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Genetics of membranous nephropathy

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ABSTRACT

An HLA-DR3 association with membranous nephropathy (MN) was described in 1979 and additional evidence for a genetic component to MN was suggested in 1984 in reports of familial MN. In 2009, a pathogenic autoantibody was identified against the phospholipase A₂ receptor 1 (PLA₂R1). Here we discuss the genetic studies that have proven the association of human leucocyte antigen class II and PLA₂R1 variants and disease in MN. The common variants in PLA₂R1 form a haplotype

that is associated with disease incidence. The combination of the variants in both genes significantly increases the risk of disease by 78.5-fold. There are important genetic ethnic differences in MN. Disease outcome is difficult to predict and attempts to correlate the genetic association to outcome have so far not been helpful in a reproducible manner. The role of genetic variants may not only extend beyond the risk of disease development, but can also help us understand the underlying molecular biology of the PLA₂R1 and its resultant pathogenicity. The

genetic variants identified thus far have an association with disease and could therefore become useful biomarkers to stratify disease risk, as well as possibly identifying novel drug targets in the near future.

INTRODUCTION

Membranous nephropathy (MN) is a kidney-specific autoimmune disease with an incidence of 10 per million persons per year [1]. It is the leading cause of nephrotic syndrome in European adults and progresses to end-stage renal disease (ESRD) in 30–40% of cases [2]. Unlike many other autoimmune disorders, males are more often affected. Approximately 25% of patients have a secondary form of MN (SMN), which is diagnosed when an alternative identifiable underlying clinical condition is present (e.g. systemic lupus erythematosus, malignancy, medication or viral infections) [2]. The remaining 75% of patients have no apparent cause and the condition is termed as 'primary' or idiopathic membranous nephropathy (IMN) [3]. IMN is caused by *in situ* binding of circulating antibodies to a podocytic antigen. Phospholipase A₂ receptor 1 (PLA₂R1) and thrombospondin type-1 domain-containing 7A (THSD7A) are the major target antigens involved in the pathogenesis of IMN [4, 5]. Subepithelial immunoglobulin-rich deposits demonstrated by electron microscopy are pathognomonic in MN [6], constituting a definitive phenotype. While IMN does not show simple Mendelian inheritance, the role of underlying genetic factors has been confirmed in recent studies.

DISCOVERY OF AUTOANTIGENS

The first autoantigen described in a rare case of antenatal MN was neutral endopeptidase (NEP), in 2002 [7]. The gene encoding NEP is metallomembrane endopeptidase. Truncated mutations were discovered in maternal DNA so the mother did not express NEP protein. When foetal NEP (paternal protein) was encountered during pregnancy, anti-NEP antibodies developed (with no consequence to the mother) that crossed the placenta to cause neonatal MN [7, 8].

The discovery of circulating antibodies to the autoantigen PLA₂R1 revolutionized our understanding of IMN as an autoimmune disease [4]. With western blots and mass spectrometry, the antibody was detected in serum from 26 of 37 patients (70%) [4]. This has been confirmed in subsequent studies and proven specific to IMN and implicated in disease progression and outcome [9, 10].

Most recently, combined immunologic and proteomic approaches identified THSD7A as another target autoantigen in MN [5]. THSD7A antibodies are found in ~2–3% of MN patients. THSD7A like PLA₂R1 is a heavily glycosylated, multi-domain transmembrane receptor located on the podocyte membrane. THSD7A resembles some of the PLA₂R1 immunological characteristics and autoantibody findings correlate with glomerular staining of the antigen. It is not understood why autoantibodies develop, however, in some THSD7A-associated cases, the development of antibodies may be linked to malignant tumours [11, 12]. Interestingly, dual positivity to both

PLA₂R1 and THSD7A is extremely rare, with only two cases identified on biopsy staining [13].

Familial clustering of MN

While all available data point towards a strong genetic component, IMN appears not to be inherited in a simple Mendelian fashion. In 1984, the first case of identical twins developing IMN was published [14]; to date, 16 families have been reported to have familial IMN [14–22], suggesting a strong genetic contribution. However, several sets of monozygotic twins with IMN had different phenotypes with a different age of onset and progression of disease [14, 20]. This suggests an environmental contribution to disease that is not yet well established.

There is a strong male preponderance in IMN [23], unlike other autoimmune diseases [24]. An X-linked recessive pattern of inheritance was suggested based on the clustering of disease between non-identical brothers [14, 16, 17, 19]. Autosomal inheritance was also apparent in other families, with male-to-male transmission [18, 22] and affected members of both genders [16, 21]. Further support for the theory of an underlying genetic mechanism was provided by two brothers with a rare syndromic form of IMN [17]. These brothers had both IMN and deafness but no linked HLA alleles [17]. To date the involvement of antibodies against phospholipase A₂ receptor 1 (aPLA₂R1ab) in cases of familial MN is unknown.

Two studies of paediatric primary MN report much lower positivity for PLA₂R1 staining of immune complexes on biopsy (6% and 45%) compared with adult studies (70–80%) [25, 26]. As yet, the genetic background to paediatric MN has not been confirmed.

CONCEPT OF GENOME-WIDE ASSOCIATION STUDIES AND CONFIRMATION OF GENETIC ASSOCIATION

The biggest breakthrough in the contribution of genetic factors in IMN so far was with three genome-wide association studies (GWASs) published in 2011 [15]. GWASs work with the hypothesis that the phenotype is associated with variations in a subset of several genes. These variations will be demarked by haplotypes/alleles that display frequency differences in the cases and controls. GWASs examine all chromosomes and their simplest form compares allele frequencies of given variations in cases to allele frequencies of controls (basic allele test). GWASs most often use common single-nucleotide polymorphisms (SNPs), which are defined by an allele frequency in a given population of >5%. The tenet is that any given disease, as long as there is no heterogeneity, will show a difference in the frequency of genetic variation within disease-associated genomic regions compared with unaffected controls. Thus SNPs utilized as genetic markers identify a chromosomal location of interest associated with disease. If the phenotype is clearly described and unique, then GWASs can be powerful for discovery of associated alleles, even with few cases [27, 28]. The first GWAS published in macular degeneration utilized 96 cases and 50 controls [27]. Of all SNPs genotyped, 105 980 were analysed and an intronic and common variant in the *complement factor H* gene that increased the likelihood of macular degeneration by a

factor of 7.4 was discovered [27]. This is contrary to the opinion (misconception) often presented in public that GWASs always need thousands or tens of thousands of samples to be able to identify genetic causes. When a phenotype is complex (i.e. hypertension and kidney failure), then indeed many more samples are needed to be able to identify regions of interest (i.e. associated alleles).

GWASs in MN

The GWAS published in 2011 investigated European populations with renal biopsy-proven IMN [15]. Three independent GWASs were performed, using 75 French European cases, 146 Dutch European cases and 335 British European cases. Despite the small number of cases even in the smallest cohort (French), a significant association in three SNPs in an *HLA-DQA1* allele on chromosome 6 was found. The 146 Dutch cases demonstrated a significant allelic association of 191 SNPs in *HLA-DQA1*. Additionally, six SNPs located within the *PLA2R1* gene on chromosome 2 were associated with IMN, the strongest being SNP rs4664308. Finally, the British study found a significant association with 144 SNPs in the *HLA-DQA1* allele and 2 SNPs in the *PLA2R1* allele. Combining the three cohorts in a meta-analysis with a total case population of 556 further strengthened the association of IMN, with 20 SNPs in *HLA-DQA1* and 13 SNPs in *PLA2R1*. The effect size of the risk SNPs was examined and even in a heterozygous state of the risk allele the odds ratio (OR) was increased in both *HLA-DQA1* and *PLA2R1*. The strongest association was with the *HLA-DQA1* region (the most significantly associated SNP being rs2187668) [15]. In a homozygous state of the *HLA-DQA1* risk allele the OR of IMN was 20.2 [15]. The OR in a homozygous state for *PLA2R1* was 4.2 [15]. Combining these two risk alleles further increased the risk of IMN to an OR of 78.5 [15]. This association was very robust for such a modest cohort [29], which is unusual for a GWAS [16]. In addition, no association was found with immunoglobulin G chains that were previously identified with a candidate gene approach on chromosome 14 [30, 31].

Imputation

The SNP coverage of these initial GWASs is low compared with the coverage available with more modern technology, particularly of the HLA alleles [32, 33]. To further assess the strength of the SNP associations that were found in the British study, an imputation study was performed [34]. Imputation is a method to increase the statistical power of association studies and potentially identify additional associated alleles [35, 36]. This technique is based on knowledge about short stretches of shared haplotypes to provide information and predict untyped alleles [37]. Imputation takes advantage of haplotype composition to match known SNPs to other SNPs that are in linkage disequilibrium with one another. In this way, it was possible to impute and examine 8.9 million SNPs in the British cohort. The strongest signals remained in *HLA-DQA1* and *PLA2R1*, and no additional loci were found as independent risk factors. The *PLA2R1* signal was somewhat weaker and *HLA-DQA1* somewhat stronger

than originally described; with homozygous risk alleles at both loci the combined OR was greater, at 79.4 [34]. In addition, imputation of classic HLA alleles was performed, with the DRB1*0301-DQA1*0501-DQB1*0201 haplotype showing the strongest association but providing little information beyond the lead SNP in *HLA-DQA1*. Subgroup analyses were undertaken and there was no significant gender-specific genetic difference and no additional loci were found on the X chromosome [34], which may have been unexpected given the unusually strong male preponderance in IMN, but statistical power for these analyses was limited. The HLA region was analysed in much more detail and this demonstrated a several hundred kilobase pair linkage disequilibrium around *HLA-DQA1* as well as other HLA class II genes [34].

M-type PLA₂R1

To investigate whether specific variants within the *PLA2R1* gene are causing this previously mentioned strong genetic association, sequencing of the 30 *PLA2R1* coding exons was performed. This was also an ethnically homogeneous group, as all 95 affected patients were white Europeans and only 45% had circulating aPLA₂R1ab [38]. All exons and splice sites of *PLA2R1* were sequenced by Sanger sequencing and all observed variants, including rare variants (minor allele frequency <1%), were analysed. To our initial surprise, no rare genetic variants causing a conformational change in PLA₂R1 structure were found. Of the variants found, six were common and three were in splice sites (exon–intron boundaries). One of these non-synonymous (causing amino acid alteration) common variants (i.e. M292V) encodes an amino acid located within CTLD1, but this is far removed from the immunodominant epitope in the N-terminal cys-rich domain and unlikely to have a contributory role in the pathogenesis of IMN [38, 39]. One reason for the lack of exonic (i.e. coding) differences may be that the true causal variant(s) lie(s) in the regulatory (i.e. intergenic or intronic) regions of the gene. For this to be examined, sequencing of the whole genomic region would need to be done. A second reason for the lack of significant results was that only 45% of the cohort had detectable aPLA₂R1ab. The remaining patients were aPLA₂R1ab negative and we now know that the association is strongest in aPLA₂R1ab-positive patients.

It is therefore most interesting to note that despite IMN being a rare disease, the variants found in *PLA2R1* were common. An explanation for this would be that the common variants recognized together create a rare haplotype [38]. Additionally, an interaction between the *PLA2R1* variants and the *HLA-DQA1* haplotype in individuals predisposed to developing IMN might be infrequent in causing autoimmunity and may therefore account for the rarity of disease and suggest a mechanism for how IMN develops [40]. Genotyping of hundreds of thousands of individuals will provide an answer to whether there is a unique genetic fingerprint of individuals developing IMN and what proportion of individuals having this genetic fingerprint actually present with IMN (i.e. show penetrance).

Antibody and gene interplay

The presence of circulating antibodies against PLA₂R1 and THSD7A is variable between patients and throughout the different stages of disease [41]. During active nephrosis and disease these levels tend to be high and remission is predated by reducing antibody titres [41]. Serologically, antibody-negative MN patients may have glomerular PLA₂R1 positivity [9]. The underlying pathological mechanism in tissue or serological PLA₂R1 positivity is the same and they represent a spectrum of the same disease. A hypothesis is these patients have the same genetic *PLA2R1* risk variants yet are demonstrating incomplete penetrance of disease manifestation. Studies were undertaken to elucidate the association of genetic variants and circulating antibodies, as the antibody titres have been associated with the severity of disease and long-term outcome [10].

PLA2R1 risk alleles are positively correlated with positivity of the pathogenic aPLA₂R1ab [42]. When patients were divided into low- or high-risk *PLA2R1* genotypes, only 4% of those with the low-risk genotype had detectable aPLA₂R1ab compared with 76% of those with the high-risk genotype [42]. This association was further strengthened for the detection of aPLA₂R1ab after combination with the low- or high-risk *HLA-DQA1* genotypes, with 0 versus 73%, respectively [42]. A larger study compared glomerular PLA₂R1 antibody staining (positivity) with negative patients and found the *PLA2R1* association only in patients with PLA₂R1 positivity. In PLA₂R1-negative patients compared with controls there was no association with *PLA2R1* SNPs [43].

This is relevant since increased aPLA₂R1ab correlates with clinical progression of disease, with higher titres associated with ESRD at 5 years and lower rates of spontaneous remission [10]. In an Indian cohort, however, there was no significant association between aPLA₂R1ab status and *PLA2R1* SNPs. Instead, there was an association of the *HLA-DQA1* risk allele with aPLA₂R1ab positivity [44]. This was subsequently replicated in a European cohort and the presence of the risk alleles in either a heterozygous or homozygous state in *HLA-DQA1* and *-DQB1* was significantly associated with higher circulating aPLA₂R1ab [10]. Neither the SNPs in intron 1 or exon 5 in *HLA-DQA1* alone had an effect on aPLA₂R1ab titres [10]. Two recent Chinese studies demonstrated the strong HLA association with aPLA₂R1ab positivity [45, 46]. One had an association with *HLA-DRB1* and the other had *HLA-DRB3*, both of which share a haplotype and thus may represent a common mechanism in Chinese patients [45–47]. The risk alleles in *PLA2R1* are said to be present in patients with systemic lupus erythematosus (SLE) albeit with lower ORs [48] and aPLA₂R1abs are occasionally found in patients with SLE [49].

Ethnic differences

Our findings from the first IMN GWAS [15] have been replicated in other studies, however, different techniques were used. These studies use a candidate gene approach whereby a specific variant alone is genotyped [50]. These SNPs are chosen as the candidate gene based on prior knowledge about PLA₂R1 or previously described SNPs [50, 51]. This is a major limitation of the candidate gene approach; they can only confirm or refute an association with a variant and cannot detect new

associations [50, 51]. Another limitation is findings are often not replicated in subsequent independent studies, rendering the results potentially unreliable [50, 51]. Table 1 provides a summary of genotyping studies to date in MN.

In MN, the first study utilizing the candidate gene approach was a small Spanish cohort of 89 patients where only a single SNP in both the *HLA-DQA1* and *PLA2R1* genes was investigated [54]. This study too found the same association in both alleles in their cohort, with an added effect of homozygous risk alleles in both genes increasing the OR of IMN to 7.3 [54]. As these studies were performed in European populations, it was of interest to investigate if these associations held true in other ethnicities.

In a cohort of 114 Indian patients the same risk alleles were identified as by Stanescu *et al.* [15, 44]. The strongest association was with the homozygous genotype in the *HLA-DQA1* SNP rs2187668. Three SNPs were associated within *PLA2R1*, one of which was the same SNP described in the GWAS [15], rs4664308 with the AA risk genotype [44]. The risk of IMN was increased by 58.4 with all four risk alleles in *HLA-DQA1* and *PLA2R1* [44]. This is a strong association with a small sample size.

The only study undertaken in African Americans so far examined 243 African American and 467 European cases of IMN [43]. Targeted sequencing of candidate genes using conventional polymerase chain reaction was performed, with genotyping of six *PLA2R1* SNPs and a single SNP in the *HLA-DQA1* region [43]. Further, they differentiated between patients who had PLA₂R1 positivity on renal biopsy (using immunofluorescence) (115 African American cases) and those who did not (128 African American cases) [43]. No association was found in African Americans with the *HLA-DQA1* SNP rs2187668, suggesting that this SNP is tagging the causal variant(s) in individuals of European and East Asian ancestry but not in African Americans. In the European subgroup analysis however, the strong association was present with *HLA-DQA1* [43]. Further, the *PLA2R1* signal was associated with glomerular PLA₂R1 positivity in the African American cohort but not in PLA₂R1-negative patients [43]. The strength of this association was lower than that found in Europeans, with the strongest association in Europeans with detectable PLA₂R1 [43].

Chinese patients demonstrated a similar association, with *PLA2R1* risk alleles increasing the risk of IMN but without any effect on outcomes and response to treatment [52]. Liu *et al.* [52] analysed two SNPs in 129 Chinese IMN patients. The risk allele increased the rates of IMN [52]. There was no difference in the different genotypes relating to progression to ESRD, although the patient numbers were too small to identify such a difference. A heterozygous state for the risk allele in the exonic *PLA2R1* region conferred a lower success rate of achieving remission [52]. A larger study including 1112 Chinese patients with IMN genotyped three SNPs in *PLA2R1* and three SNPs in *HLA* genes and found that both were associated with IMN [42]. Interestingly, in the Chinese population, the association with *HLA-DQA1* was lower than in Europeans and there was no association with HLA Class II alleles apart from *HLA-DOB* or *-DQB2* [42]. A study of 261 Chinese IMN patients linked *HLA-DRB1*1501* most significantly with IMN [45]. After correction for *HLA-DRB1*0301*, the *HLA-DQA1* association was

diminished, as these two loci are in strong linkage disequilibrium with one another [45]. The additive effect of homozygous risk alleles in *HLA-DQA1* and *PLA2R1* increased the OR of IMN to 11.13, which is considerably lower than that found in the European studies [15, 42]. However, with the newly discovered association of *HLA-DRB1* and *PLA2R1*, the OR is considerably higher, at 32.4 in the Chinese population [45]. Another Chinese study in patients phenotyped by *PLA₂R1* positivity demonstrated a stronger association with *HLA-DRB3*0202* and *HLA-DRB1*1501*, with ORs of 24.9 and 17.7, respectively [46]. Both studies have identified the same allele in *HLA-DRB1*1501*, which may truly represent the causative allele in Chinese patients. Difficulties arise with the analysis of such data, as the allele frequencies vary between ethnic groups [56]. The Chinese are genetically heterogeneous, and within a control population there were different minor allele frequencies in *HLA-DQA1* and *PLA2R1* dependent on their geographical location [56].

A study of four SNPs in *PLA2R1* in 199 Korean patients also confirmed an association of disease with rs35771982 and rs3828323 (different from the Stanescu *et al.* SNPs [15, 53]). Patients with SLE had the same genotype as controls [53].

Finally, a Japanese study performed genotyping of 15 SNPs in the *PLA2R1* gene and six *HLA* genes—*A*, *B*, *C*, *DRB1*, *DQB1* and *DPB1* [55]. The discovery sample had 53 patients and the replication study had 130 [55]. After corrections for multiple testing and correlation in the replication study, four SNPs in *PLA2R1* were associated with IMN, two of which were intronic [55]. None of the class I *HLA* genes (*A*, *B* or *C*) were significantly associated with IMN, however, *HLA-DRB1*15:01* was the most strongly associated with an OR of 2.85, followed by *HLA-DQB1* with an OR of 2.6. These ORs increased in the replication study and then subsequently in the combined analysis to 3.09 and 3.1, respectively [55]. Interactions between the *HLA* and *PLA2R1* homozygous risk alleles further increased the risk of developing IMN, with the largest OR of 17.53 in the *HLA-DRB1*15:01-DQB1*06:02* and rs2715928 *PLA2R1* combination. While these interactions are statistically significant, they are still considerably lower than the strength of interactions found in the European GWAS [15]. The differences may be due to sample size differences or because *HLA-DQA1*, which is a larger contributor to the cumulative risk in the European study, was not genotyped in this Japanese study or because of differences in linkage disequilibrium with the causal variant across different ethnic groups.

Functional effect of genes

The underlying genetic risk alleles that have been identified to date are different between individual studies, but universally there is an association of IMN with the human genes encoding leucocyte class II antigens and *PLA₂R1*. Functional studies to ascertain how these genetic variants increase the risk for disease development are required. It is also possible that the previously identified risk alleles do not affect disease onset, but instead disease severity [40].

It is unclear how the genetic risk alleles of class II *HLA* (e.g. *DQA1*) and *PLA2R* are translated through the

pathophysiological disease mechanism, but antigen presentation to T cells to initiate T cell help for a*PLA₂R1*ab production is one possibility. These risk alleles encode protein receptors that interact during antigen presentation to stimulate T cells. In this situation, *PLA₂R1* protein processed in macrophage/dendritic cells is displayed on the cell surface as *PLA₂R1* peptides bound to the class II receptor (*DQA1*) groove. The genetics of *DQA1* will shape the amino acid structure of its receptor groove, thus defining and restricting the possible 15-mer peptide sequences available from *PLA₂R1* that will fit the groove. The genetics of *PLA₂R1* may control the possible enzyme fragmentation pattern of *PLA₂R1* by a change in amino acid either creating or destroying an enzyme cut site, a change in splice sites controlling the protein species available for fragmentation or a change in the level of transcript, leading to higher levels of peptide.

As yet these T cell peptides (the *PLA₂R1* peptides presented on *DQA1*) have not been described experimentally, but studies are in progress. A recent study predicted possible T cell epitopes in *PLA₂R1* and attempted to model the interaction with known class II risk alleles [45]. It is important to emphasize that *DQA1* may not be the causal allele, particularly in non-European ethnicities [45, 46].

To elucidate the *HLA* causal alleles further, larger multi-ethnic GWASs combined with larger-scale *HLA* sequencing and fine-mapping studies are necessary. It is vital to do this before modelling their functional effects; however, it would be useful to have transcriptomic and proteomic studies to ascertain if *PLA2R* expression is modified and if this is due to an increase or decrease in transcriptional or post-transcriptional events.

Remission status

A comparison of 23 spontaneously remitting to 55 non-remitting IMN patients found no difference in genetic variants in *HLA-DQA1* or *PLA2R1* [54]. In contrast, Liu *et al.* [52] reported an association between lower rates of remission after treatment and the *PLA2R1* SNPs rs6757188 (CT genotype) and rs35771982 (CG genotype).

Response to treatment

Patients with the risk genotypes in *HLA-DQA1* and *PLA2R1* respond to immunosuppression, although the OR is low at only 0.12 [54]. The total number of patients assessed was small, with 27 responders and 28 non-responders [54]. After adjustment for baseline proteinuria, the predictive value of the risk genotype increased [54]. Analysis of two different *PLA2R1* SNPs revealed no difference between the outcomes of patients treated either conservatively or with immunosuppression [52].

Decline in renal function

The high-risk alleles (AA genotype) in *HLA-DQA1*, despite being strongly associated with IMN, are potentially protective against declining renal function [54]. High-risk genotype patients had a longer time to doubling of their serum creatinine (16.3 versus 13 years), although this was a small subgroup of only 83 patients [54]. No association was found in the eight Japanese patients who had a 50% increase in their serum

Table 1. Summary of genotyping studies of the HLA region and PLA2R1 in IMN arranged by date of publication

Study authors	SNPs	Ethnicity	IMN (n)	Serum ab positivity (n)	Glomerular PLA2R1 positivity (n)	Allele frequency (%)	Odds ratio	P-value	Controls (n)	Allele frequency (%)
Liu <i>et al.</i> [52]	PLA2R1-rs6757188 PLA2R1-rs35771982	Taiwanese Chinese	129	Unknown	Unknown	67.80 84.10	1.18 1.9	0.4 0.005	106	64.20 73.60
Kim <i>et al.</i> [53]	PLA2R1-rs35771982 PLA2R1-rs3828323	Korean	199	Unknown	Unknown	73.60 73.90	2.6 1.35	<0.001 0.09	356	68.90 71
Stanescu <i>et al.</i> [15, 31] French study	HLA-DQA1 rs2187668 PLA2R1 rs4664308	French European	75	Unknown	Unknown	31.30 23.30	4.48 1.87	1.80E-09 5.10E-03	157	9.20 36.30
Dutch study	HLA-DQA1 rs2187668 PLA2R1 rs4664308	Dutch European	146	Unknown	Unknown	37 26	3.76 2.27	5.60E-27 1.00E-09	1832	13.50 44.40
British study	HLA-DQA1 rs2187668 PLA2R1 rs4664308	British European	335	Unknown	Unknown	41.90 25.30	5.33 2.1	5.20E-36 2.10E-10	349	11.90 41.60
Joint study	HLA-DQA1 rs2187668 PLA2R1 rs4664308	European	556	Unknown	Unknown	39.20 25.20	4.32 2.28	8.00E-93 8.60E-29	2338	13 43.40
Ly <i>et al.</i> [42]	PLA2R1-rs35771982 PLA2R1-rs3749117 PLA2R1-rs4664308 HLA-DQA1-rs2187668	Chinese Han	1112	36 of 71 patients (subgroup)	Unknown	15.50 15.60 84.50 12.10	2.36 2.32 2.35 2.42	1.90E-30 2.23E-29 4.17E-30 1.11E-14	1020	30.10 30 70 5.40
Saeed <i>et al.</i> [43]	HLA-DQA1-rs2187668	Caucasian African All		Only ab positive analysed Only ab positive analysed Only ab positive analysed	280 115 530	44 20 20	3.03 2.17 2.17	1.30E-33 9.84E-07 9.84E-07	337 218 218	20 10 10
Bullich <i>et al.</i> [54]	PLA2R1-rs35771982	Caucasian African All	813 466 1512	Only ab positive analysed Only ab positive analysed Only ab positive analysed	280 115 530	26 7 21	1.98 1.74 1.53	1.44E-14 0.03 1.39E-10	337 218 556	49 17 36
Ramachandran <i>et al.</i> [44]	HLA-DQA1-rs2187668 PLA2R1-rs4664308	Spanish European South Asian-Indian	89 114	Unknown 76	Unknown 86	29 26	3.7 2	<0.001 0.05	286	14 36
Thiri <i>et al.</i> [55] Discovery analysis	HLA-DQA1-rs2187668 PLA2R1-rs3749119 PLA2R1-rs35771982 PLA2R1-rs4664308	Japanese	94 114	Either Only ab positive analysed Only ab positive analysed Only ab positive analysed	Either 94 94 94	39.50 85.20	4.73 5.36 Unknown 3.17 3.1	<0.0001 <0.0001 9.40E-05 <0.0001 0.0003	95 95 95 95	12.20 69 69 3.1
	PLA2R1-rs1511223 PLA2R1-rs35771982 PLA2R1-rs2203053 PLA2R1-rs10196882 PLA2R1-rs16844706 PLA2R1-rs877635		53	Unknown	Unknown	83 82.10 52.90 28.80 43.70 49.10	2.24 3.58 1.58 2.25 1.55 3.07	3.08E-03 2.99E-07 6.17E-03 4.41E-03 8.27E-03 1.10E-06	419	68.60 56.10 41.50 15.30 33.40 23.90

PLA2R1-rs2715928	67.30	2.12	5.84E-04	49.40
PLA2R1-rs16844715	72.10	3.12	6.21E-07	45.30
PLA2R1-rs3749119	15.70	4.02	7.02E-08	57.20
HLA-A*3303	3.80	0.39	3.00E-02	9.10
HLA-B*0702	0.90	0.13	6.33E-03	6.80
HLA-B*3501	14.20	1.9	3.00E-02	8.00
HLA-B*4403	2.80	0.33	2.00E-02	8.10
HLA-Cw*0102	8.50	0.47	1.00E-02	16.60
HLA-Cw*0704	4.70	5.89	5.79E-03	0.80
HLA-Cw*1403	2.80	0.33	2.00E-02	8.20
HLA-DRB1*0101	1.90		0.02	6.80
HLA-DRB1*0405	6.60		0.01	14.60
HLA-DRB1*1302	2.80		0.03	7.80
HLA-DRB1*1501	19.80		7.72E-05	8.00
HLA-DRB1*1602	2.80		0.01	0.20
HLA-DQB1*0401	6.60		0.01	14.60
HLA-DQB1*0501	2.80		0.03	7.50
HLA-DQB1*0602	17.90		5.12E-04	7.80
HLA-DQB1*0604	2.80		0.03	7.50
HLA-DQB1*0401	1.90		0.04	6.10
PLA2R1-rs1511223	79.30	1.57	1.57E-01	386
PLA2R1-rs35771982	78.70	2.57	1.88E-08	70.90
PLA2R1-rs10196882	20.80	1.41	ns	59.10
PLA2R1-rs877635	27.20	1.03	ns	15.70
PLA2R1-rs2715928	71.70	2.36	4.56E-07	26.50
PLA2R1-rs16844715	66.10	2.23	5.16E-07	51.70
PLA2R1-rs3749119	79.20	2.61	1.63E-08	46.70
HLA-DRB1*1501	20.20	3.36	4.97E-08	59.30
HLA-DQB1*0602	19.80	3.56	7.20E-09	7
				6.50
Replication analysis				
Japanese	130	Unknown	Unknown	
Chinese, Nanjing region	99	Dual positivity all		100
HLA-DRB1*1501	81.80			Single positivity excluded
HLA-DRB3*0202	60.60	16.93	2.75E-15	21
		3.96	5.73E-06	28
Chinese, Nanjing region	293	Dual positivity all		285
HLA-DRB1*1501				Single positivity excluded
HLA-DRB3*0202		8.32	3.44E-28	
		7.72	2.28E-27	
Chinese, Nanjing region	392	Dual positivity all		385
HLA-DRB1*1501	72.20			Single positivity excluded
HLA-DRB3*0202	69.90	24.9	2.30E-35	21
		17.7	8.00E-29	26.50
Chinese Han	261	66.3% positive		
HLA-DRB1*1501	37.55	4.65	<0.001	599
HLA-DRB1*0301	12.07	3.96	<0.001	3.84
Discovery analysis				
Chinese, Nanjing region	99	Dual positivity all		100
HLA-DRB1*1501	81.80			Single positivity excluded
HLA-DRB3*0202	60.60	16.93	2.75E-15	21
		3.96	5.73E-06	28
Chinese, Nanjing region	293	Dual positivity all		285
HLA-DRB1*1501				Single positivity excluded
HLA-DRB3*0202		8.32	3.44E-28	
		7.72	2.28E-27	
Chinese, Nanjing region	392	Dual positivity all		385
HLA-DRB1*1501	72.20			Single positivity excluded
HLA-DRB3*0202	69.90	24.9	2.30E-35	21
		17.7	8.00E-29	26.50
Chinese Han	261	66.3% positive		
HLA-DRB1*1501	37.55	4.65	<0.001	599
HLA-DRB1*0301	12.07	3.96	<0.001	3.84

ab, antibody.

creatinine with *HLA-DRB1* and *-DQB1* over an 11-year period, nor with patient survival [55]. The association with *PLA2R1* risk alleles and declining renal function has been investigated in different ethnicities and no association was found [53, 54]. In addition, there was no association with ESRD or death [52]. As yet, there has been no conclusive evidence associating genetic variants to remission status, response to treatment or a decline in renal function. These factors are difficult to determine, as studies are often done in retrospective cohorts where confounders such as immunosuppression or disease severity have a significant effect on outcome. The decline in renal function is multifactorial and is related to blood pressure control, severity of proteinuria, renal function at disease onset, age and gender, among others. These factors, themselves are likely to be independent risk factors which is why studies to date have not been significant. It may be argued that these factors are caused or influenced by genetics, thereby further complicating the potential genetic risk profile with IMN.

CONCLUSION

There has been an exponential increase in our understanding of IMN since 2009, when *PLA₂R1* was identified as the most significant pathogenic autoantigen in IMN. IMN may occur when three independent risk factors combine: unique polymorphisms in *PLA2R1* and the *HLA-DQA1* region and environmental factors. There are ethnic-specific differences in these alleles and the potential that risk alleles may contribute in predicting disease outcomes. The complex pathomechanisms of disease development highlight some of the potential problems in analysing and predicting the risk for disease progression. The genetic variants may alter the expression or function of the target antigens and enable autoantibody formation. While no rare variants (i.e. mutations) were found in the coding region of *PLA2R1*, the role of intronic variants needs to be investigated given their large regulatory role. As shown before, non-coding SNPs (i.e. intergenic or intronic genetic variations) are associated with ESRD [57] and other autoimmune conditions [58].

Whole-genome sequencing is becoming more affordable and faster and may help illuminate the true role of intergenic and intronic genetic variants in IMN. The genomic studies could be augmented with epigenomic, transcriptomic and proteomic studies to ascertain the functional effect of gene variants. The regulatory regions that control autoantibody production, such as transcription factors or microRNA, could be altered by the identified risk SNPs in a mechanism analogous to psoriasis [29]. If upstream and downstream regulatory region variants were found, these would be potential therapeutic drug targets, possibly preventing the deleterious effects of current immunotherapy. Given the large OR with joint homozygosity, genotyping could be utilized to stratify disease risk and outcomes. The utility of genetic profiling in IMN could prove to be vital for non-invasive screening or risk stratification [16, 29]. The tools (a*PLA₂R1*lab) available to us are of assistance, but by understanding the genetics we may be able to explain why the autoantibodies develop in the first place [16]. Current studies have been limited by small sample size and thus there may be a lack of appreciation of other potential associations. Expanding the horizons further, there may

even be a role for ascertaining epidemiologic risk for IMN with risk alleles and seeing if people in the general population have a genetic predisposition to disease [16]. There may be an indirect interaction between genetics and disease, such as molecular mimicry, whereby a microbe or environmental antigen resembles a *PLA2R1* variant and causes autoimmunity in patients carrying the *HLA-DQA1* risk alleles [40]. The reported homology of part of the major epitope sequence in *PLA₂R1* with a clostridial carboxypeptidase enzyme illustrates how antibodies raised during infection may potentially cross react with an autoantigen [39]. Normal control populations without IMN but with the risk alleles will be the most useful in identifying the triggers or environmental factors that contribute to eventual disease acquisition, which may further our understanding of this complex genetically predisposed disease.

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CONFLICT OF INTEREST STATEMENT

P.B. has a patent (WO2015/185949A1) issued. The other authors have no conflicts of interest to be declared. The results presented in this article have not been published previously in whole or part.

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