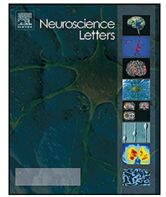
Neuroscience Letters 575 (2014) 80–84

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



The association of single nucleotide polymorphism of the Fyn gene with sporadic Alzheimer's disease in the Chinese Han population

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HIGHLIGHTS

- Evidence suggests Fyn is a potential candidate gene for Alzheimer's disease (AD).
- No association was revealed between five target SNPs of Fyn and AD risk.
- Haplotypes containing four SNPs of Fyn did not affect AD risk.
- It is the first study on association between Fyn gene and AD in the Chinese population.

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form 19 April 2014
Accepted 7 May 2014
Available online 20 May 2014

Keywords:
Fyn
Tau
Alzheimer's disease
Single nucleotide polymorphism

ABSTRACT

Recent studies suggested genetic factors contribute to the pathogenesis of sporadic Alzheimer's disease (sAD). Fibroblast Yes related novel (Fyn), a tau kinase, has been reported to be associated with aberrant phosphorylated tau and neurofibrillary tangles formation. Fyn gene may be a potential candidate gene for AD. To investigate the association of the polymorphisms in Fyn gene with the susceptibility to sAD, we conducted a case–control study in a Chinese Han cohort including 200 sAD patients and 243 control participants. Four single nucleotide polymorphisms (SNPs) (rs111787668, rs1057979, rs6916861 and rs12910) within the promoter region of Fyn gene and one (rs7768046) in intron were selected and genotyped with a polymerase chain reaction–ligase detection reaction (PCR–LDR) method. Logistic regression under four genetic models was used to analyze the association between target SNPs and the risk of sAD. After adjusting for age, sex and APOE ε4 status, no association was revealed between these SNPs or the haplotypes containing four SNPs and the risk of sAD ($P > 0.05$). The SNPs in the selected regions of the Fyn gene are unlikely to confer the susceptibility of sAD in the Chinese Han population. Further studies with a larger sample size and different ethnic populations are needed to reveal the role of Fyn gene in the pathogenesis of sAD.

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1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder characterized by progressive dementia and becomes the major health issue along with cerebrovascular diseases affecting the increasing elderly population nowadays [1,2], yet the underlying mechanism remains elusive. In the recent years, studies suggested that genetic factors also contribute to the pathogenesis of sporadic Alzheimer's disease (sAD). There is no doubt that APOE ε4 has been proven to increase the risk of sAD and familial AD (fAD) [3]. Genome-wide association studies have revealed that several SNPs within different genes, such as CLU, PICALM and CR1 are closely related to the risk of sAD [4], which strongly implicate the important role of genetic variation in the development of AD.

Neurofibrillary tangles (NFTs) is an important neuropathological hallmark of AD [5]. It has been known that NFTs are mainly composed of hyperphosphorylated tau aggregated in neuron [6,7]. Fyn, a tyrosine kinase, has been implicated to contribute to aberrant tau phosphorylation in AD pathogenesis [8,9]. The first evidence suggesting Fyn may be involved in AD was that Fyn was elevated in AD brain compared to controls [10]. Another study indicated that the increased expression of Fyn in AD brain was directly correlated with cognitive scores of the patients [11].

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http://dx.doi.org/10.1016/j.neulet.2014.05.020
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Animal studies also support the important role of Fyn in tau phosphorylation. Exposure to insulin transiently increased the activation of tau phosphorylation in SH-SY5Y neuroblastoma cells in a pathway involving Fyn [12]. Lee et al. [13] found that the SH3 domain of Fyn bound to tau in SH-SY5Y cells and induced an increase in tau phophorylation. Additionally, when tau was co-expressed with Fyn in COS-7 cells, tyrosine phosphorylation of tau was increased [14,15]. Furthermore, recent evidence suggests that Fyn plays a role in the regulation of Aβ production and mediates Aβ-induced synaptic deficits and neurotoxicity [16–18].

Based on the important biological evidence, Fyn gene, located in 6q21, may be a potential candidate gene for AD. Genetic associations between Fyn SNPs and several diseases have been reported. It was found that Fyn SNPs were associated with schizophrenia cognitive performance [19], obesity [20], anxiety [21] and alcohol dependence [22]. While there are rare studies on genetic association between Fyn and sAD. Evidence [6,23] supported the genetic association between Fyn (rs7768046) and CSF tau levels. However no association between Fyn SNPs and AD was found in a Japanese population [24]. The rare results indicate that the association of Fyn gene polymorphism with the susceptibility to AD needs to be further studied.

To further investigate the possible association of Fyn gene polymorphism with sAD, for the first time in a Han Chinese population, we analyzed the four SNPs (rs111787668, rs1057979, rs6916861 and rs12910) in the promoter region of the Fyn gene which is closely related to the function of the gene, and one SNP (rs7768046) in the intron which was reported to be associated with CSF tau levels [6,23].

2. Materials and methods

2.1. Study population

A total of 200 sAD patients were consecutively recruited from Chongqing Daping Hospital, Chongqing Mental Health Center, and Guangzhou General Hospital. Age and sex matched controls (CTRL) with normal cognition were randomly recruited from health examination center of the hospitals, from January 2010 to October 2013. All of them were ethnic Han people and lived in the south of China. The subjects were not eligible if they have: (1) a family history of dementia; (2) a concomitant neurologic disorder potentially affecting cognitive function (e.g. severe Parkinson's disease); (3) severe cardiac, pulmonary, hepatic, renal diseases or any kinds of tumor; (4) declined to participate in the study. The study was approved by the Institutional Review Board of Daping Hospital, and registered in the Chinese Clinical Trial Registry (No. ChiCTR-OCC-12001966). Written consents for genetic screening were obtained from all participants or their legal representatives.

The clinical assessment and diagnosis of AD dementia were performed following the protocol described in our previous studies [25]. In brief, the demographic data, medical history and cognitive status were collected and assessed based on formal questionnaire and a neuropsychological battery. The cognitive status was assessed with the Chinese version of Mini-Mental Status Examination (MMSE), which were validated previously in Chinese elderly people [25]. For CTRL, they only needed to use MMSE to measure cognitive function. The subjects who were abnormal in MMSE assessment were further administered with neuropsychological tests [25]. Additive score of MMSE ranged from 0 to 30. The boundary score of MMSE was defined as 17 (illiteracy), 20 ($\leq$6 years of education), and 24 (>6 years of education). These procedures were administered by trained interviewers composed of experienced neurologists.

2.2. SNP selection and genotyping

The genetic variation data of the Fyn gene was obtained from the HapMap project (http://hapmap.ncbi.nlm.nih.gov/) for 45 unrelated Chinese Han people in Beijing (CHB), which was the latest data provided (release #28). A total of seven SNPs are present in the 3-kb region upstream of the transcription start site of the Fyn gene, which covers the putative promoter region. Among the seven SNPs, four (rs111787668, rs1057979, rs6916861 and rs12910) with a minor allele frequency (MAF) $\geq$ 0.05 were selected. All four were predicted to locate in the transcription factor binding site using SNPinfo online software [26]. In addition, rs7768046, a SNP in an intron was also chosen with MAF $\geq$ 0.05 because it has been frequently studied and was associated with CSF tau level [6,23].

Venous blood was sampled into sterile anti-coagulation tubes. The genomic DNA was extracted using the Wizard GenomicDNA Purification Kit (Promega, USA) according to the product instruction. A multiplex polymerase chain reaction–ligase detection reaction (PCR–LDR) method was applied for genotyping. For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were distinguished by different extended lengths at 3′ end. The primers for both PCR and LDR reactions were all designed by Primer3 online software v.0.4.0 (http://primer3.wi.mit.edu). In brief, the PCR reactions were performed with 1 μL DNA sample (10 ng/μL), 1× GC-I buffer (TaKaRa, Japan), 3.0 mM Mg^{2+} (TaKaRa, Japan), 0.3 mM dNTP (Generay Biotech, China), 1 U HotStarTaq polymerase (Qiagen, Germany), 1 μM multiple PCR primers (Sangon, China) and ddH_2O in a total volume of 20 μL. The PCR cycling program was: 95 °C for 2 min, followed by 11 cycles of 94 °C for 20 s, 65 °C (decreased 0.5 °C per cycle) for 40 s, 72 °C for 90 s plus 24 cycles of 94 °C for 20 s, 59 °C for 30 s, 72 °C for 90 s, with a final extension at 72 °C for 2 min. Then shrimp alkaline phosphatase (Promega, USA) and Exonuclease I (Epicenter, USA) were added into the PCR products for purification. The LDR reactions were performed in a final volume of 10 μL containing 1 μL 10× ligase reaction buffer (New England Biolabs, USA), 25 U Taq DNA ligase (New England Biolabs, USA), 0.4 μL 5′ ligase primer (1 μM) mixture (Sangon, China), 0.4 μL 3′ ligase primer (2 μM) mixture (Sangon, China), 2 μL purified PCR product and 6 μL ddH_2O. The LDR reactions were cycled as: 38 cycles of 94 °C for 1 min and 56 °C for 4 min, and kept at 4 °C. 0.5 μL LDR product was then sequenced with ABI3730XL sequencer (Applied Biosystems, USA). Finally the raw data was analyzed by GeneMapper 4.1 (Applied Biosystems, USA). The primers for the five target SNPs were shown in Supplementary Table 1.

APOE genotypes (rs429358 and rs7412) were determined by the restriction fragment length polymorphism (RFLP) method. The PCR reactions were performed with 1 μL DNA sample, 1× GC-I buffer (TaKaRa, Japan), 2.0 mM Mg^{2+} (TaKaRa, Japan), 0.2 mM dNTP (Generay Biotech, China), 1 U HotStarTaq polymerase (Qiagen, Germany), 2 μM multiple PCR primers (Sangon, China) and ddH_2O in a total volume of 10 μL. The cycling program was the same as above. The digestion of endonuclease was performed with AflIII or HaeII (New England Biolabs, USA) for rs429358 or rs7412 respectively. Then the products were analyzed with ABI3130XL sequencer (Applied Biosystems, USA). The PCR primers were shown in Supplementary Table 1. The genotyping was carried out in a blind way to group status. A random sample accounting for 5% (n = 23) of the total subjects was genotyped twice by different researchers for quality control, yielding a reproducibility of 100%.

2.3. Statistical analysis

The age of two groups was compared by t-test. The proportion of sex and APOE ε4 carriers between two groups were analyzed

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