The CD18 AvaII polymorphic site not associated with Henoch-Schönlein purpura

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This study was funded by the supporting program of the Ministry of Human Resource of China Overseas Returned scholars.

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Received on February 21, 2010; accepted in revised form on June 30, 2010.

Clin Exp Rheumatol 2011; 29 (Suppl. 64): S117-S120.

Key words: CD18, Henoch-Schönlein purpura, association, AvaII polymorphism

Competing interests: none declared.

ABSTRACT

Henoch-Schönlein purpura (HSP) is an immune complex-mediated systemic vasculitis. Its genetic etiology remains unknown. CD18, the subunit of integrin beta2 in leukocytes, has essential roles in the expression and function of ITGB2, mediating immune responses. CD18 has been proved to be associated with some systemic vasculitides, such as microscopic polyangiitis and Churg-Strauss syndrome. We aimed to assess the influence of CD18 AvaII polymorphism (rs235326, C->T) in the incidence of HSP and determine its possible implication in severe systemic complications by studying 73 patients with HSP and 156 controls in China. Our results showed that AvaII polymorphism was not associated with HSP susceptibility (odd ratio (OR)=0.48, 95% confidence interval (CI)=0.53–1.39, p=0.63) or with HSP nephritic syndrome (OR=0.88, 95%CI=0.35–2.06, p=0.90). Moreover, we did not observe any significant association between serum parameters, such as CRP, IgA, IgE, C3 and C4, and HSP severity. In conclusion, our results suggested that CD18 AvaII is not associated with HSP susceptibility and its clinical outcomes.

Introduction

Henoch-Schönlein purpura (HSP) is the most common primary small-vessel vaculitis in children. Compared with other primary systemic vasculitides, the incidence of HSP in adults is very low (1). Non-thrombocytopenic palpable purpura, arthritis, internal organ involvement, such as the gastrointestinal tract (GI) and the kidney, are typical manifestations. Renal involvement results in the most serious complication. The disease is generally self-limiting and most cases can fully recover in weeks to months. The pathogenesis of HSP is still unknown. The pathological and laboratory findings of the vascular deposition of immunoglobulin A (IgA)-dominant immune complexes, polymorphonuclear neutrophils (PMNs) infiltration around the vessel, elevated serum IgA levels and proinflammatory cytokines, suggest the possibility of immune-mediated mechanism. It has been speculated that the deposition of immune complexes is critical to the initiation of HSP by activating the complement system, leukocytes, and endothelial cells. Endothelial cell-activation results in the expression of adhesion molecules, inducing adhesion and transmigration of inflammatory cells through endothelial cells (2). Hence, adhesion molecules may play a crucial role in the etiology and pathogenesis of HSP. Recent publications on genetic factors predisposing to HSP have reported many of these genetic polymorphisms related to cytokines and cell adhesion molecules are involved in the pathway of immune and inflammatory responses, as well as endothelial cell activation (3).

Beta2-integrin (ITGB2) plays an important role in the recognition of inter-cellular and cellular-matrix communication as well as regulation of leukocyte migration and adhesion. The ITGB2 heterodimer molecule is composed of two subunits: CD18 and one of four CD11 subunits- CD11a, CD11b, CD11c and CD11d (4). The human *CD18* gene is located on chromosome 21q22.3 and encodes a 95-kDa glycoprotein (5). The human *CD11* genes are located on chromosome 16p11.2 and encode glycoproteins of 180, 160, 150 and 145 kDa, respectively. CD18 has essential roles in the expression and function of ITGB2 in leukocytes, mediating immune responses, including leukocyte recruitment to inflammatory sites or secondary lymphoid organs, pathogen recog-

nition, phagocytosis, and antibody or cell-mediated cytotoxicity (6).

The genetic defects in *CD18* result in leukocyte adhesion deficiency (LAD), a rare autosomal recessive disorder characterised by immunodeficiency, as defects in CD18 impaired cell surface expression of all four ITGB2 heterodimers (6, 7). The codon 441 polymorphism (*C->T*) in exon 11 of *CD18,* which induces an *Ava*II enzyme cutting site, has also been validated to confer increased risk for some systemic vasculitides, such as microscopic polyangiitis (MP) and Churg Strauss syndrome (CSS) (8-10). However, to date, whether the AvaII polymorphic site is associated with HSP has not been reported. The aim of this study was to investigate the association of *Ava*II polymorphism in *CD18* (rs235326) with the susceptibility of HSP and its severity.

Materials and methods

A total of 73 children (44 males and 29 females) (7.3±2.7 years) with HSP in Wuhan Children Hospital (WCH) were included. All patients were unrelated and fulfilled the diagnostic criteria proposed by the American College of Rheumatology 1990 criteria for the diagnosis of HSP (11). One hundred and fifty-six age-, ethnicity- and gender-matched controls were recruited from children in the same geographic area who were scheduled for routine elective surgeries for conditions such as ectropion and entropion of eyelid, phimosis and syndactyly. Informed consent from participants and the WCH Institutional Review Board approval were obtained. Genomic DNA of subjects was extracted from peripheral blood using the phenol-chloroform method.

The definitions of clinical features followed the criteria defined by the 1990 American College of Rheumatology (ACR) (11). A clinical scoring system was used to evaluate disease severity involving the GI tract, joint, and the kidney, which is as follows: (0=absent, 1=mild, 2=moderate, 3=severe), (Table I) (12). The HSP patients were divided into two groups based on clinical presentation: high clinical score (HCS) group if clinical score ≥4 and low clinical score (LCS) group if clinical score <4. GI bleeding was defined by the presence of melena, hematochezia or a positive stool test for occult blood. Haematuria, proteinuria and nephritic syndrome were defined as has previously been reported (13). Other clinical parameters, white blood cell count (WBC), serum IgA, C-reactive protein (CRP), complement protein C3 and C4, and stool blood analysis were determined in each patient in a clinical laboratory.

Table I. Clinical scoring to evaluate the severity degree of organ involvement during the acute phase of HSP.

Organ involvement	Degree of severity
Joint	0 = no symptoms 1 = pain and/or swelling of slight grade 2 = pain and/or swelling of moderate grade 3 = pain and/or swelling of severe grade
GI tract	0 = no symptoms 1 = slight pain and/or occult blood in the stool (+) 2 = moderate pain and/or occult blood in the stool (+2, +3) 3 = severe pain and/or maelena
Kidney	0 = no proteinuria and no haematuria 1 = proteinuria (+) and/or haematuria (+) 2 = proteinuria (2+/3+) and/or haematuria (2+/3+) 3 = proteinuria (>3+) and/or haematuria (>3+)

Table II. The demographic and clinical characteristics of children with HSP.

Features	HSP patients n (%)	HCS patients n (%)	LCS patients n (%)
No.	73	19	54
Gender (male/female)	44/29	16/3	28/26
Age (years)	7.3 ± 2.7	7.3 ± 2.4	7.3 ± 2.7
Pupura	73 (100)	19 (100)	54 (100)
Arthralagias and/or arthritis	26 (35.6)	14 (73.7)	12 (22.2)
Gastrointestinal manifestations (bowel angina and/or gastrointestinal bleeding)	38 (52.1)	18 (94.5)	20 (37.0)
Renal manifestations			
Haematuria	15 (20.5)	9 (47.4)	4 (7.4)
Proteinuria	25 (34.2)	16 (84.2)	8 (14.8)
Nephritic syndrome	20 (27.4)	14 (73.7)	6 (11.1)
WBC (range)	4.47-23.28	5.10-18.09	4.47-23.28
Abnormal high CRP	30 (41.1)	6 (31.6)	24 (44.4)
Abnormal high IgA	35 (47.9)	8 (42.1)	27 (50.0)
Abnormal high C3	0 (0)	0 (0)	0 (0)
Abnormal high C4	4 (5.5)	1 (5.3)	3 (5.6)
Abnormal high IgE	19 (26.0)	4 (21.1)	15 (27.8)

Genotyping analysis

The genotyping of AvaII polymorphism was determined by PCR amplification followed by restriction enzyme digestion (10). In detail, genomic DNA (50-100ng) was amplified in a total volume of 10μl containing 1.5mmol $MgCl_2$ and 0.5U Taq polymerase using primers specific for *CD18*. The thermocycling program of the PCR was as follows: Initiated denaturation was performed at 95°C for 5min, followed by 30 cycles of 95°C for 30sec, 56°C for 30sec, 72°C for 30sec, and a final extension at 72°C for 10 min. An aliquot of the PCR product was digested with AvaII at 37°C to distinguish the wild-type allele from the mutant allele. The digested PCR products were analysed on a 2.5% agrose gel and inspected under UV light.

Statistical analysis

The results were analysed using SPSS Window 13. Chi-square was used to

Table III. Distributions of CD18 *Ava*II genotypic and allelic frequencies in patients with HSP and healthy controls.

Genotype or Allele	HSP patients (n=73)	Controls (n=156)	*p*	HSP (n=53)	HSPN (n=20)	*p*	Patients with HCS (n=19)	Patients with LCS (n=54)	*p*
CC	47	90		35	12		12	35	
TC	22	60		15	7		7	15	
TT	4	6		3	1		0	4	
T Allele	20.5%	23.1%	0.527	19.8%	22.5%	0.724	18.4%	21.3%	0.696

test the difference of genotype and allele frequencies. Odds ratio (OR) and 95% confidence interval (CI) were calculated if significant association was detected. WBC count, serum IgA, IgE, C3, C4, CRP and clinical scores are present as means and standard deviations (SD). Sex and age were included as co-variants in analysing the association of genotypes and parameters. A *p*-value of less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of HSP patients and controls

In this study, a total of 73 HSP patients (44 males and 29 females) and 156 controls (84 males and 72 females) were included. There was no significant difference in the mean age and gender ratio between patients and controls. The main epidemiological and clinical data of these patients are shown in Table II. The ratio of male to female in our patient group (1.5:1) was slightly higher than that reported in Taiwan (1.2) (14). All patients presented with a palpable purpura while more than 1/3 of patients had joint complications. Approximately half of the patients had GI symptoms, and 27.4% had nephritic syndrome. At the time of diagnosis, there were high CRP recorded in 41.1%, high IgA in 47.9%, high IgE in 26.0%, and high C4 in 5.5% patients. Serum C3 level was found to be within the normal range for all patients.

Among the 73 HSP patients, 19 patients (26%) had HCS while 54 (74%) had LCS. The mean age of the two groups was quite similar. As expected, the organ involvement was more common in patients with HCS than those with LCS. Joint symptoms, GI manifestations, and nephritic syndrome were found in 73.7%, 94.5% and 73.7%, respectively in patients with HCS and 22.2%, 37.0% and 11.1%, respectively in the LCS group. However, the laboratory parameters, such as CRP, IgA, IgE, C3 and C4, were not much different between HCS and LCS group, suggesting that these parameters may not be of value as biomarkers for the severity of HSP.

CD18 AvaII polymorphism not associated with HSP and its disease severity

The genotype frequency of the CD18 AvaII was in Hardy-Weinberg equilibrium. The allele frequencies were compared between HSP and controls, HSP and HSPN, and HCS and LCS, and no significant difference was found (shown in Table III).

Discussion

The study of HSP has proved challenging, as there is no unified system or animal model applicable to research. Given the absence of a "gold standard" laboratory test, the clinical features are still the major tools to make a HSP diagnosis. Recently, Kluger *et al*. suggested that histology is mandatory to confirm the diagnosis of vasculitis to avoid a delayed and inappropriate diagnosis (15). The pathogenesis of HSP remains unclear. It is known that mutations or polymorphisms in monogenetic autoinflammatory diseases may be susceptibility markers for complicated diseases. For example, mutations or polymorphisms in *MEFV* gene, whose mutations cause Familial Mediterranean Fever, have been reported to increase the risk of HSP and also influence its clinical presentation (16). Given the critical role of *ITGB2* in leukocyte migration and adhesion and the fact that the genetic defect in its subunit, CD18, causes leukocyte adhesion deficiency, we hypothesise that the genetic variants in *CD18* may exert a proinflammatory effect and contribute to the development of HSP.

Gencik *et al*. reported that the minor *T* allele was significantly lower in anti-myeloperoxydase (MPO)-anti-neutrophil cytoplasmic antibodies (ANCA$^+$) patients (21%), mainly referring to MP and CSS patients, compared with the control group (40.4%, $p<0.005$) and PR3-ANCA$^+$ patients (63.3%, $p<0.01$), mainly including patients with Wegener granulomatosis (WG) (11). It is known that, like HSP, MP, CSS, and WG are systemic small-vessel vasculitides. Interesting, the *T* allele in our HSP patients was similar to that of MPO-ANCA$^+$ patients in Gencik's study (20.5% *vs*. 21%). MP, CSS, and WG are systematic vaculities strongly associated with ANCA, whereas ANCA is not characteristic for HSP. In the present study, the allele frequency of AvaII in *CD18* gene in HSP patients is similar to that in normal controls (20.5% *vs*. 23.1%). There is also no significant difference in this allele frequency between HSP patients and HSPN patients (19.8% *vs*. 22.5%). Hence, common genetic variant, AvaII, in *CD 18* gene may be a genetic risk factor for MPO-ANCA$^+$ diseases, such as MP and CSS, in European population but not with HSP, at least in the Chinese population. In our study, the *T* allele frequency is much lower than that in the German population in Gencik's study (23.1% *vs*. 40.4%), suggesting a heterogeneous baseline in all frequencies in these two ethnic groups. Therefore, the association of AvaII polymorphism in CD18 with systematic small-vessel vaculities may be ethnic-dependent.

In addition, we also assessed the association of abnormal serum levels of IgA, IgE, C3, C4, and CRP with the severity of HSP, which was defined according to a clinical score. The percentage of patients with abnormal parameters mentioned above was not different between the HSC and LCS group, suggesting

these markers may not be associated with the severity of HSP, and also may not be valuable in prediction of severe clinical manifestations.

Our results showed that the AvaII polymorphic site does not constitute a risk factor for HSP. Notably, the sample size in our study is not large enough, especially for the detection of the minor difference in the frequency in allele. In our study, the frequency of *C* allele of AvaII is also lower in HSP patients compared to controls, which is in same change direction as that of Gencik's study. It may not completely exclude the possibility that AvaII is protective allele for HSP but the number of subjects studied is not enough to allow us to achieve a statistical significance. Therefore, before we draw a solid conclusion, further study with a larger sample size should be done to investigate whether AvaII polymorphism may predispose to HSP or influence its clinical presentation.

Acknowledgements

We thank Dr. Samuel Dzodzomenyo from the Children's Medical Center of Dayton, School of Medicine, Wright State University, for critically reading and editing the English of this manuscript.

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