

Galactosylation of IgA1 Is Associated with Common Variation in *C1GALT1*

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ABSTRACT

IgA nephropathy (IgAN), an important cause of kidney failure, is characterized by glomerular IgA deposition and is associated with changes in O-glycosylation of the IgA1 molecule. Here, we sought to identify genetic factors contributing to levels of galactose-deficient IgA1 (Gd-IgA1) in white and Chinese populations. Gd-IgA1 levels were elevated in IgAN patients compared with ethnically matched healthy subjects and correlated with evidence of disease progression. White patients with IgAN exhibited significantly higher Gd-IgA1 levels than did Chinese patients. Among individuals without IgAN, Gd-IgA1 levels did not correlate with kidney function. Gd-IgA1 level heritability (h^2), estimated by comparing midparental and offspring Gd-IgA1 levels, was 0.39. Genome-wide association analysis by linear regression identified alleles at a single locus spanning the *C1GALT1* gene that strongly associated with Gd-IgA1 level ($\beta=0.26$; $P=2.35 \times 10^{-9}$). This association was replicated in a genome-wide association study of separate cohorts comprising 308 patients with membranous GN from the UK ($P<1.00 \times 10^{-6}$) and 622 controls with normal kidney function from the UK ($P<1.00 \times 10^{-10}$), and in a candidate gene study of 704 Chinese patients with IgAN ($P<1.00 \times 10^{-5}$). The same extended haplotype associated with elevated Gd-IgA1 levels in all cohorts studied. *C1GALT1* encodes a galactosyltransferase enzyme that is important in O-galactosylation of glycoproteins. These findings demonstrate that common variation at *C1GALT1* influences Gd-IgA1 level in the population, which independently associates with risk of progressive IgAN, and that the pathogenic importance of changes in IgA1 O-glycosylation may vary between white and Chinese patients with IgAN.

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IgA nephropathy (IgAN) is the commonest GN worldwide and is a major cause of kidney failure.¹ The prevalence of IgAN shows marked differences across different ethnic groups, being more prevalent in people with East Asian ancestry and less prevalent in people with African ancestry compared with whites.² In addition, differences in the clinical features of IgAN in white compared with Chinese patients have been recognized for a long time,³ most notably the clear male preponderance of IgAN studies of IgAN in whites that is absent (or even reversed) in East Asian populations, suggesting that important and incompletely understood

differences in disease pathophysiology exist across different populations.^{4–8} Recent work has identified a number of genetic factors, mostly associated with mechanisms of defense against

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infection, that are associated with altered risk of disease,^{9–11} and although the prevalence of the known genetic factors vary across different ethnic groups, the observed differences fall some way short of explaining the differences in prevalence of the disease in different regions.^{2,12}

The human IgA1 molecule differs from the conserved IgA2 subclass by the presence of an additional 13-residue motif in the hinge region that undergoes O-linked glycosylation. The function of this post-translational modification is incompletely understood, but it is known that IgA1 O-linked glycans lacking a galactose moiety are more abundant in the circulation of patients with IgAN,^{13–15} and that such galactose-deficient IgA1 (Gd-IgA1) is disproportionately found in IgAN glomerular immune deposits.¹⁶ In Tn syndrome, a similarly undergalactosylated protein is present on the surfaces of blood cells that leads to autoantibody generation and disease.¹⁷ A plausible hypothesis is that Gd-IgA1 plays an important role in the pathophysiology of IgAN by functioning as an autoantigen leading to autoantibody production and formation of circulating immune complexes in susceptible individuals.^{18,19} *In vitro* evidence supports a role for these IgA-containing immune complexes in driving glomerular injury through mesangial cell proliferation and secretion of cytokines, chemokines, growth factors, and extracellular matrix components promoting glomerular inflammation and glomerulosclerosis.^{20–22} Consistent with previous data, we show that Gd-IgA1 level is a heritable trait^{23–26} and use a genome-wide approach to identify the common genetic

factor that influences Gd-IgA1 levels in white and East Asian populations.

RESULTS

Gd-IgA1 Is Elevated in IgAN, Associated with Disease Severity, and Heritable

In the discovery UK cohort, Gd-IgA1 levels were normally distributed (Supplemental Figure 1) and elevated in patients. Follow-up data were available for the majority of patients allowing classification into 154 “progressors,” defined as doubling of serum creatinine or needing renal replacement therapy, and 123 “nonprogressors,” defined as serum creatinine <1.35 mg/dl and <20% increase over at least 5 years of follow-up, with the remainder “indeterminate.” Gd-IgA1 levels were significantly higher in progressors compared with non-progressors ($P=0.001$; Figure 1). Correlation ($P=0.031$) was seen between Gd-IgA1 level and serum creatinine⁻¹ (Supplemental Figure 2). In a separate longitudinal cohort of patients with IgAN and healthy subjects (Leicester Research Archive) we observed that the Gd-IgA1 level in an individual did not change significantly over >5 years and changes in renal function in individuals with IgAN were not correlated with changes in Gd-IgA1 level (Supplemental Figure 3). Heritability (h^2) was 0.387, estimated by parent-offspring regression of standardized Gd-IgA1 levels (Figure 2), broadly consistent with previously published data.^{23–26}

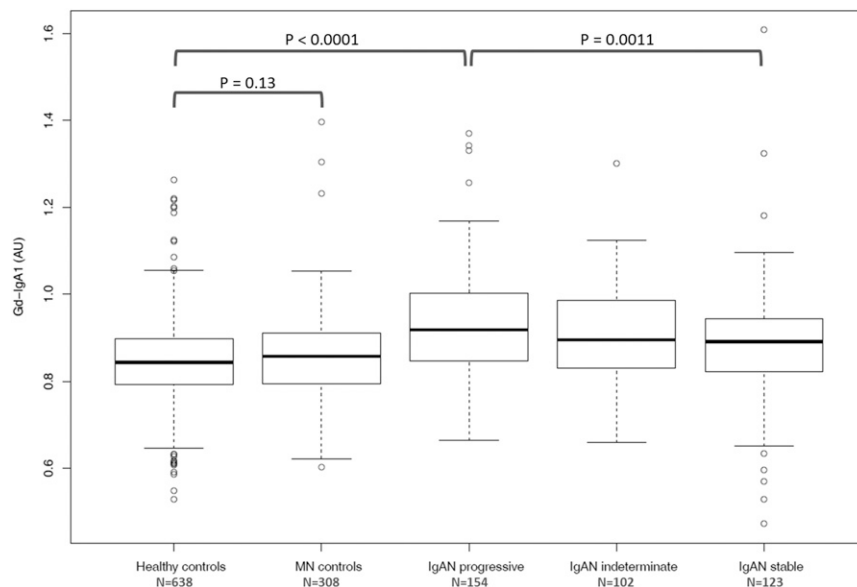


Figure 1. Gd-IgA1 levels were higher in 379 patients with IgAN compared with 638 healthy control subjects ($P<0.0001$). Levels were higher in 154 patients with progressive IgAN (defined as doubling of serum creatinine or ESRD) compared with 123 patients with IgAN in whom good renal function remained stable over >5 years ($P=0.0011$). Gd-IgA1 levels were not significantly elevated in 308 patients with Membranous Nephropathy (MN controls). AU, arbitrary units of optical absorbance.

C1GALT1 Is Principal Genetic Determinant of Gd-IgA1 Level in Multiple Cohorts

Linear regression genome-wide association study (GWAS) using standardized Gd-IgA1 levels in 513 founder members of the discovery cohort identified alleles at a single locus, spanning the *C1GALT1* gene, that were strongly associated with Gd-IgA level ($\beta=0.26$; $P=2.35 \times 10^{-9}$), with no other significantly associated alleles elsewhere in the genome (Figure 3, Table 1). Repeating the analysis conditioned on the most strongly associated SNP (rs1008897) showed no association with any independent alleles, either at *C1GALT1* or elsewhere in the genome (Supplemental Figure 4). The association with *C1GALT1* was robust to correction for age, sex, renal function, and progression, and genomic inflation factor (λ) was 1.00196, suggesting the analysis was unlikely to be confounded by unidentified population substructure. Haplotype analyses confirmed the findings of the conditional analysis, which was that the association

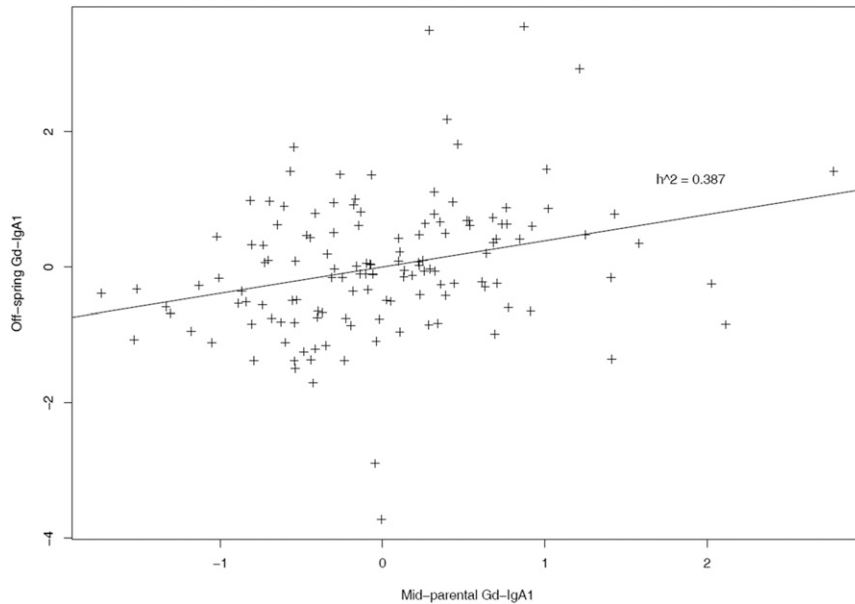


Figure 2. Heritability (h^2) of Gd-IgA1 in UK whites is 0.387. This was estimated using 134 complete trios by plotting Gd-IgA1 of patients against their mean parental Gd-IgA1. Values plotted are Gd-IgA1 levels in arbitrary units of optical absorbance standardized to mean of 0 and SD of 1 to allow narrow sense heritability estimation by calculation of the slope.

was attributable to the presence of a single haplotype, termed H1, present at a frequency of >0.3 in the UK population and strongly associated with increased Gd-IgA1 levels ($\beta=0.34$; $P=4.4 \times 10^{-11}$; Supplemental Figure 5, Table 2). Additional haplotype association analysis conditioned on the presence of H1 indicated that no other haplotypes were significantly associated with Gd-IgA1 at either

the genome-wide or nominal level corrected for multiple tests (Supplemental Table 1).

In a cohort of 318 UK white patients with biopsy-proven membranous nephropathy (MN), sera for Gd-IgA1 measurements were available for 308 and values were lower than patients with IgAN and similar to values in healthy subjects (Figure 1). Among the MN cohort, $>30\%$ of subjects had $eGFR < 60$ ml/min but, unlike in patients with IgAN, we observed no correlation between Gd-IgA1 levels and serum creatinine $^{-1}$ (Supplemental Figure 2). Genome-wide linear regression analysis of Gd-IgA1 levels within the MN cohort revealed association between Gd-IgA1 and the same alleles as observed in the discovery cohort (Supplemental Figure 6). In the combined analysis of 821 individuals, no associations at or approaching genome-wide significance (defined as $P < 5 \times 10^{-8}$) at other loci were detected. Intriguingly, weak evidence of association (P approximately 10^{-5}) was seen at the X-chromosomal

C1GALT1C1 locus encoding Cosmc (Supplemental Figure 7), which is necessary for the *C1GALT1* gene product to function.

Among 622 participants in the UK Genetic Regulation of Arterial Pressure of Humans in the Community (GRAPHIC) cohort (who all lacked evidence of renal impairment), strong association was again observed with alleles at the *C1GALT1*

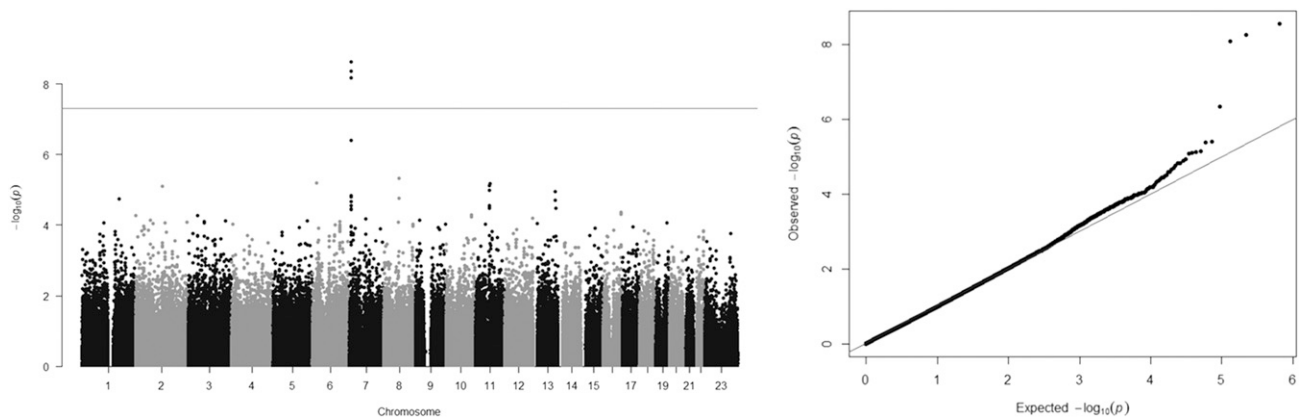


Figure 3. GWAS of Gd-IgA1 levels in 513 unrelated UK individuals showing association with alleles at a single locus on Chromosome 7. Manhattan plot (left panel) showing significance of the association of each SNP allele with Gd-IgA1 level by plotting the negative logarithm to the base 10 of the P value against the genomic position. Horizontal line indicates conventional genome-wide significance ($P=5 \times 10^{-8}$). Quantile-quantile plot (right panel) is a plot of the observed $-\log_{10}(P)$ against the $-\log_{10}(P)$ values that would be expected under the null hypothesis of no association. Deviation above the $y=x$ line indicates lower P values than would be expected to occur by chance and implies statistically significant association. The genomic inflation factor was 1.00196.

Table 1. All SNPs associated with Gd-IgA1 with $P < 5 \times 10^{-5}$ in discovery cohort

Chromosome	SNP	Raw P Value	GC	Bonferroni	GRAPHIC P Value
7	^a rs1008897	2.35E-09	2.55E-09	0.000745	5.49E-11
7	^a rs758263	4.34E-09	4.70E-09	0.001377	1.90E-07
7	^a rs13226913	6.55E-09	7.07E-09	0.002077	1.19E-08
7	^a rs4720724	3.91E-07	4.14E-07	0.1238	7.51E-09
17	rs3803780	4.06E-06	4.26E-06	1	0.982
8	rs1344616	4.62E-06	4.85E-06	1	0.510
6	rs9383456	6.48E-06	6.80E-06	1	0.755
11	rs519380	6.79E-06	7.12E-06	1	0.598
11	rs2279865	7.52E-06	7.88E-06	1	0.0545
2	rs294657	7.89E-06	8.27E-06	1	0.807
11	rs11550299	1.03E-05	1.08E-05	1	0.427
13	rs1540510	1.13E-05	1.19E-05	1	0.621
7	^a rs2108780	1.46E-05	1.53E-05	1	3.10E-06
7	rs2060163	1.59E-05	1.66E-05	1	0.00115
8	rs609760	1.73E-05	1.80E-05	1	0.605
1	rs12140760	1.82E-05	1.90E-05	1	0.0227

GC, corrected for genomic control; Bonferroni, corrected stringently for 302,210 SNPs analyzed.

^aSNPs at the *C1GALT1* locus—only these SNPs also showed significant association with Gd-IgA1 in the GRAPHIC cohort.

locus ($\beta = 0.38$, $P < 3.6 \times 10^{-11}$; Supplemental Figure 8), with no other loci implicated. Univariate and multivariate analyses demonstrated no significant association with age, sex, or other baseline characteristics including creatinine clearance and eGFR ($\beta < 0.001$ and $P > 0.5$ for both). This therefore replicates the association of the H1 haplotype with elevated Gd-IgA1 in whites, and implies that differences in renal function *per se* do not influence Gd-IgA1 level.

The Same *C1GALT1* Haplotype Is Rare but Also Associated with Gd-IgA1 in the Chinese Population

Data from the HapMap project indicate that, although common in Europeans, the H1 haplotype is less common in African and East Asian populations.²⁷ To investigate how genetic variation across this locus influences Gd-IgA1 levels in a Chinese population we measured serum Gd-IgA1 levels in a cohort of 704 Chinese patients (49% male) with IgAN, and 111 ethnicity- and age-matched controls. This demonstrated that Gd-IgA1 levels were significantly higher in Chinese patients than Chinese healthy subjects, but in both these groups Gd-IgA1 levels were lower than in UK subjects (Figure 4).

We next performed a candidate locus association study by genotyping 38 SNPs across the *C1GALT1* locus and observed association of alleles at this locus with Gd-IgA1 level ($P = 5 \times 10^{-4}$; Supplemental Figure 9). Consistent with HapMap data, we observed that the H1 haplotype was present but rare in this population, with a frequency of 0.035. The presence of this haplotype was again associated with elevated Gd-IgA1 levels (Table 2, $P = 6 \times 10^{-5}$). Multiple regression analyses showed that disease status, ethnicity, and the presence of the H1 haplotype were all independently correlated with Gd-IgA1 level, but age and sex were not: Gd-IgA1 levels remained significantly

Table 2. Association of haplotypes across *C1GALT1* with Gd-IgA1 showing a similar effect of the H1 haplotype in both populations

Cohort	CHR	SNP1	SNP2	Haplotype	Frequency	β	P Value	Bonferroni
White	7	rs4720724	rs2190935	GTCCGC ^a	0.319	0.343	4.40E-11	3.96E-10
	7	rs4720724	rs2190935	ATCTAT	0.207	0.0498	0.397	1
	7	rs4720724	rs2190935	AGCTAC	0.17	-0.315	1.36E-06	1.22E-05
	7	rs4720724	rs2190935	AGTCAT	0.134	-0.237	0.00153	0.01
	7	rs4720724	rs2190935	GGTCAT	0.0479	-0.19	0.118	1
	7	rs4720724	rs2190935	AGTTAC	0.0429	-0.258	0.0421	0.38
	7	rs4720724	rs2190935	ATCCGC	0.0154	0.058	0.78	1
	7	rs4720724	rs2190935	GTCTAT	0.012	0.304	0.214	1
	7	rs4720724	rs2190935	GTCCAC	0.0113	0.248	0.265	1
Chinese	7	rs4720724	rs2190935	AGCTAC	0.523	-0.184	0.000592	0.005
	7	rs4720724	rs2190935	AGTCAT	0.162	0.0761	0.307	1
	7	rs4720724	rs2190935	AGTTAC	0.162	0.18	0.0165	0.15
	7	rs4720724	rs2190935	GTCCGC ^a	0.0359	0.536	6.21E-05	5.59E-04
	7	rs4720724	rs2190935	GGTCAT	0.0211	0.0119	0.949	1
	7	rs4720724	rs2190935	ATTCAT	0.0195	-0.0189	0.928	1
	7	rs4720724	rs2190935	GTTCGC	0.0142	-0.392	0.102	0.92
	7	rs4720724	rs2190935	ATCCGC	0.0107	-0.148	0.542	1
	7	rs4720724	rs2190935	AGCTAC	0.01	0.224	0.431	1

P values represent the effect of testing each haplotype against all of the others. The commonest haplotype in the UK population is almost ten-fold less common in the Chinese population. SNPs defining this haplotype are rs4720724, rs758263, rs4263662, rs10259085, rs1008897, and rs2190935. Bonferroni, with correction for nine haplotypes tested.

^aH1 haplotype.

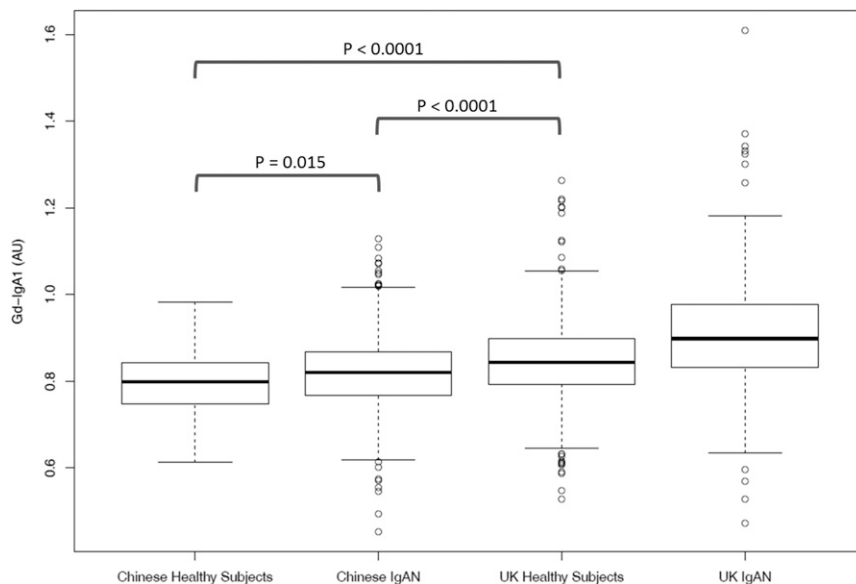


Figure 4. Gd-IgA1 levels were elevated in 704 Han Chinese patients with IgAN compared with 111 healthy Chinese subjects ($P=0.015$). Levels in both Chinese groups were significantly lower than those in UK healthy subjects and UK patients with IgAN ($P<0.0001$ for both). Ten percent of Chinese and 24% of UK subjects with IgAN exhibited Gd-IgA1 levels above the 95th percentile of their respective control populations. AU, arbitrary units of optical absorbance.

higher in white compared with Chinese subjects when stratified by number of copies of the H1 haplotype (Figure 5; $P<10^{-8}$).

DISCUSSION

Elevation of serum Gd-IgA1 is a consistent finding in IgAN that, in susceptible individuals, is associated with autoantibody production and the formation of circulating immune complexes that trigger glomerular injury through mesangial cell activation, endocapillary proliferation, podocyte injury, and tubulointerstitial inflammation and fibrosis.^{16,18,20–22} Delineating the genetic control of Gd-IgA1 production is therefore important in understanding this common GN.

In white and Chinese populations we identified association between Gd-IgA1 levels and a haplotype spanning the *C1GALT1* gene. *C1GALT1* encodes the enzyme core 1 synthase, glycoprotein-N-acetylgalactosamine 3- β -galactosyltransferase (C1GALT1), which catalyzes the transfer of galactose (Gal) from UDP-Gal to N-acetylgalactosamine (GalNAc) O-linked esters of threonine and serine residues of target proteins, including IgA1, to form the T antigen. This requires the chaperone Cosmc, encoded by the X-linked gene *C1GALT1C1*, which prevents rapid degradation of C1GALT1.²⁸ We also observed possible association between Gd-IgA1 and variation at *C1GALT1C1*, although this was not statistically significant at the genome-wide level. Mice lacking C1GALT1 develop thrombocytopenia, proteinuria, and renal failure associated with anomalous glycosylation of proteins.²⁹ Somatic

mutations inactivating Cosmc (leading to increased degradation of C1GALT1) cause Tn syndrome in humans, which is characterized by autoantibodies forming against the undergalactosylated O-linked glycoproteins on red cell membranes.¹⁷ It therefore appears likely that variation at *C1GALT1* might similarly modulate galactosylation of O-glycosylated proteins, including IgA1, resulting in generation of IgA1 neo-epitopes capable of triggering autoantibody production and disease in susceptible individuals.

There is, however, no evidence for a generalized defect in O-galactosylation in IgAN: we have previously shown that in IgAN the patterns of O-galactosylation of C1 inhibitor and IgD, the only other O-glycosylated human immunoglobulin isotype, are unaltered, implying that the reduction in IgA1 O-galactosylation in IgAN is a specific feature of IgA1-secreting cells.³⁰ This suggests that the haplotype we identified leads to elevated Gd-IgA1 through altered maturation-dependent transcriptional regulation of *C1GALT1*,

rather than *via* differences in C1GALT1 protein structure that would be present in all C1GALT1-expressing cell types. Consistent with this, imputation of all of the alleles common to the H1 haplotype using 1000 Genomes data identified no coding variants in linkage disequilibrium with the associated alleles. Furthermore, published data linking genetic variation with gene expression (eQTLs) in lymphoblastoid cell lines show that SNP alleles imputed to lie on the H1 haplotype are strongly associated with reduced *C1GALT1* transcript levels (Table 3).³¹ Additional *in silico* analyses using ENCODE data show that some strongly associated SNPs lie within consensus transcription factor binding elements, including rs7780273 (imputed $P=1.8 \times 10^{-8}$; eQTL P value of 7.5×10^{-7}), which lies at a predicted SOX2-OCT4 site,^{32,33} and rs758263, which lies within the core promoter region for *C1GALT1* and is predicted to affect binding of RUNX3, a transcription factor present in B cells that is necessary for class switching to IgA production.^{34,35} These *in silico* predictions coupled with previous *in vitro* data demonstrating modulation of IgA1 O-galactosylation by Th2 cytokines and IL-6,^{36,37} as well as our previous observation that IgA1 O-galactosylation varies depending on the site of antigen encounter and B cell activation,³⁸ are consistent with an effect of the H1 haplotype on transcriptional control of *C1GALT1*, particularly in IgA1-committed B cells.

Our data indicate that *C1GALT1* genotype explains approximately 3% of the variability in Gd-IgA1 levels, suggesting other factors are important in determining Gd-IgA1 levels in an individual. Because this is a genome-wide study it is

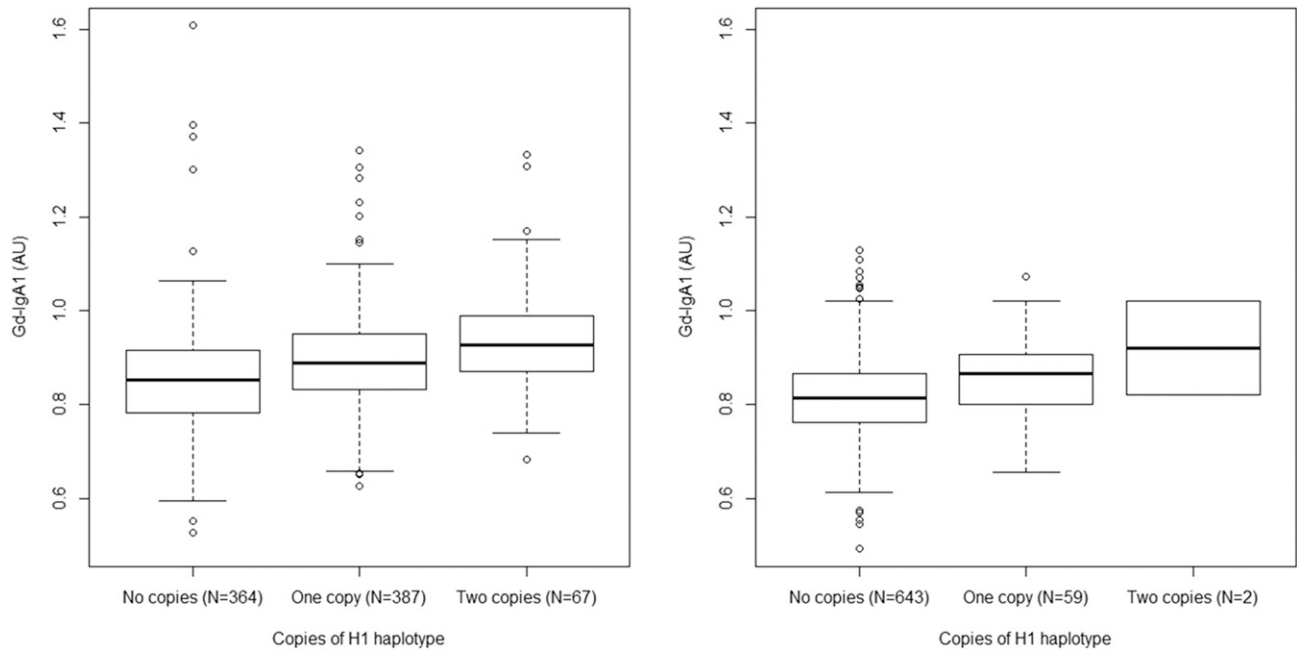


Figure 5. Gd-IgA1 levels increase with copies of the H1 haplotype in Chinese and white populations. In the combined IgA and MN (white) cohort (left panel) haplotype frequency was 0.32 ($n=821$, $R^2=0.033$, $P<0.0001$). In Chinese patients with IgAN (right panel) haplotype frequency was 0.04 ($n=704$, $R^2=0.019$, $P<0.001$). In multiple regression analysis, Chinese ethnicity was associated with lower Gd-IgA1 levels than in whites, even correcting for haplotype frequency and disease status ($P<0.0001$). AU, arbitrary units of optical absorbance.

unlikely that variation at any other single locus is responsible for this, and we also exclude the possibility that kidney function itself significantly affects Gd-IgA1 levels. Cytokines, and presumably other stimuli, acting in B cells can influence IgA1 galactosylation,³⁷ and likely contribute to the variability in

Gd-IgA1 levels. Identification of the transcriptional mechanisms influencing *C1GALT1* expression may provide insights into how Gd-IgA1 levels are controlled in health and disease.

Association between *C1GALT1* and IgAN susceptibility has previously been tested in candidate gene studies: Li *et al.*^{39,40}

Table 3. Expression quantitative trait loci of SNPs associated with Gd-IgA1³¹

SNP	Position	Gd-IgA1 <i>P</i> Value (Imputed)	GRAPHIC Replication <i>P</i> Value	Cell Type	eQTL <i>P</i> Value
rs10246303	7286445	6.488E-10	3.906E-08	LCL	3.03884E-09
rs6463656	7244181	7.576E-09	1.458E-08	LCL	3.00367E-08
rs6463657	7244254	7.576E-09	1.455E-08	LCL	3.40312E-08
rs13226913	7246846	7.576E-09	1.19E-08	LCL	3.8322E-08
rs10251505	7254489	1.561E-08	1.721E-08	LCL	2.03717E-08
rs4318980	7256490	1.767E-08	1.426E-08	LCL	1.44846E-08
rs7780273	7250449	1.883E-08	0.00010165	LCL	3.11303E-09
rs2881755	7256439	2.022E-08	1.544E-07	LCL	1.07186E-07
rs2881756	7256439	2.022E-08	N/A	LCL	1.46728E-07
rs10952047	7289543	2.358E-08	2.003E-06	LCL	1.7149E-11
rs4720726	7225451	3.287E-08	1.68E-04	LCL	7.5698E-09
rs11771259	7277215	3.691E-08	5.363E-06	LCL	1.14591E-11
rs4724958	7226553	7.369E-08	0.00017043	LCL	9.72937E-09
rs4720727	7226695	1.704E-07	2.569E-07	LCL	6.11804E-08
rs12702588	7222806	3.597E-07	N/A	LCL	7.84334E-07
rs57552003	7223104	6.837E-07	5.952E-06	LCL	5.51448E-08
rs11773545	7196878	8.911E-07	0.00176318	LCL	1.59081E-06
rs1047763	7283569	1.034E-06	2.018E-06	LCL	3.64039E-11

Gd-IgA1 *P* values are imputed, except for rs13226913 which was directly genotyped. All of these SNPs are on the H1 haplotype and the allele associated with elevated Gd-IgA1 levels is associated with lower transcript levels in all cases. eQTL, expression quantitative trait loci; LCL, lymphoblastoid cell line; N/A, not available.

genotyped nine SNPs in Asian cohorts and reconstructed haplotypes across the gene. They identified evidence of association between the disease and the presence of haplotypes containing rs1047763(G) allele (designated “YATDG” and “ATDG”). Although this allele was also associated with the disease in an Italian study,⁴¹ these associations with disease risk have not been replicated in larger genome-wide studies.^{9–11,42,43} We found that rs1047763(G) was associated with Gd-IgA1 level in both our discovery ($P=8.7 \times 10^{-6}$) and replication ($P=2 \times 10^{-6}$) cohorts, and is present on the H1 haplotype, consistent with increased disease susceptibility being mediated by increased Gd-IgA1 levels.

A limitation of this and other GWASs in IgAN is the lack of clear evidence of association with IgAN susceptibility at this locus. Our data show that for each standard deviation increase in Gd-IgA1, the odds of IgAN increased by a ratio of 1.52 (95% confidence interval, 1.35 to 1.73), and in the white discovery cohort the R^2 value associating the H1 haplotype with Gd-IgA1 level was 0.033 (implying that 3.3% of the variation in Gd-IgA1 level is explained by the number of copies of H1 haplotype). Power calculation, performed as described previously,⁴⁴ indicates that a candidate gene study (with nominal α 0.05) in the white population would need >5300 participants to have >80% power to detect an effect of the H1 haplotype on risk of IgAN. In a GWAS (where α is typically $<5 \times 10^{-8}$), a study around five times larger would be needed. In Chinese subjects the H1 haplotype is less common, and we observed an R^2 of 0.019, implying that previously published studies in this population have been underpowered to detect an association with disease risk: even the largest published meta-GWAS study, comprising 7600 cases and 13,000 controls,⁴⁵ has <10% power to detect an association between *C1GALT1* and disease.

Perhaps the most surprising finding in this study was the disparity in levels of Gd-IgA1 between white and Chinese subjects, both healthy and with IgAN. All Gd-IgA1 level measurements were performed in the same laboratory using the same batch of *Helix aspersa* lectin across all samples, with the same standards run in all plates, allowing direct comparison of Gd-IgA1 levels across the different cohorts. This is the first time such a comparison has been reported and indicates that the increased prevalence of IgAN in China cannot be attributed to differences in Gd-IgA1 levels. This, together with the differences in frequency of the H1 haplotype and the clinical manifestations of IgAN described in different continents, suggests that different pathogenic pathways may be operating in different populations. Previous studies have shown that IgAN susceptibility is associated with variation at genes involved in immune defense, and higher-risk alleles are more prevalent in East Asians.^{2,12} One possibility is that the high burden of immunologic risk alleles in East Asian populations results in a higher likelihood of a damaging immunologic response to Gd-IgA1 in the circulation, and hence a higher likelihood of kidney disease. In this paradigm, the reduced Gd-IgA1 in Chinese subjects might even have resulted from selection against

the H1 haplotype in this population due to the high prevalence of other risk alleles that increase disease susceptibility.

In summary, we demonstrate that circulating Gd-IgA1 levels are heritable and influenced by genetic variation at the *C1GALT1* gene across different populations. This provides the first direct evidence that common genetic variation can influence O-glycosylation in humans. Our observations suggest that modulation of this pathway might influence susceptibility to, or outcomes in, IgAN, and *C1GALT1* activity would be the logical enzymatic step to target in order to test this hypothesis.

CONCISE METHODS

Discovery cohort: UK Glomerulonephritis DNA Bank (UKGDB) from individuals with biopsy-proven IgAN (66% male) and healthy relatives has been previously described.¹¹ After quality control for ethnicity (using principal component analysis), genotyping rate, and excluding cryptic relatedness, estimated from identity-by-state information ($\hat{\pi} > 0.125$), serum and genotype data were available for 379 UK white patients and 309 of their parents in 134 parent-affected trios. All individuals were genotyped at 318,127 SNPs using the Illumina Sentrix HumanHap300 BeadChip, of which 302,210 passed quality control (>90% genotyping rate, minor allele frequency >0.05, Hardy-Weinberg equilibrium $P > 0.001$). Follow-up data allowed 277 of these patients to be classed as “progressors” (ESRD or doubling of serum creatinine) or “nonprogressors” (normal renal function and <20% rise in serum creatinine over >5 years’ follow-up), with the remainder classed as “indeterminate.”

UK replication cohorts: Replication was performed using serum from 308 white UK patients with biopsy-proven membranous glomerulopathy from the UKGDB,⁴⁶ genotyped using the Illumina HumanCNV370-Quad SNP chip. Further independent replication was performed using 622 samples from unrelated adults from the GRAPHIC cohort, in which all individuals had normal urinalysis and plasma urea and creatinine at recruitment.⁴⁷

Chinese replication cohort: DNA and sera were available from 704 Han Chinese patients from the Guangzhou (49% male) with biopsy-proven IgAN and 111 healthy age- and sex-matched subjects. Gd-IgA1 was measured and each patient was genotyped at 38 SNPs across the *C1GALT1* gene, selected using Haploview⁴⁸ to tag >90% of haplotypes present in the Chinese population. All subjects provided informed written consent to genetic analyses and the study was performed according to the principles of the Declaration of Helsinki with local ethical approval at each site.

Genome-wide linear regression association analyses were performed with Plink^{49,50} using Gd-IgA1 data standardized to a mean of 0 and an SD of 1 to allow interpretation of β , as detailed in the Supplemental Material. Haplotypes were visualized using Haploview.⁴⁸ Genotypes were imputed using the University of Michigan imputation server.⁵¹ ANOVA and linear regression tests were performed using R. Gd-IgA1 levels were measured using a *Helix aspersa* lectin-based ELISA method as previously described (see Supplemental Material).⁵²

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DISCLOSURES

None.

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