Journal of Human Genetics (2013) 58, 40–46

www.nature.com/jhg

ORIGINAL ARTICLE

Interaction of C1GALT1–IL5RA on the susceptibility to IgA nephropathy in Southern Han Chinese

Wei Wang[1,2,3], Yu Sun[4], Yonggui Fu[4], Xueqing Yu[1,2] and Ming Li[1,2]

IgA nephropathy is one of the most common glomerulonephritis throughout the world, which is thought to be the multifactorial complex diseases, with genetic and environmental factors contributing to this disease. The failure of replicating the single genes in previous association studies may be of that the gene–gene interaction might have more influence on the susceptibility of the complex diseases. In all, 31 single-nucleotide polymorphisms (SNPs) in 24 candidate genes (which were involved in the pathways implicated in the development or progression of IgAN) were selected to conduct a large case–control association study in 527 IgAN patients and 543 healthy controls. Traditional linear logistic regression analyses were used to detect single-locus associations in dominant, recessive and additive genetic models. Bonferroni correction was used to adjust the *P*-values for multiple testing. The gene–gene interaction effects of multiple SNPs were detected by multifactor-dimensionality reduction (MDR) method. After Bonferroni correction, no significant single-locus associations was observed between IgAN patients and controls (Pc > 0.05). The MDR analysis showed a potential interaction of C1GALT1-330G/T (rs1008898) and IL5RA31 + 197A/G (rs340833) on the susceptibility of IgAN ($P < 0.001$). Gene–gene interaction may have some influence on the susceptibility to IgA nephropathy. This finding proposed a potential gene–gene interactive model for future studies.
Journal of Human Genetics (2013) **58,** 40–46; doi:10.1038/jhg.2012.133; published online 29 November 2012

Keywords: gene–gene interactions; IgA nephropathy (IgAN); MDR (multifactor-dimensionality reduction); SNP (single-nucleotide polymorphism)

INTRODUCTION

IgA nephropathy (IgAN; MIM 161950) is one of the most common glomerulonephritis throughout the world, which is characterized by predominant IgA deposition in the glomerular mesangial area.[1,2] The pathogenesis of IgAN is not clear, but it is commonly thought to be connected with both genetic and environmental factors.[3,4] Genetic risk factors are considered to be involved in IgAN, according to the ethnic and geographic distributions, familial clustering, as well as the inter-individual variation in disease course and prognosis.[5–7]

In the past two decades, a number of single-locus genetic association studies have been performed for IgAN. The candidate genes involved in adaptive and innate immunity, glycosylation of IgA1 and renin–angiotensin system were investigated,[8] including human leukocyte antigen,[9,10] megsin,[11,12] uteroglobin,[13]selectins[14] and several cytokines and inflammatory factors.[15–17] However, most of these studies suffer the limitations of small sample size and insufficient methodologies, and the results have not always been reproducible in independent samples.[18] This lack of reproducibility may be due to genetic heterogeneity among different ethnic groups, or may reflect inadequate statistical power of IgAN cohorts.

In addition, individual single-nucleotide polymorphism (SNP) may not exhibit large independent increments in disease risk. The emerging concept suggested that multiple genes may interact with each other in complex ways to induce the occurrence and phenotypic variability of common complex diseases. Gene–gene interactions may be of particular importance in complex diseases.[19] Comparing to a single candidate gene approach, the analysis of multiple candidate gene variants and their interactions might present a more powerful approach to study complex diseases.[20]As for IgAN, till now, there was only one study using the method of gene–gene interaction (FAMHAP and DBMA), which found the interaction between variants of two glycosyltransferase genes (C1GALT1 and ST6GALNAC2) in IgAN.[21]

MDR (multifactor-dimensionality reduction), as a novel computational algorithm for testing gene–gene interactions,[22] has been used in many common complex multifactorial diseases, such as breast cancer, lupus nephritis and rheumatoid arthritis,[22–24] which is thought to be the reliable method to study the gene–gene interaction.

In our study, to understand the genetic effect on IgAN in Chinese Han population, we surveyed the SNPs of 24 candidate

[1]Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China; [2]Key Laboratory of Nephrology, Ministry of Health, Guangzhou, Guangdong, China; [3]Department of Nephrology, Sichuan Provincial People's Hospital, Chengdu, Sichuan, China and [4]Department of Biochemistry, School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China
Correspondence: Dr M Li, Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, 58th, Zhongshan Road II, Guangzhou, Guangdong 510080, China.
E-mail: li.ming1122@yahoo.com
Received 29 June 2012; revised 8 October 2012; accepted 29 October 2012; published online 29 November 2012

genes that involved in the pathways implicated in the development and progress of IgAN, in a large IgAN cohort comprising of 527 cases and 543 age- and gender-matched controls, investigated the interactive effect of these genes on IgAN by using the MDR method. Our results indicated that the variants in C1GALT1 and IL5RA had a potential genetic interactive effect in terms of predisposition of IgAN.

MATERIALS AND METHODS

Study population

Patients were recruited from the Department of Nephrology, The First Affiliated Hospital of Sun Yat-sen University in Guangzhou, China. A total of 527 patients with primary IgAN were enrolled in this study. IgAN was diagnosed by renal biopsy following the criteria of the World Health Organization. Patients with evidence of systemic diseases such as diabetes, chronic liver disease and systemic lupus erythematosus were excluded. Pregnant women were also excluded. The 543 healthy gender- and age-matched unrelated subjects with no history of renal disease or hypertension were recruited as controls on voluntary basis from Guangdong province. The study was approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) and all participants gave an written informed consent to participate in our study.

Selection of candidate genes and SNPs

Totally 73 SNPs in 24 candidate genes with potential involvement in the development and progression of IgA nephropathy were selected. In all, 48 tagSNPs of them were chosen to perform the multiplex genotyping with SNP stream Ultra-High Throughput machine (Beckman Coulter, Fulleron, CA, USA), according to the primer compatibility of multiplex PCR.

DNA extraction and genotyping

Venous blood samples were obtained from all subjects. The genomic DNA was extracted using QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. SNP stream Ultra-High Throughput machine (Beckman Coulter) was used to genotype 48 SNPs simultaneously. Sequences surrounding the SNPs were obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) and submitted to Autoprimer.com (Beckman Coulter). For each SNP, three primers were designed, two for PCR amplification and internal primer with a 5′ DNA sequence tag. Pairs of primers were used to initiate PCR amplification. The internal primers were used to initiate a sequencing reaction that added one labeled base for the alternative nucleotides of each SNP to have distinct labels. The labeled products were separated on an SNP-IT plate consisting of 384 mini-arrays with 48-spots each (Beckman Coulter).

Association testing

The Hardy–Weinberg equilibrium was tested by χ^2 statistic. Only SNPs that passed the HWE test in controls were further analyzed. The allele and genotype frequencies in patients and controls were also compared by χ^2-test. Logistic regression analyses were performed to detect single-locus associations with dominant, recessive and additive genetic models. Corrected *P*-values (P_c) were calculated using the Bonferroni inequality method. All of these analyses were conducted with R software, version 2.10.1 (http://www.R-project.org/).

Interaction analysis

The evaluation of gene–gene interactions was performed using the method of MDR, as outlined by Moore,[25] which has been implemented in the open-source MDR software package, version 0.6.1 (Hanover, NH, USA), available at http://www.epistasis.org.

Table 1 Characteristics of the study population

Variable	*IgAN*	*Healthy subjects*
Age, years	31.18 ± 10.80	33.82 ± 13.26
Gender		
Male	235 (44.59)	240 (44.19)
Female	292 (55.41)	303 (55.81)
Blood pressure		
SBP (mmHg)	127.91 ± 19.63	119.26 ± 14.23
DBP (mmHg)	80.85 ± 13.55	78.59 ± 12.45
Scr (μmol l^{-1})	120.52 ± 38.89	88.76 ± 23.35
Proteinuria (g per 24 h)	1.05 ± 1.23 g per 24 h	Negative
Gross hematuria		*Negative*
Yes	101 (20.69)	
No	387 (79.31)	
Cresent formation		*Negative*
Yes	140 (28.68)	
No	348 (71.32)	

Abbreviations: DBP, diastolic blood pressure; GFR, glomerular filtration rate; IgAN, IgA nephropathy; SBP, systolic blood pressure; Scr, serum creatinine.
Figures in parentheses are percentages.

RESULTS

Allele and genotype frequencies in IgAN patients and controls

The demographic information of recruited cohort of IgAN patients and healthy controls were shown in Table 1. There were no significant differences between the cases and healthy controls with regard to mean age or gender distribution. Totally, 31 SNPs were successfully genotyped, and detailed information of the SNPs was shown in Table 2. All of them were in Hardy–Weinberg equilibrium in each of the groups. Table 3 summarized the allele and genotype frequencies in IgAN patients and controls, respectively. There were three SNPs that exhibited suggestive association with IgAN by the genotypic test, including tumor necrosis factor (TNF)-α (rs1800629, $P = 0.014$), ST6GALNAC2 (rs9890158, $P = 0.031$) and C1GALT1 (rs1008898, $P = 0.027$). However, none of the associations was found after using the Bonferroni's inequality method.

Association of SNPs with inheritance models in IgAN

We also tested for association according to different inheritance models using logistic regression (Table 4). We found that the G allele at rs9890158 (ST6GALNAC2) was significantly associated with IgAN under an additive model ($P = 0.008$) and the T allele at rs1008898 (C1GALT1) was significantly associated under a recessive model ($P = 0.008$). However, none of the associations could withstand a multiple comparison adjustment using the Bonferroni's inequality method.

Gene–gene interaction study (MDR) of IgAN

We explored whether SNP–SNP interactions among our candidate loci contribute to the susceptibility of IgAN in this cohort. Table 5 summarized the results of the MDR analysis. The best model in each dimension was shown along with its TA, CVC consistency and significance *P*-value, as determined by permutation testing. The two-way combination of C1GALT1 (rs1008898 G/T) and IL5RA (rs340833 A/G) was significantly associated with IgAN ($P < 0.001$). In this model, the combination of C1GALT1-GT, IL5RA-GG and coexistence of C1GALT1-TT, IL5RA-GG or AG genotypes were the

Table 2 Detailed information of the 31 successfully genotyped SNPs

Gene	*Description*	*Chromosome*	*dbSNP*	*Allele*	*References*
Megsin	Serpin peptidase inhibitor, clade B (ovalbumin), member 7	18q21.33	rs1055901	C/T	Li *et al.*[11]
			rs1055902	C/T	Li *et al.*[11]
MCP-1	Monocyte chemoattractant protein-1	17q11.2–q12	rs1024611	A/G	Mori *et al.*[27]
CCR5	Chemokine receptor type 5	3p21.31	rs2857657	G/C	Berthoux *et al.*[63]
			rs4586	T/C	Berthoux *et al.*[63]
			rs13900	C/T	Berthoux *et al.*[63]
ET-1	Endothelin-1	6p24.1	rs1800541	T/G	Maixnerova *et al.*[28]
EDNRA	Endothelin receptor type A	4q31.23	rs5335	G/C	Reiterova *et al.*[29]
UG	Uteroglobin	11q12.3–q13.1	rs3741240	A/G	Narita *et al.*[13]
TGF-β1	Transforming growth factor-β1	19q13.1	rs1800469	C/T	Carturan *et al.*[15]
SELE	Selectin-E	1q24–25	rs5368	C/T	Takei *et al.*[14,31]
SELL	Selectin-L	1q24–25	rs4987130	C/T	Takei *et al.*[14,31]
			rs2205849	G/A	Takei *et al.*[14,31]
SELP	Selectin-P	1q24–25	rs6131	G/A	Takei *et al.*[14,31]
			rs6128	G/A	Takei *et al.*[14,31]
IL-10	Interleukin-10	1q31–q32	rs1800896	G/A	Bantis *et al.*[16]
NOS3	Nitric oxide synthase	7q36	rs1799983	G/T	Burg *et al.*[41]
TNF-α	Tumor necrosis factor-α	6p21.3	rs1800629	G/A	Tuglular *et al.*[32]
			rs361525	G/A	Tuglular *et al.*[32]
CD89	IgA Fc Receptor	19q13.2–q13.4	rs3816051	C/T	Narita *et al.*[33]
CD14	CD14	5q31.1	rs2569190	C/T	Yoon *et al.*[35]
AGT	Angiotensin	1q42–q43	rs699	M/T	Bantis *et al.*[34]
IL1A	Interleukin 1, alpha	2q14	rs1800587	C/T	Shu *et al.*[36]
ADD1	Adducin1	4p16.3	rs4961	G/T	Narita *et al.*[38]
ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	17q25.1	rs2304921	G/A	Li *et al.*[37]
			rs9890158	G/A	Li *et al.*[37]
NEU1	Sialidase 1 (lysosomal sialidase)	6p21.3	rs13118	T/A	Li *et al.*[37]
C1GALT1	Core 1 synthase, glycoprotein- N-acetylgalactosamine 3-beta-galactosyltransferase, 1	7p14–p13	rs1008898	G/T	Li *et al.*[64]
STAT4	Signal transducer and activator of transcription 4	2q32.2–q32.3	rs8179673	G/A	Park *et al.*[30]
PTPN22	protein tyrosine Phosphatase22	1p13.3–p13.1	rs2488457	C/G	Bottini *et al.*[65]
IL5RA	Interleukin 5 receptor, alpha	3p26–p24	rs340833	A/G	Liu *et al.*[26]

only high-risk combinations (Figure 1). A higher-level interaction models (third- and fourth-level interaction terms) did not reveal other significant patterns of genetic interactions.

DISCUSSION

In this study, we selected SNPs of 24 candidate genes, which were related to the development and progression of IgAN, to perform an association study in southern Chinese Han population. Most of these selected genes have been studied previously in different populations (Caucasian and Asian) in IgAN, and many of these genes were found having some association with the susceptibility and progression of IgAN.[11,13,16,26–38] However, in our study, we could not replicate any of the previously described association signals in the 24 high-priority candidate genes, although the relatively larger sample size (>1000 individuals) were used in our study comparing to the previous studies, which tended to produce more reliable results owing to increasing the power to detect authentic associations and decreasing the rates of type I errors.[39,40] In addition, through extensive literature review, the associations of some candidate genes have not always been reproducible in previous studies. For example, polymorphisms in the selectin gene cluster were found to be associated with IgAN in Japanese patients, but not in Caucasians from Canada, France and Finland.[26] This lack of reproducibility may be due to genetic heterogeneity among different ethnic people, or may reflect inadequate statistical power of IgAN cohorts.

The analyses of alternative inheritance models in our study detected a suggestive association of rs9890158 in ST6GALNAC2 under an additive model (risk allele: G, $P=0.008$) and rs1008898 in C1GALT1 under a recessive model (risk allele: T, $P=0.008$). Recently published association study in Han Chinese population (including 670 IgAN patients and 494 healthy controls) identified the association of ST6GALNAC2 and C1GALT1 genes with IgAN.[37,41] Our results also showed marginal association of these two genes. However, considering a large number of SNPs and models tested, our findings were not robust to Bonferroni's correction for multiple testing. The reason for the failure of replication here may be due to the potential genetic heterogeneity between different ethnic population as well as different geographic populations of China. Further studies will be needed to investigate this possibility.

As we know, IgAN is the multifactorial complex disease where genetic and environmental factors contributed equally to this disease. It is thought that there were multiple genes involved in the genetics of IgAN, where some of the candidate genes may have the strong effects as 'susceptibility loci' for development and progression, and some genes may have modest effects as 'modifier genes' for endophenotypic expression.[3] Thus, gene–gene interaction perhaps has significant effect on the genetic background of IgAN. Till

Table 3 Allele and genotype frequencies in IgAN patients and controls

Gene	SNP	Allele A minor/B	IgAN (n = 527)		Controls (n = 543)		Allelic test P	Genotypic test P
			H-W p	A count %	H-W p	A count %		
Megsin	rs1055901	T/C	0.701	23.62	0.611	21.45	0.137	0.396
	rs1055902	C/T	0.744	23.71	0.611	21.45	0.129	0.381
MCP-1	rs1024611	A/G	0.302	46.48	0.611	44.19	0.295	0.516
CCR5	rs2857657	G/C	0.218	7.49	0.088	6.81	0.283	0.439
	rs4586	T/C	0.265	46.58	0.737	44.19	0.284	0.497
	rs13900	T/C	0.285	46.48	0.611	44.19	0.989	0.533
Endothelin-1	rs1800541	T/G	0.158	21.06	0.819	22	0.275	0.602
EDNRA	rs5335	G/C	0.335	43.73	0.571	46.86	0.094	0.193
Uteroglobin	rs3741240	A/G	0.121	35.57	0.238	37.01	0.239	0.708
TGF-β1	rs1800469	T/C	0.211	41.08	0.558	44.56	0.944	0.226
E-Selection	rs5368	T/C	0.117	22.77	0.352	22.46	0.256	0.197
L-Selection	rs4987310	T/C	0.107	27.32	0.993	27.53	0.328	0.496
	rs2205849	G/A	0.259	27.6	0.903	27.62	0.279	0.667
P-selection	rs6131	A/G	0.211	21.72	0.014	22.46	0.759	0.462
	rs6128	G/A	0.086	31.3	0.436	30.75	0.259	0.198
IL-10	rs1800896	G/A	0.773	5.59	0.222	4.97	0.758	0.232
NOS3	rs1799983	T/G	0.349	10.72	0.511	9.94	0.153	0.819
TNF-α	rs1800629	A/G	0.172	9.29	0.063	7.73	0.167	0.014[a]
	rs361525	A/G	0.546	2.56	0.536	2.94	0.272	0.969
CD89	rs3816051	C/T	0.458	35.95	0.205	34.89	0.772	0.8
CD14	rs2569190	C/T	0.082	43.07	0.592	44.19	0.929	0.592
AGT	rs699	T/C	0.837	14.32	0.263	14.36	0.083	0.789
IL1A	rs1800587	T/C	0.407	6.54	0.232	7.45	0.155	0.672
ADD1	rs4961	T/G	0.082	43.92	0.197	45.3	0.491	0.081
ST6GALNAC2	rs2304921	A/G	0.409	13.18	0.435	13.07	0.228	0.981
	rs9890158	G/A	0.238	18.31	0.302	13.9	0.886	0.031[b]
NEU1	rs13118	A/T	0.01	1.51	0.092	2.02	0.137	0.63
C1GALT1	rs1008898	G/T	0.1	39.75	0.868	49.07	0.297	0.027[c]
STAT4	rs8179673	G/A	0.278	34.34	0.305	34.16	0.559	0.998
PTPN22	rs2488457	C/G	0.498	38.14	0.992	39.31	0.588	0.755
IL5RA	rs340833	A/G	0.368	24.57	0.865	30.47	0.087	0.08

Abbreviations: IgAN, IgA nephropathy; SNP, single-nucleotide polymorphism.
[a] $P_{corrected} = 0.014 \times 31 = 0.434$.
[b] $P_{corrected} = 0.031 \times 31 = 0.961$.
[c] $P_{corrected} = 0.027 \times 31 = 0.837$.

now, there was only one study about the gene–gene interaction of IgAN,[21] which also have the limitation of only focusing on the IgA1 glycosylation in IgAN. So it is important for us to perform the study of the gene–gene interaction in IgAN by checking relatively more candidate genes.

To explore this possibility we utilized the MDR approach,[22] a novel computational algorithm for testing gene–gene interactions. MDR as a method for reducing the dimensionality of multi-locus information has reasonable power to identify interactions among two or more loci in relatively small samples and can improve the identification of polymorphism combinations associated with disease risk.[20,42] This method has been used in some common complex diseases and has discovered that the genes jointly contribute to the susceptibility of these diseases.[22–24]

In our study, when pairwise interactions were analyzed, a higher risk for susceptibility of IgAN was observed for the simultaneous presence of C1GALT1-GT and IL5RA-GG genotypes, as well as for C1GALT1-TT and IL5RA-GG or AG genotypes. Three- and four-way interaction models did not lead to more specific genetic patterns of interaction. As outlined by Moore and Williams,[43] it is difficult to make inferences about the biologic significance of a statistical model of interactions; nonetheless, these two genes (C1GALT1 and IL5RA) have more biologic plausibility with IgAN, which indicated that they were potentially important for the susceptibility of IgAN.

IL5RA encodes the α chain of the interleukin 5 receptor, and signaling through this receptor may be related to modulating Th1/Th2 polarity and immune regulation.[44,45] Also, a study found that IL-5RA expressing B-1a B cells were involved in innate mucosal IgA antibody responses.[46] Although there were only one study that showed the association with IL-5RA and IgAN, the polymorphisms of this gene were found having association with some auto-immune diseases, such as asthma[47,48] and atopic dermatitis,[49] suggesting that this gene may have some influence on the pathogenesis of immune response for IgAN.

C1GALT1 (core 1 synthase, glycoprotein-*N*-acetylgalactosamine 3-β-galactosyltransferase) were the coding genes for the key glycosyltransferase (β1,3-galactosyltransferase) in IgA1 O-glycosylation process, which mediate the addition of galactose onto GalNAc residues of IgA1.[50] Accumulating evidence suggests that aberrant glycosylation of the hinge region of IgA1 has the influence on the pathogenesis of IgAN,[51,52] including glomerular deposition[53,54] and injury to renal intrinsic cells.[55,56] Till now, there were several studies that found the polymorphism of C1GALT1 related to IgAN, in both the Caucasian and Asia population.[21,57,58]

Table 4 Analysis of IgAN risk according to the mode of inheritance of the alleles

Gene	SNP	Allele A/B	IgAN (n = 527) AA	IgAN AB	IgAN BB	Control (n = 543) AA	Control AB	Control BB	P-value Dominant	P-value Recessive	P-value Additive
Megsin	rs1055901	T/C	31	187	309	23	187	333	0.218	0.368	0.229
	rs1055902	C/T	31	188	308	23	187	333	0.336	0.218	0.209
MCP-1	rs1024611	A/G	120	250	157	109	262	172	0.474	0.274	0.277
CCR5	rs2857657	G/C	1	77	449	0	74	469	0.233	0.582	0.527
	rs4586	T/C	121	249	157	108	264	171	0.243	0.56	0.296
	rs13900	T/C	120	250	157	109	262	172	0.504	0.282	0.295
Endothelin-1	rs1800541	T/G	18	186	323	26	187	330	0.892	0.318	0.646
EDNRA	rs5335	G/C	106	249	172	116	277	150	0.07	0.625	0.146
Uteroglobin	rs3741240	A/G	59	257	211	68	266	209	0.586	0.447	0.439
TGF-β1	rs1800469	T/C	82	269	176	104	276	163	0.251	0.114	0.098
E-Selection	rs5368	T/C	21	198	308	31	182	330	0.185	0.415	0.844
L-Selection	rs4987310	T/C	32	224	271	41	217	285	0.332	0.704	0.93
	rs2205849	G/A	35	221	271	42	216	285	0.488	0.727	0.993
P-selection	rs6131	A/G	20	189	318	16	212	315	0.438	0.441	0.665
	rs6128	G/A	43	244	240	55	224	264	0.263	0.313	0.779
IL-10	rs1800896	G/A	2	55	470	0	54	489	0.092	0.64	0.513
NOS3	rs1799983	T/G	4	105	418	4	100	439	0.966	0.53	0.548
TNF-α	rs1800629	A/G	8	82	443	2	80	461	0.177	0.017	0.345
	rs361525	A/G	0	27	500	2	28	513	0.969	0.553	0.969
CD89	rs3816051	C/T	64	251	212	59	261	223	0.76	0.518	0.59
CD14	rs2569190	C/T	88	278	161	103	274	166	0.994	0.331	0.59
AGT	rs699	T/C	10	131	386	8	140	395	0.583	0.773	0.907
IL1A	rs1800587	T/C	2	65	460	2	77	464	0.983	0.377	0.389
ADD1	rs4961	T/G	111	241	175	104	284	155	0.417	0.09	0.517
ST6GALNAC2	rs2304921	A/G	7	125	395	8	126	409	0.847	0.957	0.848
	rs9890158	G/A	14	165	348	7	137	399	0.014	0.135	0.008[a]
NEU1	rs13118	A/T	1	14	512	1	20	522	0.353	0.983	0.392
C1GALT1	rs1008898	G/T	89	241	197	132	269	142	0.218	0.008[b]	0.019
STAT4	rs8179673	G/A	57	248	222	58	255	230	0.985	0.959	0.976
PTPN22	rs2488457	C/G	73	256	198	84	259	200	0.454	0.802	0.573
IL5RA	rs34833	A/G	31	197	299	49	233	261	0.766	0.095	0.014

Abbreviations: IgAN, IgA nephropathy; IL, interleukin; SNP, single-nucleotide polymorphism; TNF, tumor necrosis factor.
[a] $P_{corrected} = 0.008 \times 31 = 0.248$.
[b] $P_{corrected} = 0.008 \times 31 = 0.248$.

Table 5 Results of multifactor-dimensionality reduction analysis of interactions between SNPs–SNPs contributing to susceptibility IgAN

	Best combination in each dimension	TA	CVC	P-value
IgAN *vs* control	C1GALT1-330G/T	0.558	10/10	0.01
	C1GALT1-330G/T, IL5RA-A/G	0.576	10/10	0.001
	C1GALT1-330G/T, STAT4-A/G, TGF-β1-509C/T	0.609	3/10	0.054
	C1GALT1-330G/T, UG38G/A, ADD1G460/W, PTNP22-1123G/C	0.644	3/10	0.623

Abbreviations: CVC, cross-validation consistency; IgAN, IgA nephropathy; SNP, single-nucleotide polymorphism; TA, testing accuracy.

In our study, although the selected candidate genes have no association with IgAN individually, but after using the MDR method, we can find that the interaction of these two genes (IL5RA and C1GALT1) contribute to the susceptibility of IgAN, which from another aspect demonstrated that IgAN is a complex disease, where the genetic factor contributed to this disease. The genes involved in the disease may have some minor effect, but the interaction of these genes may have more influence on the genetics of IgAN. Also, these two genes indicated that immune response and glycosylation of IgA1 may have some effect on IgAN.

The limitation of our study is that we only selected limited SNPs of some candidate genes from the pathogenesis of IgAN, which may lose some information of other potential susceptibility genes. As of now several GWAS (genome-wide association study) for IgAN have emerged[59–62] and we can comprehensively and accurately get more information about the susceptibility genes for IgAN. More candidate genes will be selected to study the gene–gene interaction through the comprehensive aspect from the whole genome level, where we can provide more solid information of the genetic background of IgAN. This will be the future work for us to do.

In conclusion, using MDR method, we explored the genetic interactive effect of variants in IL5RA and C1GALT1 on IgAN in a large Southern Chinese Han population. Our results suggested that variants in IL5RA and C1GALT1 had interaction on the genetic susceptibility of IgAN, which also demonstrated that IgAN may be associated with specific genetic polymorphisms involved in immune regulation and IgA1 O-glycosylation.

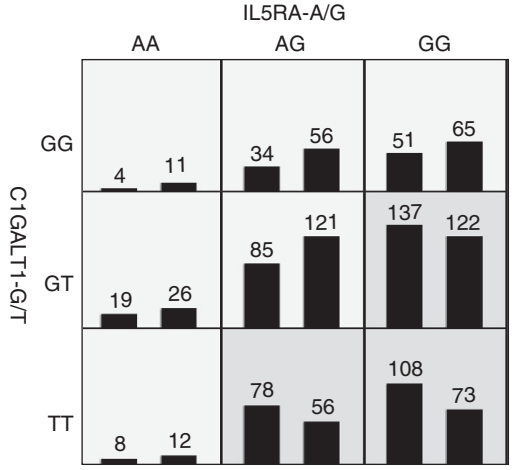

Figure 1 IL5RA-A/G and C1GALT1-G/T combinations associated with IgAN. Each multi-locus genotype combination is considered high risk when the ratio of cases to controls exceeds a threshold T, equal to the ratio of cases to controls in each population; otherwise, the cell is classified as low risk. High-risk combinations are depicted as darkly shaded cells, low-risk combinations as lightly shaded cells. For each cell, the left bar indicates the cases, the right bar the controls. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to all the subjects and healthy volunteers who participated in this work. This work was funded by Major State Basic Research Development Program of China (973 program) (2012CB517700-2012CB517706), Key Program of National Natural Science Foundation of China (81130012), National Natural Science Foundation of China for Distinguished Young Scholars (30925019), Young Scientists Fund of National Natural Science Foundation of China (81200489) and Natural Science Foundation of Guangdong Province, China (S2012040006453).

1 Barratt, J. & Feehally, J. IgA nephropathy. *J. Am. Soc. Nephrol.* **16,** 2088–2097 (2005).
2 Donadio, J. V. & Grande, J. P. IgA nephropathy. *N. Engl. J. Med.* **347,** 738–748 (2002).
3 Hsu, S. I. Racial and genetic factors in IgA nephropathy. *Semin. Nephrol.* **28,** 48–57 (2008).
4 Hsu, S. I., Ramirez, S. B., Winn, M. P., Bonventre, J. V. & Owen, W. F. Evidence for genetic factors in the development and progression of IgA nephropathy. *Kidney Int.* **57,** 1818–1835 (2000).
5 Beerman, I., Novak, J., Wyatt, R. J., Julian, B. A. & Gharavi, A. G. The genetics of IgA nephropathy. *Nat. Clin. Pract. Nephrol.* **3,** 325–338 (2007).
6 Izzi, C., Sanna-Cherchi, S., Prati, E., Belleri, R., Remedio, A., Tardanico, R. *et al.* Familial aggregation of primary glomerulonephritis in an Italian population isolate: Valtrompia study. *Kidney Int.* **69,** 1033–1040 (2006).
7 Scolari, F. Inherited forms of IgA nephropathy. *J. Nephrol* **16,** 317–320 (2003).
8 Yu, H. H., Chu, K. H., Yang, Y. H., Lee, J. H., Wang, L. C., Lin, Y. T. *et al.* Genetics and immunopathogenesis of IgA nephropathy. *Clin. Rev. Allergy. Immunol.* **41,** 198–213 (2011).
9 Cao, H. X., Li, M., Nie, J., Wang, W., Zhou, S. F. & Yu, X. Q. Human leukocyte antigen DRB1 alleles predict risk and disease progression of immunoglobulin A nephropathy in Han Chinese. *Am. J. Nephrol.* **28,** 684–691 (2008).
10 Fennessy, M., Hitman, G. A., Moore, R. H., Metcalfe, K., Medcraft, J., Sinico, R. A. *et al.* HLA-DQ gene polymorphism in primary IgA nephropathy in three European populations. *Kidney Int.* **49,** 477–480 (1996).
11 Li, Y. J., Du, Y., Li, C. X., Guo, H., Leung, J. C., Lam, M. F. *et al.* Family-based association study showing that immunoglobulin A nephropathy is associated with the polymorphisms 2093C and 2180T in the 3' untranslated region of the Megsin gene. *J. Am. Soc. Nephrol* **15,** 1739–1743 (2004).
12 Lim, C. S., Kim, S. M., Oh, Y. K., Joo, K. W., Kim, Y. S., Han, J. S. *et al.* Megsin 2093T-2180C haplotype at the 3' untranslated region is associated with poor renal survival in Korean IgA nephropathy patients. *Clin. Nephrol.* **70,** 101–109 (2008).
13 Narita, I., Saito, N., Goto, S., Jin, S., Omori, K., Sakatsume, M. *et al.* Role of uteroglobin G38A polymorphism in the progression of IgA nephropathy in Japanese patients. *Kidney Int.* **61,** 1853–1858 (2002).
14 Takei, T., Iida, A., Nitta, K., Ohnishi, Y., Yamada, R., Maeda, S. *et al.* Association between single-nucleotide polymorphisms in selectin genes and immunoglobulin A nephropathy. *Am. J. Hum. Genet.* **70,** 781–786 (2002).
15 Carturan, S., Roccatello, D., Menegatti, E., Di Simone, D., Davit, A., Piazza, A. *et al.* Association between transforming growth factor beta1 gene polymorphisms and IgA nephropathy. *J. Nephrol* **17,** 786–793 (2004).
16 Bantis, C., Heering, P. J., Aker, S., Klein-Vehne, N., Grabensee, B. & Ivens, K. Association of interleukin-10 gene G-1082A polymorphism with the progression of primary glomerulonephritis. *Kidney Int.* **66,** 288–294 (2004).
17 Syrjanen, J., Hurme, M., Lehtimaki, T., Mustonen, J. & Pasternack, A. Polymorphism of the cytokine genes and IgA nephropathy. *Kidney Int.* **61,** 1079–1085 (2002).
18 Kiryluk, K., Julian, B. A., Wyatt, R. J., Scolari, F., Zhang, H., Novak, J. *et al.* Genetic studies of IgA nephropathy: past, present, and future. *Pediatr. Nephrol* **25,** 2257–2268 (2010).
19 Ordovas, J. M., Robertson, R. & Cleirigh, E. N. Gene-gene and gene-environment interactions defining lipid-related traits. *Curr. Opin. Lipidol.* **22,** 129–136 (2011).
20 Moore, J. H. Detecting, characterizing, and interpreting nonlinear gene-gene interactions using multifactor dimensionality reduction. *Adv. Genet.* **72,** 101–116 (2010).
21 Zhu, L., Tang, W., Li, G., Lv, J., Ding, J., Yu, L. *et al.* Interaction between variants of two glycosyltransferase genes in IgA nephropathy. *Kidney Int.* **76,** 190–198 (2009).
22 Ritchie, M. D., Hahn, L. W., Roodi, N., Bailey, L. R., Dupont, W. D., Parl, F. F. *et al.* Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am. J. Hum. Genet.* **69,** 138–147 (2001).
23 Gong, R., Liu, Z. & Li, L. Epistatic effect of plasminogen activator inhibitor 1 and beta-fibrinogen genes on risk of glomerular microthrombosis in lupus nephritis: interaction with environmental/clinical factors. *Arthritis. Rheum* **56,** 1608–1617 (2007).
24 Julia, A., Moore, J., Miquel, L., Bailey, L. R., Dupont, W. D., Parl, F. F. *et al.* Identification of a two-loci epistatic interaction associated with susceptibility to rheumatoid arthritis through reverse engineering and multifactor dimensionality reduction. *Genomics* **90,** 6–13 (2007).
25 Moore, J. H. Computational analysis of gene-gene interactions using multifactor dimensionality reduction. *Expert. Rev. Mol. Diagn.* **4,** 795–803 (2004).
26 Liu, X. Q., Paterson, A. D., He, St N., George-Hyslop, P., Rauta, V., Gronhagen-Riska, C. *et al.* IL5RA and TNFRSF6B gene variants are associated with sporadic IgA nephropathy. *J. Am. Soc. Nephrol.* **19,** 1025–1033 (2008).
27 Mori, H., Kaneko, Y., Narita, I., Goto, S., Saito, N., Kondo, D. *et al.* Monocyte chemoattractant protein-1 A-2518G gene polymorphism and renal survival of Japanese patients with immunoglobulin A nephropathy. *Clin. Exp. Nephrol.* **9,** 297–303 (2005).
28 Maixnerova, D., Merta, M., Reiterova, J., Stekrová, J., Rysavá, R., Obeidová, H. *et al.* The influence of three endothelin-1 polymorphisms on the progression of IgA nephropathy. *Folia. Biol. (Praha)* **53,** 27–32 (2007).
29 Reiterova, J., Merta, M., Stekrova, J., Maixnerová, D., Obeidová, H., Kebrdlová, V. *et al.* The influence of endothelin-A receptor gene polymorphism on the progression of autosomal dominant polycystic kidney disease and IgA nephropathy. *Folia. Biol. (Praha)* **53,** 134–137 (2007).
30 Park, B. L., Cheong, H. S., Kim, L. H., Choi, Y. H., Namgoong, S., Park, H. S. *et al.* Association analysis of signal transducer and activator of transcription 4 (STAT4) polymorphisms with asthma. *J. Hum. Genet.* **50,** 133–138 (2005).
31 Takei, T., Hiraoka, M., Nitta, K., Uchida, K., Deushi, M., Yu, T. *et al.* Functional impact of IgA nephropathy-associated selectin gene haplotype on leukocyte-endothelial interaction. *Immunogenetics* **58,** 355–361 (2006).
32 Tuglular, S., Berthoux, P. & Berthoux, F. Polymorphisms of the tumour necrosis factor alpha gene at position -308 and TNFd microsatellite in primary IgA nephropathy. *Nephrol. Dial. Transplant* **18,** 724–731 (2003).
33 Narita, I., Goto, S., Saito, N., Sakatsume, M., Jin, S., Omori, K. *et al.* Genetic polymorphisms in the promoter and 5' UTR region of the Fc alpha receptor (CD89) are not associated with a risk of IgA nephropathy. *J. Hum. Genet* **46,** 694–698 (2001).
34 Bantis, C., Ivens, K., Kreusser, W., Koch, M., Klein-Vehne, N., Grabensee, B. *et al.* Influence of genetic polymorphisms of the renin-angiotensin system on IgA nephropathy. *Am. J. Nephrol.* **24,** 258–267 (2004).
35 Yoon, H. J., Shin, J. H., Yang, S. H., Chae, D. W., Kim, H., Lee, D. S. *et al.* Association of the CD14 gene -159C polymorphism with progression of IgA nephropathy. *J. Med. Genet.* **40,** 104–108 (2003).
36 Shu, K. H., Lee, S. H., Cheng, C. H., Wu, M. J., Lian, J. D. *et al.* Impact of interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphism on IgA nephropathy. *Kidney Int.* **58,** 783–789 (2000).
37 Li, G. S., Zhu, L., Zhang, H., Lv, J. C., Ding, J. X., Zhao, M. H. *et al.* Variants of the ST6GALNAC2 promoter influence transcriptional activity and contribute to genetic susceptibility to IgA nephropathy. *Hum. Mutat.* **28,** 950–957 (2007).
38 Narita, I., Goto, S., Saito, N., Song, J., Ajiro, J., Sato, F. *et al.* Interaction between ACE and ADD1 gene polymorphisms in the progression of IgA nephropathy in Japanese patients. *Hypertension* **42,** 304–309 (2003).
39 Ioannidis, J. P., Trikalinos, T. A., Ntzani, E. E., Contopoulos-Ioannidis, D. G. *et al.* Genetic associations in large versus small studies: an empirical assessment. *Lancet* **361,** 567–571 (2003).

40 Gordon, D. & Finch, S. J. Factors affecting statistical power in the detection of genetic association. *J. Clin. Invest.* **115,** 1408–1418 (2005).
41 Burg, M., Menne, J., Ostendorf, T., Kliem, V. & Floege, J. Gene-polymorphisms of angiotensin converting enzyme and endothelial nitric oxide synthase in patients with primary glomerulonephritis. *Clin. Nephro* **48,** 205–211 (1997).
42 Motsinger, A. A. & Ritchie, M. D. Multifactor dimensionality reduction: an analysis strategy for modelling and detecting gene-gene interactions in human genetics and pharmacogenomics studies. *Hum. Genomics* **2,** 318–328 (2006).
43 Moore, J. H. & Williams, S. M. Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis. *Bioessays* **27,** 637–646 (2005).
44 Szabo, S. J., Sullivan, B. M., Peng, S. L. & Glimcher, L. H. Molecular mechanisms regulating Th1 immune responses. *Annu. Rev. Immunol.* **21,** 713–758 (2003).
45 Ansel, K. M., Djuretic, I., Tanasa, B. & Rao, A. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu. Rev. Immunol.* **24,** 607–656 (2006).
46 Kataoka, K., Fujihashi, K., Sekine, S., Fukuiwa, T., Kobayashi, R., Suzuki, H. *et al.* Nasal cholera toxin elicits IL-5 and IL-5 receptor alpha-chain expressing B-1a B cells for innate mucosal IgA antibody responses. *J. Immunol* **178,** 6058–6065 (2007).
47 Cheong, H. S., Kim, L. H., Park, B. L., Choi, Y. H., Park, H. S., Hong, S. J. *et al.* Association analysis of interleukin 5 receptor alpha subunit (IL5RA) polymorphisms and asthma. *J. Hum. Genet.* **50,** 628–634 (2005).
48 Lee, J. H., Chang, H. S., Kim, J. H., Park, S. M., Lee, Y. M., Uh, S. T. *et al.* Genetic effect of CCR3 and IL5RA gene polymorphisms on eosinophilia in asthmatic patients. *J. Allergy. Clin. Immunol.* **120,** 1110–1117 (2007).
49 Namkung, J. H., Lee, J. E., Kim, E., Cho, H. J., Kim, S., Shin, E. S. *et al.* IL-5 and IL-5 receptor alpha polymorphisms are associated with atopic dermatitis in Koreans. *Allergy* **62,** 934–942 (2007).
50 Narita, I. & Gejyo, F. Pathogenetic significance of aberrant glycosylation of IgA1 in IgA nephropathy. *Clin. Exp. Nephrol.* **12,** 332–338 (2008).
51 Coppo, R. & Amore, A. Aberrant glycosylation in IgA nephropathy (IgAN). *Kidney Int.* **65,** 1544–1547 (2004).
52 Barratt, J. & Feehally, J. Primary IgA nephropathy: new insights into pathogenesis. *Semin. Nephrol.* **31,** 349–360 (2011).
53 Iwase, H., Tanaka, A., Hiki, Y., Kokubo, T., Sano, T., Ishii-Karakasa, I. *et al.* Aggregated human serum immunoglobulin A1 induced by neuraminidase treatment had a lower number of O-linked sugar chains on the hinge portion. *J. Chromatogr. B Biomed. Sci. Appl* **1,** 724–727 (1999).
54 Allen, A. C., Bailey, E. M., Brenchley, P. E., Buck, K. S., Barratt, J. & Feehally, J. Mesangial IgA1 in IgA nephropathy exhibits aberrant O-glycosylation: observations in three patients. *Kidney Int.* **60,** 969–973 (2001).
55 Amore, A., Conti, G., Cirina, P., Peruzzi, L., Alpa, M., Bussolino, F. *et al.* Aberrantly glycosylated IgA molecules downregulate the synthesis and secretion of vascular endothelial growth factor in human mesangial cells. *Am. J. Kidney Dis.* **36,** 1242–1252 (2000).
56 Amore, A., Cirina, P., Conti, G., Brusa, P., Peruzzi, L. & Coppo, R. Glycosylation of circulating IgA in patients with IgA nephropathy modulates proliferation and apoptosis of mesangial cells. *J. Am. Soc. Nephrol.* **12,** 1862–1871 (2001).
57 Bertinetto, F. E., Calafell, F., Roggero, S., Chidichimo, R., Garino, E., Marcuccio, C. *et al.* Search for genetic association between IgA nephropathy and candidate genes selected by function or by gene mapping at loci IGAN2 and IGAN3. *Nephrl. Dial. Transplant* **27,** 2328–2337 (2012).
58 Pirulli, D., Crovella, S., Ulivi, S., Zadro, C., Bertok, S., Rendine, S. *et al.* Genetic variant of C1GalT1 contributes to the susceptibility to IgA nephropathy. *J. Nephrol* **22,** 152–159 (2009).
59 Gharavi, A. G., Kiryluk, K., Choi, M., Li, Y., Hou, P., Xie, J. *et al.* Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nat. Genet.* **43,** 321–327 (2011).
60 Feehally, J., Farrall, M., Boland, A., Gale, D. P., Gut, I., Heath, S. *et al.* HLA has strongest association with IgA nephropathy in genome-wide analysis. *J. Am. Soc. Nephrol.* **21,** 1791–1797 (2010).
61 Yu, X. Q., Li, M., Zhang, H., Low, H. Q., Wei, X., Wang, J. Q. *et al.* A genome-wide association study in Han Chinese identifies multiple susceptibility loci for IgA nephropathy. *Nat. Genet.* **44,** 178–182 (2011).
62 Kiryluk, K., Li, Y., Sanna-Cherchi, S., Rohanizadegan, M., Suzuki, H., Eitner, F. *et al.* Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. *PLoS Genet.* **8,** e1002765 (2012).
63 Berthoux, F. C., Berthoux, P., Mariat, C., Thibaudin, L., Afiani, A., Linossier, M. T. *et al.* CC-chemokine receptor five gene polymorphism in primary IgA nephropathy: the 32 bp deletion allele is associated with late progression to end-stage renal failure with dialysis. *Kidney Int* **69,** 565–572 (2006).
64 Li, G. S., Zhang, H., Lv, J. C., Goto, S., Sakatsume, M., Gejyo, F. *et al.* Variants of C1GALT1 gene are associated with the genetic susceptibility to IgA nephropathy. *Kidney Int.* **71,** 379–381 (2007).
65 Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M. *et al.* A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat. Genet.* **36,** 337–338 (2004).