Original Article
Association of pro-inflammatory cytokines gene polymorphisms with Parkinson’s disease in Chinese population

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Abstract: The progressive neurodegenerative process in Parkinson’s disease (PD) is accompanied by chronic inflammation, including activation of microglia and astrocytes that express pro-inflammatory cytokines. Up to now, abundant evidence from genetic epidemiology have been assessed the association of pro-inflammation cytokines gene polymorphisms and risk of PD in different ethnicity, but conflicting results were obtained due to the heterogeneity of the genetic background among populations. Here, we recruited 248 PD patients, and 226 matched healthy controls from Jiangsu Province to estimate the influence of IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 polymorphism on patients with Parkinson’s disease. Single nucleotide polymorphism locus was genotyped using PCR-RFLP. The genotypic and allelic frequency of TNF-α, IL-6 did not show significant difference between PD and normal controls. However, the frequency of wild (AA) and homozygous mutant (CC) genotype IL-10 rs1800896 genotypes in cases and controls was found more in controls (43% and 5.7% respectively), but that of the heterozygous genotype was higher (60.15%) in cases with PD patients. In conclusion, no relationship between IL-6, and TNF-α genotypes or alleles and PD susceptibility was revealed. However, IL-10 rs1800896 GA genotype confers genetically susceptibility to PD in Chinese population.

Keywords: Parkinson’s disease, IL-6, IL-10, TNF-α, polymorphism

Introduction

Parkinson’s disease (PD) is a degenerative disorder of the central nervous system mainly affecting the motor system [1-3]. Early in the course of the disease, the most obvious symptoms are movement-related; these include shaking, rigidity, slowness of movement and difficulty with walking and gait. Later, thinking and behavioral problems may arise, with dementia commonly occurring in the advanced stages of the disease [4, 5], and depression being the most common psychiatric symptom. Other symptoms include sensory, sleep, and emotional problems. The main motor symptoms are collectively called “parkinsonism”, or a “parkinsonian” syndrome [6]. Several genetic and environmental mechanisms are thought to be involved in the pathogenesis of PD [7, 8]. While the contribution of genetic factors to the pathogenesis of the more common sporadic form of PD is still unclear.

Recently findings indicate that that the inflammatory process is an important pathological factor associated with PD [9-14]. In neurodegenerative diseases, activated microglia affect neuronal injury and cell death through the production of glutamate, pro-inflammatory factor, and reactive oxygen species, as well as by mobilizing adaptive immune response and perpetuating neural damage [15]. One hypothesis regarding the cause of the degeneration of nigrostriatal neurons is that PD is triggered by microglial activation because of increased cytokine levels [16, 17]. The genes encoding pro- and anti-inflammatory cytokines may influence cytokine production and thus account for variability in the development of inflammation [18].

The functional promoter polymorphism of nucleotide variations in genes encoding inflammatory molecules, such as IL-6, IL-10 and tumor necrosis factor-α (TNF-α) may influence their biological activities and thus might influ-
ence the risk of PD. For example, Both experimental and clinical data indicate that brain expression, plasma and cerebrospinal fluid levels of IL-6 may affect PD progression [19-24], cognitive decline or dementia both in cross-sectional and longitudinal follow-up studies [25, 26]. The IL-6 gene in humans is located on chromosome 7 (7p21). The (-174 C/G) also known (rs1800795) polymorphism in the promoter region of the IL-6 gene was reported to affect the IL-6 gene transcription rates and IL-6 plasma levels in PD patients implicating its role in development of PD. Meanwhile clinical research has suggested that there is a correlation between the IL-10 SNPs and levels of serum IL-10 with disease severity in PD patients. Many studies have focused mainly on 3’ UTR, -1082 G/A (rs1800896) in the IL-10 gene [27].

Meanwhile, several polymorphisms in the promoter region of TNF-α have been associated with different TNF-α expression levels. Of these, the TNF-α -308G/A (also referred to as rs1800629) is the best studied. It involves the substitution of a guanine (G) by a PDeine (A) and is associated with an increase in TNF-α expression levels [28, 29]. Up to now, numerous studies of genetic epidemiology have assessed the association of pro-inflammation cytokines gene polymorphisms and risk of cancer in different populations, but conflicting results were obtained due to the heterogeneity of the genetic background among populations. Furthermore, this support the need for replication studies among all ethnic groups.

Taken together the association of pro-inflammation cytokines and PD, the aim of the present study was to evaluate the influence gene polymorphisms of IL-6, IL-10 and TNF-α on the susceptibility to PD patients in Chinese population.

Patients and methods

Ethics statement

The Medical Ethics Committee of the Second Affiliated Hospital of Soochow University approved this study. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from each participant prior to the study.

Participants

A total of 248 PD cases, and 226 matched healthy controls fulfilling the criteria for PD were recruited from departments of neurology of the Second Affiliated Hospital of Soochow University from January 2013 to January 2015. All subjects are Han Chinese. All patients underwent a standardized battery of examinations. Subjects with family history of neurodegenerative diseases were excluded. The control group was recruited from the Healthy Examination Center of the Second Affiliated Hospital of Soochow University and confirmed healthy and neurologically normal by medical history, general examinations and laboratory examination.

Genotyping

Genome DNA from whole blood cells of each sample was extracted by using Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer’s instructions. DNA samples were stored at -20°C until analysis. Genotyping for the IL-6 -174 G/C, IL-10 -1188 A/C and TNF-α-308G/A polymorphisms in genomic DNA were performed using the PCR and restriction fragment length polymorphism (RFLP). The genomic region encompassing polymorphism was amplified using the following primers: IL-6 F: 5’-ACT TTT CCC CCT AGTTGTGTCTTTC-3’, R: 5’-AGAATGAGCCTCAGACATCTCCAGT-3’, IL-10 F: 5’-GGC ATTCTCTTCCAGGTCTTG-3’, R: 5’-CCATGCGCAACTTGAGAGCTG-3’, and TNF-α F: 5’-AGGCATTAGGGTTGGGAGGCAT-3’, R: 5’-TCCCTGCTCCAGATTCCG-3’. Polymerase chain reaction products were generated in a 10 μL reaction volume containing 50 ng of genomic DNA, 1X PCR buffer, 2 mmol/L MgCl2, 0.2 mmol/L of each dNTP, 1 μmol/L of each primer, and 0.25 U of Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA). Cycling conditions consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds and a final elongation step at 72°C for 1 minute. Polymerase chain reaction products were digested with 2 U of Ncol restriction enzyme at 37°C, according to the manufacturer’s instructions (New England BioLabs, Ipswich, MA). IL-6 the amplified fragment of 204 bp was cleaved into two fragments of 24 and 180 bp. The uncut product of 204 bp was identified as CC genotype; the GC genotype was revealed by two fragments of 204 and 180 bp, and the GG by a fragment of 180 bp. And the IL-10 PCR products resulted in either two fragments of 173 and 70 bp (allele C) or a single fragment of 243 bp (allele A). Finally, the -308G allele contains an Ncol restriction site not present in the -308A
allele; thus, in the presence of the -308G allele, the PCR product (107 bp) is cut into 2 fragments of 80 and 27 bp in length.

**Statistic analysis**

Data were statistically described in terms of mean ± standard deviation (SD), or frequencies (number of cases) and percentages as required depending on their distribution. The Hardy-Weinberg equilibrium (HWE) was assessed for each variation to identify the deviation. The differences of the genotypes and alleles of detected genes between patients and normal controls were evaluated by using Pearson Chi-square test. Exact test was used in the presence of the -308G allele when the expected frequency is less than 5. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. Unpaired Student’s t test or Mann-Whitney tests were used for two-group comparisons. Statistical analysis of data was performed using the SPSS software package 18.0 (SPSS Inc. USA). *P*-value less than 0.05 was considered statistically significant.

**Results**

In this study, 248 PD patients (108 males and 140 females) and 226 controls (112 males and 114 females) were screened for IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 polymorphisms using PCR-RFLP methods. No statistically significant differences were observed in age (age at patients compared with age at examination for control subjects) (*P*=0.342) and gender (*P*=0.765) between PD patients and control subjects (Table 1). In addition, MMSE scores were significantly lower in PD patients than in control subjects (*P*<0.001).

Firstly, the frequencies of genotypes and alleles of IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 were detected in case and control groups. HWE of rs1800795, rs1800896 and rs1800629 in patients and controls were listed in Table 2, and the results showed allelic distribution of detected SNP were not deviated from HWE in both case and control populations. IL-6 rs1800795 in the study population were as follows: 7.4% CC, 33.5% CG and 59.1% GG for the case study group and 4.9% CC, 30.4% GC, and 64.7% GG for the controls, indicating that the genotypes distributions were similar between the cases and the control groups. Also, genomic analysis did not reveal a difference between PD patients and healthy controls in allelic frequency at the -174 position for the IL-6 gene promoter. Similarly, the genotypic and allelic frequency of rs1800629 did not show significant difference between PD and normal controls. Then, genotype and allele frequency of rs1800629 were detected in PD patients and normal control (Table 2). The genotypic and allelic frequency of rs1800629 between cases and health controls did not show significant difference.

Whereas, the frequency of wild (GG) and homozygous mutant (AA) genotype IL-10 rs1800896 genotypes in cases and controls was found more in controls (43% and 5.7% respectively), but that of the heterozygous genotype was higher (60.15%) in cases with PD patients. Significant risk of PD was observed for GA (OR=1.74, 95% CI=1.10-2.41, *P*=0.025) genotype of IL-10. The genomic analysis did not reveal differences in allelic frequencies of the IL-10 (G/A) gene PD patients and healthy controls.

**Discussion**

The gene single nucleotide polymorphisms (SNP) have been thought to alter expressions or influence certain genes; thus, SNPs could be associated with an altered risk of multiple diseases [30-36]. Up to now, the important role of pro-inflammatory cytokines during tumor development and prognosis is increasingly gaining interest. Several lines of evidence point to the involvement of interleukin-6 (IL-6) in pathogenesis of PD. Both experimental and clinical data indicate that brain expression [37], plasma [38-40] and cerebrospinal fluid levels of IL-6 may affect plaque formation, cognitive decline or dementia both in cross-sectional and longitudinal follow-up studies [41-43].

We did not find any evidence of an association between the IL-6 (-174 C/G) polymorphism and PD in the China population sample. The distri-
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Table 2. Genotype and allele frequency of IL-6 rs1800795, IL-10 rs1800896 and TNF-α rs1800629 and Pearson’s chi-square test in PD patients and normal controls

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>Patients (n=248)</th>
<th>Controls (n=226)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-6 rs1800795</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>147</td>
<td>146</td>
<td>0.620</td>
<td>0.905 (0.626-1.320)</td>
</tr>
<tr>
<td>GC</td>
<td>83</td>
<td>69</td>
<td>0.077</td>
<td>0.822 (0.681-1.484)</td>
</tr>
<tr>
<td>CC</td>
<td>18</td>
<td>11</td>
<td>0.122</td>
<td>4.560 (0.556-37.412)</td>
</tr>
<tr>
<td>G</td>
<td>377</td>
<td>361</td>
<td>0.314</td>
<td>0.841 (0.498-1.192)</td>
</tr>
<tr>
<td>C</td>
<td>119</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL-10 rs1800896</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>106</td>
<td>81</td>
<td>0.0521</td>
<td>0.805 (0.526-1.220)</td>
</tr>
<tr>
<td>GA</td>
<td>127</td>
<td>140</td>
<td>0.025</td>
<td>1.749 (1.104-2.417)</td>
</tr>
<tr>
<td>AA</td>
<td>15</td>
<td>5</td>
<td>0.0519</td>
<td>0.972 (0.653-1.521)</td>
</tr>
<tr>
<td>G</td>
<td>339</td>
<td>302</td>
<td>0.275</td>
<td>0.571 (0.328-0.981)</td>
</tr>
<tr>
<td>A</td>
<td>157</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TNF-α rs1800629</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>172</td>
<td>169</td>
<td>0.559</td>
<td>0.869 (0.542-1.392)</td>
</tr>
<tr>
<td>GA</td>
<td>68</td>
<td>51</td>
<td>0.921</td>
<td>1.258 (0.787-2.010)</td>
</tr>
<tr>
<td>AA</td>
<td>8</td>
<td>6</td>
<td>0.350</td>
<td>3.358 (0.347-32.543)</td>
</tr>
<tr>
<td>G</td>
<td>412</td>
<td>389</td>
<td>0.356</td>
<td>0.813 (0.524-1.267)</td>
</tr>
<tr>
<td>A</td>
<td>84</td>
<td>63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test for deviation from the Hardy-Weinberg equilibrium (a value of P<0.001 was regarded as a deviation from the HWE).

Bution of the studied polymorphism was similar to that observed in countries at the same geographic latitude, but different when geographical longitude was considered [44]. Previous studies (12 case-control and 2 prospective studies) assessing the connection between the IL-6 polymorphism and the risk of PD brought equivocal results. Faltraco et al. [45] reported risk reducing association of the IL-6 C allele in PD. Pola et al. [46] found that the G/G polymorphism was associated with increased risk of PD. In other studies on Italian populations the IL-6 C allele increased the risk of PD [47, 48], the C/C genotype increased the risk of PD in women [49], and the G/G genotype was lower in PD than in healthy controls [50]. Differences in study design and the geographical variations of IL-6 frequency may in part explain the different patterns of association between this polymorphism and PD in various studies. In most studies patients with PD neuropsychological examination but in controls no test battery was used, apart from MMSE; therefore some cases with incipient PD might have been included. Moreover, all studies published to date analyzing the role of the IL-6 (-174 C/G) polymorphism in PD have been underpowered. That is why we performed a meta analysis assessing the significance of the studied polymorphism on all available data from 3107 PD patients and 10 014 controls. This meta-analysis, however, was not able to show the significance of the IL-6 (-174 C/G) polymorphism for the risk of PD [39].

IL-10 is an important antitumor cytokine that plays an important role in the development and progress of canc. Variation in the DNA sequence lead to altered IL-10 production, and this can alter individual’s susceptibility to cancer. The IL-10 3’ UTR G>A polymorphism is a functionally important SNP that alters IL-10 production and it has been a reported potential biomarker for risks of numerous disease, such as hepatitis, psoriasis, Barrett’s esophagus, asthma, and arteritis. More importantly, genetic variation in IL-10 was revealed to affect susceptibility to multiple sclerosis, another neurodegenerative disease with evident inflammatory responses. Considering the potential role of IL-10 in PD pathogenesis as well as the involvement of IL-10 polymorphisms in the predisposition to many inflammatory diseases [51-53]. In our study, increased frequency of IL-10 rs1800896 GG and AA homozygous genotype among controls but that of the heterozygous AC genotype
was higher in cases with PD; thus, a significant risk of PD was observed for AC genotype of IL-10 rs1800896, which is consistent with the results of Li [51].

TNF-α gene is located in the class III region of the human major histocompatibility complex (MHC) on chromosome 6p21 [54, 55]. Among the several single nucleotide polymorphisms (SNPs) identified in TNF-α, TNF-α rs1800629 is the most extensively studied. The A allele of this polymorphism can lead to high binding affinity of nuclear factors to the TNF promoter, resulting in a high level of transcription activity and secretion levels of TNF-α. So, it was suggested to have a significant functional effect [56]. A variety of SNPs located in the promoter region of TNF-α gene has been investigated in PD patients by different groups with contrasting results. So, The aim of the present study was to better define the role of TNF-α polymorphisms in PD; In the present study, our results suggested that the genotypes distributions of TNF-α rs1800629 was almost the similar in the cases and the control groups. Previous association studies on the TNF-α gene polymorphism have either shown no association between the -308A allele and PD [57, 58] or a positive association [59] between this allele and early PD onset [57]. Some of these differences may be as a result of the genetic heterogeneity of the population studied [60]. In particular, the TNF gene may exert different effects upon different ethnic groups, depending on its interaction with other unknown susceptibility genes and/or risk factors in another nearby locus. Indeed, the TNF gene is part of a large genomic region on chromosome 6 that contains other genes encoding proteins involved in immune inflammatory responses.

Our results indicate a lack of association between pro-inflammatory cytokines SNP and PD in our China sample, suggesting that genetic, clinical and population heterogeneity are probably responsible for conflicting results in association studies.

In summary, though no any relationship between IL-6, and TNF-α genotypes or alleles and PD susceptibility was revealed, we first identified IL-10 rs1800896 GA genotype confer genetically susceptibility to PD in Chinese population.

Disclosure of conflict of interest

None.

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