SNPs and Mutations in C1GALT1C1 Gene and the Susceptibility of Henoch–Schönlein Purpura in a Chinese Population

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Abstract

Background: Our group has found significant association between an SNP in C1GALT1 gene and the risk of Henoch–Schönlein purpura (HSP). Cosmc, encoded by C1GALT1C1 gene, is the chaperone of C1GALT1. We designed this study to investigate the association between the SNPs and mutations in C1GALT1C1 gene and the genetic susceptibility to HSP.

Methods: A total of unrelated 542 Northern Chinese, including 268 patients with HSP and 274 healthy controls were enrolled in the study. Three SNPs (rs17261572; rs5957424 and rs3810744) in C1GALT1C1 gene were analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) for further case-control association analysis. Somatic mutations of DNAs from peripheral blood B lymphocytes were detected in 10 patients and 10 normal controls.

Results: Significant association was observed between different genotypes of rs17261572 A>T and HSP. People with TT genotype have lower risk of HSP compared with people with AA genotype (OR=0.46; 95% CI: 0.24-0.90, P=0.03). There was no somatic mutation detected in total 188 clones of 20 individuals.

Conclusion: The C1GALT1C1 polymorphism was significantly related to the genetic susceptibility to HSP in Northern Chinese population. Further studies are warranted to validate our findings, and to investigate into its underlining mechanism.

Keywords: HSP; Susceptibility; C1GALT1C1; Case-control study; SNP

Introduction

Henoch–Schönlein purpura (HSP) is the most common systemic vasculitis of childhood [1]. The incidence of HSP is reported as ranging from 10 to 20 cases per 100,000 children in different populations [2]. It is characterized by a systemic leukocytoclastic angiitis, mainly affecting the small blood vessels of the skin, joints, gastrointestinal tract and kidneys. Other organs are occasionally involved, such as the brain, lungs and scrotum [3]. HSP is classically considered a mild, self-limiting vasculitis, but there are various rare and sometimes life-threatening complications.

The etiology of HSP remains unknown, although many antigens, such as infective agents, vaccinations, drugs, insect bites and foods have been found to trigger the disease [3,4]. Genetic factors, such as gene variation are maintained to play important roles in the development of HSP. C1GALT1 is a gene coding the enzyme core 1 β1,3-galactosyltransferase, which activates the attachment of Gal to GaINAc by associating with its chaperone, Cosmc [5,6], encoded by C1GALT1C1 gene.

Thus, the reduced activity of C1Galt1 and Cosmc could lead to aberrant glycosylation and affect the development of HSP. Our previous study has found the association between a tagging SNP, SNP7 (-292 C/-, rs5882115) in C1GALT1 and HSP risk in a Chinese population [7]. However, whether C1GALT1C1 gene affects the susceptibility of HSP is worthy of study. In the present study, we investigate the polymorphisms and mutations in this gene to the susceptibility of HSP in a Chinese population.

Materials and Methods

Subjects

A total of 542 unrelated Northern Chinese were enrolled in this study, including 268 patients with HSP and 274 sex and age matched healthy controls. The HSP patients were new cases that met the diagnostic criteria for HSP [8]. The controls were selected from the participants of physical examination in our hospital.

The protocol for this genetic study was approved by the medical ethics committee of Mudanjiang Medical College, and informed written consent for the genetic studies was obtained from all participants. Twenty individuals (10 patients who were recently diagnosed as HSP and 10 controls) were selected for somatic mutation detection.
SNPs selection and genotyping

The C1GALT1C1 gene was located in the chromosome X. SNPs in C1GALT1C1 were obtained from National Center for Biotechnology Information (NCBI) SNP database http://www.ncbi.nlm.nih.gov/entrez. Three SNPs were found to have frequency data with variant homogeneous genotype more than 1%, including rs3810744 (G>A) in the promoter region, rs5957424 (T>A) in the intron region, and rs17261572 (A>T) in the exon.

The three SNPs were genotyped for further association analysis in all 542 subjects by the standard PCR-RFLP procedures. Genomic DNA was extracted from whole blood using the phenol-chloroform method. For SNP rs3810744, the genomic DNA samples were amplified by PCR using the following primers, forward: 5’-ACGCGAGGTTACATCAGAAG-3’, reverse: 5’-TGACCGAGCTTCTAGCTG-3’. The products of 420 base pairs (bps) were digested by restriction endonuclease Hpy8I (Fermentas International Inc., Hanover, USA). For SNP rs5957424, the genomic DNA samples were amplified by PCR using the following primers, forward: 5’-GAATTTTCTGTTTCATTTGT-3’, reverse: 5’-AAGAGCTTTCGAGTATACTG-3’. The products of 223 bps were digested by restriction endonuclease Bse8I (Fermentas International Inc., Hanover, USA).

B lymphocyte DNA extraction and PCR amplification

Peripheral blood B lymphocytes from 20 participants (10 patients and 10 controls) were isolated by using lymphocyte isolation sterile solution (Amersham Biosciences, Uppsala, Sweden) and CD19 magnetic beads (Dynal Biotech ASA, Oslo, Norway), and then DNA of B lymphocytes was extracted by salting out procedure [9]. The whole coding region was amplified by PCR with following primers, forward: 5’-GGTTTCTGGATCTCTAGTT-3’, reverse: 5’-AAGAGGGTTCGAGTATACTG-3’. The products of 223 bps were digested by restriction endonuclease Bse8I (Fermentas International Inc., Hanover, USA).

Gene cloning and somatic mutation detection

PCR products from B lymphocyte DNA of 20 individuals were subcloned into PGEM-T vector (Promega Corporation, Madison, WI, USA) after purification and adding adenine to them. Then ligation productions were transformed to Ecoli Top 10 competent cells and cultured in Luria-Bertani (LB) solid medium for 14 hours at 37°C. More than 8 clones per individual were randomly selected and amplified in LB liquid medium for 14 hours at 37°C. Plasmids were extracted and digested with PST1 restriction enzyme (Promega Corporation, Madison, WI, USA) to verify the insertion of PCR productions [10]. Total 188 clones, including 8 to 10 clones per individual, were directly sequenced to detect somatic mutation.

Statistical Analysis

Genotype and allele frequencies were determined with the gene counting method. Significant deviation from the Hardy–Weinberg equilibrium was tested using Chi-squared test. The distributions of genotype frequencies were calculated and compared with Chi-squared test and Logistic regression using the SAS system. Odds ratios (OR) are presented with 95% confidence intervals (CI), a P value of less than 0.05 was considered to be statistically significant.

Results

This case-control study included 268 HSP cases and 274 controls. The average age at diagnosis of patients was 11.4 years (ranging from 3 to 17 years), and the gender ratio (male to female) was 1.33:1. Clinical characteristics of the patients were shown in Table 1.

Table 1: Clinical characteristic of patients with HSP.

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>n</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Cutaneous palpable purpuric rash</td>
<td>268</td>
<td>100</td>
</tr>
<tr>
<td>Internal organ involvement</td>
<td>143</td>
<td>53.4</td>
</tr>
<tr>
<td>Previous URI</td>
<td>116</td>
<td>43.3</td>
</tr>
<tr>
<td>Arthritis and/or arthralgia</td>
<td>84</td>
<td>31.3</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>59</td>
<td>22.0</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>33</td>
<td>12.3</td>
</tr>
<tr>
<td>Scrotum edema</td>
<td>9</td>
<td>3.4</td>
</tr>
<tr>
<td>HSP: Henoch-Schonlein purpura, URI: upper respiratory tract infection, Renal involvement is included in internal organ involvement.</td>
<td></td>
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</tbody>
</table>

The distributions of the C1GALT1C1 genotypes (rs17261572, rs5957424, and rs3810744) are shown in Table 2. The SNPs were all in Hardy-Weinberg equilibrium in controls (P=0.11, 0.32, and 0.59, respectively).

Table 2: Logistic regression analysis of associations between C1GALT1C1 polymorphisms and the risk of HSP.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Cases (n=200)</th>
<th>Controls (n=200)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3810744</td>
<td>64 (23.88)</td>
<td>61 (22.6)</td>
<td>1.08 (0.71, 1.66)</td>
</tr>
<tr>
<td>rs17261572</td>
<td>79 (29.48)</td>
<td>84 (30.66)</td>
<td>0.97 (0.66, 1.44)</td>
</tr>
</tbody>
</table>

aTwo-sided χ² test for difference in frequency distribution of genotypes between cases and controls.

bAdjusted for age and sex.

Discussion

HSP was considered as a polygenic and multifactorial disorder. There were extensive evidences suggested the genetic components were involved in the susceptibility and progression of HSP. The pathogenesis of HSP was still unclear, as far as we knew. Recently, more and more evidences suggested that deficient β1,3 galactosylation of hinge region of IgA1 molecule played an important role in the pathogenesis of HSP [11,12]. The galactosylation of GalNAcα1-R in hinge region of IgA1 molecule depended on the activity of C1GALT1 gene and C1GALT1C1 gene [13]. Furthermore, diseases resulted from deficiency of β1,3 galactose, such as Tn syndrome, weren’t a result of decreased expression of C1GALT1 gene, but resulted from the mutations of C1GALT1C1 gene [6]. These results suggested that it was rather the variants of C1GALT1C1 gene in influencing the galactosylation of IgA1 hinge-region than the variants of C1GALT1 gene. It implies that the variants of C1GALT1C1 gene could contribute to susceptibility of HSP by influencing β1,3 galactosylation of IgA1 molecule. One previous study revealed that there was only one SNP, c.393T>A (rs17261572), in coding region of C1GALT1C1 [14]. It was a nonsynonymous SNP. The MAF of c.393T>A was only 0.069. Therefore, association study to investigate whether the mutations (including somatic mutation) in the promoter region of C1GALT1C1 important in the pathogenesis of HSP in China.

We firstly screened the polymorphisms of C1GALT1C1 gene. Three SNPs with high minor allele frequency (MAF) were identified, including rs5957424, rs3810744, and rs17261572. Therefore, association between these SNPs and HSP risk was explored in a case-control association study in a large population sampled from the Northern Chinese. The association analysis revealed that there was significant difference of the alleles (rs17261572) between the controls and the HSP patients. These results suggested that polymorphisms of C1GALT1C1 gene might be related to the susceptibility of HSP.

In present study, we further analyzed the association between the SNP of C1GALT1C1 gene and clinical parameters of HSP. The results revealed that there was no significant difference of blood pressure, proteinuria, and renal function among the HSP patients with different genotypes. These data suggested that the genotypes of the C1GALT1C1 gene did not influence the clinical manifestations of HSP.

In previous studies of Tn syndrome, three somatic mutations of C1GALT1C1 gene were identified in two patients. The three somatic mutations, c.202C>T, c.393T>A and c.454G>A, were all in the coding region of C1GALT1C1 [8]. Except the c.393T>A mutation, both of the other two somatic mutations could impressively inhibit chaperone activity and lead to inactivation of C1β3Gal-T, and the expression of autoantibody Tn antigen on blood cells of all lineages [6]. Galactosylation deficiency was already proved in patients with HSP. Therefore, we tested whether somatic mutations exist in C1GALT1C1 gene in patient with HSP.

We performed a somatic mutation screening in the patients with HSP. DNAs from B lymphocytes where IgA molecule was produced were isolated from 20 individuals. And then the coding region of
C1GALT1C1 gene was amplified, cloned and sequenced. Except the c.393T>A, no other mutations were detected. The mutation, c.393T>A, was only found in the patients whose mutations were demonstrated in genomic DNA by routinely sequencing. Furthermore, c.393T>A was proved not to be a somatic mutation in these HSP patients. The result indicated that the variation of coding region of C1GALT1C1 gene might be of little importance in the processing of aberrant glycosylation of IgA1 molecule in patients with HSP. Mutations in other regions of C1GALT1C1 gene, which may influence the glycosylation process of IgA1 molecule, were needed to be clarified in patients with HSP. In fact, in a recent study, Malycha et al. detected mutations in whole blood DNA and in B cell DNA separately in a relative small European sample. They didn't found any important mutations of C1GALT1C1 gene in patients with IgAN. These results suggested that the C1GALT1C1 gene might influence the susceptibility to HSP by pathways other than mutation.

Our results suggested that the SNP rs17261572 was significantly associated with the risk of HSP, while the mutation (including somatic mutation) of C1GALT1C1 gene did not significantly contribute to the genetic susceptibility or clinical manifestations of HSP in Chinese population. Further studies are warranted to investigate into the mechanism of the association, which may shed light on the etiology of HSP.

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References
