RETRACTED ARTICLE: Association between nucleotide-binding oligomerization domain protein 2 (NOD2) gene polymorphisms and Parkinson’s disease (PD) susceptibility

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ABSTRACT

Objective: This study aimed to explore the association between single nucleotide polymorphisms (SNPs) of nucleotide-binding oligomerization domain protein 2 (NOD2) gene and Parkinson’s disease susceptibility, including IVS + 10A > C (rs72796353) and a missense mutation at exon 9 (c.2857A > G p.K953E).

Methods: Rs72796353 and c.2857A > G p.K953E polymorphisms of NOD2 gene were genotyped via polymerase chain reaction-restriction fragment length polymorphism in 125 cases with PD and 120 healthy controls. Genotype and allele frequencies differences of gene polymorphisms between the case and control groups were analyzed by the Chi-square test. Odds ratio (OR) and 95% confidence interval (95%CI) were used to indicate the relative susceptibility to PD. Furthermore, Hardy–Weinberg equilibrium (HWE) was evaluated by the \( \chi^2 \) test in controls.

Results: Neither genotypes nor allele of rs72796353 was significantly different in cases and control groups (\( \rho > .05 \)). Differently, AG/GG genotype of NOD2 2857A > G polymorphism were associated with the increased risk of PD (OR = 2.486, 95%CI = 1.223–5.056), and G allele carriers were 2.563 times risk to suffer from PD (OR = 2.563, 95%CI = 1.310–5.013). Besides, AG genotype might be also a risk factor for PD.

Conclusion: NOD2 c.2857A > G p.K953E polymorphism may be correlated with PD susceptibility in Chinese Han population, but not rs72779653. Further study should be conduct to certify this conclusion.

Introduction

Parkinson’s disease (PD) is a chronic neurodegenerative disorder which mainly attacks middle-aged people with a prevalence of approximately 1–2% at over 60 and 5% by age 85, behind Alzheimer’s disease (AD) [1,2]. The clinical symptoms of PD are rest tremor, rigidity, bradykinesia and postural instability [3]. PD is characterized by progressive deterioration, which severely influences the activity ability of patients and reduces living quality. PD is once reported as a non-genetic neurodegenerative disorder [4], but increasing evidences which genes, mutations involves in the onset of PD overthrow the conclusion [5–8]. What’s more, a lot of researches have confirmed that the occurrence of PD derives from polygenic inheritance, aging and environmental factors. But so far, the aetiology and pathogenesis of PD are still not completely clear.

The nucleotide-binding oligomerization domain protein 2 (NOD2), also known as the caspase activating recruitment domain 15 (CARD15), is a member of the pattern-recognition receptor family and encoded by NOD2 gene located on chromosome 16q [9,10]. NOD2 can recognize muramyl dipeptide (MDP) in cytomembrane to activate nuclear factor kappa B (NF-κB) pathway and stimulate inflammatory factor response [11–13]. Recent research has found that inflammation reaction caused by NF-κB pathway may be associated with pathogenesis of PD [14]. IVS + 10A > C (rs72796353), a known NOD2 polymorphism, is a SNP in intron 4 and has been found to involved in several diseases [15]. c.2857A > G p.K953E polymorphism is in exon 9 of NOD2, a novel missense variant. It could change the normal function of this receptor which plays a key role in the pro-inflammatory response and bacterial sensing [16].

Up to now, the relative researches about the relationship between these two mutations and PD has been not reported. Therefore, in the present study, we evaluate the effects of NOD2 rs72796353, c.2857A > G p.K953E polymorphisms on PD susceptibility were explored in a total of 245 subjects. We hope that our research can provide some clues for explaining the pathogenesis of PD and find the risk marker to early diagnose and timely treat PD.

Materials and methods

Subjects’ selection

This research adopted a case–control design, including 125 PD patients and 120 healthy controls. The patients with PD were confirmed by pathological in the First Hospital and Clinical College of Harbin Medical University. The healthy...
controls were recruited from healthy check-up centre of the same hospital with the cases at the same time. They had no extrapyramidal or other neurological degenerative diseases.

All subjects were Chinese Han population ruled out the existence of blood relationship. Written informed consent was obtained from all participants before enrolment. This research was reviewed and consented by Ethics Committee of First Hospital and Clinical College of Harbin Medical University. Sample collection was based on ethics criteria of national human genome research.

Sample collection

About 2 ml peripheral venous blood samples were collected from all subjects and anticoagulated by 0.5% EDTA (pH 8.0) in blood collection tube, and then stored at -80 °C. Genomic DNA was extracted by TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering CO., LTD, Beijing, China) according to the manufacturer’s instruction and stored at -20 °C for standby application.

Genotyping

NOD2 rs72796353, c.2857A > G p.K953E polymorphisms were genotyped via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR primer of polymorphisms were designed by Primer Premier 5.0 and synthesized by Shanghai Sangon Biotech Co., Ltd (Shanghai, China). The detailed information of PCR primers sequences are listed in Table 1.

PCR system was a volume of 25 µl mixture, including 2 µl genotic DNA template, each 0.5 µl of former and reverse primers, 12.5 µl PCR Master Mix and finally added redistilled water to 25 µl. PCR procedures were as follows: 94 °C initial denaturation for 5 min; followed by 32 cycles with denaturing at 94 °C for 30 s, annealing at 61 °C for 35 s and extension at 72 °C for 40 s; final extension for 5 min at 72 °C was performed, and preserved at 4 °C finally. The PCR products were examined by 1% agarose gel electrophoresis (AGE).

PCR amplified products of rs72796353 and 2857 A > G polymorphisms were digested with restriction enzymes MspI and Hin6I, respectively. A total volume of 20 µl enzyme digestion system were performed containing: 2.0 µl 10 × Buffer solution, 1.0 µl Taq enzyme, 10 µl PCR products, and 7.0 µl deionized sterile water. And then put into 37 °C water bath overnight. Enzyme digestion products were separated by 2% AGE and the results were observed in an ultraviolet transilluminator.

Statistical analysis

PASW statistics 18.0 software was employed for data analysis. Hardy–Weinberg equilibrium (HWE) was analyzed to test the representativeness of our study population. Genotype and allele frequencies of rs72796353 and 2857 A > G polymorphisms were calculated through direct counting. Genotypes and alleles distributions differences of NOD2 gene polymorphisms between the case and control groups were assessed by the χ² test. PD susceptibility intensity was evaluated with odds ratio (OR) and its 95% confidence interval (CI). The difference had statistical significance when p < .05.

Results

Characteristics of study subjects

A total of 245 subjects involving 125 cases and 120 controls were enrolled in this study, all subjects were Chinese Han population. The case group included 50 females and 75 males (age range 40–84 with the mean age of 67.52 ± 12.53). Among them, 19 patients were early-onset (onset age <50), the rest 101 were late-onset (onset age ≥ 50). The duration of PD patients was 4.59 ± 3.82. All patients had no family history of PD. The healthy control group included 47 females and 73 males (age range 36–85 with the mean age of 66.89 ± 13.24). The two groups had no statistical significance in age and gender (p > .05), which indicated a good match degree of selected subjects. Moreover, we found Smoking and drinking were not the risk factor of PD in this study (p > .05). MMSE (mini-mental state examination) score was respectively 28.51 ± 11.13 and 28.01 ± 3.42 in PD patients and the controls, the significant difference was found between the two groups (p = .031), but not UPDRS (“ON” or “OFF”). The detailed data are showed in Table 2 and Figure 1.

Genotypes distributions of NOD2 polymorphisms between two groups and the correlation to PD risk

Genotype and allele distributions of NOD2 rs72796353 and 2857 A > G polymorphisms are revealed in Table 3 and Figure 2. The genotypes distributions of rs72796353 and 2857 A > G in patients and the controls, the significant difference was found between the two groups (p = .031), but not UPDRS (“ON” or “OFF”). The detailed data are showed in Table 2 and Figure 1.

Statistical analysis

PASW statistics 18.0 software was employed for data analysis. Hardy–Weinberg equilibrium (HWE) was analyzed to test the

| SNP   | Primer sequences Location | SNP   | Primer sequences Location |
|-------|---------------------------|-------|---------------------------|
| rs72796353 For. S'-AGATCAGAGCGCCTCCTGT- 3’ | 5'-CAGGCTCTGGCCCTACAC- 3’ | 2857A > G For. S'-CCCGAGCTCCTCCTCCTGT- 3’ | 5'-AAGTCTGTATAATGTAAGCCAC- 3’ |

Note: MMSE: mini-mental state examination; UPDRS: Unified Parkinson’s Disease Rating Scale.

Table 1. Primer sequences of Table 1.

| SNP    | Primer sequences | Location |
|--------|------------------|----------|
| rs72796353 | For. 5'-AGATCAGAGCGCCTCCTGT- 3’ | Intron4 |
|         | Rev. 5'-CAGGCTCTGGCCCTACAC- 3’ |          |
| 2857A > G | For. 5'-CCCGAGCTCCTCCTCCTGT- 3’ | Exon9    |
|         | Rev. 5'-AAGTCTGTATAATGTAAGCCAC- 3’ |          |

Table 2. The demographic information of subjects in the case and control group.

| Characteristic | Case, n = 125 | Control, n = 120 | p     |
|---------------|---------------|-----------------|-------|
| Age (year)    |               |                 |       |
| The range    | 40–84         | 36–85           | .562  |
| Mean age     | 67.52 ± 12.53 | 66.89 ± 13.24   | .562  |
| Gender        |               |                 | .894  |
| Male          | 75            | 73              |       |
| Female        | 50            | 47              |       |
| Smoking       |               |                 | .120  |
| Current or ever | 38        | 26              | .231  |
| Never         | 87            | 94              |       |
| Drinking      |               |                 |       |
| Current or ever | 41         | 31              |       |
| Never         | 84            | 89              |       |
| Onset age (year) |          |                 |       |
| <50           | 24            |                 |       |
| ≥ 50          | 101           |                 |       |
| Duration of PD (year) | 4.59 ± 3.82 | 28.01 ± 3.42   | .031  |
| MMSE score   | 26.89 ± 3.11  | 28.51 ± 11.13   |       |
| UPDRS (“ON”) | 17.92 ± 9.63  |                 |       |
| UPDRS (“OFF”) |            | 28.51 ± 11.13   |       |

Note: MMSE: mini-mental state examination; UPDRS: Unified Parkinson’s Disease Rating Scale.
polymorphisms conformed to HWE \((p > .05)\), insuring the reliability of our study population.

Obviously, three genotypes were all detected in NOD2 gene rs72796353. As shown, AA, AC and CC genotype frequencies of rs72796353 were 74.40%, 21.60% and 4.00% in the case group and 81.67%, 15.83% and 2.50% in the control group, respectively. However, the significant distribution difference was not found between the two groups \((p > .05)\), so was allele.

In terms of c.2857A \(>\) G p.K953E polymorphism, GG genotype did not detect in controls, but the frequency was 4.00% in cases. The other genotypes AA, AG accounted for 76.80%, 19.20% in the case group and 89.17%, 10.83% in controls. AG/GG genotype had a significant frequency difference between the case and control groups, compared with AA genotype \((p = .010)\) and it was correlated to the occurrence risk of PD \((OR = 2.486, 95\% CI = 1.223–5.056)\). Similarly, G allele was also detected that it increased 1.563 times higher risk of PD development than A allele \((OR = 2.563, 95\% CI = 1.310–5.013)\).

In addition, we also analyzed the distributions of NOD2 rs72796353 and 2857 A \(>\) G polymorphisms among the PD patients according to their onset age. As displayed in Figure 3, rs72796353 and 2857 A \(>\) G variants had no close association with onset age of PD \((p > .05\) for all).

**Table 3.** Genotype and allele distributions of NOD2 rs72796353, c.2857A \(>\) G p.K953E polymorphisms in case and control groups.

| Genotype/allele | Case, \(n = 125\) (%) | Control, \(n = 120\) (%) | \(\chi^2\) | \(p\) | OR (95% CI) |
|-----------------|------------------------|--------------------------|----------------|--------|----------------|
| rs72796353      |                        |                          |                |        |                |
| AA              | 93 (74.40)             | 98 (81.67)               | –              | 1.00   | 1.00 (Ref.)    |
| AC              | 27 (21.60)             | 19 (15.83)               | 1.48           | .223   | 1.497 (0.780–2.874) |
| CC              | 5 (4.00)               | 3 (2.50)                 | 0.586          | .444   | 1.756 (0.408–7.556) |
| A               | 213 (85.20)            | 215 (89.58)              | –              | –      | 1.00 (Ref.)    |
| C               | 37 (14.80)             | 25 (10.42)               | 2.129          | .145   | 1.494 (0.869–2.568) |
| 2857A \(>\) G   |                        |                          |                |        |                |
| AA              | 96 (76.80)             | 107 (89.17)              | –              | –      | 1.00 (Ref.)    |
| AG              | 24 (19.20)             | 13 (10.83)               | 3.866          | .049   | 2.058 (0.993–4.266) |
| GG              | 5 (4.00)               | 0                        | –              | –      | –              |
| AG/GG           | 29 (23.20)             | 13 (10.83)               | 6.592          | .010   | 2.486 (1.223–5.056) |
| A               | 218 (87.20)            | 227 (94.58)              | –              | –      | 1.00 (Ref.)    |
| G               | 32 (12.80)             | 13 (5.42)                | 8.003          | .005   | 2.563 (1.310–5.013) |

**Discussion**

PD is a common neurological disease among old people, and the prevalence of the disease increases with the growth of the age. The morbidity of PD in China aged is 1.7% in over age 65, which severely affects the living quality of patients and their family [17]. The majority of PD cases are sporadic, and 10–15% of PD patients have a positive family history [18]. The generation and progression of PD result from genetic susceptibility and environmental factor [19]. Looking for PD susceptible genetic maker is an effective means to adjust the living environment of risk population and avoid the occurrence of PD greatly.

However, the pathogenesis of PD is not completely clear. The research on PD patients and various animal models have prompted that inflammatory responds especially neuroinflammation promote the occurrence and progression of PD, which are caused glial reaction, T cell infiltration, high expression of inflammatory factors and virulence factors from glial cells [20]. Two recent genome-wide association studies find that human leucocyte antigens HLA-DRA, HLA-DRB5 and HLA-DRB1 are associated with PD, which further proved inflammation-related genes involved in PD progression [21–23]. Inflammatory response initiated by NF-κB pathway may play an important role in pathogenesis of PD [14]. In animal models of PD, the continuous NF-κB activation is proved to increase inflammatory responses and dopaminergic neurons degeneration, which indicates the relationship...
between NF-κB pathway and PD pathogenesis [24,25]. NOD2 is expressed in brain tissue and may play an important role in activating NF-κB pathway. Among the variants identified in NOD2 gene, three polymorphisms R702W, G908R, 3020insC have been confirmed to be associated with Crohn’s disease (CD), meanwhile show racial diversity of polymorphism distribution [26]. Furthermore, NOD2 polymorphisms are proved to be associated with PD risk [27]. Ma et al. find that NOD2 P268S polymorphism might play a role in sporadic PD susceptibility based on Chinese population and meanwhile prove that inflammatory response involve in PD [28].

In 2002, a French team published an analysis of NOD2 gene by direct DNA sequencing in 453 patients with CD and detected NOD2 SNP rs72796353, which was identified as potential disease-causing mutation [29]. Subsequently, Schnitzler et al. further explore the association of NOD2 rs72796353 with diseases susceptibility and phenotype in a large, well-characterized German population with inflammatory bowel disease (IBD), which suggests that rs72796353 may be as an additional genetic marker for the CD disease behaviour [15]. Interestingly, a novel missense variant c.2857A > G p.K953E at exon 9 of NOD2 gene is first found in a child (7 years old) with an early ulcerative colitis (VEO-UC) c.2857A > G p.K953E polymorphism had no obvious association with onset years of PD (p > 0.05 for all).

In this study, we combined NOD2 rs72796353, c.2857A > G p.K953E polymorphisms with PD susceptibility for the first time. On the one hand, the homozygous genotype of the minor allele in c.2857A > G p.K953E polymorphism was not detected in the control group and it was significantly correlated to PD susceptibility. Specifically, AG/GG genotype carriers had the more risk to be subject to PD than AA carriers and G allele was a risk factor in our study population. However, the study result of rs72796353 with PD risk showed that neither genotypes nor alleles was significantly different between two study groups and it might be associated with PD susceptibility indirectly. However, whether the identified polymorphism could be employed as a diagnostic biomarker of PD remained unclear. Further investigations are required to address the issue.

In previous studies, c.2857A > G p.K953E polymorphism was speculated to determine a cumulative dysfunctional effect with other gene variants such as IL10R, and rs72796353 was as a predictive marker for some diseases; but these factors had not been considered in our study. Therefore, a more detailed analysis of these two mutations should be performed. Deeper investigations of different populations are warranted to clarify the present results with selecting the study groups strictly, increasing sample quantity and enlarging research genes.

Disclosure statement

No potential conflict of interest was reported by the authors.

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