Genetic analysis of the S100B gene in Chinese patients with Parkinson disease

Yi Guo\textsuperscript{a,b,1}, Huarong Yang\textsuperscript{a,c,1}, Xiong Deng\textsuperscript{a}, Zhi Song\textsuperscript{b}, Zhijian Yang\textsuperscript{a}, Wei Xiong\textsuperscript{d}, Lamei Yuan\textsuperscript{a}, Hongbo Xu\textsuperscript{a}, Sheng Deng\textsuperscript{c}, Hao Deng\textsuperscript{a,b,∗}

\textsuperscript{a} Center for Experimental Medicine, The Third Xiangya Hospital, Central South University, Changsha, China
\textsuperscript{b} Department of Neurology, The Third Xiangya Hospital, Central South University, Changsha, China
\textsuperscript{c} Department of Neurology, The Second Hospital, Guiyang Medical College, Kaili, China
\textsuperscript{d} Key Laboratory of Carcinogenesis of Ministry of Health and Key Laboratory of Carcinogenesis and Cancer Invasion of Ministry of Education, Cancer Research Institute, Central South University, Changsha, China

HIGHLIGHTS

- Mutations in the S100B coding region play little or no role in Chinese PD.
- The first study of the S100B gene in a large cohort of Chinese PD patients.
- The result will stimulate researches about pathogenic role of the S100B gene.

ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form
11 September 2013
Accepted 14 September 2013

Keywords:
Parkinson disease
S100 calcium-binding beta
Mutation
The coding region

ABSTRACT

There is growing evidence that genetic abnormalities play an important role in the pathogenesis of Parkinson disease (PD). At least 18 genetic loci and 13 disease-related genes for parkinsonism have been identified. The S100 calcium-binding beta (S100B), which is expressed and secreted by astrocytes, has been found to be associated with PD. To evaluate whether the S100B variants are related to PD in Chinese Han population, we conducted genetic examination of the S100B gene in 502 PD patients from Mainland China. We did identify two known variants c.279+4T>C (rs187503470) and c.99C>G (p.Leu33Leu, rs1051169) in our patients. Neither of these two variants is predicted to change amino acid or splice site, indicating that they are not pathogenic mutations. Our results suggest that mutations in the coding region or intron/exon boundaries of the S100B gene play little or no role in the development of PD in Chinese population.

1. Introduction

Parkinson disease (PD; MIM 168600) is the second most common progressive neurodegenerative disorder after Alzheimer’s disease, and it affects approximately 1% of people over the age of 65 years [1]. It is characterized by loss of dopamine-producing neurons, aggregation of \( \alpha \)-synuclein in neurons of particular brain regions, including the brainstem and cortical regions, and presentation of troublesome symptoms and signs, including rest tremor, bradykinesia, rigidity, postural instability, and other motor and non-motor manifestations [2,3]. There is growing evidence that genetic abnormalities play an important role in the pathogenesis of PD [4,5]. Research in PD genetics has been prolific with the discovery of a series of genes that are responsible for monogenic parkinsonian disorders. The past 16 years has witnessed dramatic progress in the genetic basis of PD by the discovery of at least 18 genetic loci and 13 disease-related genes for parkinsonism through linkage analyses of families with typical PD (PARK1-15) or genome-wide association studies of index patients (PARK16-18) [4]. The S100 calcium-binding beta (S100B), a calcium-binding protein, expressed and constitutively secreted by astrocytes, has been reported to be associated with the development of PD [6–9]. To determine whether coding mutation in the coding region of the S100B gene is related to PD in Chinese Han population, we conducted genetic analysis of 502 patients with PD from Mainland China.

2. Materials and methods

Five hundred and two unrelated Chinese Han patients with PD from the south of Mainland China were enrolled in this study. The
diagnosis of PD was made according to common diagnostic criteria [10]. The mean age of these PD patients was 65.9 ± 10.2 years (male/female: 310/192) and the mean age at onset of PD symptoms was 62.5 ± 7.9 years. Among the 502 PD patients, 119 (23.7%) had first- or second-degree relatives affected with PD (familial PD; male/female: 72/47), 383 cases (76.3%) had no family history (sporadic PD; male/female: 238/145). Mutation analysis of several genes that are associated with PD was conducted in some of the patients first. Mutation analysis of the vacuolar protein sorting 35 gene (VPS35) was done in 40.2% (202/502) of the patients, and no mutation was identified [11]. Mutation analysis of the F-box only protein 48 gene (FBXO48) was carried out in 67.5% (339/502) of the patients, and no mutation was found [2]. Mutation analysis of two point mutations (p.Ala502Val and p.Arg1205His) in the eukaryotic translation initiation factor 4-gamma 1 gene (EIF4G1) was done in 80.3% (403/502) of the patients and none of these patients carry the two mutations [3]. Every patient who participated the study signed an informed consent. The study was approved by the Ethics Committee of the Third Xiangya Hospital, Central South University, China.

Genomic DNA was extracted from lymphocytes using standard phenol-chloroform method [12]. Genetic analysis of the coding region of the S100B gene was performed in 502 PD patients and the method was described previously [13]. The primers used for PCR amplification are listed in Table 1. PCR products were generated with 100 ng of gDNA in 2.5 μL 10× PCR buffer, 2.0 μL of 2.5 mmol/L each dNTP, 1.5 μL of 25 mmol/L MgCl2, 1 μL of 10 μmol/L each primer and 1 U Taq polymerase in a total volume of 25 μL. PCR amplified the S100B gene by using a GeneAmp 9700 thermal cycler system (Applied Biosystems, Foster City, CA, USA), and PCR conditions were 95 °C for 3 min, followed by 35 cycles of 95 °C for 40s, 58 °C for 35s, 72 °C for 45s, and a final extension step at 72 °C for 5 min. 8.5 μL of PCR products were digested by 0.8 U shrimp alkaline phosphatase (SAP, Fermentas) and 8 U exonuclease 1 (Ferments) in a 10 μL reaction volume, and was sequenced directionally using an 8-capillary 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA)[13].

3. Results

In this study, we identified two nucleotide variants in 502 Chinese Han patients with PD. A known c.279+4T>C variant (rs187503470, 0.0005 in the frequency in dbSNP from 1000 Genomes project) in the 3'-untranslated region (3'-UTR) of the S100B gene was found in a sporadic PD case. The patient was a 73-year-old man and his age of onset was 70 years. A known variant c.99C>G (p.Leu33Leu, rs1051169) in exon 2 was also observed with the G-allele frequency of 39.9% in our patients. These two variants are not predicted to change either amino acid or splice site (http://www.fruitfly.org/seq_tools/splice.html). Therefore, neither of these two variants is likely to be pathogenic mutation.

4. Discussion

Genetic factors are well-known to play an important role in the pathogenesis of PD [4,14]. Human S100B gene, located on chromosome 21q22.2-q22.3, spanning over 6.5 kb, is a 3-exon gene, which includes two coding exons encoding 92 amino acids. S100B is a glial-derived protein that functions as a neurotrophic factor and a neuronal survival protein during development of the central nervous system [15]. This protein, expressed in high abundance in the brain [16], belongs to the large superfamily of S100 proteins, which are broadly involved in cell cycle regulation, differentiation, growth and mobility. As an intracellular regulator, S100B interacts with target proteins thereby altering their functions via the multiligand receptor RAGE (receptor for advanced glycation end products), affecting energy metabolism, protein phosphorylation, the dynamics of cytoskeleton constituents (and hence, of cell shape and migration), Ca2+ homeostasis, cell proliferation and differentiation [16]. Increased S100B levels, caused by increased secretion of S100B from astrocytes or release from damaged astrocytes, have been detected in neurodegenerative, neuroinflammatory and psychiatric diseases [17]. An association of the S100B rs3788266 variant with schizophrenia and psychosis in bipolar affec- tive disorders indicates the potential involvement of S100B in dopaminergic dysfunction [18,19].

The aggregative evidence shows that glial cells may be involved in the development of PD, for example, astrocytes may effectively endocytose α-synuclein proteins secreted from neurons [20], treatment with high concentrations of S100B may induce neuronal cell apoptosis through nitric oxide release from astrocytes [16,21], and blockade of microglial activation led to a significant attenuation of PD-like disease process caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [22]. Increase of S100B-positive astrocytes in the substantia nigra of mice treated with MPTP, an animal model for PD, and the autoimmun e responses to S100B antigen in serum of patients with PD indicate that S100B may also be involved in the pathogenesis of PD [9,23,24]. Overexpression of S100B in the brain of S100B transgenic mice promoted the pathogenesis and development of PD by reducing the expression of dopamine D2 receptor (D2DR) and G protein-coupled receptor kinase 2 (GRK2), ultimately affecting the synthesis and metabolism of neurotransmitters dopamine and 5-hydroxytryptamine and enhancing oxidative stress [25]. Additionally, a recent study reported increased S100B protein levels in the substantia nigra of post-mortem patients with PD, and unregulated S100B messenger RNA and protein levels in MPTP-treated mice. Deletion protects against MPTP-induced toxicity through the RAGE and tumor necrosis factor-α (TNF-α) pathway [7]. An age-dependent and site-specific increase in co-localization of A30P α-synuclein and S100B within astrocytes that persisted after doxycycline treatment was observed in a recent study, supporting the association of the S100B and PD [26].

Based on these findings and the hypothesis that S100B is involved in PD pathogenesis, we set out to study the S100B variants in Chinese Han patients with PD. In this study, we screened the mutations of the coding region and intron/exon boundaries of the S100B gene, and identified two nucleotide variants in 502 PD patients. The known non-coding c.279+4T>C transition in the 3'-UTR does not change splicing, and c.99C>G substitution in exon 2 does not change amino acid (http://www.fruitfly.org/seq_tools/splice.html), suggesting neither of these variants is pathogenic mutation.

To the best of our knowledge, this is the first study designed to assess the role of the S100B gene variants in the coding region and intron/exon boundaries in a large cohort of Chinese patients with PD from Mainland China. However, our study does not exclude
completely the possibility of variants in the non-coding region of the S100B gene that may cause PD in our subjects. Further studies are needed to appraise the contribution of S100B in clinical evaluation of PD and the role of astrocytes in PD [8]. Although a defect in the S100B gene may be involved in severity of PD, our study suggests that mutation in the coding region of the S100B gene probably does not play a major role in the pathogenesis of PD. Further analysis of variants in the regulatory regions of this gene [17], and in other genes involved in the dopamine system, especially RAGE and TNF-α pathways, may provide further understanding of the molecular and cellular mechanism underlying PD pathogenesis.

Acknowledgments

This study was supported by National Natural Science Foundation of China (81101339, 81271921, 81001476), Research Foundation for the Doctoral Program of Higher Education of China (20111062110026), Natural Science Foundation of Hunan Province, China (10JJ4020), Sheng Hua Scholars Program of Central South University, China (H.D.), Construction Foundation for Key Subjects of the Third Xiangya Hospital, Central South University, China (H.D.), and Students Innovative Pilot Scheme of Central South University, China (YC1241, CL11280).

References