Upsala J Med Sci 112 (3): 289–295, 2007

Chondroitin Sulfate Proteoglycan 2 (CSPG2) Gene Polymorphisms rs173686 and rs251124 are not Associated with Intracranial Aneurysms in Chinese Han Nationality

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Abstract

Background. There is evidence suggesting that genetic variants in the chondroitin sulfate proteoglycan2 (CSPG2, also known as versican) gene are involved in the pathogenesis of intracranial aneurysms (IAs). Some authors have demonstrated that single nucleotide polymorphisms (SNPs) rs173686 and rs251124 in the promoter region of the CSPG2 gene are associated with IAs. We performed a case-control study to investigate whether these SNPs might affect the development of IAs in Chinese Han nationality.

Methods. The study group comprised 240 Chinese Han nationality patients with at least one intracranial aneurysm and 240 healthy Han nationality controls. Genomic DNA was isolated from blood leukocytes. The SNPs rs173686 and rs251124 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.

Results. Genotype and allele frequencies of the SNPs rs173686 and rs251124 were both demonstrated to be in Hardy-Weinberg equilibrium. No significant difference in genotype or allele frequencies between case and control groups was detected at either of the two SNPs.

Conclusions. The data do not support the hypothesis that the two SNPs (rs173686 and rs251124) in the promoter region of the CSPG2 gene influence the development of intracranial aneurysms in Chinese Han nationality.

Introduction

Intracranial aneurysm (IA) is responsible for about 80% of spontaneous subarachnoid hemorrhage (SAH). The pathogenesis of IA is unclear. It is widely believed that it has a multifactorial etiology. Among all the factors, genetic variation is one that has been increasingly researched.

Many genetic variations have been found to be associated with IAs. Of all the genes studied, some might underlie inherent defects of structural molecules, e.g. ELN(1, 2) and COL1A2(3); some might involve degradation and remodeling of the vascular wall matrix, e.g. MMP-9(4, 5), TIMP and Endoglin(6); and some others might cause hemodynamic changes, e.g. ACE(7, 8). But not all researches confirmed the association between these genetics variations and IAs (9–14).

Chondroitin sulfate proteoglycan 2 (CSPG2, also known as versican) plays an important role in the assembly of extracellular matrix (ECM); and diminished

Received 17 August 2007
Accepted 21 August 2007

maintenance of the ECM has been increasingly regarded as an important factor in the development of IAs (15). Besides, CSPG2 is located at chromosome 5q14.3 and very close to a locus which has shown suggestive evidence of linkage to IAs in a Japanese cohort (1). So it is a good candidate gene for IAs. In premier literatures, some authors have reported CSPG2's role in the pathogenesis of aortic aneurysm, but no study has related it to IA, until recently Ruigrok et al reported two single nucleotide polymorphisms (SNPs) of CSPG2 gene (rs173686 and rs251124) were associated with IAs (16). But as the authors have said, "The demonstrated association of versican with IAs should be replicated by studies in other populations." So we conducted this research to find out whether they are associated with IAs in Chinese Han population.

Materials and Methods

Study Population

This study was approved by the local ethics committee, and all participants gave written informed consent. The patient group consisted of 240 unrelated, consecutively recruited patients with sporadic intracranial aneurysms (104 men, age range, 23 to 69 years; mean age, 49 years. 136 women, age range, 16 to 75 years; mean age, 42 years). All patients presented with at least one aneurysm, which was confirmed by cerebral angiography, and they were all operated on at the departments of neurosurgery of Beijing Tiantan hospital. All patients were of Chinese Han nationality. They had no history of previous subarachnoid hemorrhage, nor was there a familial history of such hemorrhages. Autosomal dominant polycystic kidney disease (ADPKD), neurofibromatosis type 1 (NF1) and hereditary connective tissue diseases such as Ehlers-Danlos syndrome type IV (EDS IV) and Marfan syndrome were excluded in all patients.

The control groups consisted of 240 subjects who presented with headache or other neurological complaints (116 men, age range, 18 to 69 years; mean age, 41 years. 124 women, age range, 27 to 72 years; mean age, 42 years). Control subjects met the following criteria: (a) confirmation that they did not harbor IA by digital subtraction angiography, 3-dimensional computed tomography, or by magnetic resonance angiography, (b) age at diagnosis≥40 years old, (c) no medical history of any stroke including IA and SAH, and (d) 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.

Sequence Analysis

The DNA sequence containing the target site was amplified by polymerase chain reaction (PCR). Primer pairs were designed on the basis of the genomic sequence of the CSPG2 gene published in NCBI. (http://www.ncbi.nlm.nih.gov/).

For rs173686, the upper primer was 5'-CCCTCAGCATCTTCTCCAAC-3'; the lower primer was 5'-GGTTAGAAGGGCAGGCAAAT-3'. The product was 236bp in length. For rs251124, the upper primer was 5'-CAGTGCAGTTGGAGAAG-CAG-3'; the lower primer was 5'-GCCAACAGTTTTCCCTGAGA-3'. The product was 211bp in length.

All polymerase chain reactions (PCR) were performed in 96-well microplates with a thermal cycler (GeneAmp® PCR System 9700, Applied Biosystems). Blank controls, using water instead of genomic DNA, were also run with each set of amplification.

The PCR mixture contained 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 400 μmol/L each of the four deoxynucleotides, 1 μmol/L each of the primers, and 1.25 U of DNA Polymerase (TaKaRa Taq®) in a final volume of 25 μL.

The PCR reaction conditions were optimized in several preliminary experiments. After an initial denaturation at 94 °C for 5 min, the target sequence was amplified by 35 PCR cycles—each consisting of denaturation at 94 °C for 30 sec, annealing for 30 sec at 61°C for rs173686 and 63°C for rs251124, and extension at 72 °C for 1 min—followed by a final extension at 72 °C for 7 min.

PCR products were electrophoresed on a 1.2% agarose gel, and bands were cut out and eluted. Then the products were directly sequenced by Applied Biosystems 3730/3730xl DNA Analyzers. No PCR product was detected from any of the negative control reactions. No unreported SNP was found among study groups.

Statistical Analyses

We performed chi-square tests to examine differences in genotype and allele frequencies between patients and controls. A P-value of <0.05 was taken as statistically significant. For both rs173686 and rs251124, deviations from Hardy-Weinberg equilibrium were evaluated by comparing observed and expected genotype frequencies by an exact goodness-of-fit test separately in cases and controls.

Results

As to clinical characteristics, 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, as shown in Table 1.

The genotype and allele counts in patients and controls, as well as $\chi 2$ and p values are presented in Table 2. All genotype distributions were consistent with

Table 1. Characteristics of Cases vs. Controls

	Aneurysms	Controls	χ^2/t value	p value
No	240	240		
male	104	116		
female	136	124	χ^2=1.208	0.272
Age at diagnosis (years)				
Mean±SD	45.14±11.66	41.82±8.96	t=0.341	0.496
Range	16-75	18-72		
Hypertension (%)	84(35.0%)	76(26.7%)		
Non-hypertension (%)	156(65.0%)	164(73.3%)	χ^2=0.600	0.439
Current or ex-smoker (%)	48(20.0%)	36(15.0%)		
Non-smoker (%)	192(80.0%)	204(85.0%)	χ^2=2.078	0.149

Table 2. Genotype and allele frequencies of SNP rs173686 and rs251124

Genotype	Aneurysms n (%)	Controls n (%)	χ^2	p value
rs173686 Genotype				
AA	8 (3.3%)	8 (3.3%)		
AG	72 (30.0%)	60 (25.0%)		
GG	160 (66.7%)	172 (71.7%)	1.525	0.467
rs173686 Allele				
G	392 (81.7%)	404 (84.2%)		
A	88 (18.3%)	76 (15.8%)	1.059	0.303
rs251124 Genotype				
CC	124(51.7%)	132(55.0%)		
CT	104(43.3%)	96(40.0%)		
TT	12(5.0%)	12(5.0%)	0.570	0.752
rs251124 Allele				
C	352 (73.3%)	360 (75%)		
T	128(26.7%)	120 (25%)	0.348	0.555

Hardy-Weinberg equilibrium. For either of the two SNPs rs173686 and rs251124, there was no statistically significant difference in genotype or allele frequencies between the patient and control groups.

Discussion

In our research, we studied whether the two SNPs rs173686 and rs251124 of CSPG2 gene were susceptible factors of IAs, but the data didn't show there was any association between them.

Our research relied on direct DNA sequencing technique, which guaranteed the validity of the data. Every patient underwent DSA and craniotomy for clipping of IAs, which could make sure of the diagnosis.

Our study on CSPG2 might contribute to the comprehension of its role in the pathogenesis of cerebral vascular diseases. The proteoglycan CSPG2 is one of the several extracellular matrix (ECM) molecules. It is mainly produced by smooth muscle cells (SMCs). The synthesis of CSPG2 is highly regulated by specific growth factors and cytokines. However, genetic variation of CSPG2 gene may also have impact on the level of expression. CSPG2 could bind growth factors, enzymes, lipoproteins, and a variety of other ECM components to influence the assembly and remodeling of ECM. Its coding gene is localized on chromosome 5q14.3 and very close to a previously implicated locus for IAs noted in a Japanese cohort (1).

Many authors studied the change of CSPG2 in aortic aneurysms, and proved the significant role of CSPG2 in the maintenance of vascular wall. The levels of mRNA and the concentration of CSPG2 in abdominal aortic aneurysm (AAA) in vivo was found to be significantly decreased.(17) However, CSPG2 in SMC monolayer cultures from tissue affected by AAA was found to be obviously elevated (18). These results provided evidence that the decline in density of SMCs led to decreased production of CSPG2, which in turn caused weakness of the vascular wall. In other researches, the fine molecular structure and organization of CSPG2 in human AAA was studied and found to be extensively fragmented (19).

Guo et al conducted a genome-wide search for the location of the defective gene for thoracic aortic aneurysms and dissections (TAA/dissections) and found a major locus for familial TAAs and dissections maps to 5q13-14 (20), where the CSPG2 gene was located. Ruigrok showed two SNPs of CSPG2 gene (rs173686 and rs251124) were associated with IAs (16). These researches revealed the possible role of CSPG2 genetic variations in pathogenesis of IAs. But our data didn't support the association between these two SNPs and IAs. So it was questionable whether these two SNPs definitely impacted on the transcription of CSPG2 gene and the expression of protein. More researches should be done to ascertain the intrinsic correlation.

One of the underlying causes of the discrepancy between our result and research by Ruigrok might be ethnic-related differences of allele frequency between the two populations. Besides, our study group was relatively small. As a general rule in statistics, the smaller the sample size was, the more the sampling error would be. The conclusion would seem to be more convincing if the study group could be larger.

The disagreement also indicated that some SNPs might be minor effective on the predisposition of IAs. While they were singled out for investigation, the results might be negative. Nevertheless, if they were combined with other SNPs, the re-

sults could be positive due to synergic effects. Future researcher should pay more attention to the association between haplotype and pathogenesis.

To sum up, these conflicts indicate that the genetic nature of IAs is much more complicated than we had ever thought. The possible impact of these two SNPs (rs173686 and rs251124) in the promoter region of the CSPG2 gene on susceptibility to intracranial aneurysms is still controversial, with our data conflicting from former reported studies. More researches are needed in the future. In those population showing associations between these SNPs and IAs, more attention should be paid to the correlations between genetic variation and protein expression.

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