RESEARCH ARTICLE

Genetic Screening for Mutations in the Chip Gene in Intracranial Aneurysm Patients of Chinese Han Nationality

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Abstract

We performed a case-control study to investigate whether SNPs of CHIP might affect the development of IA in Chinese Han nationality. We believe we are the first to have screened IA patients for mutations in the CHIP gene to determine the association with these variants. The study group comprised 224 Chinese Han nationality patients with at least one intracranial aneurysm and 238 unrelated healthy Han nationality controls. Genomic DNA was isolated from blood leukocytes. The entire coding regions of CHIP were genotyped by PCR amplification and DNA sequencing. Differences in genotype and allele frequencies between patients and controls were tested by the chi-square method. Genotype and allele frequencies of the SNP rs116166850 was demonstrated to be in Hardy-Weinberg equilibrium. No significant difference in genotype or allele frequencies between case and control groups was detected at the SNP. Our data do not support the hypothesis of a major role for the CHIP gene in IA development in the Chinese Han population.

Keywords: CHIP - genetic screening - intracranial aneurysms - subarachnoid hemorrhage

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Introduction

Intracranial aneurysm (IA) is currently one of the most devastating brain diseases, and its rupture can cause subarachnoid hemorrhage (SAH). Although the etiology of intracranial aneurysm is still unknown, but lots of research reports indicating genetic factors play an important role in IA, a number of potential candidate genes has been found associated with IA (Sun et al., 2007). Besides, genome research has prompted some susceptible loci of genes (Yasuno et al., 2010). Of all the genes studied, some might involve degradation and remodeling of the vascular wall matrix, e.g. MMP-9 (Peters et al., 1999; Pannu et al., 2006) and TIMP (Takenaka et al., 1999); and some are concerned with immune inflammatory response in vascular walls, e.g. NF-κB (Aoki et al., 2007); some associated with cell signal transduction, e.g. TGF-β signaling (Lindsay et al., 2011).

Carboxyl terminus of Hsc70-interacting protein (CHIP, also known as STUB1 or UBOX1), is a U-box-dependent E3 ubiquitin ligase and primarily expressed in human striated muscle, aortic SMCs, endothelial cells, and other tissue cells (Ballinger et al., 1999). CHIP facilitates the ubiquitination and subsequent proteasome-dependent degradation of several chaperone-bound client proteins such as p53 (Esser et al., 2005), ErbB2 (Zhou et al., 2003), cystic fibrosis transmembrane conductance regulator (Meacham et al., 2001), tau (Goryunov et al., 2007), Ask1 (Hwang et al., 2005), and ataxin-1 (Choi et al., 2007). Thus, these observations indicate that CHIP plays an important role in the regulation of cell growth, apoptosis, inflammation and neurodegeneration. Given that apoptosis and inflammation and pathological vascular remodeling are major events in the process of IA (Kataoka et al., 2010). For the first time, we investigated the role of CHIP in IA from the viewpoint of genetics. So we screened IA patients for mutations in the CHIP gene (STUB1; GenBank accession no.: NM_005861).

Materials and Methods

Study population

We studied 224 sporadic IA patients (clinical details summarized in Table 1). We recruited participants from the Department of Neurology, the first affiliated Hospital, Baotou Medical College of China, and all participants originated from the Chinese Han population in Inner Mongolia autonomous region. All patients underwent a standardized neurological examination. All patients presented with at least one aneurysm, which was confirmed by cerebral angiography. Neurofibromatosis type 1 (NF1), autosomal dominant polycystic kidney disease (ADPKD), and hereditary connective tissue diseases such as Marfan syndrome were excluded in all...
patients. They had no history of previous SAH, nor was there a familial history of such hemorrhages.

The control groups consisted of 238 subjects (details summarized in Table 1) was recruited from the first affiliated Hospital of Baotou Medical College. The control subjects were matched with the IA patients for gender, age, ethnicity and origin (origin: Inner Mongolia autonomous region, China). Control subjects met the following criteria: (a) confirmation that they did not harbor IA by digital subtraction angiography, 3-dimensional computed tomography, and magnetic resonance angiography, (b) no medical history of any stroke including IA and SAH, and (c) no family history of IA and SAH in first-degree relatives.

There is no statistical significant difference in age, sex or the prevalence of risk factors like hypertension and cigarette smoking between the patient and control groups. The study was approved by the local ethics committee, and all participants gave written informed consent.

**Methods**

Genomic DNA was extracted from peripheral blood using standard protocols. Therefore, we screened the coding regions (exons 1-7) of the CHIP gene (GenBank accession no.: NM_005861) for mutations. The above-mentioned exons were amplified by polymerase chain reaction (PCR) carried out in a thermocycler (GeneAmp® PCR System 9700, Applied Biosystems). Primer pairs were designed on the basis of the genomic sequence of the CHIP gene published in NCBI. (http://www.ncbi.nlm.nih.gov/). The PCR conditions are available upon request. The PCR products were sequenced in both directions on an ABI3730XL automated sequencer (Applied Biosystems).

**Statistical analysis**

The Hardy–Weinberg equilibrium for the polymorphism c.-112 C > G was analyzed with the online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php). Statistical analyses were conducted using the SPSS 13.0 statistical software. We performed chi-square tests to compare the allelic and genotypic distributions between patients and controls. A P < 0.05 was considered statistically significant.

**Results**

The genotype and allele counts in patients and controls, as well as x² and p-values are presented in Table 2. All genotype distributions were consistent with Hardy-Weinberg equilibrium. For the one SNP rs116166850, there was no statistically significant difference in genotype or allele frequencies between the patient and control groups.

**Discussion**

There is strong evidence suggesting that vascular remodeling coupled with inflammation is responsible for IA. Focalized vascular remodeling continues with increasing inflammation. When the inflammation is intense, major structural components of the vascular wall may be destroyed and lead to aneurysm rupture. Genetic factors, such as certain single nucleotide polymorphisms may have a role in accentuating the sustained vascular remodeling and inflammation (Peters et al., 1999; Ruigrok et al., 2005). While observational studies suggests CHIP was involved in not only vascular remodeling but also inflammation (Kumar et al., 2012). So in this population-based study, we examined whether sequence variations in the CHIP gene contribute to the development of IA.

TGF-β signaling pathways contribute to migration and proliferation of vascular smooth muscle cells involved in significant vascular remodeling in response to injury (Muratoglu et al., 2011). Hong et al. found that CHIP can modulate the sensitivity of TGF-β signaling by regulate the basal levels of multiple Smads through a proteasome degradation mechanism (Xin et al., 2005). It has been suggest that CHIP may contribute to vascular remodeling concerned to intracranial aneurysms development. The major collagenases in intracranial aneurysm tissues seem to be matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) known to be produced by inflammatory cells (Kim et al., 1997). Jang et al. found the overexpression of CHIP suppressed expression of MMP-9 (Jang KW et al., 2011). Meanwhile, CHIP strongly inhibited the nuclear localization and the transcriptional activity of NF-κB. The activation of the IkappaB kinase complex (IKK) was also blocked by CHIP overexpression (Jang et al., 2011). In
summary, these studies indicate CHIP may play a potential role in the development of intracranial aneurysms. We selected the study participants from an independent Chinese Han population and matched the controls to the patients for age, gender, ethnic background and origin. No mutation was found in the coding regions in our IA patients; only the one variations (c.-112 C > G) was identified. Further there was no association between the allelic and genotypic distribution frequencies of c.-112C > G and IA. The most likely explanation for why no mutations were found in the CHIP gene on mutation screening is as follows: there is strong evidence suggesting that multifactorial interactions (gene–gene, and gene–environment or a combination of the two actions) may contribute to the etiology of IA, different factors might contribute to different risks for developing IA, and CHIP might only confer a very slight risk for the development of IA. That is, CHIP may not be the primary cause of IA. In addition, because we did not screen potential promoter, enhancer and silencer regions and the 3’UTR of CHIP, we still cannot exclude the influence of CHIP variations on the a etiology of IA. In future investigations, the above-mentioned regions should be included in the screening. In addition, the sample size involved in our study was insufficient to provide a definitive answer as to whether the CHIP gene is associated with the development of IA, and a larger sample size should be used in future studies for replication. Finally, considering the influence of genetic heterogeneity, the contribution of CHIP to the pathogenesis of IA may be markedly influenced by the geographic and ethnic origin of the IA subjects. Therefore, it is necessary to screen mutations in CHIP in other ethnic populations.

Currently, the most cautious statement is that our data do not support a major role for the CHIP gene in the development of IA in the Chinese population.

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