing at 94°C for 30 seconds, annealing at 57.7°C for 30 seconds, and extension at 72°C for 30 seconds. The last extension was at 72°C for 7 minutes, and then reaction samples were stored at 4°C . The PCR product was 491 bp. The PCR reaction conditions for the Ala1144Thr mutation were as follows: the initial denaturation was at 94°C for 3 minutes, followed by 25 cycles of reactions: denaturing at 94°C for 30 seconds, annealing at 60.3°C for 30 seconds, and extension at 72°C for 30 seconds. The last extension was at 72°C for 7 minutes, and then reaction samples were stored at 4°C . The PCR product was 323 bp.

We electrophoresed 7 μ L of PCR products on a 2% agarose gel at 120V for 30 minutes, and then observed under a UV gel imager. When the correct length band was observed, the PCR reaction was digested using restriction enzymes. The total volume of enzymatic reaction included 10 μ L PCR product,

Table 1. Comparison of the general clinic data of Uyгур and Han PD patients.

General data	Uyгур	Han	χ^2	t	P
Sex (male: female)	60; 40	50; 50	0.012	–	>0.05
Age (average)	58.45±11.74	60.13±11.59	–	1.947	>0.05
Early – onset group age	46.17±3.52	46.74±3.08	–	0.983	>0.05
Late – onset group age	66.01±8.04	66.74±7.68	–	0.718	>0.05

2 μ L 10 \times buffer solutions, 0.5 μ L restriction endonucleases (10 U/ μ L), and double-distilled water added to bring the final volume to 20 μ L. The reaction was carried out in a 0.2 mL high-quality sterilized Elmendorf PCR tubes and incubated at 37°C overnight (approximately 16–24 hours). The enzymatic products were subjected to polyacrylamide gel electrophoresis and analyzed by a UV gel imaging system. The DNA specimens were sequenced by the Golden Standard PCR product sequencing system.

Restriction endonuclease Nirimaii was used to identify the Thr12Met site mutation. When there was no mutation, there were 2 RFLP bands at 330 bp and 161 bp. This result was defined as GG type. If there was a mutation at the Thr12Met site, there were 3 or 4 RFLP bands. These results were defined as AA type or AG type. AA type was a homozygous mutation with 3 RFLP bands at 105 bp, 161 bp, and 330 bp. AG type was a heterozygous mutation with 4 RFLP bands at 105 bp, 225 bp, 161 bp, and 330 bp.

Restriction endonuclease Faquir was used to identify the Ala1144Thr site mutation. When there was no mutation, there were 4 RFLP bands at 49 bp, 59 bp, 96 bp, and 119 bp. This result was defined as CC type. If there was a mutation at the Ala1144Thr site, there were 3 or 5 RFLP bands. The TT type was a homozygous mutation with 3 RFLP bands at 49 bp, 59 bp, and 215 bp. The CT type was a heterozygous mutation with 5 RFLP bands at 49 bp, 59bp, 96 bp, 119 bp, and 215 bp.

Statistical analyses

Experimental data were analyzed using SPSS 17.0 statistical analysis software. Mean \pm standard deviation was used to describe the central tendency of the measured data. Homogeneity of variance test was performed for the comparison of 2 data groups. The Hardy-Weinberg equilibrium test was performed for the representative sample groups. The chi-squared test was used for comparison, and $p>0.05$ indicates population genetic equilibrium. The test standard was set to $\alpha=0.05$. Differences were considered statistically significant when $p<0.05$.

Results

Comparison of the general data of Uyгур and Han PD patients

The chi-squared test showed no statistically significant differences between Uyгур and Han PD patient males and females ($p>0.05$), and the t test demonstrated no statistically significant differences in ages ($p>0.05$). The comparisons between groups are shown in Table 1.

Detection and analysis of Thr12Met mutation of *ATP13A2* gene subtypes

To determine the gene mutation of Thr12Met, total RNA was extracted from patients with PD and PCR-RFLP was performed. As shown in Figure 1A, the PCR amplification fragment of Thr12Met sites in *ATP13A2* gene was 491bp. Thr12Met sites of *ATP13A2* gene was cut into 3 bands, and it was called AA type (homozygotic type): 105//161//330 (Figure 1B). As shown in Figure 1C, the sequence of Thr12Met sites in *ATP13A2* gene was analyzed, and T was mutated into C.

Detection and analysis of Ala1144Thr mutation of *ATP13A2* gene subtypes

We analyzed the expression of Ala1144Thr Mrna. As shown in Figure 2A, the PCR amplification fragment of Ala1144Thr sites in *ATP13A2* gene was 323 bp. Sequence analysis of Ala1144Thr sites in *ATP13A2* gene (Figure 2B) showed there was no mutation, whereas when rs3170740 gene mutation was found, CC was changed into TT or CT (Figure 2C, 2D).

Discussion

Previous studies have demonstrated that the incidence of PD has great ethnic and geographic variation around the world [10]. Specifically, most PD patients are Caucasians, followed by Asians, and then African-Americans. The prevalence rate in Caucasians is 10.6–30.2 million, with an incidence rate of

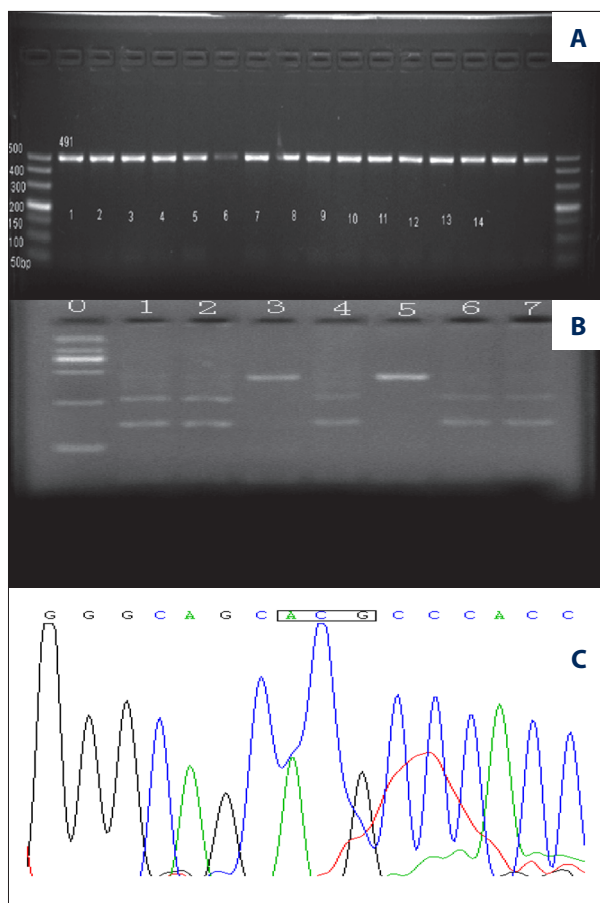


Figure 1. Detection and analysis of Thr12Met mutation of *ATP13A2* gene subtypes. (A) the PCR amplification fragment of Thr12Met sites in *ATP13A2* gene was 491 bp; (B) Thr12Met sites of *ATP13A2* gene was cut into 3 bands; (C) sequence of Thr12Met sites in *ATP13A2* gene was analyzed, and T was mutated into C.

1.2–2.0 million. The prevalence rate in Asians is 4.4–8.2 million, with an incidence rate of 1.0 million. The prevalence rate in African-Americans is 3.1–5.8 million, with an incidence rate of 0.45 million [11]. Moreover, the prevalence rates among different countries in Europe and America are also different. Besides racial factors, studies have shown that the incidence and prevalence of PD varies in people of the same race living in different environments. These findings indicate that environmental factors may also play an important role in the development of PD [6]. Primary PD patients with a distinct familial history represent 10–15% of all PD cases, revealing significant genetic susceptibility and the involvement of familial and genetic factors [12,13]. The vast majority of PD patients have no apparent genetic predisposition, suggesting that the development of PD may be the combined action of environmental factors and genetic susceptibility [14].

In different populations, the *ATP13A2* gene mutant site types and frequencies are different [7]. The overall frequency of

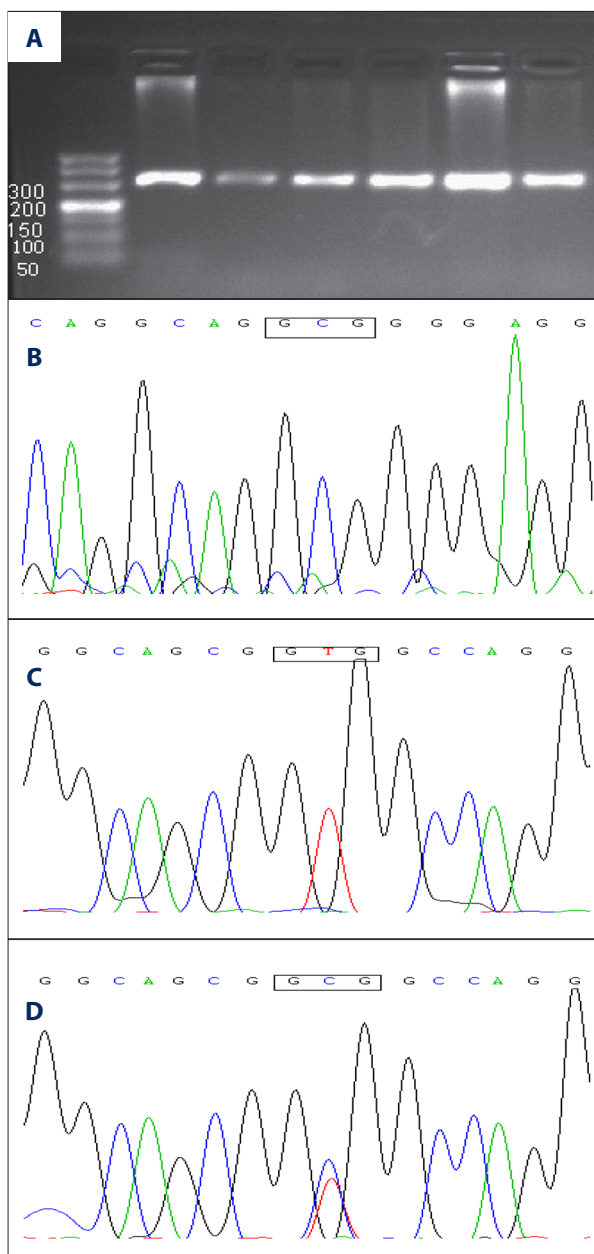


Figure 2. Detection and analysis of Ala1144Thr mutation of *ATP13A2* gene subtypes. (A) The PCR amplification fragment of Ala1144Thr sites in *ATP13A2* gene was 323 bp; (B) Sequence analysis of Ala1144Thr sites in *ATP13A2* gene; (C) there was no mutation; (D) whereas when rs3170740 gene mutation was found, CC was changed into TT or CT.

ATP13A2 gene mutations is low [15]. Several studies analyzing PD patients (primarily EOPD) showed negative mutation rates at the *ATP13A2* gene [16]. There were no *ATP13A2* gene mutations found in late-onset PD patients [17]. Consequently, *ATP13A2* mutations may only be related to specific types of PD pathogenesis [18].

Xinjiang is an area inhabited by various ethnic groups. The climate conditions, lifestyle, diets, education level, and the disease spectrums of different ethnic groups vary in different regions [19]. In this study, the PCR-RFLP method was used to investigate the PD gene mutations in patients of the Uygur and Han ethnic groups in Xinjiang. The results showed that 2 of the 200 PD cases had Thr12Met mutations.

One of these was a 50-year-old male Han patient who had the disease for 2 years. Limb stiffness and slow movement characterized the disease's onset. The limb tremor symptom was relatively mild. His parents and siblings did not have similar disease history. The progression of the disease was slow. The total score of the UPDRS-III was 8; H-Y grading was the second period. The cranial MRI showed brain ischemia, normal nerve sensory conduction velocity, and no f-wave. The visual and auditory evoked potentials were normal. Sleep polysomnography showed a sleep apnea index of 4, indicating slightly disordered sleep architecture, and increased light sleep stages. MMSE score was 24; SAS score was 46; SDS score was 34. The electrolytes were normal. EMG (bicep muscle, anterior tibial muscle) showed fibrillation potential. EEG was normal. Another patient with the mutation was a Han female, who had the disease for 5 years with an onset at 30-years-old. Slow movement, muscle rigidity, tremors, and response to Dopamine treatment were found. The patient also showed emergence of symptoms fluctuation, with no illusion. She had supranuclear gaze palsy and dementia. The cranial MRI and other examinations were normal.

Di Fonzo et al. [20] (2007) reported Thr12Met and Gly533Arg heterozygous mutations in 2 Italian sporadic PD patients (<50 years old). Both patients had similar clinical manifestations. Consistent results were reported in the present study, suggesting that the Thr12Met heterozygous mutation may be an

EOPD disease-related risk factor. Several scholars have also reported that the Thr12Met mutation, EOPD, and familial PD might be related [14].

Ala1144Thr of the *ATP13A2* gene is one of the latest mutant sites under study, but is not well documented [17]. Our research results showed no Ala1144Thr mutation in the 200 cases of Uygur and Han PD patients. It is worth noting that a few mutations at the rs3170740 position (CGAGAGCTGGCCGAGCAGCCCTGGCC[A/G]CCGCTGCCGCCGCCCTGAGGT) were discovered. The middle "C" was mutated to a "T". The sequencing results showed a heterozygous mutation with 2 peaks. The serial number of the mutation is rs3170740, which needs to be verified in further studies.

Conclusions

Only 2 cases of *ATP13A2* gene Thr12Met mutations were found in this study, with both belonging to the Han ethnic group. There were no site mutations found in the Uygur PD patients, indicating that the gene mutation at this site may be rare in Xinjiang Uygur PD patients. Overall, the mutation rates of Thr12Met and Ala1144Thr were low in the PD patients in the Uygur and Han ethnic groups of the Xinjiang area. This result is consistent with previous studies, but there are also differences that may be attributed to the insufficient sample size, the detection methods affected by many external factors, or the geographical differences. Therefore, more studies and replicates are needed to further understand the *ATP13A2* gene function and provide experimental evidence for individualized treatment of PD patients.

Declaration of conflict of interest

None.

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