Association of Nurr1 gene mutations with Parkinson’s disease in the Han population living in the Hubei province of China☆

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Abstract

Nurr1 defects could in part underlie Parkinson’s disease pathogenesis, and Nurr1 gene polymorphism has been found in Caucasian patients with Parkinson’s disease. In this study, heteroduplex technology was applied to compare the DNA sequences of eight exons of Nurr1 among 200 sporadic Parkinson’s disease patients and 200 healthy controls in the Han population in the Hubei province, China. One allele amplified from exon 3 of Nurr1 was polymorphic in five Parkinson’s disease patients (2.5%, 5/200), and two individuals had a polymorphic allele amplified from exon 2 (1%, 2/200). The anomalous electrophoresis fragment in exon 3 of Nurr1 gene contained a 709C/A missense mutation, and a polymorphic single nucleotide polymorphism at 388G/A was identified in exon 2. Compared with the control group, the Nurr1 gene expression level in the Parkinson’s disease group was decreased, and the Nurr1 gene expression levels in Parkinson’s disease patients carrying the polymorphisms at exons 2 and 3 were significantly decreased. Our data indicate that the single nucleotide polymorphism 388G/A in exon 2 and the 709C/A missense mutation in exon 3 of the Nurr1 gene in the Chinese population might affect the pathogenesis of Parkinson’s disease.

Key Words

Nurr1 gene; Parkinson’s disease; gene mutations; gene polymorphism; pathogenesis; neurodegenerative disease; neural regeneration

Research Highlights

(1) Heteroduplex analysis was used to conduct gene screening of eight exons of Nurr1 gene among Parkinson’s disease patients and controls.
(2) Missense mutations in exons 2 and 3 of the Nurr1 gene were found in Chinese patients with Parkinson’s disease.

Abbreviations

DA, dopaminergic neurons; TH, tyrosine hydroxylase
INTRODUCTION

Parkinson’s disease manifests as many involuntary movement disorders, including tremor, reduced action and raised muscle tension. It is estimated that the number of Parkinson’s disease patients aged > 65 years in China is > 2 000 000, and the morbidity is increasing. The molecular mechanisms underlying this disease are still not fully understood. A series of Parkinson’s disease-related genes have been identified, such as α-synuclein, Parkin 1–18 and others. However, the rare mutation rates of these genes still cannot account for most Parkinson’s disease molecular pathogenesis. Therefore, identification of gene polymorphisms controlling the production of dopaminergic neurons (DA), as well as their development and maintenance of function after maturity will provide new insights into the pathogenesis of Parkinson’s disease.

The Nurr1 gene consists of eight exons and seven introns. The gene’s upstream 5’ flanking region contains a transcription promoter region and an adjusting zone. The first ATG codon from the 5’ end in exon 3 is the translation start point. A stop codon is located in the upstream region of exon 8, while the 3’ untranslated region of exon 8 contains multiple repetitive ATTTA sequences, and plays a stabilizing role in mRNA transcription. Nurr1 is an immediate early gene and acts as gene transcription factor. The Nurr1 gene plays a dominant role in DA neuronal development, differentiation and the maintenance of function after maturation. Zetterström et al. reported that Nurr1 knockout rats died 1 day after birth, and that DA neurons were completely lacking in the ventral area of the brain. By contrast, tyrosine hydroxylase (TH) was detected in other regions, demonstrating that the Nurr1 gene plays a key role in maintaining the functions of mature DA neurons in the midbrain. Grimes et al. detected a Nurr1 gene polymorphism in the patients with Parkinson’s disease, and showed that the Nurr1 expression level was decreased by 45% in blood lymphocytes. Studies revealed that Parkinson’s disease patients carry exon 1 and intron 6 polymorphisms in the Nurr1 gene.

It was reported that the Nurr1 gene participates in the control of central dopamine metabolism, and that the Nurr1 expression level was significantly decreased with age in mesencephalic substantia nigra cells of the exon 3 heterozygous mouse, accompanied by decreased dopamine levels. Nurr1 regulated TH metabolism and induced DA neuron formation. Transfection of embryonic stem cells with the Nurr1 gene can significantly improve their differentiation into DA neurons. Thus, it can be speculated that Nurr1 is a key gene for DA neuron development, differentiation and maintenance of functions, and that a lack of Nurr1 will increase the environmental toxin sensitivity of neurons. Therefore, we used heteroduplex technology to conduct gene polymorphism analysis of eight exons of the Nurr1 gene in Parkinson’s disease patients from the Han population living in the Hubei province, China.

RESULTS

Quantitative analysis and clinical information of involved subjects

In this study, 200 sporadic Parkinson’s disease patients, including 110 males and 90 females (average age 62.03 ± 0.67 years) and 200 healthy controls consisting of 100 males and 100 females (average age 60.08 ± 0.82 years) were included. All subjects entered the final analysis. Clinical information for the Parkinson’s disease patients and healthy controls is shown in Table 1.

Table 1 Clinical characteristics of the participants

| Item                        | Parkinson’s disease | Control |
|-----------------------------|---------------------|---------|
| n                           | 200                 | 200     |
| Gender (n, male/female)     | 110/90              | 100/100 |
| Nationality                 | Han                 | Han     |
| Age (mean±SD, year)         | 62.03±0.67          | 60.08±0.82 |
| Sickness (mean±SD, year)    | 52.91±9.07          | NA      |
| Course of disease (mean±SD, year) | 10.87±4.83       | NA      |
| Family history              | None                | None    |

NA: Not applicable.

Exons 2 and 3 of the Nurr1 gene containing gene polymorphisms in Parkinson’s disease patients

We applied heteroduplex technology to conduct fragment analysis of eight exons of Nurr1; abnormal electrophoresis fragments were subcloned and DNA sequencing was performed. We identified one allele amplified from exon 3 of Nurr1 that was polymorphic in five Parkinson’s disease patients (5/200). Two individuals presented a polymorphic allele amplified from exon 2 (2/200), but no polymorphic allele existed in the healthy controls.

Gene mutation of exons 2 and 3 of Nurr1 in Parkinson’s disease patients

By DNA sequencing, we found that the anomalous electrophoretic fragment in exon 3 of the Nurr1 gene contained a 709C/A missense mutation (Figure 1). This mutation would change the 125th serine into tyrosine, affecting Nurr1 serine phosphorylation. However, further analysis needs to be carried out to determine the effect of the 388G/A polymorphism in exon 2 (Figure 2) on Nurr1 function, as exon 2 is part of the Nurr1 promoter region.
Nurr1 gene level decreased in Parkinson’s disease patients

Compared with the control group, the Nurr1 gene expression level of Parkinson’s disease group was decreased (P < 0.05). The Nurr1 gene expression levels in Parkinson’s disease patients carrying the polymorphisms at exons 2 and 3 were significantly decreased compared with the control group (P < 0.01; Table 2).

Table 2  Nurr1 gene expression level (Ct value by real-time PCR) in blood

| Group                 | n  | Nurr1 gene expression level |
|----------------------|----|----------------------------|
| Control              | 200| 3.12±0.68                  |
| Parkinson’s disease  | 200| 1.29±0.15                  |
| Exon 2 (92G/A)       | 2  | 0.93±0.26                  |
| Exon 3 (709C/A)      | 5  | 1.01±0.21                  |

The level of Nurr1 gene expression was analyzed by real-time PCR. The expressive level of Nurr1 was assessed as the average Ct value in real-time PCR reactions. Data are expressed as mean ± SEM. *P < 0.05, **P < 0.01, vs. control group (Student’s t-test).

DISCUSSION

Nurr1 is highly expressed in the developing and adult ventral midbrain and is required for the acquisition and maintenance of the dopaminergic phenotype in nigrostriatal neurons. It has been reported that, besides the central nervous system, Nurr1 is expressed in many other tissues including peripheral blood lymphocytes. Using quantitative real-time PCR amplification, we analyzed the Nurr1 gene level in the peripheral blood lymphocytes of 200 sporadic Parkinson’s disease patients and 200 healthy controls and found that the Nurr1 expression level in the de novo Parkinson’s disease group was significantly decreased compared with that in the healthy control group, especially in several patients carrying two polymorphisms in exons 2 and 3 of Nurr1, indicating that these mutations exert a down-regulating effect on the expression of Nurr1 in peripheral blood lymphocytes.

Studies on postmortem brains have found that an age-related decline in the levels of DA phenotypic markers is associated with down-regulation of Nurr1.
expression in the human substantia nigra\textsuperscript{[28–29]}.

Chu and colleagues reported that the optical density of Nurr1 immunofluorescence was significantly decreased in nigral neurons containing a-synuclein-immunoreactive inclusions in Parkinson’s disease patients\textsuperscript{[30–31]}. Therefore, we investigated whether the Nurr1 gene level in peripheral blood lymphocytes could exactly reflect the change in the disease stage in Parkinson’s disease patients. In the present study, we obtained similar results, similar to Le’s report that the Nurr1 gene level in human peripheral blood lymphocytes revealed a significant decrease in individuals with Parkinson’s disease and parkinsonian syndromes\textsuperscript{[32]}. In conclusion, our present data showed that a single nucleotide polymorphism 388G/A in exon 2 and a 709C/A missense mutation in exon 3 of the Nurr1 gene exist in the Chinese population and might affect the pathogenesis in Parkinson’s disease patients.

SUBJECTS AND METHODS

Design
A gene polymorphic analysis.

Time and setting
The study was performed at the Zhongnan Hospital Affiliated to Wuhan University, China from January 2009 to October 2011.

Subjects
Parkinson’s disease patients were recruited from the Out-Patients Facility of Parkinson Clinic Center in Zhongnan Hospital, Wuhan University, China from 2009 to 2011.

Inclusion criteria
(1) Patients were diagnosed according to the UK Parkinson’s Disease Society Brain Bank Clinical Diagnostic Criteria for Parkinson’s Disease\textsuperscript{[33]}.
(2) All came from the Hubei Province of China.
(3) All patients were of Han ethnicity.
(4) Patients and their families signed an informed consent form.

Healthy physical examination controls were randomly selected from the Health Examination Center in Zhongnan Hospital, Wuhan University, China.

In total, 200 sporadic Parkinson’s disease patients and 200 healthy controls were enrolled in this study, and matched for age, sex and ethnicity.

Methods

Nurr1 DNA analysis

Whole blood samples were collected from the upper limb veins of Parkinson’s disease patients and healthy controls. Genomic DNA was extracted from 4 mL of whole blood using the QIAamp DNA Mini Kit (QIAGEN, Düsseldorf, Germany) according to the manufacturer’s protocols. Extracted genomic DNA samples were stored at \(-80^\circ\text{C}\) until gene analysis was carried out. The primer pairs used to amplify Nurr1 alleles were designed using the Primer3 (v. 0.4.0) system (PREMIER Biosoft International, Palo Alto, USA).

Nurr1 PCR amplification primers and conditions are as follows:

| Fragment | Sequence (5’–3’) | Annealing temperature (°C) | Fragment size (bp) |
|----------|------------------|---------------------------|-------------------|
| Exon1    | Sense primers: CAT CTG TAC GCT CT TCC GCT AA <br>Antisense primers: CAT CCT TCG GTC CCA CTC T | 59 | 430 |
| Exon2    | Sense primers: CAT ATG CCC AGC TGA ATC TC <br>Antisense primers: GTT ACA GGG TTT GCC TTG GC | 58 | 579 |
| Exon3    | Sense primers: TAA GGT TTG CCC GCC CCA TC <br>Antisense primers: CTA CTG GCA CCA AGG CAG AG | 60 | 305 |
| Exon4    | Sense primers: TTC TCC GAG TTG CCT GAT <br>Antisense primers: TCC AAA TGG GTC GTA TAG TT | 59 | 396 |
| Exon5    | Sense primers: TAA CAG GGC TCT TCC TTT GC <br>Antisense primers: CCT TGC TTG CCT TCT TTA CC | 59 | 487 |
| Exon6    | Sense primers: GCT GGA TGG CAC TGT ATT <br>Antisense primers: AGC CTC CCT GGA TTG TCT | 58 | 406 |
| Exon7    | Sense primers: ATG GAA TGG AGG TGG GAT AG <br>Antisense primers: GTA CTG ACC TGT GAC CAT AG | 60 | 438 |
| Exon8    | Sense primers: ATT GAT TCC ATT GTG GAA TTC TCC T <br>Antisense primers: TGT GTA GTC CAT GTA AAT CCA G | 60 | 513 |

Briefly, eight exon-specific primer pairs were used to amplify exons 1–8 of Nurr1 using a thermal cycler (model 9700, Applied Biosystems, Foster City, CA, USA). PCR products were electrophoresed through 4% acrylamide gels for 2 hours and abnormal alleles were sequenced using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequence data were analyzed using Match Tools and Navigator software (Match Tools Allele Identification package, Applied Biosystems). The expression level of Nurr1 gene was assessed as the average Ct.
value using real-time PCR in all Parkinson’s disease patients and healthy controls.

**Statistical analysis**

Data are expressed as mean ± SEM and were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA). Comparisons between groups were performed using the chi-square test and Student’s t-test.

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**Author contributions:** Xiaoliang Lou was responsible for the data acquisition, and analysis, drafted the manuscript, conducted statistical processing, and was head of funds. Weijing Liao provided technical information, and supervised the experiment.

**Conflicts of interest:** None declared.

**Ethical approval:** The protocol for this study was approved by the Ethics Committee of Zhongnan Hospital, Wuhan University, China.

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