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Variation in *IGHMBP2* is not associated with IgA nephropathy in independent studies of UK Caucasian and Chinese Han patients

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Abstract

Background. IgA nephropathy is a major cause of end-stage renal disease worldwide. Its aetiology is poorly understood but there is good evidence for a major genetic component, although to date, no gene has been conclusively identified. We describe a new UK multicentre DNA collection assembled to investigate this. A Japanese genome-wide analysis recently reported that common genetic variation in *immunoglobulin mu-binding protein 2* (*IGHMBP2*) was associated with IgA nephropathy. We sought to replicate this using the new UK collection, and through an independent parallel analysis of a Han Chinese population.

Methods. In the UK collection, haplotype-tagging (tag) single-nucleotide polymorphisms (SNPs) and haplotypes were analysed in a case-control study (349 cases, 605 controls) and family-based analysis (162 complete and 23 partially complete family trios), which was performed using the transmission disequilibrium test. In parallel, 663 cases of IgA nephropathy and 663 controls from a Chinese population were analysed: coding and flanking regions of the gene were re-sequenced in a subset, and SNP and haplotype association analysis was performed in the whole collection using the identified tagSNPs and all the coding and exonic flanking SNPs.

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Results. Case-control studies in UK and Chinese populations, and family-based tests in the UK population provided no evidence for association between variation in *IGHMBP2* and IgA nephropathy. The A allele of SNP G34448A was not present in the UK collection. It was present but not associated with the disease in the Chinese population.
Conclusion. Variation in *IGHMBP2* does not confer significant susceptibility to IgA nephropathy in UK Caucasian or Chinese Han populations.

Keywords: Caucasian; Chinese; genetic association study; IgA nephropathy; *IGHMBP2*

Introduction

IgA nephropathy (IgAN) is the most common form of glomerulonephritis worldwide and is an important cause of end-stage renal disease (ESRD) [1]. Clinical presentation is usually with haematuria with a variable degree of proteinuria, and progression to ESRD occurs in approximately in 25–50% of those affected in the 20 years following diagnosis [2–4]. Pathologically, IgAN is characterized by deposition of polymeric immunoglobulin A in the renal mesangium, accompanied by proliferation of mesangial cells and expansion of matrix.

The aetiology of IgAN is poorly understood, but a number of observations suggest there is a significant genetic contribution to the disease. Firstly, incidence and prevalence are markedly different between ethnic groups. For example, higher rates are observed in East Asian populations which are unlikely to be explained by differences in ascertainment [5]. Secondly, limited studies of relatives of patients with IgAN have reported an increased incidence of urinary abnormalities, increased serum IgA levels and increased risk of renal disease compared with the general population [6]. Thirdly, in some families, IgAN occurs with autosomal dominant inheritance, sometimes with incomplete penetrance, or with marked familial aggregation. Linkage analysis of these families has implicated discrete genetic loci, but as yet, no responsible gene has been cloned [7–11]. In the great majority of cases, IgAN is not familial, which is consistent with a multifactorial complex disease model. It is hoped that identifying genetic variants that contribute to susceptibility and progression in IgAN will improve understanding of the pathogenesis of this important and poorly understood condition, and here, we report findings from a recently established UK multicentre family-based DNA collection, the Medical Research Council (MRC)/Kidney Research UK National DNA Bank for Glomerulonephritis (UKDBG).

Recently, genome-wide association studies have provided a powerful tool to identify genes involved in determining risk in polygenic diseases [12], however, interpretation of these results has proved difficult in the absence of replication, and the most robust association study in IgAN was of 465 Japanese patients and used a stepwise case-control genome-wide approach to identify an association between IgAN and the non-synonymous single-nucleotide polymorphism (SNP) G34448A (rs2275996) of *immunoglobulin mu-binding protein 2* (*IGHMBP2*, Entrez GeneID: 3508) [13]. The SNP is predicted to result in a glutamate to lysine amino acid change at residue 928 (E928K), and the A allele was associated with IgA nephropathy with an odds ratio of 1.85 (95% confidence interval 1.39–2.50, $P = 0.00003$) using a dominant model [13].

As yet, we are not aware of attempts to replicate the initial interesting finding from the Japanese population, and there is no functional data to suggest how genetic variation in *IGHMBP2* might result in altered susceptibility to IgAN. In view of this, our groups have investigated whether genetic variation in the *IGHMBP2* gene contributes to IgAN susceptibility in populations from the UK and China. In addition to the UKDBG, we used samples from three centres in southern China and Hong Kong [14]. The studies were performed independently using different genotyping platforms.

Materials and methods

Both studies were conducted in accordance with the Declaration of Helsinki and were approved by the appropriate research ethics committees. Informed consent was obtained from all participants.

UK study

Patients and controls. The MRC/Kidney Research UKDBG is a multicentre UK collection of DNA from patients with biopsy-proven glomerular diseases and their relatives. For this study of IgAN, DNA was collected through four UK centres (Glasgow, Leicester, London and Oxford), from 349 unrelated individuals (67% male) affected with biopsy-proven IgAN together with DNA from 343 of their parents and 75 of their siblings (comprising 162 complete parent-affected trios and 23 incomplete trios, Table 1). Inclusion required that a renal biopsy had been performed which showed diffuse mesangial IgA deposition. Non-Caucasians and individuals with evidence of liver disease or Henoch–Schonlein purpura were excluded. Baseline demographic and clinical details are shown in Table 2.

DNA samples from 605 control individuals (57% male; all of self-reported white ethnicity) were selected at random from the UK 1958 Birth Cohort (58BC, described previously [12]), and SNPs were genotyped using the same protocol as samples from the UKDBG collection.

SNPs. Eight haplotype–tagging SNPs (tagSNPs) were selected using HAPLOVIEW v3.32 and data from the International HapMap Database (www.hapmap.org). These tagSNPs were predicted to capture haplotypes occurring at >1% frequency at this locus in the Caucasian population. In addition, the SNP rs2275996/G34448A, previously associated with IgAN [13], was typed in all individuals. Genotyping was performed using KASPar, (Kbioscience, Essex, UK) which utilizes a competitive allele-specific PCR with 3′-5′ exonuclease-deleted DNA polymerase and a fluorescence resonance energy transfer quenching reporter oligo; details available from www.kbioscience.co.uk.

Statistical analysis. The chi-square test with 1 degree of freedom (df) was used to test for deviation from Hardy–Weinberg equilibrium (HWE). Tests for genotype differences between case and control populations were performed using a non-additive genotype test with 2 df, and allelic case-control association was tested using a trend test with 1 df as previously described [15]. HAPLOVIEW was used to perform haplotype association

Table 1. Composition of UK family trios

Composition	Number
Case and both parents	162
Case, mother and one or more siblings	15
Case, father and one or more siblings	4
Case and one or more siblings only	4

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Table 2. Clinical and demographic data (number or mean ± standard deviation)[a]

	Chinese study	UK Study	*P*
Gender (F/M)	382/281	114/234	<0.01
Age (year)	32.5 ± 10.3	33 ± 10.56	0.578
Duration of disease (month;)	19.2 ± 31.8	89.6 ± 82.2	<0.01
Systolic BP (mmHg)	121 ± 19	138.0 ± 24.0	<0.01
Diastolic BP (mmHg)	77 ± 13	82.0 ± 15.8	0.055
Documented haematuria (%)	82.4	78.1	0.110
Proteinuria at diagnosis (%)	76.5	49.3	<0.01
Proteinuria (g/d)	1.05 ± 1.71	n/a	n/a
Serum creatinine (umol/L)	111 ± 131	110 ± 122	0.818
Serum CHOL (mmol/L)	5.51 ± 2.68	n/a	n/a
Serum TG (mmol/L)	1.68 ± 3.93	n/a	n/a
IgA (mmol/L)	2.82 ± 1.10	3.72 ± 1.58	<0.01

[a]Clinical data was available for 70–100% for all categories except serum IgA, where data was recorded for 50% of UK cases. The two populations were matched for age and serum creatinine while gender, systolic blood pressure and serum IgA levels were significantly different between the two studies. The Chinese clinical data was gathered at time of biopsy in all cases.

and transmission disequilibrium tests (TDTs) [16]. FBAT v2.0.2c was used for family-based tests using incomplete trios [17]. No correction for multiple testing was performed. Population substructure was estimated using the program STRUCTURE [18].

Chinese study

Patient and control recruitment. Six hundred sixty-three patients were included in this study, recruited according to criteria described previously [14] from three renal units in southern China and Hong Kong (the First and the Third Affiliated Hospitals of Sun Yat-Sen University and Queen Mary Hospital, University of Hong Kong). IgAN was diagnosed by renal biopsy according to the World Health Organization criteria. All cases were unrelated. Baseline clinical data are shown in Table 2. In addition, 663 healthy southern Chinese volunteers, confirmed by detailed clinical and laboratory examination, matched for age, gender and ethnicity, were recruited as control subjects in parallel with the collection of the cases. Details of age and gender-matched data are shown in Table S1. All patients and controls are the Han ethnicity.

Re-sequencing of the IGHMBP2 gene and SNP identification. Genomic DNA was extracted with QiAamp Maxi kits (Qiagen, Germany). Seventy patients and 45 controls were randomly selected from the collection (the discovery population) for re-sequencing. Primers to amplify the coding regions including the intron–exon boundaries (extending 200 bp into intronic regions), and the 5′ and 3′ flanking regions of the gene (1 kb at each end) were designed using Primer Premier 5 (http://www.premierbiosoft.com/). The PCR products were sequenced in both directions using standard techniques (ABI PRISM 3730XL, Applied Biosystems, Foster City, CA). Results were analysed against sequences of *IGHMBP2* (Entrez GeneID: 3508) retrieved from the University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/).

Linkage disequilibrium test and haplotype construction. SNPs with minor allele frequencies ≥5% identified from the 45 healthy controls of the discovery population were selected for the linkage disequilibrium (LD) test (Figure 1A and Table 3). The SNP rs2275996 (originally reported as G34448A), previously reported to be associated with IgAN in the Japanese population [13], was also included in the analysis, although the minor allele frequency in the discovery control population was 0.04. The LD structure was determined by HAPLOVIEW v3.32 (http://www.broad.mit.edu/mpg/haploview) [19]. In addition, haplotype construction and tagSNP selection were performed with HAPLOVIEW using the 45 controls.

Genotyping and association analyses. SNPs were genotyped by real-time PCR using TaqMan assays or by direct sequencing of the PCR products. Primers and probes (Table S2) were designed using Primer Express 2.0 (Applied Biosystems) or Primer Premier 5.0 with the exception of SNPs g.68432276C>T and g.68457277G>C (rs645436) which were typed using TaqMan™ Assays on Demand™ and Assays by Design™, respectively. In the Taqman assay, 10 ng DNA was used in a total volume of 25 μl containing TaqMan Universal PCR Master Mix (Applied Biosystems). Amplification and post-PCR end point plate read were carried out according to the manufacturer's instructions using the Applied Biosystems 7500 System and the SDS software version 3.1 (Applied Biosystems) with the following thermal profile: 95°C for 15 minutes and 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Four negative and six positive controls were included on each plate. Samples which could not be scored using the 7500 System were re-genotyped by direct sequencing using an ABI PRISM 3730XL genetic analyser (Applied Biosystems). All samples were genotyped 'blind' with regard to the case/control status.

The seven tagSNPs, in coding and 100-bp flanking intronic regions, selected from both LD blocks, were genotyped, and separate association tests were performed on each of the tagSNPs in the case and control populations.

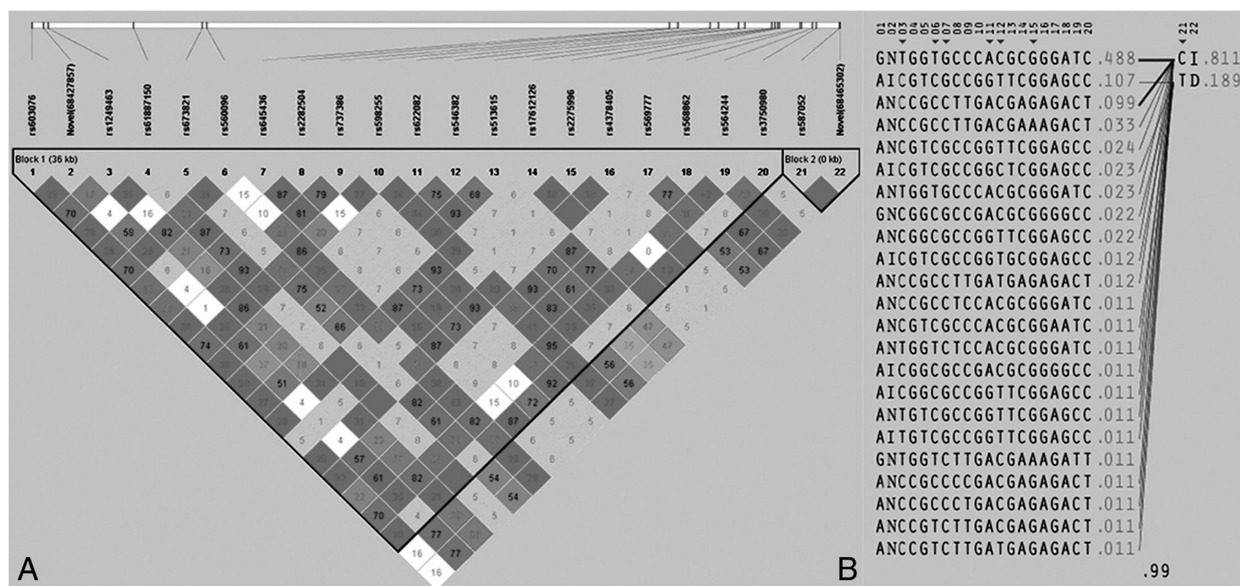

Fig. 1. (**A**) LD in 22 SNPs of *IGHMBP2* gene in 45 control individuals of the Chinese study (the numbers in each box correspond to the r^2 parameter between respective SNPs; the block structure was estimated using Haploview). (**B**) Haplotypes and tagSNPs used in the association studies in each block.

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Haplotypes were constructed based on the six tagSNPs in block 1 and two SNPs (including one tagSNP and the SNP g.68465302I/D) identified in block 2 using HAPLOVIEW, and haplotype association tests were performed on both LD blocks. In addition, the remaining 15 SNPs in block 1 with minor allele frequencies ≥0.01 located in all exons (cSNPs) and their 100-bp flanking regions (which included SNP g.68432276C>T, only identified in the case population in our re-sequencing) and SNPs g.68435294/rs673821G>T and SNP g.68459585 /rs598255C>G (which are located between 100 and 200 bp from the nearest exon in introns 4 and 12, respectively) were genotyped in all cases and controls for further association tests.

Data validation and statistical analyses. The assay results were 100% concordant when confirmed by direct sequencing in 5% of randomly selected samples. Chi-square tests demonstrated that the distributions of the genotypes were in HWE and were also used to compare allele and genotype frequencies of patients and controls. Haplotype data were analysed by logistic regression. Population admixture was estimated using the STRUCTURE and STRAT programs [18,20].

Results

UK study

Data quality and DNA bank validation. In the UKDBG samples, SNP assay failure rate was 0.8% (0.3–2.4%). One hundred twenty randomly selected samples were analysed as blind internal duplicates in all assays, and the results were entirely concordant. There were 10 Mendelian errors in six SNPs occurring in five families which were excluded from family-based tests. In the 58BC DNA collection, the SNP assay failure rate was 1.3% (0.5–3.9%) for all SNPs. No SNP deviated from HWE (i.e. $P > 0.05$).

Single-SNP association tests. No significant differences in SNP allele or genotype frequencies were observed between the 349 patients and 605 controls in the UK study.

Table 3. SNPs identified by re-sequencing in 70 cases and 45 controls from the Chinese population

Chromosome location	dbSNP ID	Position in gene	Alleles Major/minor	Amino acid Change	Minor allele frequency	
					70 Cases	45 Controls
Block 1						
Tagging SNPs						
68428053	rs1249463	exon 1	T/C	—	0.43	0.44
68435538	rs560096	exon 5	T/C	L202S	0.42	0.44
68457277	rs645436	intron 8	G/C	—	0.22	0.22
68460535	rs622082	exon 13	A/G	T671A	0.23	0.22
68460840	rs546382	exon 13	C/T	—	0.22	0.22
68462396	rs2275996	exon 14	G/A	E928K	0.06	0.04
Non-tagging SNPs						
68427288	rs603076	upstream	G/A	—	0.42	0.48
68427857	Novel	upstream	N/I [a]	—	0.17	0.17
68427929	rs57258566	upstream	T/C	—	0.01	0.01
68427995	rs4930624	5′ UTR	C/T	—	0.00	0.03
68432088	rs61887150	intron 2	G/C	—	0.23	0.20
68432276	Novel	exon 3	C/T	T115M	0.03	0.00
68435294	rs673821	intron 4	G/T	—	0.24	0.20
68435400	Novel	intron 4	5C/6C	—	0.06	0.02
68438978	rs10896380	exon 6	A/G	I276V	0.00	0.02
68439176	Novel	intron 6	C/T	—	0.00	0.01
68457694	rs2282504	intron 9	C/T	—	0.17	0.20
68458155	rs751879	intron 9	T/C	—	0.01	0.01
68458391	rs591841	intron 10	C/T	—	0.01	0.03
68458524	rs11228413	exon 11	C/T	—	0.01	0.03
68459208	rs737386	intron 11	C/T	—	0.20	0.19
68459228	Novel	intron 11	C/T	—	0.02	0.02
68459585	rs598255	intron 12	C/G	—	0.38	0.46
68460604	rs2236654	exon 13	C/T	R694W	0.01	0.01
68460846	rs11228414	exon 13	A/G	—	0.05	0.02
68462080	rs513615	intron 13	G/T	—	0.22	0.21
68462103	rs3794031	intron 13	C/T		0.01	0.03
68462250	rs17612126	exon 14	C/A	T879K	0.20	0.20
68462452	rs4378405	intron 14	G/A	—	0.20	0.20
68463424	rs569777	intron 14	G/A	—	0.22	0.23
68463511	rs568862	intron 14	A/G	—	0.22	0.26
68464030	rs564244	3′ UTR	T/C	—	0.44	0.44
68464202	rs3750980	3′ UTR	C/T	—	0.21	0.19
Block 2						
Tagging SNPs						
68465301	rs587052	Downstream	C/T	—	0.21	0.19
Non-tagging SNPs						
68465302	Novel	Downstream	I/D [b]	—	0.21	0.19
Non-tagging SNPs located between the blocks						
68464456	rs9095	3′ UTR	T/C	—	0.01	0.03
68464741	Novel	Downstream	CTT/-	—	0.01	0.01

[a]N/I: no insertion/insertion of CCGCCGCCATCTTC.
[b]I/D: insertion/no insertion of AGGGGC.

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Table 4A. UK allelic case-control association data

SNP	Allele	Frequency		Case-control	
		Cases	Controls	Chi^2	*P*
rs1249528	G	0.599	0.618	0.657	0.418
rs497149	T	0.269	0.282	0.325	0.568
rs11608044	A	0.133	0.112	1.792	0.181
rs4930624	C	0.760	0.769	0.211	0.646
rs10896379	C	0.821	0.814	0.145	0.703
rs7123974	C	0.321	0.328	0.111	0.740
rs2275996	G	1.000	1.000	n/a	n/a
rs4378405	A	0.320	0.329	0.163	0.686
rs568862	A	0.481	0.481	0.001	0.982

Table 4B. UK allelic (TDTs)[a]

SNP	Allele	Transmitted: untransmitted	TDT		TDT (n = 180[b])
			Chi^2	*P*	*P*
rs1249528	G	72:58	1.508	0.220	0.230
rs497149	T	55:54	0.009	0.924	0.853
rs11608044	A	33:30	0.143	0.706	0.710
rs4930624	C	57:47	0.962	0.327	0.340
rs10896379	C	44:41	0.106	0.745	0.673
rs7123974	C	67:56	0.984	0.321	0.599
rs2275996	G	00:00	n/a	n/a	n/a
rs4378405	A	66:56	0.82	0.365	0.660
rs568862	A	80:72	0.421	0.516	0.633

[a]Transmitted/untransmitted refers to heterozygous parent to affected offspring.
[b]Includes 157 complete and 23 incomplete trios.

The SNP previously associated with IgAN in the Japanese population (rs2275996/G34448A) was monomorphic for the G allele in this UK population (Table 4A).

Haplotype association tests. All haplotypes occurring at >1% in the HapMap CEU database also occurred in this UK population, and at similar frequencies (Table 5). No haplotypes were significantly over- or under-represented in cases compared with controls (Table 6).

Family-based study using TDTs. Of the 349 patients with IgAN, both parents were available in 162 cases. The five trios in which there were Mendelian errors were excluded leaving 157 complete trios for family-based analysis. TDTs indicated that no alleles tested were over- or under-transmitted to affected individuals from heterozygous parents (Table 4B). Moreover, all haplotypes identified were equally transmitted to affected offspring (Table 6). In addition to the 157 complete trios, there were 23 incomplete families (Table 1) in which genotypes from siblings were used to infer genotypes and haplotypes transmitted from the missing parents. Analysis of all 180 families demonstrated no over- or under-transmission of alleles or haplotypes to affected individuals (Tables 4B and 6).

Population structure. Analysis with the program STRUCTURE provided no evidence of significant population substructure in the UK samples.

Table 5. Haplotype frequencies in the HapMap database (CEU) and UK study

Block[a]	Haplotype	CEU HapMap frequency	UKDBG/58BC frequency
1	GAGCCCG	0.275	0.323
1	GTGCTTG	0.175	0.183
1	AAGCCTG	0.100	0.153
1	AAATCTG	0.092	0.120
1	AAGTCTG	0.156	0.114
1	GTGCCTG	0.083	0.092
1	GAGCCTG	0.025	0.011
2	AA	0.295	0.324
2	GG	0.525	0.517
2	GA	0.153	0.157

[a]Block 1: rs1249528, rs497149, rs11608044, rs4930624, rs10896379, rs7123974, rs2275996; Block 2: rs4378405, rs568862.

Chinese study

Re-sequencing and SNP identification. Re-sequencing studies were first executed in a discovery population consisting of 70 cases and 45 healthy controls which were randomly selected from the collection (which comprises 663 cases and 663 controls). This identified 37 SNPs with minor allele frequencies from 0.01 to 0.48. Eleven of these were coding SNPs (cSNPs), of which seven were non-synonymous and four synonymous (Table 3). Three SNPs (g.68427995C>T, g.68438978A>G and g.68439176C>T) were only identified in the controls, and one SNP (g.68432276C>T) was identified only in patients in the discovery population.

LD pattern and haplotypes. Two LD blocks were identified spanning the gene. Block 1 constitutes the major block which spans a 36.9-kb genomic region from the 5′ upstream region to the 3′ untranslated region (UTR) of the gene. Twenty-three haplotypes with frequency ≥1%, and 20 SNPs were identified in block 1 (Figure 1A and B). Block 2 of the gene is smaller and is located at the 3′ flanking region (Figure 1A and B) with only two identified SNPs within a single PCR product: one tagSNP g.68465301C>T and the other SNP g.68465302I/D.

Association analyses

Single-tagSNP association tests. The six tagSNPs located in block 1 and the tagSNP in block 2 were selected for initial association analysis. Chi-square tests indicated that, comparing all cases with all controls, there were no significant differences in allele or genotype (genotype data not shown) frequencies of these SNPs (Table 7). No association with IgAN of the allele rs2275996 (A) (originally reported as the cSNP G34448A which was associated with IgAN in the Japanese population) was detected. The other SNP (g.68465302I/D) in block 2 also showed no significant association with IgAN (Table 7). In addition, there was no association observed between tagSNP genotypes and IgA level at diagnosis (Table S3).

Haplotype association tests. The six tagSNPs in block 1 captured seven haplotypes with frequencies >0.01 (Table 8). In block 2, three haplotypes were captured by SNPs g.6845301C>T and g.68465302I/D. No significant differences in haplotype frequencies were found between patient

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Table 6. UK haplotype case-control association test and TDT[a]

Block[b]	Haplotype	Frequency (cases, controls)	Transmitted:untransmitted	Case-control		TDT	
				Chi^2	P	Chi^2	P
1	GAGCCCG	0.319, 0.326	66.0:54.0	0.080	0.777	1.201	0.273
1	GTGCTTG	0.180, 0.185	40.3:45.0	0.087	0.768	0.257	0.612
1	AAGCCTG	0.159, 0.149	35.9:40.0	0.320	0.572	0.221	0.638
1	AAATCTG	0.133, 0.112	34.0:31.1	1.956	0.162	0.131	0.717
1	AAGTCTG	0.106, 0.118	26.5:39.3	0.641	0.423	2.473	0.116
1	GTGCCTG	0.089, 0.094	28.0:21.3	0.089	0.765	0.905	0.342
1	GAGCCTG	0.011, 0.010	5.0:5.1	0.067	0.795	0.000	0.983
2	AA	0.319, 0.327	66.0:55.0	0.135	0.713	1.000	0.317
2	GG	0.517, 0.517	73.0:81.0	0.000	0.992	0.415	0.520
2	GA	0.163, 0.154	38.0:41.0	0.254	0.615	0.114	0.736

[a]Transmitted/untransmitted refers to heterozygous parent to affected offspring.
[b]Block 1: rs1249528, rs497149, rs11608044, rs4930624, rs10896379, rs7123974, rs2275996; Block 2: rs4378405, rs568862.

Table 7. Single-locus association analysis in the Chinese population

SNP location	dbSNP ID	Allele	Frequency (number tested)		Chi^2	P	Adjusted P[a]
			Patients	Controls			
Block 1							
TagSNPs							
68428053	rs1249463	T	0.572 (657)	0.541 (656)	2.580	0.108	0.275
68435538	rs560096	T	0.516 (662)	0.508 (660)	0.150	0.699	0.997
68457277	rs645436	G	0.776 (662)	0.772 (658)	0.050	0.823	0.991
68460535	rs622082	A	0.790 (663)	0.789 (662)	0.013	0.908	0.996
68460840	rs546382	C	0.793 (663)	0.790 (661)	0.053	0.817	0.986
68462396	rs2275996	G	0.931 (662)	0.928 (660)	0.062	0.803	0.990
Other SNPs with minor allele frequency ≥0.05							
68435294	rs673821	G	0.789 (553)	0.795 (553)	0.099	0.753	0.964
68459585	rs598255	C	0.544 (540)	0.546 (540)	0.007	0.931	1.000
68462250	rs17612126	C	0.776 (662)	0.772 (660)	0.075	0.784	0.990
68462452	rs4378405	G	0.776 (662)	0.772 (660)	0.075	0.784	0.994
68463511	rs568862	A	0.771 (654)	0.773 (660)	0.016	0.899	1.000
68464030	rs564244	T	0.539 (655)	0.536 (662)	0.019	0.890	1.000
68464202	rs3750980	C	0.771 (654)	0.765 (657)	0.159	0.690	0.968
Other SNPs with minor allele frequency between 0.01 and 0.05							
68427995	rs4930624	C	0.978 (657)	0.983 (656)	0.969	0.325	0.356
68432276	Novel	C	0.979 (660)	0.979 (656)	0.013	0.910	0.987
68435400	Novel	5C	0.955 (662)	0.967 (660)	2.866	0.090	0.096
68438978	rs10896380	A	0.979 (663)	0.980 (663)	0.019	0.892	0.980
68458524	rs11228413	C	0.972 (663)	0.979 (661)	1.250	0.264	0.306
68460604	rs2236654	C	0.974 (663)	0.979 (661)	0.775	0.379	0.429
68460846	rs11228414	A	0.958(663)	0.969 (660)	2.338	0.126	0.146
68464456	rs9095	T	0.973 (654)	0.975 (659)	0.077	0.781	0.860
Block 2							
TagSNP							
68465301	rs587052	C	0.797 (659)	0.791 (657)	0.181	0.671	0.942
Other SNP							
68465302	Novel	I[b]	0.776 (659)	0.776 (657)	0.000	0.996	1.000

[a]*P*-values adjusted using STRAT.
[b]I, AGGGGC insertion.

and control groups (Table 8). In addition, no association was observed when the analysis was repeated using fewer tagSNPs for each block.

Further association tests. As no association was found with the above SNPs and haplotypes, and in order to further confirm the lack of association, we genotyped and tested the 13 remaining SNPs located within exons and 100-bp flanking regions with allele frequency ≥0.01 and two additional SNPs (g.68435294/rs673821G>T and SNP g.68459585/rs598255C>G) located between 100 and 200 bp from the exon-flanking regions. No significant differences were observed between allele or genotype (genotype data not shown) frequencies in cases and controls in any of the single SNPs tested (Table 7).

Population stratification tests. Analysis using the STRUCTURE program suggested that the population is significantly structured and is composed of four subpopulations. Analysis with the STRAT program from the structured population showed that none of the alleles of the 23 SNPs tested were significantly associated with IgAN (Table 7).

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Table 8. Haplotype frequency and association in cases and controls from Chinese study

Haplotype[a]	Haplotype count (frequency)			
	Case	Control	Chi^2	*P*
Block 1				
TTGACG	639 (0.487)	628 (0.480)	0.127	0.721
CCGGTG	225 (0.171)	231 (0.176)	0.100	0.751
CCCACG	183 (0.139)	200 (0.153)	0.990	0.320
CCCACA	74 (0.056)	79 (0.060)	0.204	0.651
CCGACG	58 (0.044)	61 (0.046)	0.069	0.793
TCGGTG	35 (0.027)	26 (0.020)	1.281	0.258
TCGACG	34 (0.026)	27 (0.021)	0.828	0.363
Block 2				
CI[b]	1022 (0.779)	1020 (0.779)	0.000	0.983
TD[c]	262 (0.200)	271 (0.206)	0.208	0.648
CD	28 (0.021)	19 (0.015)	1.740	0.187

[a]Block 1: rs1249463, rs560096, rs645436, rs622082, rs546382, rs2275996; Block 2: rs587052, SNP 68465302.
[b]I, AGGGGC insertion.
[c]D, no AGGGGC insertion.

Discussion

We used two independent collections of DNA from individuals with IgA nephropathy, one from the UK and one from China. While age and renal function were similar in affected individuals between the two studies, there were differences in other demographic and clinical characteristic (Table 2). This could be due to differences in ascertainment, or could reflect genuine differences in disease phenotype between the two populations. This observation, together with acknowledged genetic divergence between the respective populations, mandated the separate genetic analyses which we performed.

We did not detect any association between different *IGHMBP2* alleles or haplotypes and IgAN in the UK or Chinese collections that were studied. Power calculations [21,22] indicated that the UK study (which used unscreened controls) was predicted to have >80% likelihood of detecting a risk allele occurring at or above a frequency of 3.5% which confers a genotype relative risk (GRR) of similar magnitude (1.85) to that reported from G34448A in the Japanese population [13]. The Chinese study had >99% likelihood of detecting an effect of alleles with similar GRR and frequency and was predicted to have >80% power to detect alleles conferring a GRR of 1.39 (the lower 95% confidence interval reported in [13]) occurring at similar frequency (power calculation assumed allelic association, risk allele frequency of 0.108, genotype relative risk of 1.39 and 663 cases and controls). Clearly, our study cannot completely exclude smaller GRRs, or effects from alleles occurring at lower frequencies.

Consistent with the lack of genetic association, existing knowledge of *IGHMBP2* and its mouse orthologue (termed S mu bp2 or cardiac transcription factor 1, Entrez GeneID: 20589) has not yet suggested a mechanism by which variation would predispose to IgAN. It is predicted to encode a 993-amino acid protein with DNA binding, ATP binding and helicase domains [23]. The messenger RNA is ubiquitous, but while humans and mice with mutations in the gene show evidence of defective motor neuron function, they exhibit no recognized renal or immunological phenotype. Thus mutations in *IGHMBP2* underlie autosomal recessive distal spinal muscular atrophy type 1 (MIM 604320) and distal hereditary motor neuronopathy type VI (HMN6) (MIM 604320) [24], and a mutation in the mouse orthologue of the gene underlies the neuromuscular degeneration (nmd) mouse [25].

In the UK part of this study, the A allele of rs2275996/G34448A, which was previously associated with an increased risk of IgAN in the Japanese population, was not present, so the current study cannot exclude that this allele predisposes to IgAN in some individuals in the UK population, although the allele is likely to be very rare. In addition, in the Chinese population studied here, the minor (A) allele frequency of rs2275996/G34448A was 0.072 (g.68462396 (A) in Table 7), which is not significantly different from the previously reported frequency of 0.083 in control individuals from the Japanese population [13]. We found that this allele was not associated with increased risk of IgAN in the Chinese population, which implies that any functional change in the protein caused by the E928K substitution predicted by rs2275996/G34448A does not confer increased risk of IgAN.

The previously reported association of variation in *IGHMBP2* and IgAN may be a false positive finding, but it remains possible that a risk allele, in strong LD with rs2275996/G34448A, exists in the Japanese population but is not present in the populations studied here. If this is the case, then identifying this allele and its functional consequences may provide insight into the pathogenesis of IgAN, at least in the Japanese population. Nevertheless, the current study demonstrates that none of the tested variants of *IGHMBP2* in either the UK or Chinese populations studied are significantly associated with altered risk of IgAN, and a logical next step will be to undertake genome-wide association studies in these populations.

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Conflict of interest statement. None declared.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Cerebral microbleeds in predialysis patients with chronic kidney disease

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Abstract

Background. Gradient-echo T2*-weighted magnetic resonance imaging (T2*-weighted MRI) is highly sensitive for detecting cerebral microbleeds (CMBs). CMBs have been reported to be a risk factor for future cerebrovascular events and a marker of cerebral small vessel disease in the general

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