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Mutation analysis of microRNA-7 gene in Chinese patients with Parkinson's disease

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ABSTRACT

Objective: To investigate the mutation of small sequence changes in microRNA-7 gene in Chinese patients with Parkinson's disease (PD).

Methods: We analyzed miR-7 variants in 225 PD patients from Chinese Han group by DNA sequence.

Results: None of the patients had miR-7 variants.

Conclusion: MiR-7 variation is not associated with PD in Chinese patients.

KEY WORDS Parkinson's disease; microRNA-7 gene; gene mutation; DNA sequence

DOI:10.3969/j.issn.1672-7347.2012.12.001

中国汉族帕金森病人 microRNA-7 变异分析

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[**摘要**] **目的：**调查中国大陆帕金森病（Parkinson's disease，PD）患者 microRNA-7 基因的变异。**方法：**对 225 例汉族 PD 患者应用 DNA 测序技术进行 microRNA-7 基因突变分析。**结果：**在本组 PD 患者中未发现 microRNA-7 变异。**结论：**MicroRNA-7 基因突变不是导致中国人群罹患 PD 的主要因素。

[**关键词**] 帕金森病；microRNA-7 基因；基因突变；DNA 测序

Parkinson's disease (PD) is a neurodegenerative disorder that affects 2% of the people >65 years[1–2]. PD is characterized by a loss of dopaminergic neurons (DNs) within the substantia nigra and both, acquired and inherited risk factors have been implicated in DN death [3–4]. The existence of affected relatives is a risk factor to develop PD, and linkage analysis in families identified at least 16 loci/genes implicated in PD. Most of the PD patients

Date of reception: 2011–10–10

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Foundation items: This work was supported by the Major State Basic Research Development Program of China ("973" Program, 2011CB510000) and the National Natural Science Foundation of China (81130021, 30900469, and 30971035).

are sporadic cases, and the genetic predisposition resides in the variation (polymorphisms) at several candidate genes. α-Synuclein is the main component of the Lewy bodies, the pathological hallmark of PD [5]. Mutations in the α-synuclein gene (SNCA), including gene duplications, cause familial autosomal-dominant PD [6–8]. In addition, over-expression of α-synuclein increases the vulnerability of DNs to neurotoxins and promotes cell death [9–10]. Recent report shows that cerebrospinal fluid (CSF) α-synuclein could be a diagnostic biomarker for PD and related dementias [11].

MicroRNAs are small (approximately 22–24 nucleotides long) non-coding RNAs that bind to the 3'UTR of mRNAs and negatively regulate translation. The miRNA genes encode a large RNA transcript, the pri-miRNA, that are first processed to the approximately 90 nucleotides long precursor microRNA (pre-miRNA), and these are in turn processed to the single-strand mature miRNA. Mature miRNAs are incorporated into the RNA-induced silencing complex that regulates mRNA expression [12]. Through this process, miRNAs regulate cellular processes such as differentiation, growth, proliferation, and apoptosis [13–14]. Point mutations in the seed region of miR-96, a miRNA expressed in hair cells of the inner ear, result in autosomal dominant, progressive hearing loss. This is the first study implicating a miRNA in a mendelian disorder [15]. Hu and colleagues [16] discovered common variants in pre-miRNA sequences played a role in the prediction of non-small cell lung cancer (NSCLC) survival. These observations raise the possibility that mutations in miR genes can trigger human being disease. Junn and colleagues [6] showed that microRNA-7 (miR-7), which is expressed mainly in neurons, represses α-synuclein protein levels through the 3'UTR of SNCA mRNA, and miR-7-induced down-regulation of α-synuclein protects cells against oxidative stress. Further, in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced neurotoxin model of PD in cultured cells and in mice, miR-7 expression decreases, possibly contributing to increased α-synuclein expression. These findings provide a mechanism by which α-synuclein levels are regulated in neurons, have implications for the pathogenesis of PD, and suggest miR-7 as a therapeutic target for PD and other α-synucleinopathies. To evaluate the pathogenic role of miR-7 in PD, we have analyzed miR-7 mutation in a cohort of Chinese Han patients with sporadic late-onset PD.

1 Materials and methods

1.1 Materials

Genomic DNA samples were obtained from 225 Chinese patients with sporadic PD, including 124 male and 101 female patients with onset at or after the age of 50 years. The age at onset ranged from 50 to 76 years and the average age of disease onset was (58±13) years. Patients with an onset of symptoms at an age ⩾50 years were classified as late-onset PD (see http://www.ninds.nih.gov/ disorders/ parkinsons disease for the definition of early- and late-onset PD). Patients were recruited through the Xiangya Hospital of Central South University. Patients were the Han ethnic group and from the South of China (Hunan, Hubei, Guangdong, and Jiangxi). All patients are genetically unrelated and without family history of parkinsonism, and gave their informed consent to participate in the study, approved by the Ethical Committee of Xiangya Hospital. Genomic DNA was extracted from freshly blood leukocyte using hydroxybenzene-chloroform extraction.

1.2 Identification of miRNA mutations

Sequence and genomic location of human miR-7 were obtained from the Sanger Institute miRBase (http://microrna.sanger.ac.uk/sequences/index shtml). A computer program was developed to download the surrounding genomic DNA sequences of miR-7 gene and design primers for polymerase chain reaction (PCR). A 203-bp DNA fragment was PCR-amplified from 225 patients with primers that flanked the whole pre-miRNA sequence (reference sequence ENSG00000384970, www.esemble.org): AAAACTGCTGCCAAAACCAC (forward) and GCTGCATTTTACAG CACCAA (reverse). Reactions were performed in a total volume of 20 μL containing 1×Q.PCR buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L of each dNTP (Sangon, Shanghai, China), 0.5 μmol/L of both forward and reverse primers, 1.0 U Qiagen HotStar Taq DNA polymerase (Qiagen, Hilten, Germany), and 50 ng of genomic DNA. Cycle conditions were: 5 min at 94 ℃; 35 cycles of 30 s denaturing at 94 ℃, 30 s annealing at 60 ℃, and 40 s extension at 72 ℃ with a final extension 5 min at 72 ℃. Both strands were sequenced using ABI Prism® 3100 Sequence Detection System (www.appliedbiosystems.com). All sequencing chromatograms were compared to the published cDNA sequence using DNAstar software.

2 Results

To investigate the potential neurol degenaration role of

mutations/SNPs in miR-7 gene, we screened 225 Chinese late-onset PD patients. The results showed that there was no nucleotide change in miR-7 gene among these patients. The PCR gel electrophoresis of PCR products of the DNA samples and DNA sequencing diagram of the whole pre-miRNA sequence of miR-7 gene are showed in Figure 1 and Figure 2.

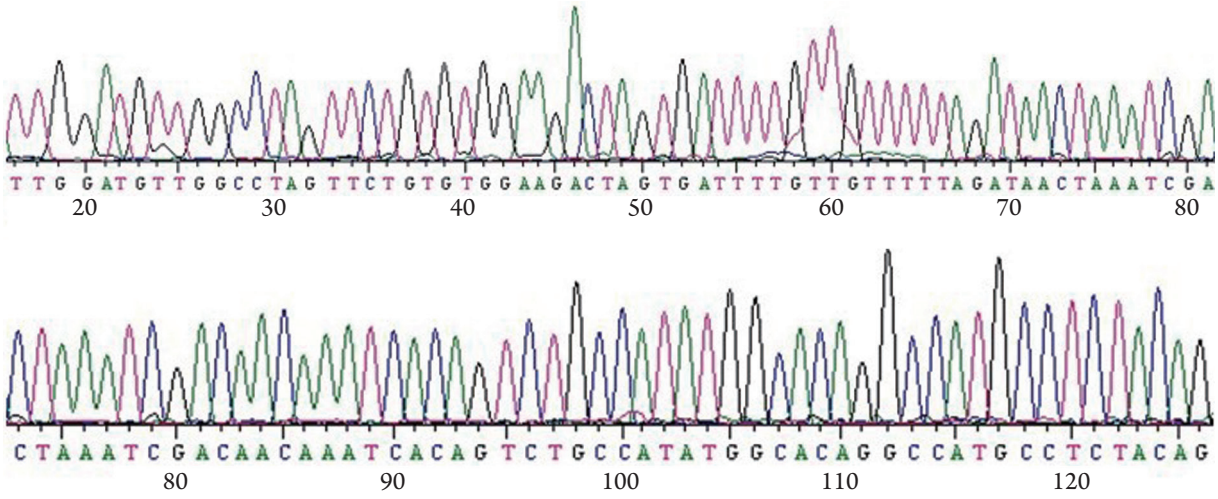

Figure 1 DNA sequencing diagram of miR-7 gene.

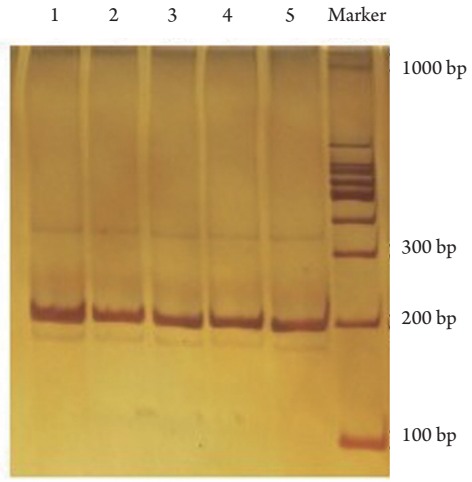

Figure 2 MiR-7 gene was successfully amplified. 1–5: PCR products of the samples; Marker: 100-bp DNA ladder.

3 Discussion

Many miRNAs are up- or down-regulated in various human neurodegenerative disorder, indicating that miRNAs play a role in neurodegeneration[6,16–25]. However, the mechanism through miRNAs to regulate the expression of the pathogenic genes in neurodegenerative disorders is largely unknown because the biogenesis of miRNAs involves in multiple steps[12–13]. Junn and colleagues [6] discovered that miR-7 represses α-synuclein protein levels through the 3'-UTR of α-synuclein mRNA to protect cells against oxidative stress. To further explore the potential link of miR-7 with PD, we screened sequence miR-7 variations in 225 Chinese PD patients. An analysis of mutation patterns showed that no nucleotide change was found in any individual, suggesting that the sequence variations in miR-7 gene might not be PD specific. However, it's necessary to investigate in larger PD patients group and to screen the 3'UTR of SNCA gene which including miR-7 target sites. In addition, the decrease of miR-7 expression possibly contributes to increased α-synuclein expression [6]. It's important to investigate miR-7 expression level in PD patients' brain tissue or CSF in future.

In conclusion, we did not find nucleotide changes in miR-7 (that binds to the 3'UTR of SNCA mRNA) among our PD patients, suggesting that miR-7 DNA change is rarely linked to PD-risk in Chinese patients.

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(Edited by CHEN Liwen)