Research paper

A homozgyous parkin p.G284R mutation in a Chinese family with autosomal recessive juvenile parkinsonism

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\textbf{HIGHLIGHTS}

\begin{itemize}
\item A homozgyous variant, c.850G>C (p.G284R), in the parkin gene was identified by exome sequencing and Sanger sequencing.
\item Both patients in the pedigree presented with typical but heterogeneous clinical features of autosomal recessive juvenile parkinsonism and with different ages of disease onset.
\item The homozgyous variant co-segregated with the disease in the family and was absent in 800 controls.
\end{itemize}

\textbf{ARTICLE INFO}

\textbf{Article history:}
Received 11 April 2016
Received in revised form 4 May 2016
Accepted 8 May 2016
Available online 10 May 2016

\textbf{Keywords:}
Autosomal recessive juvenile parkinsonism
Exome sequencing
Parkin
Mutation
Genetic counseling

\textbf{ABSTRACT}

Autosomal recessive juvenile parkinsonism (AR-JP) is a distinct clinical and neuropathologic entity characterized by early onset parkinsonism and localized neuronal degeneration in the substantia nigra without Lewy bodies. The purpose of this study is to identify the genetic defect in a Chinese pedigree with familial AR-JP and to explore genotype-phenotype correlation. A three-generation Chinese Han pedigree with familial AR-JP was recruited in this study, and the patients in the pedigree presented with typical but heterogeneous clinical features of AR-JP and with different ages of disease onset. Exome sequencing and Sanger sequencing were conducted in the index case diagnosed as juvenile parkinsonism and a homozygous variant, c.850G>C (p.G284R), in the parkin gene was identified. The homozygous variant co-segregated with the disease in the family and was absent in 800 controls. The homozygous variant, c.850G>C (p.G284R), in the parkin gene is possibly responsible for AR-JP in this pedigree. Heterozygous c.850G>C mutation carriers were free of any neurological symptoms, consistent with a loss-of-function mechanism of the parkin mutations. These findings may provide new insights into the cause and diagnosis of AR-JP and have implications for genetic counseling.

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1. Introduction

Parkinson’s disease (PD) is one of the most common neurodegenerative disorders, with a prevalence of 1–2% in individuals over 65 years of age [1]. It is characterized pathologically by selective degeneration of nigral dopaminergic neurons and the presence of Lewy bodies. The cause of the disease remains unknown, while mitochondrial dysfunction, environmental and genetic factors might all be involved [2]. Although less than 10% of PD patients exhibited a classical Mendelian type of inheritance, dramatic progress in identification of monogenically inherited PD genes had been made in recent two decades and it provided tremendous insight into pathogenesis of this complex disorder [3]. At least 23 loci and 18 disease-causing genes have been described to be involved in monogenic transmission of PD [4–7].

Autosomal recessive juvenile parkinsonism (AR-JP; MIM 600116) is a distinct disease entity among the broad spectrum of PD. Clinically, AR-JP is characterized by some typical features of parkinsonism with onset usually before age 40, a good response to levodopa treatment, sleep benefit (amelioration of parkinsonian symptoms after sleep or napping), a susceptibility to dopa-induced...
dyskinesia, motor fluctuation and slow progression. The parkinsonian triad, including tremor, rigidity and Bradykinesia, was all mild, while gait freezing, foot dystonia, retropulsion and hyperreflexia were relatively prominent [8]. Neuropathologically, AR-JP is characterized by localized neuronal loss with gliosis in the ventrolateral and medial regions of the substantia nigra pars compacta without Lewy bodies [9]. Mutations in the parkin gene (PARK2, MIM 602544) are robustly associated with AR-JP [10]. Homozygous or compound heterozygous mutations in the parkin gene have been described in up to 49% of familial and 18% of sporadic early-onset PD, including autosomal recessive juvenile disease [11]. Here, we report a homozygous mutation, c.850G>C (p.G284R), in the parkin gene that causes AR-JP in a Chinese Han family. Heterogeneous phenotype in this family draws attention to the broad spectrum of phenotypes in AR-JP caused by mutations in parkin gene.

2. Materials and methods

2.1. Pedigree and subjects

A three-generation Chinese family with AR-JP was recruited in this study (Fig. 1A). Both patients and the other unaffected family members were evaluated by two independent neurologists from the Third Xiangya Hospital. The diagnosis of AR-JP was made based on PD diagnostic criteria [12], combined with the age at onset and the mode of inheritance. Recessive inheritance was suggested in this pedigree where two members of one generation were affected, especially siblings, but not their parents or their children. Clinical data and venous blood samples were obtained from two affected individuals (II:1 and II:3) and four unaffected family members (I:1, I:2, II:4 and III:1). Blood samples were also taken from one hundred unrelated ethnically-matched normal controls without clinical features of PD (male/female: 50/50, age 39.5 ± 4.6 years). This study was approved by the Institutional Review Board of the Third Xiangya Hospital of Central South University, and all participants signed consents to participate in the study.

2.2. Variant analysis

Genomic DNA from peripheral venous blood lymphocytes was extracted from all participants using standard phenol-chloroform extraction method as previously described [13]. To systematically search for the disease-causing gene, exome sequencing in index case (II:3) was performed using the Agilent’s SureSelect Human All Exon V5 Kit on the Illumina HiSeq 2000 platform (Novogene Bioinformatics Technology Co., Ltd). At least 1.5 micrograms (μg) genomic DNA from the index case (II:3) was used to establish the exome library according to the manufacturer’s instructions [14].

Paired-end reads were aligned to the human reference genome sequence obtained from UCSC database (build 37.1 version hg19, http://genome.ucsc.edu/) using Burrows-Wheeler ALIGNMENT tool [15]. High-quality alignment was required to guarantee variant calling accuracy (greater than 0) for detecting single nucleotide polymorphisms (SNPs) and insertions-deletions (indels). After applying a series of filtering strategies as previously described [16], the analysis-ready BAM alignment result was collected, ANNOVAR (Annotate Variation) was conducted to annotate SNPs and indels, including variant position, variant type, and conservative prediction. All potential variants were filtered against the public database as previously described to remove the polymorphism loci [16]. Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping version 2 (PolyPhen-2) were performed to evaluate whether the amino acid substitutions affect protein function [17]. Sanger sequencing was then performed in family members and 100 normal controls to validate the identified potential disease-causing variant [13]. Sequences of the primers used for amplifying the causative variant of parkin gene (NM_004562.2) were as follows: 5'-GCCACCTTGATTTGCTTAG-3' and 5'-TTTGTATCGACGATCGAA-3'.

MutationTaster (http://www.mutationtaster.org/) was applied to test the possible impact of amino acid substitution on protein function. The Basic Local Alignment Search Tool (http://blast.st-va.

![Fig. 1](image-url) (A) Pedigree of the family with AR-JP showing affected cases (fully shaded). N, normal; M, the parkin p.G284R mutation. The arrow indicates the proband with AR-JP. Fig. B, C, D. Mutation analysis of the parkin p.G284R mutation. (B) Homozygous p.G284R mutation patient (II:3). (C) Heterozygous p.G284R mutation carrier (III:1). (D) Normal control.
3. Result

3.1. Clinical findings

Detailed clinical characteristics are summarized in Table 1. Both patients in the family displayed early onset parkinsonism. The index case (II:3) had been well until about the age of 8 years when gait disturbance appeared. Mild tremor started at age 13, and became progressively more prominent over time. He also had been slow-moving and had shown frozen gait. He had taken levodopa since 15 years of age. His symptoms improved significantly with the medication. About 2 years after the initiation of levodopa administration, he showed wearing-off phenomenon. His sister (II:1) firstly presented with dystonia of the feet at 22 years of age. She prominently displayed tremor and retropulsion with age. She also responded well to levodopa in combination with trihexyphenidyl. Motor fluctuations, amelioration of the symptoms after sleep or nap and slow progression are observed in both patients [18].

3.2. Exome sequencing and identification of pathogenic mutation

A total of 43.46 million reads with an average read length of 150 bp were generated from gDNA of the index patient, and 43.29 million reads (99.61%) were mapped to the human reference genome. 4018.28 Mb of effective sequences were mapped to the target region, and the average sequencing depth on target region was 79.74. About 99% of the region were covered by the target sequence at 10× or greater. A total of 36,783 SNPs, including 17,543 in the exon regions and 1,596 in the splicing sites, were identified. In addition, 2,542 indels, including 417 in the exon regions and 209 in the splicing sites, were detected. A prioritization scheme similar to what has been described in recent studies was utilized to detect the possible pathogenic variant in the patient [16,19]. We eliminated common known variants in public databases, including database of SNPs build 137 (MAF >1%), 1000 genomes project with a frequency >0.5%, and NHLBI Exome Sequencing Project 6500 (ESP6500), and synonymous variants. SIFT and PolyPhen-2 analyses were used to predict the function of non-synonymous variants. Using the above filtering criteria, only 252 variants were suspected to be possible disease-causing variants and were prioritized for further analysis. Except a known c.850G > C (p.G284R) variant in the parkin gene, no other variants in known PD disease-causing genes were found in the index case (II:3, Fig. 1A). The homozygous c.850G > C (p.G284R) variant was confirmed by Sanger sequencing (Fig. 1B), and the same homozygous variant was also detected in his affected sister (II:1) and asymptomatic young brother (aged 13 years, II:4). Heterozygous c.850G > C (p.G284R) variant was identified in three unaffected family members (I:1, I:2 and III:1, Fig. 1C). Mutation-Taster predicted that the substitution was disease-causing with a high probability near 1, indicating a high possibility. The glycine at position 284 (p.G284) is shown to be highly conserved among various vertebrates based on multiple sequence alignments (Fig. 2). Additionally, this variant was also absent in a total of 800 controls, including 100 ethnically matched unrelated controls (Fig. 1D) in this study and 700 Chinese controls with medical conditions other than parkinsonism from exome sequencing by Novogene Bioinfor-

<table>
<thead>
<tr>
<th>Subject</th>
<th>I:1</th>
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<th>II:3</th>
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yr. years; Het, Heterozygote; Hom, Homozygote.

![Fig. 2. Conservation analysis of the parkin p.Gly284 amino acid residue.](image)
matics Technology Co., Ltd. All these data suggest that p.G284R be a pathogenic mutation.

4. Discussion

AR-JP was first recognized in 1973 in patients presented with early onset parkinsonian symptoms with marked diurnal fluctuation [20]. In 1997, Ishikawa and Tsuji thoroughly described AR-JP as a distinct disease entity from clinical, neuropathologic, and genetic perspectives by analyzing 17 patients from 12 Japanese families [18]. Subsequently, Kitada and collaborators identified the parkin gene as responsible for AR-JP [10]. The parkin gene, containing 12 coding exons, encodes a protein of 465 amino acids with an N-terminal ubiquitin-like domain and a C-terminal RING-finger motif [10]. To date, at least 259 different mutations in the parkin gene, including exon deletion, insertions, multiplications, missense mutations and truncating mutations, have been identified based on the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) [21]. Most of point mutations cluster in the RING-in-between-ring-RING region of the protein as well as in the ubiquitin-like region in European families [22].

The parkin gene is associated with the ubiquitin proteasome system as an E3 ubiquitin ligase, and works in conjunction with E2 and E3 of the ubiquitin proteasome system to ubiquitinate misfolded or aggregated proteins [23]. Parkin has been found to be selectively recruited to impaired mitochondria and promote their autophagy, thus plays a vital role in mitochondrial quality control [24]. Furthermore, accumulating evidence shows that parkin may share a common biological pathway with the PTEN-induced kinase 1 gene (PINK1) and DJ-1, the other two genes responsible for autosomal recessive PD, being involved in mitophagy, mitochondrial fusion and fission, mitochondrial trafficking. Loss of function of any of the three gene products may cause nigral degeneration, a pathological hallmark of PD [25,26].

In this study, a homozygous variant, c.850G>C (p.G284R), in the parkin gene was identified by exome sequencing and Sanger sequencing in our family. Though the parkin p.G284R variant (NP_004553.2) is recorded in SNP database (rs751037529), the quite low frequency (<0.001%) in SNP database, co-segregation with the AR-JP phenotype in the family, localized in the RING1 domain (238–293 amino acids), in silico prediction of deleterious effect and the absence in 800 Chinese controls suggest that it is a pathogenic mutation. The 13-year-old boy with homozygous c.850G>C (p.G284R) mutation remains asymptomatic. It may be due to relatively young age and/or incomplete penetrance. Though heterozygous carriers of parkin mutations have been reported to have minor motor signs or present with late-onset parkinsonism [27], two elder heterozygous c.850G>C carriers (1.1, aged 56 years and 1.2, aged 51 years) are free from PD, consistent with a loss-of-function mechanism of the parkin gene and findings from our previous studies [28–30]. Heterozygous parkin mutations were reported in only a few patients and may be caused by the second pathogenic mutation escaping detection or digenic inheritance [31].

Consanguineous mating was denied in this family and a homozygous parkin mutation was found in both symptomatic patients. Therefore, the possibility of founder effect should be considered as the parents resided within the same geographic area. Additionally, homozygous or compound heterozygous mutations involving p.G284R mutation has been reported in unrelated Chinese patients with AR-JP [21,32–35]. These findings further support that p.G284R mutation should be a founder mutation instead of a hot spot mutation.

In summary, our data support that the homozygous mutation, c.850G>C (p.G284R), is the causative genetic factor for AR-JP in this family. Heterogeneous phenotype in this family reflects the broad spectrum of phenotypes in AR-JP that is caused by mutations in parkin gene. Further functional studies of how the single gene defect leading to selective nigral neuronal degeneration as well as interactions of parkin, PINK1 and DJ-1 may help unravel pathogenesis of PD.

Conflict of interest

All authors declare no conflicts of interest.

Acknowledgements

We thank the participating members and investigators for their cooperation and efforts in collecting clinical and genetic information and DNA specimens. We also wish to thank Prof. Zhi Song and Associated Prof. Wen Zheng for their clinical diagnosis and comments. This work was supported by grants from New Xiangu Talent Project of the Third Xiangu Hospital of Central South University, China (JY201501, Han Chen).

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