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Research article

Association of AKT1 gene polymorphisms with sporadic Parkinson's disease in Chinese Han population

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HIGHLIGHTS

- AKT1 gene rs2498799, rs2494732 and rs1130214 polymorphisms were examined in Han Chinese cohort.
- The rs2498799 in AKT1 gene is associated with risk of PD patients in Han Chinese female population.
- No association was found between in rs2494732 and rs1130214 polymorphism and PD.

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ABSTRACT

Genetic variants of AKT1 have been shown to influence brain function of Parkinson's disease (PD) patients, and in this paper our aim is to investigate the association between the three single-nucleotide polymorphisms (rs2498799; rs2494732; rs1130214) and PD in Han Chinese. 413 Han Chinese PD patients and 450 healthy age and gender-matched controls were genotyped using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR–RFLP) method. Both the patient and control groups show similar genotype frequencies at the three loci: rs2498799, rs2494732 and rs1130214. We are able to identify a significant difference in the frequencies of genotype ($p = 0.019$) and G allele (OR = 0.764, 95% CI = 0.587–0.995, $p = 0.045$) both at rs2498799 between the patient and control groups. Furthermore, the association of subjects with GG genotypes versus those with GA + AA genotype remain significant after adjusting for age in the Han Chinese female cohort (OR = 0.538, 95%CI = 0.345-0.841, $p = 0.006$), which is especially evident in the late-onset cohort (OR = 0.521, 95%CI = 0.309-0.877, $p = 0.012$). In contrast, allele frequencies at rs2494732 and rs1130214 were similar between patients and controls in all subgroup analyses. These results suggest that polymorphism of AKT1 locus is associated with risk of PD and that the G allele at rs2498799 may decrease the risk of PD in the North-eastern part of Han Chinese female population.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic (DA) neurons in the substantia nigra of the midbrain. Substantial progress has been made in the study of genetic basis of PD [3]. Mutations expression contributes to the damage and subsequent loss of DA neurons. In particular, the vast majority of PD cases are considered idiopathic, whose specific pathogenesis remains to be elucidated. By identifying genes that show association with sporadic disease, the understanding of genetic architecture of PD may provide valuable insights into individual risk prediction and gene therapy for PD in the near future [29].

AKT1 is a serine/threonine protein kinase, belonging to the AKT/PKB family comprised of three genes in mammals: AKT1/PKB, AKT2/PKB and AKT3/PKB, which is critical for cell survival [10], and particularly for neuronal and non-neuronal cells [7,32]. AKT1 positively regulates transcription factors, CREB and NF-κB [9,20,28], which promote expression of several pro-survival genes, such as brain-derived neurotrophic factors. Continually enriched data suggests that AKT1 may be associated with neurological disorders, such as Huntington disease [15], schizophrenia [22], Alzheimer's disease [13], amyotrophic lateral sclerosis [8], and most importantly, Parkinson's disease [5,23,30].

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AKT1, who plays a major role in intracellular signaling pathways, is postulated as of etiological importance in Parkinson's disease of the dopamine system. Proteins from the AKT family are found to be phosphorylated to a lesser extent in dopaminergic neurons, as well as in small non-dopaminergic cells of the substantia nigra in Parkinson patients [27]. Functional studies investigating specific phosphorylation of AKT1 in Parkinson patients versus controls are warranted. Non-surprisingly, enhancing AKT1 kinase activity has been demonstrated to be protective in cellular and animal models of neurodegenerative conditions [14,19]. AKT1 is down regulated in the midbrain of PD patients [31] and specifically in the neurons in the substantia nigra pars compacta [21], which is reflected in both neurotoxin-induced cellular and genetic models of Parkinsonism [35]. More recently, oxidative modification of AKT1 increased its association with phosphatase PP2A, resulting in loss of its kinase activity in the ventral midbrain in a mouse model of PD [11].

Altered AKT1 signaling may influence cell survival in brain function. Genetic variants of AKT1 have been shown to influence neurodegenerative diseases. An association study performed in a Greek PD cohort [34] identified a certain protective SNP haplotype of T-T-G-G (rs2494743, rs2498788, rs2494746, rs1130214) in AKT1. On the contrary, one Swedish research does not support the hypothesis of genetic variants in AKT1 conferring protection against Parkinson's disease [26]. As such, these findings suggest that the effects of AKT1 polymorphism on PD risk depend on ethnicity, highlighting the need for further study on these polymorphisms in other populations.

Notably, these studies do not include the Han Chinese population from mainland China. Therefore, in view of the population-specific heterogeneity, we conducted the case-control study explores the role of three identified genetic variants of the AKT1 gene (rs2498799, rs2494732 and rs1130214), which are associated with the North-eastern part of mainland China. These include SNPs (whether alone or in haplotypes) previously reported to be associated with neurological diseases in the European and Japanese samples [17,22]. Furthermore, we also performed stratified analyses according to the age and gender of onset and to investigate whether they are related to the risk of PD in Han Chinese population.

2. Subjects and methods

2.1. Subjects

With an aim to provide more evidence into genuine loci contributing to PD across diverse populations, we genotyped 413 Parkinson patients (59.2 ± 11.32 years) and 450 healthy controls (59.0 ± 9.95 years; without any history of neurologic or psychiatric disorders) from our sample population for the following three SNPs in the AKT1 gene: rs2498799, rs2494732 and rs1130214. While all the PD patients were recruited from the Department of Neurology of the Affiliated Hospital of the Medical College of Qingdao University, the controls were selected from the Health Examination Center of the same, who were matched to the PD patients by sex and age. The characteristics of the PD patients and healthy control (HC) subjects are listed in Table 1.

Our study was approved by the Institute Ethical Committee and all subjects provided written informed consent. All patients fulfilled the London Brain Bank Clinical Diagnostic Criteria for idiopathic PD [6].

2.2. Methods

DNA was obtained from blood samples using standard protocols. We isolated genomic DNA from peripheral blood lymphocytes using a Genomic DNA extraction kit (Tiangen, Beijing) following the manufacturer's instructions. Polymorphisms of AKT1 gene (rs2498799, rs2494732, rs1130214)were detected in all subjects by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR–RFLP). The lengths of PCR-amplified fragments and restriction fragments are shown in Table 2. Moreover, to validate genotyping results, we performed sequencing analysis in randomly-selected 10% of the samples.

PCR amplification mixtures for AKT1 were carried out in a total volume of 10 μL, containing 37.5 ng of genomic DNA, 0.25U Hotstar-Taq DNA polymerase, 2 pmol primers of each, 200 μM of dNTP, and 1 μL 10 × PCR Buffer (100 mM of Tris–HCl, pH 8.3, 500 mM of KCl, 15 mM of MgCl2) (Applied Takara).

The conditions for rs2498799 and rs1130214 polymorphisms were as follows: 94 °C for 4 min, 35 cycles were performed with denaturation for 30 s at 94 °C, hybridization for 30 s at 61 °C, and extension for 30 s at 72 °C, followed by a final extension of 72 °C for 7 min. For rs2494732, the condition was 94 °C for 4 min, 35 cycles were performed with denaturation for 30 s at 94 °C, hybridization for 30 s at 64 °C, and extension for 30 s at 72 °C, followed by a final extension of 72 °C for 7 min.

The PCR products of rs2498799 were digested with 5 units of restriction endonuclease HpyCH4IV (New England BioLabs NEB, Beijing) at 37 °C for 2 h. And the PCR products of rs2494732 and rs1130214 were digested with 5 units of restriction endonuclease Pvu II and Xcml (New England BioLabs NEB, Beijing) at 37 °C for 2 h. All the digestion products were resolved on a 2% agarose gel stained with ethidium bromide and visualized in ultraviolet light.

In our study, patients and controls are subdivided into two groups: first, patients with early-onset PD (EOPD, diagnosed <50 years of age) and late-onset PD (LOPD, diagnosed ≥50 years of age) matched with the same-age control subgroups; Second, male and female PD patients arematched with the same-gender control subgroups. Male and female PD patients, and each healthy matched control subgroup. Means and standard deviations for the continuous variables, and frequencies and percentages for the categorical variables were analyzed, respectively. Significance for differences in gender and age between the PD and control groups were assessed using the chi-squared test and *t*-test, respectively. Genotype distributions were tested for concordance with Hardy-Weinberg equilibrium (HWE). Allele and genotype frequencies were determined by direct counting of alleles. Univariate and multivariate analyses were used to determine the risk factors for PD. The allelic and genotypic frequencies of each polymorphism were compared between the patients and the controls using Chi-square test or Fisher's exact test as deemed appropriate. Odds ratios (OR) and 95% confidence intervals (95% CIs) were calculated to assess the strength of polymorphisms and PD susceptibility. Logistic regression analysis was performed using PD as the dependent variable, while age, gender, and genotype (allele) of the three SNPs were used as independent variables. All statistical tests were carried out using SPSS version 17.0 (IBM, Chicago, USA) based on a two-tailed probability and P-values less than 0.05 were considered to be statistically significant. Haplotype analysis was performed using Haploview 4.2.

3. Results

Data on a total of 863 subjects including 413 patients and 450 controls were used for analysis. Genotype data were in Hardy Weinberg equilibrium for both cases and controls for all SNPs. No significant differences between patients and controls were observed for any single markers by allele or genotype. The genotype and allele frequencies of each SNP for both groups are summarized in Table 3.