This study was designed to investigate the frequency of estrogen receptor (ER) gene polymorphism in Chinese patients with Parkinson's disease (PD). Polymerase chain reaction (PCR) method and restriction fragment length polymorphism (RFLP) were used to detect the ER gene polymorphisms in 158 PD patients and 146 healthy controls. 'x' accounted for 83.5% and 80.8%, respectively (P> 0.05). 'xx' was found in 77.2% of the PD group and in 69.9% of the control group (P> 0.05). The frequency of 'p' in the PD and control group was 67.7% and 64.0%, respectively (P> 0.05). 'pp' was 51.9% in the PD group and 43.8% in the control group (P> 0.05). 'ppxx' was found in 49.4% of the PD and 43.0% of the control subjects (P> 0.05). There was no significant difference in the 'x', 'xx', 'p', 'pp' or 'ppxx' between males and females within the PD or control groups. In conclusion, we found no significant differences in the genotype or allele frequencies between patients with Parkinson's disease and healthy subjects. These findings suggest that the estrogen receptor gene polymorphism may not play a key role in the pathogenesis PD in Chinese patients.
Does estrogen receptor gene polymorphism play a role in Parkinson’s disease?

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Abstract

This study was designed to investigate the frequency of estrogen receptor (ER) gene polymorphism in Chinese patients with Parkinson’s disease (PD). Polymerase chain reaction (PCR) method and restriction fragment length polymorphism (RFLP) were used to detect the ER gene polymorphisms in 158 PD patients and 146 healthy controls. In the PD and control groups, “x” accounted for 83.5% and 80.8%, respectively ($P>0.05$). “xx” was found in 77.2% of the PD group and in 69.9% of the control group ($P>0.05$). The frequency of “p” in the PD and control group was 67.7% and 64.0%, respectively ($P>0.05$). “pp” was 51.9% in the PD group and 43.8% in the control group ($P>0.05$). “ppxx” was found in 49.4% of the PD and 43.0% of the control subjects ($P>0.05$). There was no significant difference in the “x”, “xx”, “p”, “pp” or “ppxx” between males and females within the PD or control groups. In conclusion, we found no significant differences in the genotype or allele frequencies between patients with Parkinson’s disease and healthy subjects. These findings suggest that the estrogen receptor gene polymorphism may not play a key role in the pathogenesis PD in Chinese patients.

Key words: Receptor; estrogen; gene polymorphisms; Parkinson’s disease; neurology.
Introduction

There is a tendency of polygenes in Parkinson’s disease (PD). In recent years, there has been a growing interest in the neuroprotective effects of estrogen and the possible beneficial effects of estrogen in the neurodegeneration of dopaminergic neurons. Epidemiological investigations [1], laboratory studies in vivo and in vitro [2-5] and clinical trials [6] indicate that endogenous and exogenous estrogen may modify the risk of PD. Estrogen receptors, which act as the mediator of estrogen, play a key role in the estrogen-induced protection against PD [7]. A recent study showed that interaction of polymorphisms in the genes encoding interleukin-6 and estrogen receptor-beta is closely related to the susceptibility to Parkinson's disease [8].

This study is to investigate the relationship between the estrogen receptor gene polymorphism, and the susceptibility of PD, and clarify the frequency of estrogen receptor gene polymorphism in healthy Chinese subjects and in patients with PD.

Subjects and Methods

Patient selection

The institutional review board for human research at Liaocheng People’s Hospital approved this study. Written informed consent was obtained from all participants. From September 2001 to October 2002, 158 PD patients were recruited from hospitalized patients and from patients attending the outpatient clinics of the Department of Neurology. There were 86 males (54.4%) and 72 females with an average age of 64.9±12.3 (range 52 to 76) years.
The clinical manifestations of PD were assessed according to the Unified Parkinson’s Disease Rating Scale (UPDRS). PD was diagnosed on three main features: resting tremor, rigidity, bradykinesia and postural instability.

Patients were excluded if they had any one of the features: pyramidal or cerebella signs; abnormal eye movements; dementia; autonomic dysfunction; parkinsonism due to other neurologic disease, drugs or toxins; uncontrolled hypertension and unstable ischemic heart disease.

During the same study period, 146 healthy controls were also selected from our hospital clinics. There were 67 males (45.9%) and 79 females with an average age of 61.5±10.7 (range 50 to 72) years. These participants underwent a thorough physical examination in our hospital clinics.

All the study subjects in the PD and control groups were natural population of Hans (the largest ethnic group in China), and there was no consanguinity relationship between each other among the study participants.

**Reagents**

Taq DNA polymerase, 10×Taq DNA polymerase buffer, mixture of 4dNTPs, MgCl₂ (25mmol/L), 10mg/ml ethdium bromide, 2.5% agarose gel, PCR markers (All from Promega company), Xba-I, Pvu-II restriction enzyme (MBI co.); other reagents were all of analytically quality.

**The construction and synthesis of Primers**

A pair of primers (Sangon Co. Canada) was contrived according to the methodology reported by Kobayashi and colleagues [9]. Primer 1: 5’
CTGCCACCCTATCTGTATCTTTTCCTATTCTCC 3’;
Primer 2: 5’ TCTTTTCTGACCACCCTGGCTCGATTATCTGA 3’. We amplified the part of the intron and the second exon specifically. The length of the product was 1.3 Kb.

Isolation of DNA

DNA was isolated from a human whole blood sample using DNA Isolation Kit (Supergene biotechnology Co.,LT, Wuhan, China). 100μl of fresh blood was collected in a tube containing EDTA-k3, with addition of 100μl sterile ddH₂O, Mix well and 200μl 6mol/L NaI. Also added were an equal volume of chloroform-isoamylalcohol (24:1) and vortex 30s. The supernatant was removed after centrifuge. After adding isopropanol the sample was incubated at room temperature for 15 min. After removing the supernatant, the pellet was rinsed once with 70% ethanol. The DNA was dissolved in 3ml of TE buffer and quantified by ultraviolet spectrometry photometer.

Amplification of DNA by polymerase chain reaction (PCR)

The DNA was mixed in a sterile eppendorf tube on ice. The following reagents were added to the mixed solution: 10×Taq DNA polymerase buffer, 5μl mixture of 4dNTPs (each at a concentration of 2.5mM) 4μl, 8μl primers (mixture of upstream and downstream primers), 0.1μg isolated DNA, and 2-3U Taq DNA polymerase. The mixtures were set in the PTC-100™ PCR instrument (ASTEC, Japan). The reaction mixtures were heated for 5 min at 95⁰C to denature the DNA, and processed 30 cycles as this procedure: denatuation at 94⁰C for 30s, anneal at 61⁰C for 60s, extension (polymerization) at 72⁰C for 90s. After the last cycle, extension was at 72⁰C for 10min. The products were identified by 2.5% agarose gel
electrophoresis, dying with ethidium bromide (EB), and the amplification results were observed under hand-held ultraviolet lamp. Photographs were taken by specific ultraviolet photographic equipments.

**Digestion of DNA with restriction enzyme**

10µl PCR products were mixed with 8u Pvu II and 16u Xba I restriction enzyme, incubating at 37°C for 6h. The products were identified by 2.5% agarose gel electrophoresis, stained with ethidium bromide (EB). The amplification results were observed with photographs taken under hand-held ultraviolet lamp.

Estrogen receptor genotype was identified by the following processes: using Xba I restriction enzyme to distinguish the following genotypes: XX (final products were 1.3kb), Xx (final products were 1.3kb, 910bp, 390bp) and xx (final products were 910bp, 390bp); using Pvu II restriction enzyme to distinguish the following genotypes: PP (1.3kb), Pp (850bp, 450bp, 1.3kb) and pp (850bp, 450bp). Capital letters represent the disappearance of the locus of restriction enzyme because of mutation, whereas lower case letters represent the presence of the locus of restriction enzyme.

Estrogen receptor genotypes were classified as a P or p allele on the basis of a Pvu II-RFLP; and X or x allele on the basis of an Xba I-RFLP. Nine types of genotypes were investigated: PPXX, PPXx, PPxx, PpXX, PpXx, Ppxx, ppXX, ppXx and ppxx. “P” and “p” represent “with” and “without” Pvu II restriction enzyme locus respectively; “X” and “x” represent “with” and “without” represent “with” and “without” Pvu II restriction enzyme locus respectively.
Statistical analysis

The degree of subjects conforming to Hardy-Weinberg balance, single genotype and the frequency of allele between each group were tested by Chi square test. The differences in the estrogen receptor allele frequency between men and women or between different groups were tested by R×C contingency table analysis. The enumeration data where the number of stylebook was less than 40 were analyzed by four-fold table method. All these tests were performed by using software package of SPSS10.0. $P<0.05$ was considered statistically significant.

Results

There was no significant difference in the male or female sex, or the average age between the PD and control groups ($P>0.05$).

The amplification product of ER gene was a band of 1.3 Kb (Fig 1). The PCR-RFLP results are shown in Fig 2. There was 50bp between two enzyme loci. The 1.3Kb products were incised to two segments 850bp、450bp by Pvu II. The 1.3Kb products were incised to two segments 910bp、390bp by Xba I.

Table 1 and 2 show the distribution of genotype and allele in the PD and control groups. As shown in Table 1, in the PD group, “x” accounted for 83.5%, and the rate in male and female groups was 77.9% and 90.3%, respectively. “xx” was the most frequent, with a rate of 77.2% in Xba-I. The rate of “xx” in the male and female groups was 69.8% and 86.1%, respectively. “p” was the most frequent (67.7%), with a rate of 62.8 % in the male and
73.6% in the female group. The frequency of “pp” was 51.9% in Pvu II, with a rate of 46.6% in the male group and 58.2% in the female group. Genotype ppxx accounted 49.4% (Table 2); the frequency in the male and female group was 41.9% and 58.3%, respectively.

In the control group, the frequency of “x”, “xx”, “p”, “pp” (Table 1) and genotype ppxx (Table 2) was similar to that in the PD group ($P > 0.05$). Within each of the two groups, the frequency of “x”, “xx”, “p”, “pp” (Table 1) and genotype ppxx (Table 2) was also similar between males and females ($P > 0.05$).

**Discussion**

In 1999 Isoe-Wada and colleagues [10] reported a positive association between an estrogen receptor gene polymorphism and Parkinson’s disease with dementia. The frequency of the P allele in the PD group was found higher than that in the control subjects, leading to the conclusion from the investigators that the estrogen receptor gene may be a common susceptibility gene for dementia in PD [10]. In 2002, a case-control study investigated the association of PD with two estrogen receptor gene polymorphisms and came to a conclusion that estrogen receptor gene polymorphisms do not seem to contribute to PD susceptibility [11]. A later study also came to the same conclusion that there was lack of association between an estrogen receptor 1 gene polymorphism and PD [12]. There has been no study on the association between estrogen gene polymorphisms and PD in Chinese patients.

PD is a polygene inherited disease influenced by numerous genetic and environmental factors. Endogenous and exogenous estrogen plays an important role to the protection of
PD [13]. Previous studies demonstrated that estrogen receptor is the key mediator in the course of the effect of estrogen [14]. Estrogen receptors lie both in cytoplasm and in nuclei, acting as transcription factors [14]. Once the estrogen receptors combined with estrogen and became dimers, they combine with estrogen react element, activating target gene transcription, and accelerating the proliferation and differentiation of cells.

There are mutations in the first intron in the human estrogen receptor gene, which can be recognized by restriction enzyme. In the present study, we distinguished different estrogen receptor genotypes through digesting the amplification products of estrogen receptor gene by Xba I or Pvu II restriction enzyme. We found that there is no significant difference of the estrogen receptor gene xba I polymorphism and Pvu II polymorphism between PD patients and the controls. These results suggest that although the estrogen receptor genotypes might decide the expression levels and functional differences of different individuals, and influence the biological effect of estrogen in vivo, there is lack of association between estrogen receptor gene polymorphism and PD.

In summary, this was the first report on the estrogen gene polymorphisms in Chinese PD patients. There was no significant difference in the estrogen gene polymorphism between patients with PD and the healthy subjects. In patients with PD, no significant difference was found in the estrogen gene polymorphisms between male and females. The results of this study are important in clarifying the role of estrogen receptor gene in the pathogenesis of PD.

Acknowledgements

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References


Table 1. Estrogen receptor Xba I and Pvu II polymorphism in the PD and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Xba I genotype</th>
<th>Xba I allele</th>
<th>Pvu II genotype</th>
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<tr>
<td></td>
<td>XX</td>
<td>Xx</td>
<td>xx</td>
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<td>PD</td>
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<tr>
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<td>16(10.1%)</td>
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<td>62(86.1%)</td>
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Table 2. ER Xba I and Pvu II polymorphism in Parkinson’s disease

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<th>PpXx</th>
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<td>6(7.0%)</td>
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<td>36(41.9%)</td>
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Figure legends

Fig 1. The amplification product of ER gene.

Fig 2. PCR-RFLP results. M. marker; 1. XX (product of 1.3 kb); 2. xx (product of 910 bp, 390 bp); 3. pp (product of 850 bp, 450 bp); 4. PP (1.3 kb); 5. Product of 1.3 kb, there is no enzyme locus.
Fig 2