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RESEARCH ARTICLE

DQB1*060101 may contribute to susceptibility to immunoglobulin A nephropathy in southern Han Chinese

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Abstract **Immunoglobulin A nephropathy (IgAN) is a common form of chronic glomerulonephritis with unknown pathogenesis. Accumulating evidences have shown the ethnic-specific association between certain human leukocyte antigen (HLA) alleles and IgAN susceptibility. This study was designed to explore the relationship between HLA-DQB1 alleles and disease susceptibility and clinical manifestations of patients with IgAN in southern Han Chinese. A PCR sequence-based typing technique was used to detect HLA-DQB1 alleles in 217 IgAN patients and 229 healthy subjects. Clinical data were collected from each patient at the time of renal biopsy. Twenty HLA-DQB1 alleles were detected in IgAN patients and healthy subjects. High frequency of HLA-DQB1*060101 and low frequency of HLA-DQB1*030101 were observed in IgAN patients compared with healthy controls. Further stratification analysis revealed that the frequency of DQB1*060101 was significantly higher in patients with urine protein ⩾ 1.0 g/24 h than in patients with urine protein < 1.0 g/24 h. In combination with our previous DRB1 results, we also analyzed the association of DRB1-DQB1 haplotypes with IgAN. We found that the frequency of haplotype DRB1*090102-DQB1*060101 was significantly higher [odds ratio (OR) = 4.409, *Pc* = 0.016], whereas that of HLA-DRB1*070101-DQB1*020101 was significantly lower (OR = 0.194, *Pc* = 0.016) compared with healthy controls. Our study indicated that HLA-DQB1*060101 alleles may be a potential predictor of high-risk IgAN susceptibility in Chinese Han population.**

Keywords DQB1; human leukocyte antigen (HLA); IgA nephropathy; haplotype; association study

Introduction

Immunoglobulin A nephropathy (IgAN), an immune complex-mediated glomerulonephritis defined immunohistologically by the deposition of IgA in the mesangial area of glomeruli, is the most common primary glomerular disease worldwide [1]. Although most individuals with IgAN have a mild form of the disease, 15%–40% of cases will progress to end-stage renal disease within 20 years of disease onset [2]. Many clinical and histological factors have been shown to be associated with progression of the disease, including high blood pressure, heavy proteinuria, renal insufficiency, and a severe histological appearance of renal biopsy [3–5].

Accumulating evidences indicated that genetic factors play important roles in the development and progression of IgAN, as demonstrated by a wide ethnic variation in prevalence, familial aggregation, clustering in isolated populations, and animal models of hereditary IgAN [6,7]. The human leukocyte antigen (HLA) genes, which have been mapped on the short arm of chromosome 6, are important determinants of the IgA-mediated immune response and possibly involved in the pathogenesis of IgAN [8]. The encoded HLA molecules are highly polymorphic transmembrane glycoproteins composed of α and β subunits, which are expressed on the surface of B lymphocytes, macrophages/monocytes, and some T lymphocytes [9]. The genes encoding the three major expressed HLA class II products are arranged in three main subregions: DP, DQ, and DR.

Our previous study discovered that HLA-DRB1 polymorphisms are related to the occurrence and disease progression of IgAN patients in Han Chinese, with HLA-DRB1*140501 being a susceptible allele, HLA-

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DRB1*070101 being a resistant allele, and HLA-DRB1*030101 possibly serving as a predictor of disease progression and renal damage of IgAN in Han Chinese [10]. To date, five genome-wide association studies (GWAS) of IgAN were performed in different ethnic populations, and all of these studies discovered that HLA alleles are related to susceptibility of IgAN [11–15]. In addition, HLA alleles may play an important role in the pathogenesis of IgAN. GWAS merely implicated the locus of HLA alleles in IgAN susceptibility and analyzed HLA alleles imputed from single nucleotide polymorphisms (SNPs). Such studies can only provide limited information about the diversity of the polymorphisms within this region. Therefore, the genotyping of HLA alleles via a precise and accurate method is necessary for further study.

In this work, we investigated the association of HLA-DQB1 genes and IgAN in ethnic southern Han Chinese using high-resolution sequence-based typing (SBT) in a case–control study. In combination with our previous DRB1 typing results [10], we also analyzed the association of DRB1-DQB1 haplotypes with IgAN.

Methods

Patients and healthy subjects

Patients were recruited from the Department of Nephrology, The First Affiliated Hospital of Sun Yat-sen University in Guangzhou, China. A total of 217 primary IgAN patients diagnosed by renal biopsy were enrolled in this study. The diagnosis of IgAN was confirmed by histological and immunological examinations following renal biopsy and met the diagnostic criteria of the World Health Organization [16]. Patients with evidence of systemic diseases, such as diabetes, chronic liver disease, and systemic lupus erythematosus, were excluded. Pregnant women were also excluded. In addition, 229 healthy gender- and age-matched unrelated subjects with no history of renal disease or hypertension were recruited from Guangdong Province. Ethical approval of the study was obtained from the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). Written informed consent was signed by each participant.

All patients underwent detailed medical and biochemical examinations. Clinical data were collected from each patient at the time of renal biopsy. Specific clinical variables screened for genotype correlations included the following: serum IgA level (measured clinically at the time of biopsy), history of gross hematuria (by history), proteinuria (defined by urine protein to creatinine ratio or 24 h protein excretion when available), hypertension (defined as SBP $>$ 140 mmHg, DBP $>$ 90 mmHg, or history of antihypertensive medication use), eGFR (calculated at the time of biopsy using the MDRD formula), and crescent formation (pathological characteristics measured at the time of biopsy).

DNA extraction

Venous blood samples were obtained from the participants. The genomic DNA was extracted from peripheral blood lymphocytes by using QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.

Genotyping of HLA-DQB1

HLA-DQB1 genes were genotyped to six-digit resolution using PCR-SBT as previously described [17–19]. Amplified PCR products were sequenced directly on an ABI 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data from HLA class II were typed using an internet automatic analytical program (http://www.ncbi.nlm.nih.gov/mhc/sbt).

Statistical analysis

Allele frequencies of DQB1 in patients and controls were counted. Hardy–Weinberg equilibrium was calculated using Arlequin software 2.0 (University of Geneva, Geneva, Switzerland) [20]. The frequency distribution of HLA-DQB1 alleles in patients and controls was compared using the χ^2 test with Yates correction and Fisher's exact test. Odds ratios (OR), confidence intervals (CIs), and P values were calculated using the Epi-Info program [21,22]. Corrected P values (Pc) were calculated using the Bonferroni inequality method [23].

In combination with our previous DRB1 typing results [10], the frequencies of two-locus (DRB1-DQB1) haplotypes were estimated in patients and controls using the expectation–maximization algorithm implemented in Arlequin 2.0.

Results

Frequency distribution of HLA-DQB1 alleles in patients and healthy controls

The demographic information of the recruited cohort of IgAN patients and healthy controls is shown in Table 1. No significant differences were observed between the cases and healthy controls in terms of mean age or gender distribution. Allele frequencies of the HLA-DQB1 gene in IgAN patients and healthy subjects of Han Chinese ethnicity are summarized in Table 2. Up to 20 different alleles were detected in IgAN patients and healthy subjects. The most common DQB1 allele of the IgAN

patients was DQB1*060101 with a frequency of 20.73%, followed by DQB1*030302 (14.51%) and DQB1*050201 (13.36%). As for the detected DQB1 alleles among controls, the most predominant one was DQB1*030101/0309 (20.30%), followed by DQB1*050201 (17.46%) and DQB1*020101/0202 (11.35%). The frequency of HLA-DQB1*060101 was significantly higher in the patient's group than in healthy subjects (OR = 2.088, $P < 0.0001$, $Pc = 0.0001 \times 20 = 0.002$), whereas the frequency of HLA-DQB1*030101/0309 was significantly lower in patients than in healthy subjects (OR = 0.534, $P = 0.001$, $Pc = 0.001 \times 20 = 0.02$).

Table 1 Basic clinical characteristics of IgAN patients

Variable	IgAN (*n*, total = 217)	Healthy subjects (*n*, total = 229)
Age (year)	33.7±16.00	34.2±17.80
Sex		
Male	91 (42.30%)	106 (46.40%)
Female	126 (57.70%)	123 (53.60%)
Blood pressure		
Hypertension	54 (24.88%)	
Norma blood pressure	163 (75.12%)	229 (100%)
Content of urine protein		
⩾1.0 g/24 h	80 (36.86%)	
<1.0 g/24 h	137 (63.14%)	Negative
Gross hematuria		
Yes	70 (32.25%)	
No	147 (67.75%)	229 (100%)
Serum IgA level		
> 3.45 mmol/L	61 (28.11%)	
⩽3.45 mmol/L	156 (71.89%)	
Renal function		
GFR ⩽ 60 ml/min	48 (22.11%)	
GFR > 60 ml/min	169 (77.89%)	229 (100%)
Crescent formation		
Yes	59 (27.18%)	
No	158 (72.82%)	229 (100%)

Association of polymorphisms of HLA-DQB1 alleles with clinical manifestations of IgAN patients

We analyzed the effect of HLA-DQB1 alleles on clinical manifestations of IgAN patients. The IgAN patients were divided into different subgroups for further stratified analysis. The frequency of HLA-DQB1*060101 in patients with urine protein ⩾ 1.0 g/24 h was significantly higher than that in patients with urine protein < 1.0 g/24 h (OR = 2.367, $P < 0.0001$, $Pc = 0.0001 \times 19 = 0.0019$; Table 3). The frequencies of HLA-DQB1*0401 and HLA-DQB1*050101 in patients with crescent formation were significantly higher than those in patients without crescent formation (HLA-DQB1*0401: OR = 7.701, $P < 0.0001$, $Pc = 0.0001 \times 19 = 0.0019$; HLA-DQB1*050101: OR = 4.266, $P = 0.007$, $Pc = 0.007 \times 19 = 0.013$; Table 4). The frequency of HLA-DQB1*0402 in patients with gross hematuria was also higher than that in patients without gross hematuria (OR = 13.119, $P = 0.003$, $Pc = 0.003 \times 19 = 0.057$; Table 5). However, this association was not significant after adjustment using the Bonferroni's inequality method. No significant difference was observed in the frequencies of the HLA-DQB1 alleles when adjusted by other variables, such as hypertension, serum IgA level, and renal function (Tables 6, 7, and 8, respectively).

DRB1-DQB1 haplotype analysis

In a previous report [10], we identified 37 distinct HLA class II alleles of the DRB1 locus in 139 IgAN patients and 143 healthy controls. We found that HLA-DRB1*140501 was significantly common in IgAN patients than in controls (OR = 3.938; $P = 0.001$, $Pc = 0.037$), whereas the DRB1*070101 allele was less common (OR = 0.205; $P < 0.0005$, $Pc < 0.0185$). Using these data, we calculated DRB1-DQB1 haplotypes in the present study and obtained a total of 136 haplotypes. The most frequent DRB1-DQB1 haplotype in patients was DRB1*090102-DQB1*060101 with a frequency of 8.63%, followed by DRB1*150101-DQB1*030302 (5.03%) and DRB1*080302-DQB1*060101 (4.31%) (Table 9). In controls, the most frequent DRB1-DQB1 haplotype was DRB1*070101-DQB1*020101 with a frequency of 6.99%, followed by DRB1*090102-DQB1*030101 (5.24%) and DRB1*080302-DQB1*060101 (4.89%). Compared with the healthy controls, the frequency of DRB1*090102-DQB1*060101 was significantly higher in patients with IgAN (OR = 4.409; $P = 0.001$, $Pc = 0.016$), whereas the frequency of DRB1*070101-DQB1*020101 was significantly lower (OR = 0.194; $P = 0.001$, $Pc = 0.016$) in patients with IgAN.

Discussion

In the past three decades, the association of the HLA region with IgAN has been widely investigated. However, many studies with small sample size yielded different results, so the association of these regions with IgAN remains unclear. The important role of the HLA region has received extensive attention with the emergence of GWAS in recent years. GWAS can only detect the association of tag SNPs within the HLA region and identify the HLA allele using imputation methods. Such methods cannot distinguish the precise HLA alleles (such as the four digital HLA alleles). Therefore, we performed SBT for the accurate determination of the significant HLA allele in IgAN to overcome this

Table 2 Distribution of HLA-DQB1 allele frequencies in IgAN patients and healthy controls

DQB1 allele	IgAN (Total = 217 × 2)		Control (Total = 229 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	52	11.98	52	11.35	0.035	0.835	1.063	0.706	1.600
*030101/0309	52	11.98	93	20.30	10.139	0.001[a]	0.534	0.370	0.772
*030201	19	4.37	15	3.27	0.469	0.390	1.352	0.678	2.690
*030302	63	14.51	49	10.69	2.62	0.085	1.417	0.951	2.112
*0304	1	0.23	3	0.65	ND	ND	ND	ND	ND
*0310	2	0.46	1	0.21	ND	ND	ND	ND	ND
*0312	0	0	2	0.42	ND	ND	ND	ND	ND
*0401	18	4.14	13	2.83	0.782	0.361	1.481	0.717	3.061
*0402	7	1.61	6	1.31	0.100	0.784	1.235	0.412	3.704
*050101	15	3.45	17	3.71	0.010	0.859	0.929	0.458	1.883
*050201	58	13.36	80	17.46	2.564	0.096	0.729	0.505	1.052
*050202	2	0.46	4	0.87	0.932	0.687	0.525	0.096	2.884
*050301	25	5.76	34	7.42	0.747	0.347	0.762	0.447	1.300
*050302	2	0.46	3	0.65	ND	ND	ND	ND	ND
*0505	1	0.23	4	0.87	ND	ND	ND	ND	ND
*060101	90	20.73	51	11.13	14.725	<0.0001[b]	2.088	1.439	3.030
*0602	13	2.99	13	2.83	0	1	1.057	0.484	2.306
*0604	4	0.92	8	1.74	0.606	0.387	0.523	0.156	1.750
*060502	1	0.23	0	0	ND	ND	ND	ND	ND
*0609	9	2.07	10	2.18	0	0.910	0.949	0.382	2.358

ND, not determined; [a]$Pc = 0.001 \times 20 = 0.02$; [b]$Pc = 0.0001 \times 20 = 0.002$.

Table 3 Distribution of HLA-DQB1 alleles in IgAN patients with 24 h proteinuria >1 g or ⩽1 g

DQB1 allele	24 h proteinuria >1 g (Total = 80 × 2)		24 h proteinuria ⩽1 g (Total = 137 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	20	12.50	32	11.67	0.010	0.800	1.080	0.595	1.961
*030101/0309	14	8.75	38	13.86	2.048	0.106	0.596	0.312	1.137
*030201	5	3.12	14	5.10	0.535	0.330	0.599	0.212	1.695
*030302	22	13.75	41	14.96	0.042	0.728	0.906	0.518	1.585
*0304	1	0.62	0	0	ND	ND	ND	ND	ND
*0310	1	0.62	1	0.36	ND	ND	ND	ND	ND
*0401	6	3.75	12	4.37	0.005	0.749	0.851	0.313	2.312
*0402	3	1.87	4	1.45	0	0.712	1.129	0.285	5.838
*050101	7	4.37	8	2.91	0.584	0.391	2.14	0.425	10.779
*050201	13	8.12	45	16.42	5.213	0.011[a]	0.450	0.235	0.863
*050202	1	0.62	1	0.36	ND	ND	ND	ND	ND
*050301	9	5.62	16	5.83	0	0.926	0.961	0.415	2.28
*050302	0	0	2	0.72	ND	ND	ND	ND	ND
*0505	1	0.62	0	0	ND	ND	ND	ND	ND
*060101	48	30	42	15.32	12.351	<0.0001[b]	2.367	1.437	3.794
*0602	5	3.12	8	2.91	0	0.904	1.073	0.345	3.336
*060502	0	0	1	0.36	ND	ND	ND	ND	ND
*0604	0	0	4	1.45	ND	ND	ND	ND	ND
*0609	4	2.5	5	1.82	0.016	0.634	1.379	0.365	5.213

ND, not determined; [a]$Pc = 0.011 \times 19 = 0.209$; [b]$Pc = 0.0001 \times 19 = 0.0019$.

Table 4 Distribution of HLA-DQB1 alleles in IgAN patients with or without crescent formation

DQB1 allele	Crescent formation (Total = 59 × 2)		No crescent formation (Total = 158 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	13	11.01	39	12.34	0.045	0.868	0.879	0.451	1.713
*030101/0309	10	8.47	42	13.29	1.461	0.156	0.604	0.293	1.247
*030201	5	4.23	14	4.43	0	0.93	0.954	0.336	2.711
*030302	13	11.01	50	15.82	1.235	0.224	0.659	0.344	1.263
*0304	0	0	1	0.31	ND	ND	ND	ND	ND
*0310	0	0	2	0.62	ND	ND	ND	ND	ND
*0401	13	11.01	5	1.58	16.917	<0.0001[a]	7.701	2.682	22.114
*0402	3	2.54	4	1.26	0.261	0.369	2.035	0.449	9.231
*050101	9	7.62	6	1.89	6.820	0.007[b]	4.266	1.484	12.262
*050201	17	14.40	41	12.97	0.054	0.698	1.129	0.614	2.077
*050202	1	0.84	1	0.31	ND	ND	ND	ND	ND
*050301	5	4.23	20	6.32	0.361	0.391	0.655	0.24	1.787
*050302	0	0	2	0.62	ND	ND	ND	ND	ND
*0505	1	0.84	0	0	ND	ND	ND	ND	ND
*060101	22	18.64	68	21.51	0.275	0.507	0.836	0.489	1.428
*0602	4	3.38	9	2.84	0	0.771	1.197	0.361	3.963
*0604	0	0	4	1.26	ND	ND	ND	ND	ND
*060502	0	0	1	0.31	ND	ND	ND	ND	ND
*0609	2	1.69	7	2.21	0	0.730	0.761	0.156	3.717

ND, not determined; [a]$Pc = 0.0001 \times 19 = 0.0019$; [b]$Pc = 0.007 \times 19 = 0.013$.

Table 5 Distribution of HLA-DQB1 alleles in IgAN patients with or without gross hematuria

DQB1 allele	Gross hematuria (Total = 70 × 2)		No gross hematuria (Total = 147 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	18	12.85	34	11.56	0.053	0.698	1.128	0.613	2.077
*030101/0309	14	10	38	12.92	0.517	0.380	0.749	0.391	1.432
*030201	6	4.28	13	4.42	0	1	0.957	0.324	2.824
*030302	20	14.28	43	14.62	0	1	0.973	0.548	1.726
*0304	0	0	1	0.34	ND	ND	ND	ND	ND
*0310	0	0	2	0.68	ND	ND	ND	ND	ND
*0401	8	5.71	10	3.40	0.761	0.259	1.721	0.664	4.461
*0402	6	5.45	1	0.34	6.984	0.003[a]	13.119	1.564	110.051
*050101	6	4.28	9	3.06	0.138	0.514	1.418	0.495	4.065
*050201	18	12.85	40	13.6	0.004	0.830	0.937	0.516	1.702
*050202	0	0	2	0.68	ND	ND	ND	ND	ND
*050301	7	5	18	6.12	0.062	0.639	0.807	0.329	1.979
*050302	0	0	2	0.68	ND	ND	ND	ND	ND
*0505	0	0	1	0.34	ND	ND	ND	ND	ND
*060101	31	22.14	59	20.06	0.318	0.618	1.133	0.694	1.850
*0602	2	1.42	11	3.74	1.041	0.186	0.373	0.082	1.705
*0604	0	0	4	1.22	ND	ND	ND	ND	ND
*060502	1	0.71	0	0	ND	ND	ND	ND	ND
*0609	3	2.14	6	2.04	0	0.944	1.051	0.259	4.266

ND, not determined; [a]$Pc = 0.003 \times 19 = 0.057$.

Table 6 Distribution of HLA-DQB1 alleles in IgAN patients with hypertension or normal blood pressure

DQB1 allele	Hypertension (Total = 54 × 2)		Normal blood pressure (Total = 163 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	8	7.40	44	13.49	2.304	0.123	0.513	0.233	1.126
*030101/0309	10	9.25	42	12.88	0.696	0.315	0.690	0.334	1.427
*030201	3	2.77	16	4.90	0.444	0.348	0.554	0.158	1.938
*0304	0	0	1	0.30	ND	ND	ND	ND	ND
*030302	20	18.51	43	13.19	1.452	0.173	1.496	0.836	2.677
*0310	0	0	2	0.61	ND	ND	ND	ND	ND
*0401	3	2.77	15	4.60	0.297	0.410	0.592	0.168	2.087
*0402	0	0	7	2.14	ND	ND	ND	ND	ND
*050101	4	3.70	11	3.37	0	0.871	1.101	0.343	3.533
*050201	16	14.81	42	12.88	0.121	0.609	1.176	0.631	2.190
*050202	2	1.85	0	0	ND	ND	ND	ND	ND
*050301	5	4.62	20	6.13	0.118	0.552	0.743	0.272	2.029
*050302	1	0.92	1	0.30	ND	ND	ND	ND	ND
*0505	0	0	1	0.30	ND	ND	ND	ND	ND
*060101	29	26.85	61	18.71	2.794	0.076	1.595	0.959	2.652
*0602	3	2.77	10	3.06	0	0.878	0.903	0.244	3.343
*060502	1	0.92	0	0	ND	ND	ND	ND	ND
*0604	1	0.92	3	0.92	ND	ND	ND	ND	ND
*0609	2	1.85	7	2.14	0	0.852	0.860	0.176	4.203

ND, not determined.

Table 7 Distribution of HLA-DQB1 alleles in IgAN patients with serum IgA > 3.45 or ⩽ 3.45 mmol/L

DQB1 allele	Serum IgA > 3.45 mmol/L (Total = 61 × 2)		Serum IgA ⩽ 3.45 mmol/L (Total = 156 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	11	9.01	41	13.14	1.051	0.255	0.655	0.325	1.321
*030101/0309	14	11.47	38	12.17	0.001	1	0.935	0.487	1.794
*030201	5	4.09	14	4.48	0	0.858	0.91	0.32	2.582
*030302	25	20.49	38	12.17	4.237	0.032	1.858	1.067	3.238
*0304	0	0	1	0.32	ND	ND	ND	ND	ND
*0310	1	0.81	1	0.32	ND	ND	ND	ND	ND
*0401	6	4.91	12	3.84	0.056	0.599	1.293	0.474	3.526
*0402	2	1.63	5	1.6	0	0.978	1.023	0.196	5.346
*050101	6	4.91	9	2.88	0.563	0.313	1.741	0.606	5.001
*050201	14	11.47	44	14.1	0.321	0.532	0.79	0.416	1.5
*050202	2	1.63	0	0	ND	ND	ND	ND	ND
*050301	8	6.55	17	5.44	0.047	0.660	1.218	0.511	2.900
*050302	0	0	2	0.64	ND	ND	ND	ND	ND
*0505	1	0.81	0	0	ND	ND	ND	ND	ND
*060101	20	16.39	70	22.43	1.598	0.155	0.678	0.392	1.173
*0602	4	3.27	9	2.88	0	0.763	1.141	0.345	3.777
*0604	1	0.81	3	0.96	ND	ND	ND	ND	ND
*060502	0	0	1	0.32	ND	ND	ND	ND	ND
*0609	2	1.63	7	2.24	0.001	0.684	0.726	1.149	3.545

ND, not determined.

Table 8 Distribution of HLA-DQB1 alleles in IgAN patients with eGFR ⩽ 60 or > 60 ml/min/1.73 m^2

DQB1 allele	eGFR ⩽ 60 ml/min/1.73 m^2 (Total = 48 × 2)		eGFR > 60 ml/min/1.73 m^2 (Total = 169 × 2)		χ^2	*P*	OR	95% CI	
	n	%	*n*	%				Lower	Upper
*020101/0202	8	8.33	44	13.01	1.143	0.196	0.607	0.276	1.339
*030101/0309	12	12.5	40	11.83	0	0.861	1.064	0.534	2.120
*030201	5	5.20	14	4.14	0.028	0.659	1.272	0.446	3.624
*030302	18	18.75	45	13.31	1.370	0.193	1.503	0.824	2.740
*0304	0	0	1	0.29	ND	ND	ND	ND	ND
*0310	1	1.04	1	0.29	ND	ND	ND	ND	ND
*0401	5	5.20	13	3.84	0.09	0.564	1.374	0.477	3.954
*0402	2	2.08	5	1.47	0	0.708	1.383	0.264	7.243
*050101	4	4.16	11	3.25	0.013	0.673	1.292	0.402	4.154
*050201	14	14.58	44	13.01	0.052	0.734	1.141	0.596	2.184
*050202	1	1.04	1	0.29	ND	ND	ND	ND	ND
*050301	6	6.25	19	5.62	0	0.806	1.119	0.434	2.886
*050302	0	0	2	0.58	ND	ND	ND	ND	ND
*0505	0	0	1	0.29	ND	ND	ND	ND	ND
*060101	14	14.58	76	22.48	0.829	0.377	0.717	0.386	1.332
*0602	4	4.16	9	2.66	2.380	0.082	5.89	0.316	1.096
*0604	0	0	4	1.18	ND	ND	ND	ND	ND
*060502	0	0	1	0.29	ND	ND	ND	ND	ND
*0609	2	2.08	7	2.07	0	1	1.006	0.206	4.924

ND, not determined.

Table 9 HLA-DRB1-DQB1 haplotypes in IgAN patients and healthy controls

DRB1-DQB1	IgAN patients (Total = 139) (278 alleles)		Healthy subjects (Total = 143) (286 alleles)		*P*	OR	95% CI	
	n	%	*n*	%			Lower	Upper
*030101-020101	6	2.15	9	3.14	0.603	0.679	0.238	1.933
*070101-020101	4	1.43	20	6.99	0.001[a]	0.194	0.065	0.576
*070101-030302	2	0.71	10	3.49	0.046	0.200	0.043	0.921
*080302-060101	12	4.31	14	4.89	0.842	0.876	0.398	1.930
*090102-030101	7	2.51	15	5.24	0.146	0.467	0.187	1.163
*090102-060101	24	8.63	6	2.09	0.001[b]	4.409	1.774	10.961
*090102-030302	6	2.15	8	2.79	0.828	0.767	0.263	2.238
*110101-050101	7	2.51	8	2.79	1.000	0.898	0.321	2.509
*120101-030101	2	0.71	8	2.79	0.107	0.252	0.053	1.196
*140101-050301	5	1.79	9	3.14	0.448	0.564	0.187	1.703
*140101-050201	3	1.07	10	3.49	0.103	0.301	0.082	1.106
*140501-020101	7	2.51	12	4.19	0.384	0.590	0.229	1.521
*150101-030302	14	5.03	12	4.19	0.783	1.211	0.550	2.666
*150101-060101	10	3.59	6	2.09	0.413	1.741	0.624	4.857
*150101-0602	6	2.15	10	3.49	0.482	0.609	0.218	1.698
*160201-050201	4	1.43	8	2.79	0.383	0.507	0.151	1.704
Others	159		121					

[a]$Pc = 0.001 \times 16 = 0.016$. [b]$Pc = 0.001 \times 16 = 0.016$.

deficiency.

This study analyzed the distribution of HLA-DQB1 alleles in patients with IgAN and their potential significance. A total of 20 different HLA-DQB1 alleles were detected. The frequency of HLA-DQB1*060101 was significantly higher but the frequency of HLA-DQB1*030101 was significantly lower in patients with IgAN compared with the healthy individuals in Han Chinese.

Our findings were inconsistent with previous HLA association studies. In Caucasian patients with primary IgAN, a significant increase in the HLA-DQw7 allele frequency was observed [24]. In another European study, a decreased frequency of DQB1*0201 was observed in British patients and a decreased frequency of DQB1*0602 was noted in Finnish patients, whereas no association between the DQ markers and IgAN was found in Italian patients [25]. These results have several possible explanations. First, different disease susceptibility genes may operate in different populations and account for the variation in prevalence in different geographical locations. Second, IgAN is a heterogeneous disease that may be associated with different HLA susceptibility alleles in patients from different regions. The frequency of HLA-DQB1*060101 was also reported to be increased in membranoproliferative glomerulonephritis and hepatitis B virus-associated glomerulonephritis, which may implicate that HLA-DQB1*060101 is an important allele related to the immune complex-mediated glomerulonephritis, including IgAN [26].

The mechanism by which HLA-DQB1 polymorphisms contribute to the pathogenesis of IgAN has yet to be clarified. A previous study indicated that HLA-DQB1*060101 may confer susceptibility to cervical cancer [27]. Sequence analysis revealed that DQB1*060101 allele encodes Leu at position 9 and Asp at position 37 among cervical cancer patients with a susceptible allele, and other DQB1 alleles encode Phe or Tyr and Ile or Tyr at the same two positions, respectively [27]. This result revealed that susceptibility-associated DQB1*060101 alleles shared a common motif, L/D, at positions 9 and 37 of DQB1 (95 and 123 in DQ nucleotide numbering), which are part of predicted antigen recognition sites of the class II molecule [27]. Accordingly, peptide binding and subsequent immune triggering depends critically on these single amino acid variants. HLA plays a critical role in presenting antigens to T lymphocytes, which are important determinants of the IgA-mediated immune response. The DQB1*060101 alleles may selectively influence the MHC allele–pathogen motif, which will be shown to the T cell receptor. This scenario leads to a detrimental cellular immune response responsible for the development of IgAN.

Further analysis was conducted to explore the effect of HLA-DQB1 alleles on the clinical features and histological renal lesions in IgAN patients. We found that HLA-DQB1*060101 significantly affected the occurrence of proteinuria in IgAN patients. Previous studies mostly among Caucasian patients, for example in France, demonstrated a strong association between HLA-DQB1*0301 and poor clinical outcomes of IgAN patients [28]. To our knowledge, the present study is the first report on HLA-DQB1 allele's effect on clinical phenotypes and pathological feathers in Chinese Han IgAN patients.

Although polymorphisms in the HLA class II gene have been extensively studied, the effect of HLA haplotypes on IgAN risk remains unknown. Only a previous single study has shown that HLA-A2-B5-DR5 (OR = 2.990) is a susceptibility haplotype in Caucasians [29]. Based on our results, combined with our previous DRB1 data, we found that DRB1*090102-DQB1*060101 haplotype was common in IgAN patients, whereas DRB1*070101-DQB1*020101 haplotype was less common. This analysis of HLA haplotype may provide more information to understand the extraordinary complexity and diversity of the HLA region, as well as help further reveal the pathogenesis of IgAN.

The findings in our current study are very interesting and compelling considering that the results survived multiple testing corrections. However, we do realize the limitations of this study because of the relatively small sample size analyzed. For the rare HLA alleles, we did not have sufficient means to assess the effects on IgAN susceptibility. In addition, further replication studies involving large independent Chinese samples are necessary to confirm our findings.

In conclusion, our results provide important information of the association of HLA DQB1 and DRB1-DQB1 haplotype with IgAN in Han Chinese.

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Compliance with ethics guidelines

Wei Wang, Ming Li, Li Wang, and Xueqing Yu declare that they have no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the

Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients involved in the study.

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