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Search for genetic association between IgA nephropathy and candidate genes selected by function or by gene mapping at loci IGAN2 and IGAN3

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Abstract

Background. IgA nephropathy (IgAN) is not generally considered a hereditary disease, even though extensive evidence suggests an undefined genetic influence. Linkage analysis identified a number of genome regions that could contain variations linked to IgAN.

Methods. In this case–control association study, genes possibly involved in the development of IgAN were investigated. DNA samples from 460 North Italian patients with IgAN and 444 controls were collected. Candidate genes were selected based on their possible functional involvement (6 genes) or because of their location within linkage-identified genomic regions *IGAN2* and *IGAN3* (5 and 13 genes within chromosome 4q26–31 and 17q12–22, respectively). One hundred and ninety-two tag and functional single-nucleotide polymorphisms (SNPs) were typed with Veracode GoldenGate technology (Illumina).

Results. *C1GALT1* showed an association with IgAN (rs1008898: P = 0.0019 and rs7790522: P = 0.0049). Associations were found when the population was stratified by gender (*C1GALT1*, *CD300LG*, *GRN*, *ITGA2B*, *ITGB3* in males and *C1GALT1*, *TRPC3*, *B4GALNT2* in females) and by age (*TLR4*, *ITGB3* in patients aged <27 years). Furthermore, rs7873784 in *TLR4* showed an association with proteinuria (G allele: P = 0.0091; GG genotype: P = 0.0077).

Conclusions. Age and gender are likely to evidence distinct immunological and inflammatory reactions leading to individual susceptibility to IgAN. Overall, a genetic predisposition to sporadic IgAN was found. We might hypothesize that *C1GALT1* and *TLR4* polymorphisms influence the risk to develop IgAN and proteinuria, respectively.

Keywords: association study; C1GALT1; genetics; IgA nephropathy; polymorphisms

Introduction

IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide. Nonetheless, mechanisms regulating abnormal IgA synthesis and its selective mesangial deposition, mesangial cell proliferation and extracellular matrix expansion leading to renal fibrosis are still poorly understood [1–4].

Although not generally considered a hereditary disease, IgAN ethnic variation in prevalence and familial clustering have suggested an undefined genetic influence [5–9]. IgAN does not exhibit basic Mendelian segregation patterns, although an increased risk of the disease was observed in close relatives of probands. The heterogeneity of the IgAN phenotype is more consistent with the combined effects of variation at multiple interacting genes and the environment [6, 8]. The first report on genome-wide linkage analysis in familial IgAN identified a genetic locus on chromosome 6q22–23 (*IGAN1*) [10]. Linkage analysis to *IGAN1*, in an independent group of Italian IgAN families, evidenced that the majority are not linked to this locus confirming genetic heterogeneity in familial IgAN. Bisceglia *et al.* [11] and Paterson *et al.* [12] found the existence of other candidate chromosomal regions on 4q,

7q, 12q, 17q and 2q. The regions 4q26–31 and 17q12–22 exhibited the strongest evidence of linkage from two point, multipoint parametric and non-parametric linkage analysis becoming the second (*IGAN2*) and third (*IGAN3*) genetic loci candidate to contain causative and/or susceptibility genes to familial IgAN [11].

Case–control studies have been long heralded as the optimal design to discover gene variation associated with genetic susceptibility to complex multifactorial diseases [13], and they have been successfully applied to a host of complex diseases [14]. IgAN may also follow this multifactorial paradigm, and hence, a case–control design may be successful in recovering genetic susceptibility factors.

In this study, the genotype of 23 candidate genes selected on the base of their functional possible involvement (6 genes) or because mapping in the genomic regions *IGAN2* (5 genes) and *IGAN3* (13 genes) were retrospectively evaluated in 460 Italian patients affected by IgAN, to search for single nucleotide polymorphisms (SNPs) correlated with the development and the clinical course of this disease.

Materials and methods

Patients

Four hundred and sixty DNA samples (446 successfully genotyped) of North Italian patients affected by IgAN were collected; 188 (185) of them were from Brescia, 45 (42) from Cremona, 35 (34) from Bologna, 21 (21) from Trieste and 171 (164) from Turin. The pathology was diagnosed by performing renal biopsy (RB) between the years 1966 and 2003 and patients with Henoch–Schonlein purpura were excluded. Patient characteristics, including gender, age at time of RB, serum creatinine (sCr), proteinuria (PTU) and creatinine clearance (CrCl) at time of RB, dialysis, transplantation, relapse after transplantation and Lee's classification, are shown in Table 1. This study was approved by local ethical committees of involved hospitals and informed consent was obtained from each subject. Two hundred and twenty-eight IgAN patients of Brescia, Cremona, Bologna and Trieste have already been included in the previous association study of Pirulli *et al.* [15], where eight polymorphisms of *C1GALT1* were analysed. In the present study, we selected 10 different SNPs of *C1GALT1*, with the exception of rs1008898, located in the 5′ flanking region at position −330, that was in common with the previous study. The other seven SNPs of the previous study were not considered in this work because they did not pass our criterion of selection.

In order to perform a case–control association study and to reflect a similar geographic origin of patients, a healthy North Italian population ($n = 444$; 429 successfully genotyped) was also typed as control: they belong to negative urinalysis unrelated individuals, mainly blood or bone marrow donors. We collected 86 (84 successfully genotyped) controls from Brescia, 43 (40) from Cremona, 24 (24) from Bologna, 16 (15) from Trieste and 275 (266) from Turin (Table 1). To reflect a similar geographic origin (the only difference is the lower number of controls from Brescia, that we supplied with a higher number of controls from Turin) and because of the selection within blood and marrow donors, controls could not match patients for age and sex.

Gene selection

Two main strategies were used in order to select 23 genes. The first is candidate gene approach based on their functional possible involvement in IgAN. Six genes, already analysed in previous studies, were evaluated: the core-1-β,3-galactosyltransferase (*C1GALT1*) [15, 16] and its specific molecular chaperone β1,3-GT (*C1GALT1C1*) [17, 18], GalNAc α2,6-sialyltransferase (*ST6GALNAC2*) [19, 20], transferrin receptor 1 (*TFRC*) [21, 22], immunoglobulin A Fcα receptor (*CD89*) [23, 24] and toll-like receptor 4 (*TLR4*) [25, 26] (Table 2).

By the second approach, public databases were queried to obtain the available gene maps for 4q24–31 (*IGAN2*; 21cM) and 17q12–22 (*IGAN3*; 15cM) chromosomal linked regions [11] and the information on the expression pattern of these genes in tissue and organs (www.hapmap.org, www.ensembl.org). Genes of potential interest located on *IGAN2* were selected: the transient receptor potential channel 3 (*TRPC3*) [45] and interleukin 21 (*IL21*) [46, 47], complement factor I (*CFI*) [1, 42], aminopeptidase A ectopeptidase (*ENPEP*) [43, 44] and nephronectin (*NPNT*) [41] (Figure 1). On *IGAN3* different chemokines (*CCL2*, *CCL3*, *CCL5*, *CCL7*) were selected and a chemokine receptor (*CCR7*) [27, 28], granulin (*GRN*) [31, 32], α and β subunits of the platelet adhesive glycoprotein receptor complex GPIIb/IIIa (*ITGA2B* and *ITGB3*) and integrin α3 (*ITGA3*) [33–37]. Furthermore, the hepatocyte nuclear factor-1-β (*HNF1B*) [29], CD300 antigen-like family member G (*CD300LG*) [30], *TBX21* [38, 39] and β-1,4-*N*-acetyl-galactosaminyltransferase2 (*B4GALNT2*) [40] were analysed [Figure 2, (see Supplementary data for colour version) Table 2]. The last set of genes have never been analysed in previous studies for association to IgAN (Supplementary data).

SNP selection and genotyping

Peripheral blood from patients and controls was collected in ethylene diamine tetra-acetic acid, and genomic DNA was extracted by 'salting out' method [48] or automatically (Macherey–Nagel kit). Electrophoresis was carried out using 2% agarose, stained with ethidium bromide and visualized by ultraviolet transillumination. All DNA samples were quantified with the PicoGreen method.

Within the detected genes mentioned above, 192 polymorphisms were carefully selected. Tag SNPs, that are organized into a region of the genome with high linkage disequilibrium (LD), and functional SNPs were chosen to further narrow the linked chromosomal regions. Functional SNPs were nonsynonymous and synonymous polymorphisms, SNPs in TFBS (transcription factor-binding site), ESE (exonic splicing enhancer) and ESS (exonic splicing silencers) regions, triplex and intron–exon boundaries regions (http://pupasuite.bioinfo.cipf.es). Minor allele frequency (MAF) of all the SNPs in the selected gene was analysed in CEPH (Utah residents with Northen and Western European Ancestry) population. Organizing selected polymorphisms into LD bins with pairwise $r^2 > 0.8$ (http://hapmart.hapmap.org/BioMart/martview), tag SNPs with MAF > 0.05 (www.sysnps.org) and functional SNPs with MAF > 0.01 were selected (Table 2).

The 192 selected SNPs (179 successfully genotyped) were analysed with the Veracode GoldenGate technology (Illumina), a multiplex recent testing method based on digitally encoded microbeads. A designability rank score (0–1.0) was calculated for each SNP by Illumina for the conversion of a SNP into a successful GoldenGate assay. Of the 192 SNPs, 170 with a score >0.6 (designability rank = 1, high success rate) and 20 with a score between 0.4 and 0.6 (designability score = 0.5, moderate success rate) were selected (Supplementary data: Illumina SNPs list). The Illumina GoldenGate assay was performed according to the manufacturer's protocol.

For the total number of samples, the call frequency [the frequency of the total number of genotypes in each sample with a GenCall (GC) score above the no-call threshold, from 0 to 1] was 1 for 78 SNPs, between 0.893 and 0.999 for 121 SNPs and 0 for 13 SNPs (not successfully genotyped). Seven loci of the 13 failed SNPs were not successfully genotyped because of the cluster separation, 2 because of the low intensity, 2 because >3 clusters were detected, 1 because an allele failed and 1 because detected only a single cluster was detected (Supplementary data: Illumina Results, Illumina GenomiPhi Results).

The automatic allele calling was done using the Illumina GenCall software with a GC threshold of 0.25. The software assigned three clusters on a graph based on the fluorescence obtained (Supplementary data: Illumina Results, Illumina GenomiPhi Results). The GC score, a confidence score of the genotyping of each point, depends on the intensity of fluorescence and the distance of the point from the centre of the cluster on the graph. The obtained values for each SNPs across all samples referred to as the 50 or 10% GC score is listed in Supplementary data: Illumina Results, Illumina GenomiPhi Results and detailed in Tables 3, 5 and 6 for the SNPs with a significant P-value.

Statistical analysis

Allele and genotype frequencies were calculated by direct gene counting. Hardy–Weinberg (H–W) equilibrium was calculated on the basis of expected genotype frequencies. Both simple analyses, such as the study of allele or genotyping frequencies and the H–W equilibrium, and higher scale analyses such as LD, haplotype estimations and allele and genotype association analyses were performed using the SNPator bioinformatic tool [49] and SPSS (SPSS Inc., Chicago, IL). We considered $P < 0.01$ as the significance cut-off and value. We applied the Bonferroni correction for multiple testing for all comparisons [P_c-value was adjusted for 179 SNPs

Table 1. Characteristics of successfully genotyped patients and controls[a]

Variable	*n* patients	%	*n* controls	%
Centre (n P = 446; n C = 429)				
Brescia	185	41	84	20
Cremona	42	9	40	9
Bologna	34	8	24	6
Trieste	21	5	15	3
Torino	164	37	266	62
Gender (n P = 446; n C = 429)				
M	350	78	173	40
F	96	22	256	60
Age at RB (years) (n P = 420; n C = 429)				
(P range 1–75 years)				
Mean	38		49	
Median	36		48	
Quartiles				
<27	126	31	12	3
27 < x < 48	196	48	185	49
>48	84	21	179	48
Missing	40		53	
Hypertension at B (n P = 279)				
Yes	117	42	ND	ND
No	162	58	ND	ND
Missing	167			
sCr at RB (mg/dL) (n P = 342)				
>1.5 mg/dL	150	44	ND	ND
<1.5 mg/dL	192	56	ND	ND
Missing	104			
CrCl at RB (mL/min) (n P = 226)				
>70 mL/min	153	68	ND	ND
<70 mL/min	73	32	ND	ND
Missing	220			
PTU at RB (g per day) (n P = 351)				
<3 g per day	234	67	ND	ND
>3 g per day	117	33	ND	ND
Missing	95			
Dialysis (n P = 220)				
No	26	12	ND	ND
Yes	194	88	ND	ND
Missing	240			
Renal transplantation (n P = 210)				
No	52	25	ND	ND
Yes	158	75	ND	ND
Missing	10			
Relapse after transplantation (n P = 114)				
No	89	78	ND	ND
Yes	25	22	ND	ND
Missing	44			
Lee's classification (n P = 193)				
Mild	58	30	ND	ND
Severe	135	70	ND	ND
Missing	267			

[a]n P, number of patients; n C, number of controls; sCr, serum creatinine; CrCl, creatinine clearance; PTU, proteinuria; ND, not determinable.

(179 of 192 SNPs were successfully genotyped) and P_c-values <0.05 were considered as significant], with the exception of *C1GALT1*, as it was already associated with IgAN in previous studies. Odds ratios (ORs) were adjusted for sex and age with logistic regression as implemented in SNPstats (http://bioinfo.iconcologia.net/snpstats/start.htm). Power analysis was also performed and applied to each statistical significant association. Our sample size is adequate to achieve 80% power at $P < 0.05$ to detect a positive association for each polymorphism, but not sufficient at $P < 0.01$.

Results

Four hundred and sixty DNA samples (446 successfully genotyped) of North Italian patients affected by IgAN were collected. A healthy North Italian population ($n = 444$; 429 successfully genotyped) geographically matched with patients was also typed as control. Patient characteristics are listed in Table 1.

One hundred and ninety-two SNPs were genotyped (179 successfully). Polymorphisms were carefully selected within candidate genes by function (*C1GALT1*, *C1GALT1C1*, *TFRC*, *FCAR*, *TLR4*, *ST6GALNAC2*) and by mapping in two genomic regions, *IGAN2* (*NPNT*, *CFI*, *ENPEP*, *TRPC3*, *IL21*; Figure 1, see Supplementary data for colour version) and *IGAN3* (*CCL2*, *CCL7*, *CCL5*, *CCL3*, *HNF1B*, *CCR7*, *CD300LG*, *GRN*, *ITGA2B*, *ITGB3*, *TBX21*, *B4GALNT2*, *ITGA3*; Figure 2) (Table 2). Quality control details can be found in Supplementary data. No statistically significant departures from H–W equilibrium were found.

Table 2. Selected genes and SNPs[a]

Gene	Chr	OMIM	Ensembl ID	Start Ensembl	End Ensembl	Length (bp)	No. of SNPs	No. of failed SNPs[b]	Definition	References
C1GALT1	7	610555	ENSG00000106392	7240414	7250505	10091	10	0	Core 1 synthase involved in glycolsylation of IgA1	[15, 16]
C1GALT1C1	X	300611	ENSG00000171155	119643564	119648033	4469	3	0	Specific chaperone of core 1 synthase	[17, 18]
TFRC	3	190010	ENSG00000072274	197260553	197293343	32790	7	0	Transferrin receptor	[21, 22]
FCAR	19	147045	ENSG00000186431	60077534	60095055	17521	9	0	Fcα receptor of IgA	[23, 24]
TLR4	9	603030	ENSG00000136869	119506291	119519589	13298	10	0	Toll-like receptor 4 involved in glomerular inflammation	[25, 26]
ST6GALNAC2	17	610137	ENSG00000070731	72073056	72093524	20468	7	1	Sialyltransferase involved in sialyation of IgA1	[19, 20]
CCL2	17	158105	ENSG00000108691	29606409	29608329	1920	6	0	Monocyte chemotactic protein 1	[27, 28]
CCL7	17	158106	ENSG00000108688	29621354	29623373	2019	4	1	Monocyte chemotactic protein 3	[27, 28]
CCL5	17	187011	ENSG00000161570	31222613	31231490	8877	4	0	Chemoattractant for blood monocytes	[27, 28]
CCL3	17	182283	ENSG00000006075	31439737	31441517	1780	3	3	Macrophage inflammatory protein 1α	[27, 28]
HNF1B	17	189907	ENSG00000108753	33120548	33179182	58634	17	0	Hepatocyte nuclear factor 1β	[29]
CCR7	17	600242	ENSG00000126353	35963550	35975250	11700	5	1	C–C chemokine receptor type 7 precursor	[27, 28]
CD300LG	17	610520	ENSG00000161649	39280050	39296520	16470	5	0	CD300 molecule-like family member G	[30]
GRN	17	138945	ENSG00000030582	39778017	39785996	7979	3	2	Granulin	[31, 32]
ITA2B	17	607759	ENSG00000005961	39805076	39822399	17323	2	0	Integrin α2β, platelet membrane glycoprotein IIb	[33–37]
ITGB3	17	173470	ENSG00000056345	42686207	42745076	58869	15	1	Integrin β3, platelet membrane glycoprotein IIIa	[33–37]
TBX21	17	604895	ENSG00000073861	43165609	43178484	12875	6	0	T-box 21	[38, 39]
B4GALNT2	17	111730	ENSG00000167080	44565328	44602235	36907	9	3	β-1,4 *N*-acetyl-galactosaminyl transferase 2	[40]
ITGA3	17	605025	ENSG00000005884	45488431	45522843	34412	11	0	Integrin α3, receptor for fibronectin	[36, 37]
NPNT	4	610306	ENSG00000168743	107036054	107112273	76219	11	0	Nephronectin	[41]
CFI	4	217030	ENSG00000205403	110881301	110942590	61289	8	0	Complement factor I precursor	[1, 42]
ENPEP	4	138297	ENSG00000138792	111616697	111702812	86115	14	1	Glutamyl aminopeptidase	[43, 44]
TRPC3	4	602345	ENSG00000138741	123019633	123092359	72726	10	0	Transient receptor potential cation channel 3	[45]
IL21	4	605384	ENSG00000138684	123753221	123761662	8441	3	0	Interleukin 21	[46, 47]

[a]Chr, chromosome.
[b]Failed SNPs were selected, but not successfully genotyped.

Associations

Allele, genotype and haplotype frequencies were compared between cases and controls to assess the potential role of each gene and SNP in the susceptibility to IgAN.

Overall, no SNP showed significantly different allele frequencies between the whole set of cases and controls (Supplementary data: SNPs frequency and association). However, genotype frequencies were different for two SNPs in *C1GALT1*: rs1008898 [GG + GT versus TT genotype; P = 0.0019; OR = 2.65; 95% confidence interval (CI) = 1.40–5.01], already analysed by Pirulli *et al.* [15] and Li *et al.* [16], and rs7790522 (AA + AG versus GG genotype; P = 0.0049; OR = 2.07; 95% CI = 1.24–3.46) (Figure 3, see Supplementary data for colour version). These SNPs resulted in almost complete LD (D' = 0.95, Haploview, www.hapmap.org). To verify these associations, the analysis was performed with patients (n = 232) not included in the study of Pirulli *et al.* [15] and the association was marginally replicated (rs1008898: GG + GT versus TT genotype; P = 0.0468; OR = 2.48; 95% CI = 0.99–6.22).

Overall, no significant associations in genotype frequencies were observed for the genes in the *IGAN2* and *IGAN3* genome regions (Figure 4, Figure 5, see Supplementary data for colour versions).

Gender

The patient population was stratified by gender (males: n = 361, 78%; females: n = 99, 22%). In males, various SNPs of different genes showed an association with the risk to develop IgAN: rs1008898, rs13245879 and rs7790522 of *C1GALT1*; rs12453522 of *CD300LG*, rs4792938 of *GRN*, two SNPs of *ITGB3* (rs5918 and rs4629024) and one of *ITGA2B* (rs850730) (Table 3).

Also in females, different SNPs were found to be associated with IgAN: rs10263069 of *C1GALT1*, rs3796892 of *ENPEP*, rs6820068 of *TRPC3* and two SNPs of *B4GALNT2*, rs4550490 and rs1403528; the last one remains statistical significant after Bonferroni correction (Table 3).

Different haplotypes resulted from each gene with at least one SNP associated with IgAN which were analysed in males and females. Each haplotype was compared both with the most common haplotype and with all the other haplotypes. In males, the GAAGA and GGAGC haplotypes of *CD300LG* were associated, respectively, with a higher and a lower risk to develop IgAN and GT and CT haplotypes of *ITGA2B* with a higher and a lower risk, respectively (Table 4). Furthermore, GCGATTGCCTGGGAT haplotype of *ITGB3*, when analysed versus all the other haplotypes, is associated with a higher risk to develop IgAN (Table 4).

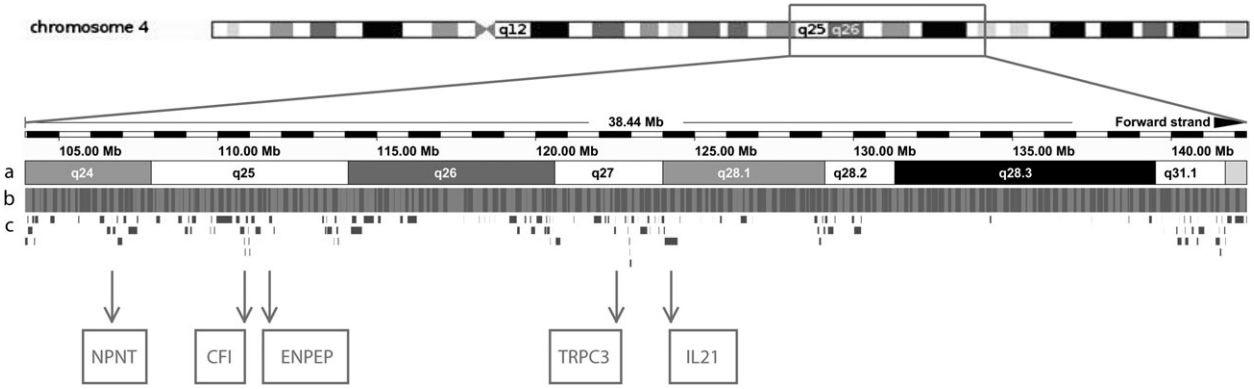

Fig. 1. Chromosome 4q24–31 (*IGAN2*) and selected genes. (**a**) Chromosome bands; (**b**) Contigs; (**c**) Ensembl/Havana genes (from www.ensembl.org).

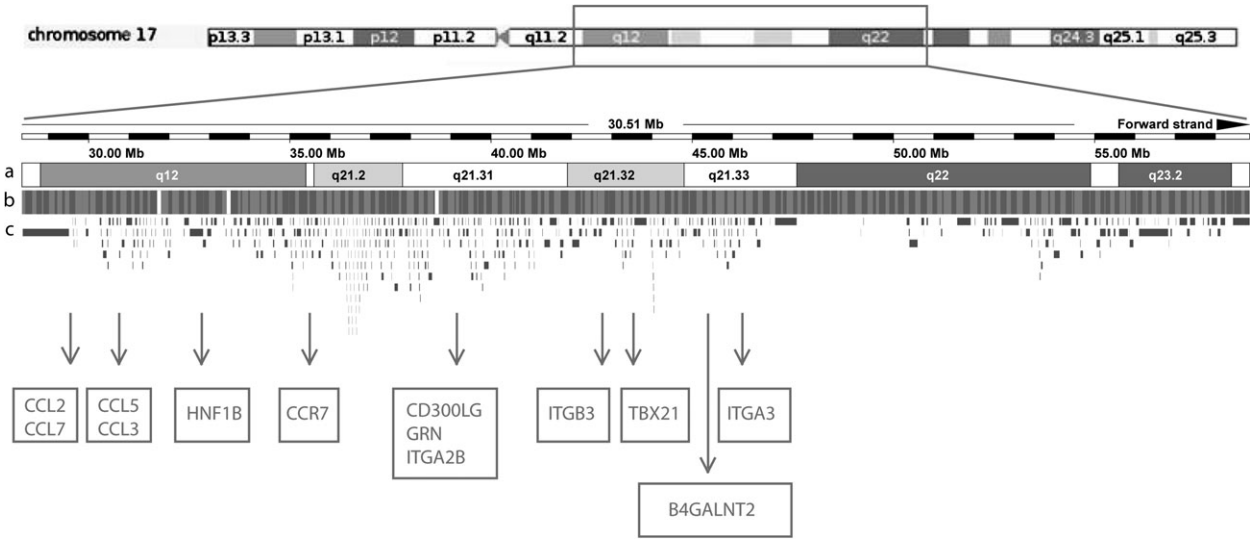

Fig. 2. Chromosome 17q12–22 (*IGAN3*) and selected genes. (**a**) Chromosome bands; (**b**) Contigs; (**c**) Ensembl/Havana genes (from www.ensembl.org).

In females, the most common haplotype of *B4GALNT2* (GAATGACGA), in both the analyses with six different haplotypes and with all the other haplotypes, is associated with a lower risk to develop IgAN (Table 4).

Age at time of RB

IgAN shows a great diversity in age at onset, which may signal heterogeneity in its genetic architecture. Therefore, patient populations were stratified by quartiles into different groups of age at RB. Frequencies between minor and major quartiles were compared: the first included patients aged <27 years ($n = 126$, 31%) and the second patients aged >48 years ($n = 84$, 21%). In patients aged <27 years, an association between the risk to develop IgAN and rs4449421 of *NPNT* was found as well as rs7873784 of *TLR4* and four SNPs of *ITGB3*, rs2015049, rs2292867, rs5918 and rs3809865 (Table 5).

In patients aged >48 years, rs3917878 of *CCL2*, rs7224013 of *CCL7* and rs2290065 of *CCR7* resulted in association with the disease (Table 5).

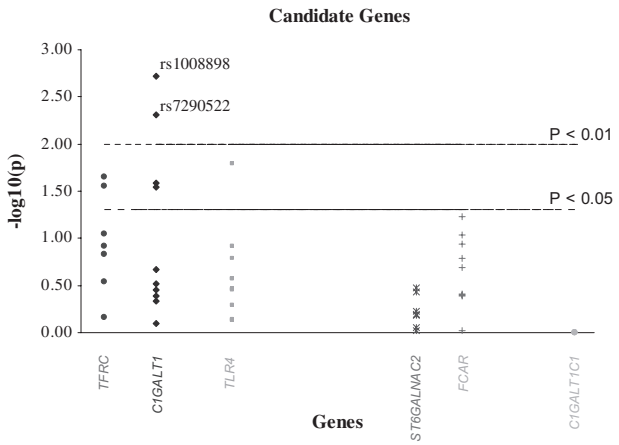

Fig. 3. SNP genotype associations of candidate genes with the development of IgAN.

Since *ITGB3* showed an association with the risk to develop IgAN both for males and young patients, an analysis in males aged <27 years was performed and an association of rs5918 SNP was found (Table 5).

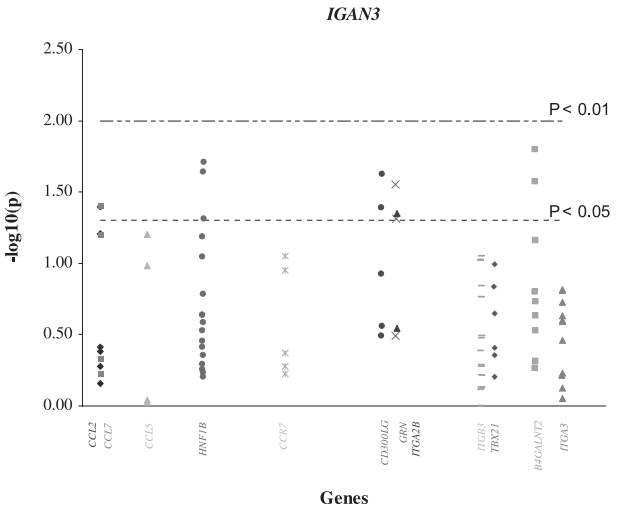

Fig. 4. SNP genotype associations of analysed genes in chromosome 17 (*IGAN3*) with the development of IgAN. Distances along the x-axis are proportional to the actual physical base pair distances.

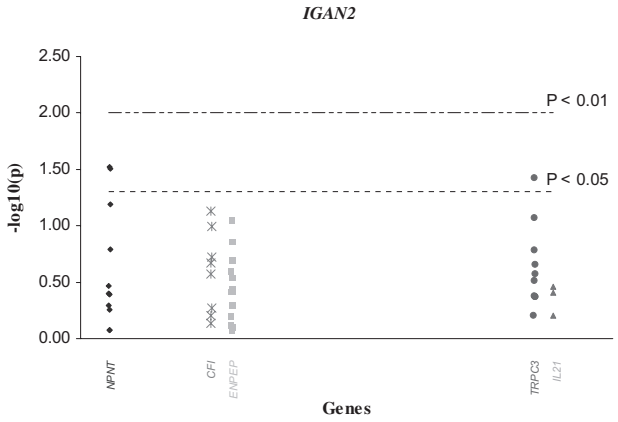

Fig. 5. SNP genotype associations of analysed genes in chromosome 4 (*IGAN2*) with the development of IgAN. Distances along the x-axis are proportional to the actual physical base pair distances.

Significant genotypic associations within sex and age classes were confirmed by means of logistic regression with both sex and age class as covariates, by means of the SNPstats programme (Table 3, Table 5) (http://bioinfo.iconcologia.net/SNPstats) [50].

Clinical characteristics

Patients were also stratified according to several clinical parameters, which could represent endophenotypes. Hypertension, serum creatinine level, creatinine clearance, proteinuria at RB and outcome such as dialysis, renal transplantation and relapse of IgAN after transplantation were analysed. An association between IgAN with mild proteinuria (<3 g per day) and three SNPs of *C1GALT1* (rs1008898, rs13245879 and rs7790522) was found as well as between IgAN with severe proteinuria (>3 g per day) and one SNP of *TLR4* (rs7873784) (Table 6).

Discussion

In this study, a comprehensive scan for genotype association in Italian IgAN patients was provided for the first time. One hundred and ninety-two selected polymorphisms were analysed in 23 genes in a large homogeneous North Italian population using high-throughput SNP genotyping. Genes were carefully selected based on their possible functional involvement in IgAN (6 genes) or because of their location within two candidate regions in linkage with the disease, the first on chromosome 17q12–22 (*IGAN3*, 13 genes) and the second on chromosome 4q26–31 (*IGAN2*, 5 genes) [11].

The heterogeneity of the IgAN phenotype seems to be consistent with the combined effects of multiple interacting polymorphic genes and environmental factors [7, 11, 51, 52]. One of the difficulties in predicting the genetic influence of the susceptibility to IgAN is the involvement of immunologic factors and extracellular matrix rearrangements, which could affect the pathogenesis of IgAN. Age and gender are likely to evidence distinct immunological and inflammatory reactions leading to individual susceptibility to IgAN. Our results suggest a genetic involvement in IgAN, especially when stratified for gender, age and proteinuria.

Firstly, candidate genes selected by function were analysed. In the cohort of North Italian patients affected by IgAN, two polymorphisms (rs1008898 and rs7790522) of *C1GALT1*, the core-1-β1,3-galactosyltransferase, already known to be involved in glycosylation of IgA1 [15, 16], showed an association with the risk to develop IgAN. Furthermore, rs1008898 is described to be in LD with rs11772919 ($D' = 0.69$), an SNP correlated with the expression of *C1GALT1* (http://eqtl.uchicago.edu). This is further evidence that the *C1GALT1* gene is associated with the disease and suggests that its modified expression, possibly due to different SNPs, might be involved in the hypoglycosylation of IgA1. *C1GALT1* also showed an association, with different SNPs, in males and females separately and in patients with mild proteinuria (<3 g per day).

When the population was stratified by age, SNP rs7873784 in the *TLR4* gene showed an association in individuals aged <27 years and in patients with severe proteinuria (>3 g per day). *TLR4* expression has been recently investigated in IgAN by Coppo *et al.* [26] and its engagement in circulating mononuclear cells has suggested a role in the development of glomerular inflammation and a possible specific involvement in IgAN. Yoon *et al.* [53] proposed that serum CD14, a TLR4 ligand on the surface of macrophages/monocytes, affect IgAN progression, modulating the mesangial responsiveness to deposited IgA [54]. It might be hypothesized that rs7873784 in *TLR4* (or other SNPs in LD with it) might up-regulate the production of TLR4, although we could not find any data correlating *TLR4* expression with its SNPs (http://eqtl.uchicago.edu).

Secondly, the selected linked regions were analysed. On *IGAN3*, an association between the risk to develop IgAN in males and rs12453522 of *CD300LG* was found. The amino acid sequence of the immunoglobulin (Ig) V-like domain of CD300LG glycoprotein shows ~35% identity to those of the polymeric Ig receptor (pIgR) and of Fcα/muR [30], generating a possible binding for IgA. SNP rs4792938 in

Table 3. SNP allele and genotype associations with the development of IgAN in males and females[a]

Gender	Gene	Chr	SNP	50% GC score	10% GC score	Risk configuration (allele/genotype)	P (%)	C (%)	P-value	Raw OR	95% CI	Adjusted OR (95% CI)
Males	*C1GALT1*	7	rs1008898	0.8201	0.8122	GG + GT	97	91	0.0031	3.13	1.42–6.90	3.23 (1.32–7.69)
			rs13245879	0.8312	0.8312	TT + GT	100	97	0.0029	12.5	1.50–104.99	11.11 (1.37–100)
			rs7790522	0.8475	0.8475	AA + AG	95	88	0.0062	2.42	1.27–4.64	2.44 (1.20–5.00)
	CD300LG	17	rs12453522	0.8437	0.8437	AA	68	57	0.0095	1.64	1.13–2.39	1.64 (1.10–2.50)
	GRN	17	rs4792938	0.6937	0.6937	GG + GC	94	86	0.0008	2.79	1.50–5.18	2.94 (1.45–5.88)
	ITGA2B	17	rs850730	0.6046	0.6046	G allele	39	29	0.0029	1.52	1.15–2.00	ND
						CG + GG	62	49	0.0051	1.69	1.17–2.44	1.76 (1.16–2.59)
	ITGB3	17	rs4629024	0.915	0.915	CC	15	7	0.0065	2.43	1.26–4.67	2.28 (1.14–4.56)
			rs5918	0.8076	0.7822	C allele	17	10	0.0021	1.87	1.25–2.81	ND
						CC + CT	31	18	0.0017	2.04	1.30–3.20	1.98 (1.21–3.23)
Females	*C1GALT1*	7	rs10263069	0.9168	0.9168	CT + TT	80	63	0.0056	2.25	1.26–4.04	1.79 (0.89–3.61)
	ENPEP	4	rs3796892	0.3566	0.3566	CC[b]	3	0	0.0045	ND	ND	ND
	TRPC3	4	rs6820068	0.8239	0.8239	CT + CC[c]	23	12	0.0084	2.24	1.22–4.12	2.47 (1.23–4.97)
	B4GALNT2	17	rs1403528	0.8967	0.8967	A allele	74	56	1.206×10^{-5}[d]	2.25	1.56–3.26	ND
			rs4550490	0.9156	0.9156	AA	54	31	0.0001[e]	2.65	1.64–4.28	2.44 (1.37–4.35)
						T allele	63	51	0.0072	1.59	1.13–2.23	ND

[a]Chr, chromosome; P, patients; C, controls; ND, not determinable.
[b]CC genotype in controls is = 0.
[c]CC genotype in both patients and controls is = 0. Adjusted OR, OR adjusted with logistic regression.
[d]Significant P-value after Bonferroni correction ($P_c < 0.01$).
[e]Significant P-value after Bonferroni correction ($P_c < 0.05$).

Table 4. Haplotype associations with the development of IgAN in males and females[a]

Gender	Gene	Haplotype	Cod.	P (%)	C (%)	Analyses	P-value	OR	95% CI
Males	*CD300LG*	GAAGA[b]	H1[b]	52	43	H1/others	0.0057	1.4411	1.11–1.87
		GGAGC	H2	17	24	H2/others	0.0071	0.6490	0.47–0.89
			H2	25	36	H2/H1	0.0018	0.5846	0.42–0.82
	ITGA2B	CT[b]	H1[b]	54	66	H1/others	0.0001[c]	0.5921	0.45–0.77
		GT	H2	39	29	H2/others	0.0029	1.5202	1.15–2.00
			H2	42	31	H2/H1	0.0007	1.6226	1.23–2.15
	ITGB3	GTGGCTACTTTGTAA[b]	H1[b]	22	22	ND	ND	ND	ND
		GCGATTGCCTGGGAT	H2	16	9	H2/others	0.0010	2.0167	1.32–3.09
Females	*B4GANT2*	GAATGACGA[b]	H1[b]	9	20	H1/others	0.0021	0.4116	0.23–0.73
		GAATGACAA	H2	42	16	H2/H1	0.0033	3.8392	1.51–9.78
		GAACGCTAG	H3	17	2	H3/H1	0.0073	8.9000	1.37–57.80
		ACGTAATAA	H4	50	20	H4/H1	0.0009	4.0455	1.72–9.51
		GCATGATAA	H5	32	9	H5/H1	0.0048	4.6148	1.49–14.27
		GCATGACAA	H6	17	2	H6/H1	0.0073	8.9000	1.37–57.80
		GAATGCTAA	H7	12	0[d]	H7/H1	0.0011	ND	ND

[a]For each gene are shown the most common haplotype and haplotypes with P-value <0.01. P, patients; C, controls; ND, not determinable.
[b]Most common haplotype.
[c]Significant P-value after Bonferroni correction ($P_c < 0.05$).
[d]Number of controls presenting H7 = 0.

granulin gene (*GRN*), which may be involved in immune response dysregulation [31, 32], also showed an association. Granulin is a pluripotent secreted growth factor that mediates cell cycle progression and cell motility [32]. This SNP might change the expression of the gene, thereby contributing to the susceptibility to IgAN. Furthermore, SNPs of *ITGA2B* (rs850730) and *ITGB3* (rs4629024 and rs5918) integrins, the α and β subunits of the platelet adhesive glycoprotein receptor complex GPIIb/IIIa, might also be involved. *ITGB3* was also found in association with IgAN in individuals aged <27 years, particularly in males. Among other functions, integrins mediate platelet aggregation through binding of plasma fibrinogen and serve as receptor for platelet adhesion to the extracellular cell matrix *in vivo* [36]. They also regulate mesangial cell proliferation and deposition of excessive extracellular matrix [33–37], which is a recognized risk factor for progression of IgAN. The detected SNPs of *ITGA2B* and *ITGB3* might enhance the expression of these genes and lead to the rearrangement of cellular matrix, triggering mesangial depositions and susceptibility to IgAN. Literature reports that, when stratified for gender, *GRN* is associated with Alzheimer disease [55] and *ITGB3* with the metabolic syndrome in female patients [56].

In females, *B4GALNT2* showed an association with IgAN. B4GALNT2 catalyses the addition of an *N*-acetylgalactosamine residue via a β-1,4 linkage to a subterminal galactose residue substituted with an α-2,3-linked sialic acid [40]. Among other functions, *B4GALNT2* was supposed to change the expression of von Willebrand factor (vWF) and may be relevant in type I von Willebrand

Table 5. SNP allele and genotype associations with the development of IgAN in patients with age <27 or >48 years[a]

Age	Gene	Chr	SNPs	50% GC score	10% GC score	Risk configuration (allele/genotype)	P (%)	C (%)	P-value	Raw OR	95% CI	Adjusted OR (95% CI)
Age <27 years	*NPNT*	4	rs4449421	0.8891	0.8891	TT	3	0[b]	0.0021	14.03	1.55–126.72	ND
	TLR4	9	rs7873784	0.8316	0.8316	G allele	91	83	0.0019	2.08	1.30–3.35	ND
						GG	83	69	0.0014	2.26	1.36–3.78	2.86 (1.52–5.26)
	ITGB3	17	rs2015049	0.7785	0.7785	G allele	87	79	0.0072	1.71	1.15–2.55	ND
			rs2292867	0.7782	0.7782	C allele	92	85	0.0031	2.07	1.27–3.40	ND
						CC	84	72	0.0050	2.09	1.24–3.52	2.56 (1.37–5.00)
			rs5918	0.8076	0.7822	C allele	21	14	0.0033	1.71	1.19–2.46	ND
						CC + CT	39	25	0.0024	1.92	1.26–2.93	2.18 (1.33–3.58)
			rs3809865	0.8225	0.8225	T allele	38	28	0.004	1.54	1.15–2.07	ND
Age >48 years	*CCL2*	17	rs3917878	0.8282	0.8282	TT	5	0[b]	0.0008	10.63	1.91–58.99	ND
	CCL7	17	rs7224013	0.8243	0.8243	AA	5	0[b]	0.0008	10.68	1.92–59.27	ND
	CCR7	17	rs2290065	0.8516	0.8516	TT	4	0[c]	0.0015	15.85	1.63–154.30	ND
Males aged <27 years	*ITGB3*	17	rs5918	0.8076	0.7822	C allele	24	14	0.0003	2.04	1.38–3.02	ND
						CC + CT	43	25	0.0004	2.32	1.45–3.73	3.47 (1.86–6.45)

[a]Chr, chromosome; P, patients; C, controls; adjusted OR, OR adjusted with logistic regression; ND, not determinable.
[b]Two controls presenting risk genotype.
[c]One control presenting risk genotype.

Table 6. SNP allele and genotype associations with proteinuria (PTU) in patients with IgAN[a]

PTU (g/day)	Gene	Chr	SNPs	50% GC score	10% GC score	Risk configuration (allele/genotype)	P (%)	C (%)	P-value	OR	95% CI
<3 g/day	*C1GALT1*	7	rs1008898	0.8201	0.8122	G allele	83	76	0.0052	1.5017	1.13–2.00
						GG + GT	99	92	0.0001[b]	9.9418	2.37–41.77
			rs13245879	0.8312	0.8312	T allele	93	88	0.0054	1.7785	1.18–2.68
			rs7790522	0.8475	0.8475	A allele	79	72	0.0053	1.4644	1.12–1.92
						AA + AG	97	89	0.0002[b]	4.4648	1.88–10.63
>3 g/day	*TLR4*	9	rs7873784	0.8316	0.8316	G allele	90	83	0.0091	1.8451	1.16–2.94
						GG	81	69	0.0077	1.9749	1.19–3.28

[a]Chr, chromosome; P, patients; C, controls.
[b]Significant P-value after Bonferroni correction ($P_c < 0.05$).

disease, characterized by reduced levels of plasma vWF [57–59]. *B4GALNT2* might influence the rearrangement of cellular matrix in IgAN and susceptibility to the disease, but further investigations on gene expression and molecular mechanisms even in IgA glycosylation are needed.

Finally, the *IGAN2* region was analysed. Genes in association with the risk to develop IgAN were found in females: *ENPEP*, that may affect the renin–angiotensin system [43, 44], *TRPC3*, the transient receptor potential channel 3 [45], and *NPNT*, in individuals aged <27 years, that may play a role in maintaining kidney filtration barrier [41].

Taken together, these findings suggest a genetic predisposition to sporadic IgAN. In this study, several genes were analysed in IgAN for the first time, tag and functional SNPs being selected with MAF >0.05 and >0.01, respectively. For this reason, rare SNPs cannot be excluded to be associated with the disease as well as polymorphisms in other genes within *IGAN2* and *IGAN3* that we did not analyse. Other variants, such as insertion/deletion and copy number variations, were excluded for technical reasons and some SNPs did not pass the Illumina bioinformatics prediction of typing. Our sample size is adequate to achieve 80% power at $P < 0.05$ to detect a positive association for each polymorphism, but not sufficient at $P < 0.01$. Population stratification for sex and age may be a potential source of spurious associations, but this study does not argue to be a genome-wide association study; we analysed a few SNPs in a small number of selected candidate genes only in North Italian population samples and it does not claim overall significance for any SNP. Finally, although the North Italian population can be considered genetically homogeneous [60], any genetic stratification would also confound the present study, with a potential to create false positives. Therefore, we could consider our results as preliminary for future studies.

Moreover, clinical data of the analysed population were partially lacking and different clinical characteristic collected at time of RB could be influenced by environmental factors and medical treatments. Excluding proteinuria, this could be a reason for the inefficiency of the analysis with clinical characteristics.

Overall, we might confirm the influence of *C1GALT1* on the susceptibility to IgAN and hypothesize a role of *TLR4* on proteinuria. A significant association between *B4GALNT2* and the disease was also found, and it might be an interesting starting point for future analyses. Expression studies and other investigations in different populations with a larger sample size should be performed in order to replicate these results and to identify a possible causative gene variant for the disease. Genome-wide association

studies, given their recent success in a number of common diseases, could also contribute to detect additional susceptibility genes for IgAN. Genetic factors possibly involved in the disease may be used for many purposes such as prognosis and to develop an IgAN 'risk index', which may improve new strategies to reduce disease risk factors and eventually lead to a better treatment of the disease.

Supplementary data

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

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Conflict of interest statement. None declared.

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