**HLA-DQA1 and PLA2R1 Polymorphisms and Risk of Idiopathic Membranous Nephropathy**

Gemma Bullich, José Ballarín, Artur Oliver, Nadia Ayasreh, Irene Silva, Sheila Santín, Montserrat M. Díaz-Encarnación, Roser Torra, and Elisabet Ars

**Summary**

**Background and objectives** Single nucleotide polymorphisms (SNPs) within HLA complex class II HLA-DQ α-chain 1 (HLA-DQA1) and M-type phospholipase A2 receptor (PLA2R1) genes were identified as strong risk factors for idiopathic membranous nephropathy (IMN) development in a recent genome-wide association study. Copy number variants (CNVs) within the Fc gamma receptor III (FCGR3) locus have been associated with several autoimmune diseases, but their role in IMN has not been studied. This study aimed to validate the association of HLA-DQA1 and PLA2R1 risk alleles with IMN in a Spanish cohort, test the putative association of FCGR3A and FCGR3B CNVs with IMN, and assess the use of these genetic factors to predict the clinical outcome of the disease.

**Design, settings, participants, & measurements** A Spanish cohort of 89 IMN patients and 286 matched controls without nephropathy was recruited between October of 2009 and July of 2012. Case-control studies for SNPs within HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) genes and CNVs for FCGR3A and FCGR3B genes were performed. The contribution of these polymorphisms to predict clinical outcome and renal function decline was analyzed.

**Results** This study validated the association of these HLA-DQA1 and PLA2R1 SNPs with IMN in a Spanish cohort and its increased risk when combining both risk genotypes. No significant association was found between FCGR3 CNVs and IMN. These results revealed that HLA-DQA1 and PLA2R1 genotype combination adjusted for baseline proteinuria strongly predicted response to immunosuppressive therapy. HLA-DQA1 genotype adjusted for proteinuria was also linked with renal function decline.

**Conclusion** This study confirms that HLA-DQA1 and PLA2R1 genotypes are risk factors for IMN, whereas no association was identified for FCGR3 CNVs. This study provides, for the first time, evidence of the contribution of these HLA-DQA1 and PLA2R1 polymorphisms in predicting IMN response to immunosuppressors and disease progression. Future studies are needed to validate and identify prognostic markers.

**Introduction**

Idiopathic membranous nephropathy (IMN) is the most common cause of nephrotic syndrome in the adult white population (1), with an incidence of approximately 1 case per 100,000 persons per year (2). IMN is defined as a histopathological entity characterized by subepithelial deposits of IgG and complement, which causes membrane-like thickening and subsequent proteinuria (3).

The M-type phospholipase A2 receptor (PLA2R1) located on podocytes has been identified as the major target antigen, which triggers the accumulation of circulating autoantibodies in more than 75% of individuals with IMN (4,5). Furthermore, autoantibodies against aldose reductase, mitochondrial superoxide dismutase 2, α-enolase and synaptopemal complex protein 65 have been discovered to be present in serum and glomeruli from patients with IMN (6–8). Therefore, IMN is considered to be an autoimmune disease. However, at least six familial cases have been reported, suggesting a genetic contribution to the disease (2,9–13).

Recently, a genome-wide association study involving three independent cohorts (British, Dutch, and French cohorts) identified a highly significant association between IMN and single-nucleotide polymorphisms (SNPs) within PLA2R1 and HLA complex class II HLA-DQ α-chain 1 (HLA-DQA1) genes (14). HLA-DQA1 is part of the heterodimer forming the antigen-binding groove that plays a central role in the immune system by presenting peptides derived from extracellular proteins to immunocompetent cells. Many genes within the HLA locus have previously been associated with IMN (15–17). Moreover, other SNPs within PLA2R1 have been associated with IMN in Taiwanese and Korean populations (18,19). Additional studies to identify and validate genetic risk factors for IMN would be valuable.
in independent populations may help to elucidate its pathogenesis.

Another important source of genetic variability is copy number variants (CNVs) consisting of gains or losses of DNA segments of at least 1 kb. The Fc gamma receptor (FCGR) locus on chromosome 1q23 is subject to CNVs, and their role in susceptibility to autoimmune diseases has been widely studied (reviewed in ref. 20).

The extremely variable clinical course of IMN and the controversial immunosuppressive therapy make treatment decisions challenging (1,22). About one third of IMN patients experience spontaneous remission (SR) of the nephrotic syndrome (23). However, a significant number of patients have a poor response to immunosuppressive therapy and progress to ESRD (24). Prognostic markers of disease progression would be very helpful tools for treatment decision making (25).

The goals of the present study were to (1) validate the association of HLA-DQA1 and PLA2R1 risk alleles with IMN in a Spanish population, (2) study, for the first time, the putative association of CNVs within FCGR3A and FCGR3B genes with IMN, and (3) assess the use of these genetic variants in predicting SR, immunosuppressive therapy response, and decline in renal function.

Materials and Methods

Study Population

Spanish patients with biopsy-proven IMN who attended our center between October of 2009 and July of 2012 were recruited (n=89). The diagnosis was achieved by renal biopsy performed between 1974 and 2011. None of the patients enrolled had any evidence of a secondary cause of membranous nephropathy. The control group consisted of 286 age- and sex-matched Spanish adults without nephropathy kindly provided by the Biobank of our institution. The study was approved by the Institutional Review Board, and all participants gave their signed informed consent.

For all genotype–phenotype correlation studies, patients referred to our center for renal transplantation (n=5) and patients with no clinical information (n=1) were excluded (Figure 1). Baseline characteristics and follow-up data of the remaining 83 patients were obtained from medical records until an end point (remission or ESRD) was reached or until July of 2012 (Table 1). Initially, patients were treated using a conservative approach based on supportive treatment with angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, diuretics, statins, and/or dietary sodium restriction. After an observational period of approximately 6 months, patients with persistent nephrotic syndrome and no significant decrease in proteinuria levels started immunosuppressive therapy. Patients with deterioration of renal function or proteinuria >10 g/d started immunosuppressive therapy at the same time as angiotensin-converting enzyme inhibitors. All patients were treated in our center, and the first-line treatment was based on existing recommendations at that time. In the event of resistance, patients were treated with an alternative immunosuppressive regimen, and in case of relapse, another course of immunosuppressive therapy was attempted.

For the association study of the genetic variants with SR, patients with a minimum follow-up of 2 years were classified according to their clinical outcome into SR or non-SR (NSR) patients, and the latter group was separated into immunosuppressive responders and immunosuppressive nonresponders (Figure 1). Of note, those patients that only received corticosteroid monotherapy (n=3) were excluded. SR patients (n=23) were defined as achieving partial or complete remission (proteinuria <3.5 or <0.3 g/d, respectively, in at least three consecutive determinations and normal renal function) in the absence of immunosuppressive therapy (23). Responders (n=27) included patients treated with one or more courses of immunosuppressive therapy who achieved partial or complete remission. Nonresponders (n=28) were defined as patients treated with one or more courses of immunosuppressive therapy who reached ESRD or had no significant and/or sustained reduction of proteinuria levels (proteinuria >3.5 g/d) and severe deterioration of renal function. For renal function decline analysis, the time from renal biopsy to doubling of serum creatinine (DSC) was calculated in 83 patients who had not reached ESRD at diagnosis (Figure 1).

HLA-DQA1 and PLA2R1 SNP Genotyping

Genomic DNA was isolated from peripheral blood using a standard method. SNPs rs2187668 (located within the first intron of the HLA-DQA1 gene) and rs4664308 (located within the first intron of the PLA2R1 gene) were genotyped using TaqMan SNP Genotyping Assays (C_58662585_10 and C_27902747_10, respectively) according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA). Amplification reactions were performed on an ABI 7000 Real-Time PCR System (Applied Biosystems). Internal controls for each genotype were included in all runs. Genotype frequencies for both SNPs were within Hardy–Weinberg equilibrium in controls.

CNV Analysis

The paralogue ratio test was used to determine CNVs at the FCGR3 locus (including FCGR3A and FCGR3B genes). Restriction enzyme digest variant ratio assay was used to distinguish between FCGR3A and FCGR3B genes based on the work by Hollox et al. (26) with small changes (Supplemental Material).

Statistical Analyses

Descriptive data were expressed as mean±SD for normally distributed variables and median (range) for skewed variables. Comparisons of baseline characteristics among clinical outcome groups were made using Kruskal–Wallis and chi-squared tests. Association analyses were assessed by means of chi-squared or Fisher’s exact test when appropriate. SNPStats software was used to decide the best
inheritance model for each SNP (27). This software uses the likelihood ratio test to compare every model with the most general model (the codominant) and calculates Akaike’s Information Criterion; the best model for a specific SNP is the one with the lowest Akaike’s Information Criterion. Unadjusted and adjusted logistic regression analyses were performed to evaluate the relationship between response to immunosuppressive therapy and genetic and clinical variables. Model performance was evaluated using the area under the receiver operating characteristics curve and the Hosmer–Lemeshow goodness-of-fit test. Leave-one-out crossvalidation was performed as an additional measure of accuracy. The odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated. Associations between genetic variants and renal survival rate were estimated by the Kaplan–Meier method and the log-rank test. DSC was considered as the primary end point. Multivariate Cox regression analysis was performed to evaluate the relationship between renal function decline and genetic and clinical variables. The hazard ratio was calculated with 95% CI. \( P < 0.05 \) was considered significant for all analyses. Statistical analyses were performed using SPSS version 17.0 software.

**Results**

**Association of HLA-DQA1 and PLA2R1 SNPs with IMN in a Spanish Cohort**

SNPs within HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) genes were genotyped in a Spanish cohort of 89 IMN patients and 286 controls. Association analysis under different genetic models showed that HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) were significantly associated with IMN under a dominant model (OR, 3.70; 95% CI, 2.25 to 6.08; \( P < 0.001 \) and OR, 2.00; 95% CI, 1.23 to 3.23; \( P = 0.005 \), respectively) (Table 2). The risk for IMN increased when combining the disease-associated genotypes of both SNPs (A/A or A/G for HLA-DQA1 [rs2187668] and A/A for PLA2R1 [rs4664308]), yielding an OR of 7.33 (95% CI, 3.55 to 15.13; \( P < 0.001 \)) (Table 3).

**Association of CNVs of the FCGR3 Locus with IMN in a Spanish Cohort**

The copy number (CN) of FCGR3A and FCGR3B genes was determined in our Spanish cohort of 89 IMN patients and a subset of 93 controls. The CN profile of FCGR3A and FCGR3B genes did not differ significantly between IMN patients and controls (Table 4). However, controls

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**Figure 1.** Flowchart for the classification of idiopathic membranous nephropathy (IMN) patients included in the genotype–phenotype correlation studies.
Table 1. Patients’ characteristics according to clinical outcome

<table>
<thead>
<tr>
<th>Patients’ Characteristics</th>
<th>Total&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Spontaneous Remission</th>
<th>Immunosuppressive Responders</th>
<th>Immunosuppressive Nonresponders</th>
<th>P Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (%)</td>
<td>83</td>
<td>23 (29.5)</td>
<td>27 (34.6)</td>
<td>28 (35.9)</td>
<td></td>
</tr>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.6 ± 14.4; n = 83</td>
<td>51.0 ± 16.8; n = 23</td>
<td>44.0 ± 12.5; n = 27</td>
<td>46.8 ± 13.6; n = 28</td>
<td>0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex (women/men)</td>
<td>25/58</td>
<td>11/12</td>
<td>6/21</td>
<td>6/22</td>
<td>0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proteinuria (g/d)</td>
<td>5.6 (0.2–20.0); n = 81</td>
<td>2.6 (0.2–15.5); n = 23</td>
<td>6.2 (2.1–18.0); n = 26</td>
<td>6.8 (1.5–20.0); n = 26</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.0 (0.5–3.8); n = 81</td>
<td>0.8 (0.5–1.9); n = 23</td>
<td>1.0 (0.6–1.9); n = 27</td>
<td>1.15 (0.7–2.7); n = 27</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>82 (10–274); n = 83</td>
<td>95 (27–123); n = 23</td>
<td>84 (38–274); n = 27</td>
<td>78 (25–120); n = 27</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Follow-up (mo)</td>
<td>86 (14–420); n = 83</td>
<td>72 (24–346); n = 23</td>
<td>93 (33–255); n = 27</td>
<td>90 (28–324); n = 28</td>
<td>0.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration of disease (mo)</td>
<td>36 (3–283); n = 77</td>
<td>22 (3–50); n = 23</td>
<td>34 (9–183); n = 27</td>
<td>82 (41–283); n = 15</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to IS therapy (mo)</td>
<td>6 (0–178); n = 52</td>
<td>—</td>
<td>6 (0–119); n = 27</td>
<td>8 (0–178); n = 25</td>
<td>0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ponticelli regimen/CNI/MMF</td>
<td>7/44/4</td>
<td>—</td>
<td>3/24/0</td>
<td>4/20/4</td>
<td>0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rapidity of response (mo)</td>
<td>—</td>
<td>22 (4–116); n = 27</td>
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</tbody>
</table>

Data are mean ± SD or median (range). GFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration formula. Duration of disease was the time between diagnostic and spontaneous remission/onset of IS-induced remission/ESRD (nonresponsive patients without ESRD were excluded). Time to IS therapy was the time between diagnostics and the start of IS therapy. Rapidity of response was the time between start of IS therapy and onset of remission. IS, immunosuppressive; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil.

<sup>a</sup>Total number considering patients included in the spontaneous remission and IS response analyses (n=78) in addition to patients with follow-up times >2 years (n=2) and patients treated with corticosteroids monotherapy (n=3).

<sup>b</sup>Kruskal–Wallis test among patients with spontaneous remission, IS responders, and IS nonresponders.

<sup>c</sup>Chi-squared test among patients with spontaneous remission, IS responders, and IS nonresponders.

<sup>d</sup>First-line treatment. Patients treated with corticosteroid monotherapy were not considered.

Table 2. Association between SNPs within HLA-DQA1 and PLA2R1 genes and idiopathic membranous nephropathy

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th>Ref</th>
<th>MAF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Allelic P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>n (Frequency)</th>
<th>Codominant Model&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dominant Model&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Recessive Model&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI); AIC; P Value</td>
<td>OR (95% CI); AIC; P Value</td>
<td>OR (95% CI); AIC; P Value</td>
</tr>
<tr>
<td>HLA-DQA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMN&lt;sup&gt;e&lt;/sup&gt; (rs2187668)</td>
<td>G</td>
<td>0.287</td>
<td>&lt;0.001</td>
<td>40 (44.9)</td>
<td>47 (52.8)</td>
<td>2 (2.2)</td>
<td>3.88 (2.34 to 6.42); 390.8</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0.033</td>
<td></td>
<td>215 (75.2)</td>
<td>65 (22.7)</td>
<td>6 (2.1)</td>
<td>3.70 (2.25 to 6.08); 387.9</td>
</tr>
<tr>
<td>PLA2R1&lt;sup&gt;e&lt;/sup&gt; (rs4664308)</td>
<td>A</td>
<td>0.264</td>
<td>0.03</td>
<td>9 (10.1)</td>
<td>29 (32.6)</td>
<td>51 (57.3)</td>
<td>2.13 (1.27 to 3.57); 410.4</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0.355</td>
<td></td>
<td>32 (11.2)</td>
<td>139 (48.6)</td>
<td>115 (40.2)</td>
<td>2.00 (1.23 to 3.23); 408.9</td>
</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism; HLA-DQA1, HLA complex class II HLA-DQα-chain 1; PLA2R1, M-type phospholipase A2 receptor; Ref, reference; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval; AIC, Akaike’s Information Criterion; IMN, idiopathic membranous nephropathy.

<sup>a</sup>Chi-squared test.

<sup>b</sup>Likelihood ratio test.

<sup>c</sup>Dominant model was considered A/A and A/G versus G/G (ref) for HLA-DQA1 and G/G and A/G (ref) versus A/A for PLA2R1.
showed a trend to low FCGR3A CN (5% versus 0%, respectively; \( P = 0.06 \)).

**Genotype–Phenotype Correlations**

**Genetic Variants and Spontaneous Remission.** We tested whether HLA-DQA1 (rs2187668), PLA2R1 (rs4664308), and FCGR3B CNVs were associated with IMN SR in a group of 23 SR and 55 NSR patients. The FCGR3A gene was not included because of its low variation in CN. No significant association was found for any of these variants. However, all patients who achieved SR (except for one patient) had two copies of the FCGR3B gene, whereas 18% of NSR patients presented either high (more than two) or low (less than two) FCGR3B CN (Supplemental Table 1).

**Genetic Variants and Immunosuppressive Therapy Response.** Association of these three genetic variants with response to immunosuppressive therapy was assessed by comparing responding (\( n = 27 \)) and nonresponding (\( n = 28 \)) patients. Genotypes were combined under a dominant model that considered the nonrisk genotypes for IMN susceptibility as a reference. In unadjusted regression analysis, the carriers of the IMN susceptibility genotypes (A/A and A/G for HLA-DQA1 [rs2187668] or A/A for PLA2R1 [rs4664308]) showed a trend to response to immunosuppressive therapy (OR, 0.12; 95% CI, 0.02 to 0.72; \( P = 0.02 \)). In the multivariate model, adjustment for baseline proteinuria significantly increased the predictive value of this genotype combination for response to immunosuppressive therapy (OR, 0.08; 95% CI, 0.01 to 0.58; \( P = 0.01 \)), whereas no association was found for the type of immunosuppressor, age, sex, or baseline serum creatinine (Table 5). This model showed moderate discrimination (area under the receiver operating characteristics curve=0.728; leave-one-out crossvalidation=61.5%), and the Hosmer–Lemeshow test indicated good fit (\( P = 0.61 \)). No significant results were found for FCGR3B CN (data not shown).

**Genetic Variants and Decline in Renal Function.** Survival analysis over a mean follow-up of 7.2 years of time to DSC was performed considering patients who had not reached ESRD at diagnosis (\( n = 83 \)). Results showed that patients carrying the A/A or A/G genotype for HLA-DQA1 had a longer mean DSC-free time than patients carrying the G/G genotype (16.3 versus 13.0 years, respectively; log-rank \( P = 0.05 \)) (Figure 2). Multivariate Cox regression analyses revealed that the A/A and A/G genotypes for HLA-DQA1 were significant protective factors after adjusting for baseline proteinuria (hazard ratio, 0.37; 95% CI, 0.15 to 0.90; \( P = 0.03 \)). No association of the PLA2R1 SNP or the FCGR3B CNVs was found.

**Discussion**

In this study, we confirmed the association of HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) with IMN

<table>
<thead>
<tr>
<th>Gene (CNV)</th>
<th>n (Frequency)</th>
<th>OR (95% CI)</th>
<th>( P ) Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FCGR3A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN&lt;2</td>
<td>CN=2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0)</td>
<td>88 (98.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (5.4)</td>
<td>86 (92.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FCGR3B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN&lt;2</td>
<td>CN=2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (11.2)</td>
<td>74 (83.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (7.5)</td>
<td>81 (87.1)</td>
<td></td>
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</tr>
</tbody>
</table>

CNV, copy number variant; CN, copy number; FCGR3, Fc gamma receptor III.

$^a$Fisher’s exact test considering CN<2 versus CN=2 and CN>2.
susceptibility in a Spanish cohort. The combination of high-risk genotypes for both SNPs was associated with higher risk of IMN, which was previously described (14). In contrast, FCGR3A and FCGR3B CNVs were not significantly associated with IMN. For the first time, we showed that HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) contribute to predict IMN prognosis.

Little is known about the contribution of HLA-DQA1 and PLA2R1 genetic variants in IMN pathogenesis. The fact that autoantibody response in IMN is restricted to a conformation-dependent epitope of PLA2R1 led to the hypothesis that modifications in the coding sequence of this gene may contribute to antibody formation (4,28). Coenen et al. (29) found no evidence to support this hypothesis;

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### Table 5. Logistic regression analyses between SNPs within HLA-DQA1 and PLA2R1 genes and idiopathic membranous nephropathy response to immunosuppressive therapy

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Univariate Analysisa</th>
<th>Multivariate Analysisa,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DQA1</td>
<td>PLA2R1</td>
<td>n R (%)</td>
<td>n NR (%)</td>
</tr>
<tr>
<td>(rs2187668)</td>
<td>(rs4664308)</td>
<td>G/G</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/G</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>G/G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>A/G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>A/A</td>
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</tbody>
</table>

Genotype combination

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>n R (%)</th>
<th>n NR (%)</th>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>G/G or A/G</td>
<td>2 (20.0)</td>
<td>8 (80.0)</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>A/A</td>
<td>6 (46.1)</td>
<td>7 (53.9)</td>
<td>0.29 (0.05–1.65)</td>
<td>0.14</td>
<td>0.20 (0.03–1.23)</td>
<td>0.08</td>
</tr>
<tr>
<td>A/A or A/G</td>
<td>G/G or A/G</td>
<td>6 (46.1)</td>
<td>7 (53.9)</td>
<td>0.12 (0.02–0.72)</td>
<td>0.02</td>
<td>0.08 (0.01–0.58)</td>
<td>0.01</td>
</tr>
<tr>
<td>A/A or A/G</td>
<td>A/A</td>
<td>13 (68.4)</td>
<td>6 (31.6)</td>
<td>0.12 (0.02–0.72)</td>
<td>0.02</td>
<td>0.08 (0.01–0.58)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

R, responder to immunosuppressive therapy; NR, nonresponder to immunosuppressive therapy.

aUnivariate analysis considering a dominant model for HLA-DQA1 and a dominant model for PLA2R1. The nonrisk genotypes for idiopathic membranous nephropathy susceptibility were considered as the reference.

bAdjusted for proteinuria at diagnosis.

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Figure 2. | Survival analysis of time without doubling serum creatinine (DSC-free) according to the HLA-DQA1 genotypes. The number of patients at risk at selected time points is shown below the plot. Log-rank test considering a dominant model for HLA-DQA1 genotypes shows $P<0.05$. |
in a cohort of 95 IMN patients, only 9 patients carried rare sequence variants in the PLA2R1 gene, and only 4 of the 9 patients were among the 60 patients who presented circulating autoantibodies against PLA2R. Our study provides additional support to the previously found associations between IMN and common coding and noncoding variants within PLA2R1 and HLA-DQA1 genes (14,18,19,29). Interestingly, the disease-associated genotype of PLA2R1 (rs4664308) is the common genotype, which was previously reported (14,30). These associations with relatively common variants, although IMN is a rare disease, raised the hypothesis that the confluence of relatively common polymorphisms in these genes may result in a rare haplotype that confers susceptibility to IMN (29,31).

In this study, we assessed, for the first time, the putative association between FCGR3A and FCGR3B CNVs and IMN. We hypothesized that low FCGR3A CN could decrease antibody-dependent cell-mediated cytotoxicity, thereby playing a protective role in IMN development. Low FCGR3A CN was only found in control individuals, supporting our hypothesis; however, statistical significance was not reached. In mice, deletion of the ortholog of human FCGR3A, fcyRIV, is protective against the development of nephrotic nephritis (32). However, in humans, either high or low FCGR3A CN was associated with susceptibility to antiglomerular basement membrane disease (33). FCGR3B CNVs have been described as a putative risk factor for several autoimmune diseases, such as GN in systemic lupus erythematosus and primary Sjögren’s syndrome (34,35). Our results indicate no contribution of FCGR3B CNVs to IMN susceptibility. Similarly, no association has been observed in Graves’ and Addison’s diseases (35,36). Additional studies may help to clarify the relationship between FCGR3A and FCGR3B CNVs and IMN.

The highly variable clinical course of IMN encourages the search for prognostic markers of clinical outcome. Age at onset<50 years, women, baseline proteinuria<8 g/d, and preserved renal function at presentation are predictors of SR (37,38). The genetic variants analyzed in this study showed no significant association with SR, although 18% of NSR patients exhibited either high (more than two) or low (less than two) FCGR3B CN compared with 4% of SR patients; this finding suggests that alterations in FCGR3B CN could hinder SR. FCGR3B CN was correlated with protein expression and immune complex clearance (39); therefore, changes in FCGR3B CN could alter the balance between Fc receptors, disrupting the tightly regulated immune system (20) and impeding achievement of SR.

More interestingly, our results showed that the risk genotypes for IMN development (A/A or A/G for HLA-DQA1 and A/A for PLA2R1) also predict response to immunosuppressive therapy and protection to renal function decline. Recently, Lv et al. (30) found that 73% of individuals carrying these IMN susceptibility genotypes had anti-PLA2R antibodies, whereas these antibodies were absent in all carriers of the protective genotypes. In our cohort, immunosuppressive therapy was more effective in patients carrying the IMN susceptibility genotype combination, likely by decreasing anti-PLA2R levels. We speculate that other genetic and environmental factors could contribute to the development of IMN in patients carrying the protective genotypes (G/G for HLA-DQA1 and A/G or G/G for PLA2R1), explaining their low likelihood of response to immunosuppressive treatment. To the best of our knowledge, this association is the first found between genetic variants and clinical outcome in IMN. Thibaud et al. (40) reported an association study of TNF-α gene polymorphisms with IMN. This group found a significant association of a SNP in the promoter region and a downstream microsatellite of the TNF-α gene with IMN susceptibility. However, no association of these polymorphisms with IMN progression was identified.

We propose that the HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) genotypes could add some predictive value to the currently used clinical and histologic markers. The two most accurate and validated markers for IMN progression to ESRD are the Toronto Risk Score and the urinary excretion of β2-microglobulin or IgG (41,42). Recently, the level of anti-PLA2R has been correlated with clinical disease activity (4,5,43,44), and high anti-PLA2R levels have been associated with a significantly reduced frequency of SR (45). The clinical complexity of the disease suggests that a combination of prognostic markers would be the best option for prediction of clinical outcome.

The small size of our cohort is the main limitation of this study. Genotype-phenotype correlation studies require large cohorts of IMN patients with long follow-up time because of their stratification depending on clinical outcome. SR and responder patients attended our center less frequently than nonresponder patients. For this reason, SR and responder patients are underrepresented in our cohort. The inclusion of patients diagnosed over a 30-year period implied the use of different treatment regimens among patients. However, our analysis showed no influence of the type of treatment in the association of HLA-DQA1 and PLA2R1 genotypes with immunosuppressive response. Nevertheless, because most patients were treated with calcineurin inhibitors as first-line treatment, our results should be confirmed for patients treated with other immunosuppressive regimens.

In conclusion, we have validated the association of HLA-DQA1 (rs2187668) and PLA2R1 (rs4664308) with susceptibility to IMN in a Spanish cohort, whereas no significant association was found for FCGR3 CNVs. For the first time, we have presented evidence of the contribution of these SNPs to the prediction of IMN response to immunosuppressive therapy and decline in renal function. This finding may help to identify potential responding and nonresponding patients and thus, provide some help in treatment decisions. Future collaborative efforts to incorporate large datasets will indeed be critical to validate the relationship between these genetic variants and IMN clinical outcome.

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