RESEARCH ARTICLE

Association of *HLA-DQA1* gene polymorphisms with the risk of children primary nephrotic syndrome in Chinese population

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Background: The association between gene polymorphisms and the risk of primary nephrotic syndrome (PNS) is uncovering recently. This study aims to investigate the relationship between single nucleotide polymorphisms (SNPs) on *HLA-DQA1* gene and the risk of PNS.

Methods: In this study, we genotyped eight single nucleotide polymorphisms (SNPs) in the *HLA-DQA1* gene in 501 PNS patients and 532 healthy people in Chinese population. Then we analyzed associations of these SNPs with the clinical features in primary nephrotic syndrome of children in Chinese population.

Results: Significant associations with PNS were found on missense SNP rs1129740 (GG vs AA, odds ratio (OR) = 1.987, 95% confidence interval (CI) = 1.468-2.652, $P = 0.00177049$) and rs1047992 (AA vs GG, OR = 1.857, 95% CI = 1.325-2.391, $P = 1.1073E-10$) of the *HLA-DQA1* gene.

Conclusions: This work suggests SNPs of *HLA-DQA1* are risk factors for PNS in Chinese population, which implies roles of immune response in the pathogenesis of PNS.

KEYWORDS

children primary nephrotic syndrome, *HLA-DQA1*, polymorphism

1 | INTRODUCTION

Primary nephrotic syndrome (PNS) is a kidney disorder that featured by edema, hypoalbuminemia, hyperlipidemia, and massive proteinuria and suffered during childhood.^{1,2} The approximate prevalence of PNS is about 16 cases per 100 000 children, which makes it the most frequent glomerular disorder of children.³⁻⁵ The risk factors and pathogenesis underlying PNS have not been well elucidated. Recent studies suggested that genetic factors might justify, a minimum of partially, such risk of children PNS.^{6,7}

The human leukocyte antigen (HLA) is a gene family encoding the major histocompatibility complex (MHC) proteins, which are liable for the regulation of the immune system in humans.⁸ HLA genes area unit extremely polymorphic, which suggests that they need many alternative alleles, permitting them to fine-tune the immune response system. HLA genes play important roles in various

diseases.⁸⁻¹¹ Major histocompatibility complex, class II, DQ alpha 1 and DQ beta 1, conjointly referred to as *HLA-DQA1* and *HLA-DQB1*, locate on short arm of chromosome 6 (6p21.3) and encode MHC class II present antigens.¹² Recent study suggested that single nucleotide polymorphisms (SNPs) (rs1129740, rs1140342, rs1071630, and rs1140343) in *HLA-DQA1* and *HLA-DQB1* were significantly associated with childhood-onset steroid-sensitive nephrotic syndrome (SSNS).¹³ However, it's still unclear whether SNPs in *HLA-DQA1* and *HLA-DQB1* are associated with PNS.

To investigate whether gene polymorphisms of *HLA-DQA1* and *HLA-DQB1* are associated with risk of PNS, we genotyped and analyzed eight SNPs (rs1129740, rs1140342, rs1047992, rs12722051, rs1071630, rs1130432, rs9273651, and rs1140343) in the *HLA-DQA1* and *HLA-DQB1* gene in 501 PNS patients and 532 healthy children, which are from the Chinese Han population. The results indicated gene polymorphisms (rs1129740 and rs1047992) of *HLA-DQA1* but not that

Table 1. Characteristics of samples

	Patients (n = 501)	Control (n = 532)
Age (years)	4.8 ± 3.76	5.16 ± 4.26
Male	297	289
Female	204	243
Body weight (kg)	39 ± 15	41 ± 19
Disease duration (years)	2.1 ± 2.9	2.9 ± 3.5

of *HLA-DQB1* are significantly associated with the risk of PNS. The results of this study suggest a potential contribution of *HLA-DQA1* gene in the pathogenesis of PNS.

2 | MATERIALS AND METHODS

2.1 | Patients

All participants (including PNS patients and healthy controls) were enrolled in the study between January 1, 2015, and

December 31, 2017, in Xuzhou Children's Hospital and in Chinese Han population. Written consent was obtained from legal guardians of all participants with a full rationalization of the procedure. The look of this study was approved by the ethics panel of Xuzhou Children's Hospital in accordance with the declaration of national capital. PNS patients were diagnosed by PNS specialists through clinical examinations. Criteria for enrollment as an impact enclosed no history of nephrosis, traditional diagnosis, and no puffiness.

2.2 | DNA genotyping

DNA of patients and control population was obtained from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA, USA). PCR system was used to capture selected SNPs. The PCR products were sequenced by ABI-PRISM 3730 genetic analyzer (Sequenom, Inc., San Diego, CA, USA). We genotyped four SNPs in the *HLA-DQA1* gene and four SNPs in the *HLA-DQB1* gene. All the eight SNPs with minor allele frequencies (MAF) > 0.1 in the Chinese population were selected from the dbSNP database (www.ncbi.nlm.nih.gov/SNP). All the eight SNPs were missense SNPs.

Table 2. Allele and genotype frequencies of SNPs

Group	Allele frequency		P	Genotype frequency			P	H-W
rs1129740	A	G		AA	AG	GG		
Patients	469	533	0.00177049	95	279	127	0.00014265	0.029963
Control	541	523		142	257	133		0.741149
rs1140342	C	T		CC	TC	TT		
Patients	516	486	0.05720511	127	262	112	0.11829002	0.577095
Control	549	515		145	259	128		0.843209
rs1047992	A	G		AA	AG	GG		
Patients	309	693	1.1073E-10	46	217	238	7.916E-10	0.942346
Control	239	825		25	189	318		0.900154
rs12722051	A	T		AA	AT	TT		
Patients	677	325	0.056872	231	215	55	0.2671501	0.896503
Control	717	347		249	219	64		0.342857
rs1071630	A	T		AA	AT	TT		
Patients	529	473	0.05298636	139	251	111	0.27128101	0.993415
Control	568	496		146	276	110		0.620356
rs1130432	C	T		CC	TC	TT		
Patients	415	587	0.05409631	75	265	161	0.18676304	0.131447
Control	446	618		89	268	175		0.727897
rs9273651	C	T		CC	TC	TT		
Patients	191	811	0.05125835	12	167	322	0.05302142	0.199128
Control	197	867		19	159	354		0.976268
rs1140343	T	G		TT	TG	GG		
Patients	537	465	0.04134722	134	269	98	0.2753932	0.20592
Control	583	481		152	279	101		0.401135

SNP, single nucleotide polymorphism.

Figure 1. The linkage disequilibrium (LD) analysis between the SNPs

Table 3. The linkage disequilibrium (R^2) among the SNPs

R^2	rs1129740	rs1071630	rs12722051	rs1130432	rs1140343	rs1140342	rs9273651	rs1047992
rs1129740	1	0.719	0.011	0.172	0.597	0.469	0.083	0.098
rs1071630	0.719	1	0.009	0.186	0.656	0.524	0.069	0.007
rs12722051	0.011	0.009	1	0.069	0.011	0.012	0.001	0.086
rs1130432	0.172	0.186	0.069	1	0.329	0.277	0.04	0.189
rs1140343	0.597	0.656	0.011	0.329	1	0.807	0.078	0.019
rs1140342	0.469	0.524	0.012	0.277	0.807	1	0.001	0.075
rs9273651	0.083	0.069	0.001	0.04	0.078	0.001	1	0.061
rs1047992	0.098	0.007	0.086	0.189	0.019	0.075	0.061	1

SNP, single nucleotide polymorphism.

2.3 | PCR

PCR system for 50 μ L was as follows: the reaction mixture contained 2 μ L DNA, 2 μ L of every primer, 4 μ L dNTP, 5 μ L PCR buffer, 0.25 μ L of Taq enzyme (Takara, Japan), and double water to a volume of 50 μ L. The sport conditions were as follows: the initial denaturation at 94°C for 5 minutes, thirty-nine amplification cycles were administered per the subsequent temperature profile: 30 second at 94°C, 45 second at 53.7°C, 50 second at 72. The ultimate extension lasted for 5 minutes at 72°C.

2.4 | Statistical analysis

Statistical analysis was performed with R (version 3.4.1; RStudio, Boston, MA, USA). The discrepancies in genotype frequencies and allele at single loci between PNS patients and healthy controls were determined by Chi-square test. Hardy–Weinberg equilibrium (HWE) test was performed using SPSS. The relative risk was determined by the odds ratio (OR), and a 95% confidence interval (CI) was estimated for each OR. Linkage disequilibrium (LD) was determined by R^2 . P -values are two-tailed, and the cut line of statistical significance was 0.05.

3 | RESULTS

3.1 | rs1129740 and rs1047992 are associated with PNS

The case–control study consisted of 501 PNS patients (297 boys and 207 girls with a mean age $\pm$ SD of 4.8 ± 3.76 years) and 532 healthy controls (289 boys and 243 girls with a mean age $\pm$ SD of 5.16 ± 4.26 years) (Table 1). The distribution of the genotypes of the eight SNPs was in HWE in all the controls and in most of the patients, except for rs1129740 and rs1047992 in PNS patients. There were no significant differences in allele frequencies or genotype frequencies between PNS patients ($n = 501$) and controls ($n = 532$) for six SNPs (Table 2). The allele frequencies and genotype frequencies of rs1129740 and rs1047992 are significantly different between PNS patients and controls ($P < 0.05$). The G allele frequency of rs1129740 was higher in PNS patients than

in controls ($P = 0.00177049$, odds ratio (OR) = 1.987, 95% confidence interval (CI) = 1.468–2.652). The A allele frequency of rs1047992 was higher in PNS patients than in controls (OR = 1.857, 95% CI = 1.325–2.391, $P = 1.1073E-10$). The genotype frequencies of rs1129740 ($P = 0.00014265$) and rs1047992 ($P = 0.7916E-10$) are significantly different between PNS patients and controls, respectively. There are no significant differences between PNS patients and controls in either allele frequencies or genotype frequencies in the other six SNPs.

3.2 | Linkage disequilibrium analysis

The result of the linkage disequilibrium (LD) analysis between the eight SNPs indicated that no SNP pairs exhibited strong LD ($R^2 > 0.8$) (Figure 1). These SNPs pairs in this study only showed modest or weak LD (Table 3). It revealed that these SNPs play their roles independently in the studied population. Therefore, no haplotypes were built in this study.

4 | DISCUSSION

Primary nephrotic syndrome (PNS) is a childhood kidney disorder that featured by edema, hypoalbuminemia, hyperlipidemia, and massive proteinuria and suffered.^{1,2} Recent studies suggested that genetic factors, such as single nucleotide polymorphisms (SNPs) of genes, might justify, a minimum of partially, the risk of many types of diseases,^{14–16} as well as children PNS.^{6,7} In this study, we found significant associations between missense SNP rs1129740 (odds ratio (OR) = 1.987, 95% confidence interval (CI) = 1.468–2.652, $P = 0.00177049$) and rs1047992 (OR = 1.857, 95% CI = 1.325–2.391, $P = 1.1073E-10$) of the *HLA-DQA1* gene and the risk of children PNS.

The human leukocyte antigen (HLA) is a gene family encoding the major histocompatibility complex (MHC) proteins, which are liable for the regulation of the immune system in humans.⁸ HLA genes area unit extremely polymorphic and play important roles in various diseases.^{17–19} Studies of smaller sample size from totally different populations have antecedently reportable association between SNPs in *HLA-DQA1* and *HLA-DQB1* and childhood-onset steroid-sensitive nephrotic syndrome¹³, which was limited by sample sizes

and was restricted to different population.^{20–22} This study aims to investigate whether SNPs of *HLA-DQA1* and *HLA-DQB1* are associated with risk of PNS in 501 PNS patients and 532 healthy controls of Chinese Han population. The results indicated that SNPs of *HLA-DQA1* but not that of *HLA-DQB1* are associated with the risk of PNS in Chinese Han population.

The lack of LD between rs1129740 and rs1047992, which are the SNPs associated with PNS, in this study suggests that the loci found in this study could represent independent mechanism. The rs1129740 is the risk locus for childhood-onset steroid-sensitive and steroid-resistant nephrotic syndrome.^{13,23} The further finding of HLA loci related to the PNS suggests that all of them share an underlying immune-mediated mechanism in illness pathological process.^{24,25} Our ability to duplicate this locus in a totally different population implied the fact that the risk factor rs1129740 might not be population specific. Meanwhile, the newly identified rs1047992 might be a new risk factor for PNS in Chinese population.

Generally, this study discovered the association of rs1129740 and rs1047992 on *HLA-DQA1* with the risk of PNS in Chinese Han population. It extends our knowledge on the pathogenesis of PNS and may promote diagnostic and therapeutic strategies for PNS.

AUTHORS CONTRIBUTION

Bingbing Zhu, Lijun Tian, and Huandan Yang performed the experiments. Tingting Yuan, Juan Lv, and Qianqian Peng analyzed the data. Ruifeng Zhang wrote and edited the manuscript.

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How to cite this article: Zhu B, Zhang R, Yang H, et al.

Association of *HLA-DQA1* gene polymorphisms with the risk of children primary nephrotic syndrome in Chinese population. *J Clin Lab Anal*. 2019;33:e22623. <https://doi.org/10.1002/jcla.22623>