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Association of *Interleukin-2* Gene Polymorphism with Henoch-schönlein Purpura Nephritis

Jiajia Cao, Junfeng Zhang, Hui Xu, Wei Li, Jianrong Shi, and Qing Ye

Department of clinical laboratory, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, National Children's Regional Medical Center, Hangzhou, China

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ABSTRACT

Henoch-Schönlein purpura nephritis (HSPN) is a common vasculitis that mostly affects children, and previous studies have indicated that genetic factors may influence disease susceptibility. The aim of this study was to evaluate a possible association of three *interleukin-2 (IL-2)* gene polymorphisms (rs3136534, rs2069776, and rs2069762) with HSPN in the Chinese population.

A total of 81 patients with HSPN and 200 healthy children were enrolled. The distribution of genotypes, allelic frequencies, and haplotype frequencies among the three *IL-2* polymorphisms were analyzed using the Sequenom MassARRAY system by means of matrix-assisted laser desorption ionization-time of flight mass spectrometry method.

Compared to the healthy controls, genotyping analysis demonstrated rs3136534 was associated with a decreased HSPN risk in the dominant inheritance model (G/T+T/T vs. G/G; OR, 0.54; 95% CI, 0.31–0.93). However, the frequency of the T allele and haplotypes of rs3136534 showed no statistical significance. For the frequency of genotype, allele, and haplotype of the rs2069776 and rs20697622 polymorphisms, no significant differences were observed between HSPN patients and controls.

Our results suggest that the rs3136534 polymorphism of the *IL-2* gene is associated with susceptibility to HSPN in Chinese children.

Keywords: Henoch-Schönlein purpura nephritis; Interleukin-2; Polymorphism

INTRODUCTION

Henoch-Schönlein purpura (HSP), also referred to as immunoglobulin (Ig) A vasculitis, occurs predominantly in childhood and is an IgA-mediated systemic small-vessel vasculitis with main manifestations of skin purpura, arthritis, gastrointestinal bleeding, and nephritis.[1] Generally, the prognosis of HSP is favorable, but kidney involvement is a severe complication. Approximately 50% of patients develop nephritis within 3 months of disease onset.[2] Kidney involvement in HSP is called HSP nephritis (HSPN). Most patients with HSPN presenting with only hematuria and/or low-grade proteinuria rarely progress to chronic kidney injury and kidney failure. However, a small percentage of patients

Corresponding Author: Qing Ye, MD;
Department of clinical laboratory, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, National Children's Regional Medical Center, Hangzhou, China. Tel: (+86 0571) 8667 0023, Fax: (+86 0571) 8667 0023, E-mail: qingye@zju.edu.cn

will develop nephritic syndrome or kidney function damage.[3]

Several studies suggest that cytokine-mediated immune and inflammatory responses play a vital role in the development of HSP and HSPN.[4,5] It is well known that IL-2 secreted by helper T cells type 1 (T_H1) is involved in the antibody-mediated immune response and promotes the activation, growth, and differentiation of various immune cells, such as B lymphocytes, T cell subsets, and natural killer cells.[6] As a multipotent cytokine, IL-2 has had a significant impact on immunology research.

To date, several single nucleotide variations (SNVs) of immune response genes have been shown to be used as susceptibility biomarkers of autoimmune diseases and inflammatory conditions.[7] Until recently, most genetic studies supported the claim that genetics is crucial in the pathogenesis of HSP.[8] Previous studies indicated that polymorphic variants in *IL-2* genes have an association with multiple autoimmune diseases, such as type 1 diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis.[9,10,11] However, association studies between the *IL-2* gene and HSPN have not yet been illuminated. This study mainly aimed to investigate the role of *IL-2* genetic polymorphisms (rs3136534, rs2069776, and rs2069762) in susceptibility to HSPN in Chinese children.

MATERIALS AND METHODS

Patients and Controls

Our study subjects consisted of 200 healthy controls and 81 patients newly diagnosed with purpura nephritis at Zhejiang University Children's Hospital. All patients in our study had at least a 6-month follow-up, and they fulfilled the diagnostic criteria for HSPN. The diagnostic criteria for HSPN were based on the International Study of Kidney Diseases in Children.[12] Nephritis was defined as the presence of hematuria and/or proteinuria. The definition of hematuria was more than 5 red blood cells in urine sediment at high power. Proteinuria was defined as more than 150 mg of protein in 24-hour urine collection. Patients with other systemic diseases or autoimmune disorders, such as IgA nephropathy, systemic lupus erythematosus, and anti-neutrophil cytoplasmic autoantibody-associated nephritis, were excluded.

A total of 200 healthy children (92 females and 108 males), with a mean age (±standard deviation) of 7.2±2.1 years, were randomly selected from the physical examination center in our hospital. All controls had no systemic inflammation, autoimmune disorders, or other diseases. All subjects in the present study were Han Chinese, and there were no genetic associations between participants.

The study was approved by the Ethical Committee of Children's Hospital of Zhejiang University. Informed consent was obtained from each participant in accordance with the Helsinki Declaration.

Genomic DNA Extraction and Genotyping

Genomic DNA was extracted from leukocytes that had been isolated from EDTA-anticoagulated blood samples using a DNA extraction kit (Tissuebank Biotechnology Co, Ltd, Shanghai, China) according to a standard protocol. The DNA extraction was kept at –20°C until genotyping and then at –80°C for long-term storage. SNVs genotyping was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA).

The primers for *IL-2* SNVs rs2069776, rs2069762 and rs31365344 are presented in supplementary Table 1. Genotype calling was performed in real time by MassARRAY RT software version 3.0.0.4 and analyzed using MassARRAY Typer software version 3.4 (Sequenom).

Statistical Analysis

The allelic, genotype, and haplotype frequencies of the *IL-2* gene between HSPN and controls were calculated by a chi-square ($\chi 2$) test. We analyzed genotype frequencies under codominant, dominant, recessive, and overdominant models. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. The $\chi 2$ test was also used to analyze Hardy–Weinberg equilibrium. Statistical analyses were performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA), and a two-tailed $p<0.05$ was considered statistically significant. Haplotype block and linkage disequilibrium were calculated using Haploview 4.2 software.[13]

RESULTS

The demographics and clinical characteristics of HSPN patients and controls, such as sex, age at diagnosis, serum levels of creatinine, white blood cell counts, and C-reactive protein, are shown in Table 1.

The mean age at disease onset of HSPN patients was 7.7±2.4 years. The children in the healthy controls had a mean age of 7.2±2.1 years (p=0.06), and there were no significant differences in sex (p=0.961) between the case and control groups. However, white blood cell counts and C-reactive protein were significantly higher in HSPN. The three *IL-2* polymorphisms and allele frequencies (rs3136534, rs2069776, and rs2069762) in the HSPN group and control group are shown in Tables 2 and 3. The distribution of these 3 polymorphisms obeyed the Hardy-Weinberg equilibrium in both HSPN patients and controls (p>0.05). The results showed that the rs3136534 polymorphism decreased the risk of HSPN in the dominant inheritance model (OR, 0.54; 95% CI, 0.31–0.93; p=0.028; G/T+T/T vs. G/G). Risk estimation using a codominant model showed that patients with G/T genotypes had a reduced risk of HSPN compared to patients with the GG genotype (p=0.042, data not shown). However, the frequency of the T alleles of 3136534, showed no statistical significance. For the rs2069776 and rs2069762 polymorphisms, no differences were observed in genotype or allele frequencies between HSPN patients and controls (p>0.05).

Linkage disequilibrium of the 3 SNVs was also analyzed for all subjects in the present study. We observed that the 3 SNVs were located in the same haplotype block. The data from haplotype analysis of the *IL-2* gene for HSPN risk are shown in Table 4. However, no significant association was found between *IL-2* haplotypes and the risk of HSPN.

Table 1. Demographic and clinical characteristics of HSPN patients and controls

Characteristics	Patients (n=81)	Controls (n=200)	*p*
Age at disease onset (years), mean±SD	7.7±2.4	7.2±2.1	0.06
Gender (male/female)	44/37	108/92	0.961
Purpuric rash, n (%）	81 (100)		
Hematuria, n (%)	76 (42.0)		
Proteinuria, n (%)	64 (35.3)		
C3 (g/L), mean±SD	1.19±0.25		
C4 (g/L), mean±SD	0.28±0.12		
IgA (g/L), mean±SD	1.99±0.78		
IgE (IU/mL), mean±SD	126.17±128.23		
White blood cell ($\times 10^9$/L), mean±SD	9.91±3.91	7.31±2.02	< 0.001
C-reactive protein (mg/L), mean±SD	7.06±14.23	3.14±3.52	0.005
Serum creatinine (mg/dL), mean±SD	0.53±0.24	0.51±0.15	0.516

HSPN, Henoch–schönlein purpura nephritis; Ig, immunoglobulin; SD, standard deviation

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Table 2. *IL-2* gene polymorphisms in HSPN and healthy controls

Polymorphisms	Controls n=200 [n (%)]	HSPN n=81 [n (%)]	OR (95% CI)	*p*
rs3136534				
Codominant model				
G/G	50 (25%)	31 (38.3%)	1	
G/T	101 (50.5%)	34 (42%)	0.54 (0.30-0.98)	0.09
T/T	49 (24.5%)	16 (19.8%)	0.53 (0.26-1.08)	
Dominant model				
G/G	50 (25%)	31 (38.3%)	1	0.028
G/T-T/T	150 (75%)	50 (61.7%)	0.54 (0.31-0.93)	
Recessive model				
G/G-G/T	151 (75.5%)	65 (80.2%)	1	0.39
T/T	49 (24.5%)	16 (19.8%)	0.76 (0.40-1.43)	
Overdominant model				
G/G-T/T	99 (49.5%)	47 (58%)	1	0.19
G/T	101 (50.5%)	34 (42%)	0.71 (0.42-1.19)	
rs2069776				
Codominant model				
A/A	154 (77%)	66 (81.5%)	1	
A/G	45 (22.5%)	15 (18.5%)	0.78 (0.41-1.49)	0.53
G/G	1 (0.5%)	0 (0%)	0.00 (0.00-NA)	
Dominant model				
A/A	154 (77%)	66 (81.5%)	1	0.40
A/G-G/G	46 (23%)	15 (18.5%)	0.76 (0.40-1.46)	
Recessive model				
A/A-A/G	199 (99.5%)	81 (100%)	1	0.41
G/G	1 (0.5%)	0 (0%)	0.00 (0.00-NA)	
Overdominant model				
A/A-G/G	155 (77.5%)	66 (81.5%)	1	0.46
A/G	45 (22.5%)	15 (18.5%)	0.78 (0.41-1.50)	
rs2069762				
Codominant model				
A/A	86 (43%)	41 (50.6%)	1	
A/C	90 (45%)	34 (42%)	0.79 (0.46-1.36)	0.36
C/C	24 (12%)	6 (7.4%)	0.52 (0.20-1.38)	
Dominant model				
A/A	86 (43%)	41 (50.6%)	1	0.25
A/C-C/C	114 (57%)	40 (49.4%)	0.74 (0.44-1.24)	
Recessive model				
A/A-A/C	176 (88%)	75 (92.6%)	1	0.24
C/C	24 (12%)	6 (7.4%)	0.59 (0.23-1.49)	
Overdominant model				
A/A-C/C	110 (55%)	47 (58%)	1	0.64
A/C	90 (45%)	34 (42%)	0.88 (0.52-1.49)	

HSPN: Henoch–schönlein purpura nephritis; OR: odds ratio; CI: confidence interval

Table 3. Allele frequencies of three SNVs in the *IL-2* gene in HSPN patients and controls

SNVs	Allele	Controls n=200 [n (%)]	HSPN n=81 [n (%)]	OR (95% CI)	*p*
rs3136534	G	201 (50.2)	96 (59.3)	1.00	
	T	199 (49.7)	66 (40.7)	1.44 (0.96-2.08)	0.052
rs2069776	A	353 (88.2)	147 (90.7)	1.00	
	G	47 (11.7)	15 (9.3)	1.30 (0.71-2.41)	0.39
rs2069762	A	262 (65.5)	116 (71.6)	1.00	
	C	138 (34.5)	46 (28.4)	1.33 (0.89-1.98)	0.16

HSPN: Henoch–schönlein purpura nephritis; SNVs: single nucleotide variations; OR: odds ratio; CI: confidence interval

Table 4. Haplotype analysis of *IL-2* gene for risk of HSPN

Haplotype			Controls (Frequency)	HSPN (Frequency)	OR (95% CI)	*p*
rs3136534	rs2069776	rs2069762				
G	A	A	199 (49.9)	96 (59.3)	1	
T	A	C	136 (34.0)	46 (28.4)	0.72 (0.48-1.09)	0.12
T	G	A	46 (11.6)	15 (9.3)	0.67 (0.35-1.30)	0.24
T	A	A	16 (4.0)	5 (3.1)	0.66 (0.23-1.89)	0.44

HSPN, Henoch–schönlein purpura nephritis; OR, odds ratio; CI, confidence interval

DISCUSSION

Henoch–schönlein purpura, sometimes referred to as IgA vasculitis, is the most common immune complex-mediated leukocytoclastic systemic small-vessel vasculitis in children.[14] HSPN is the most important chronic complication of HSP and thus may be the main cause of morbidity and mortality in this childhood vasculitis.[15]

The pathogenesis and etiology of HSPN have not been completely elucidated. However, serological reports in HSP have indicated dysregulated inflammatory cytokine production. T_H1-, as well as T_H2-related cytokines, play an important role in pediatric HSPN patients.[16,17] IL-2 promoted T-cell immune responses in vivo; previous studies showed that the level of IL-2 was reduced in HSP.[18] Regarding genetic studies, numerous pieces of evidence, such as familial clustering, ethnic differences, and regional discrepancies, support the notion that genetics are crucial in the pathogenesis of HSPN.[8,19] Tabel et al,[20] reported that the *IL-8* gene 2767 A allele was significantly associated with an increased risk of nephritis in children with HSP. López-Mejías et al.,[21] demonstrated that *IL-1β* rs16944 polymorphism could be considered a marker of severe renal manifestations and renal sequelae in HSP. In our previous study, we found that the rs2275913 polymorphism of the *IL-17A* gene was associated with susceptibility to HSP in Chinese children.[22]

IL-2 genetic polymorphisms have been proposed to have an association with susceptibility to some vasculitis and autoimmune diseases. Barış et al,[23] showed a significant correlation between the *IL-2* gene polymorphisms and the age of Behçet's disease onset. Marta Fichna[24] demonstrated that the frequency of the minor C allele of *IL-2* (rs3136534) decreased in autoimmune Addison's disease subjects and appeared protective after Bonferroni correction analysis. In another study, the genotype and allele frequencies of rs3136534 did not show any association with the course of multiple sclerosis in a Spanish population.[25] Mousa et

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al,[26] demonstrated that the *IL-2* gene polymorphisms of rs2069762 were linked with an increased risk of developing non-Hodgkin lymphoma. A study by Wang et al,[27] revealed that the *IL-2* rs2069762 GG genotype and the G allele of this polymorphism decreased the risk of chronic periodontitis. However, Yousefi A et al,[28] demonstrated that G/T alleles of *IL-2* at -330 (rs2069762) and haplotypes did not show a significant association with autoimmune hepatitis.

A study by Lee KA et al,[29] strengthens the evidence that cytokine polymorphisms of rs2069776 are involved in regulating sleep-wake cycles in a chronic illness population. Genotypes G/T and G/G of rs2069762 in patients with myasthenia gravis tended to have ineffective clinical responses to tacrolimus, while no significant differences were observed for rs2069776.[30] Genome-wide association studies showed that *IL-2* (rs2069762) polymorphisms have no correlation with multiple sclerosis.[31] In the present study, our results indicated that the rs3136534 polymorphism was significantly associated with a decreased risk of HSPN in the dominant inheritance model. In contrast, the rs2069776 and rs2069762 polymorphisms had no significant differences in susceptibility to HSPN. We could not find any studies in the literature that supported or contradicted our findings. It needs to be investigated in larger patient series to support this finding.

In conclusion, this study demonstrates an association between genetic variation in *IL-2* and HSPN in Chinese children. We found that only the rs3136534 polymorphism decreased the risk of HSPN in the dominant inheritance model. However, there are limitations to this study. First, the sample size of this study was relatively small, which may reduce the statistical power of the results. Second, because of the homogeneous Chinese-Han ethnic origin of the patient population, the results of the study should be interpreted with caution. Therefore, our results may need further confirmation of the association and the relevant mechanisms between genetic variation in *IL-2* and HSPN in diverse populations with larger sample sizes.

STATEMENT OF ETHICS

The study was approved by the Ethics Committee of Children's Hospital, Zhejiang University School of Medicine (2020-IRB-057).

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CONFLICT OF INTEREST

The authors have no competing interests to disclose.

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