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A Genetic Risk Score Distinguishes Different Types of Autoantibody-Mediated Membranous Nephropathy

Sanjana Gupta[a] Mallory Lorraine Downiea Chris Cheshire[a] Stephanie Dufek-Kamperis[a] Adam Paul Levine[a, f] Paul Brenchley[b] Elion Hoxha[c] Rolf Stahl[c] Neil Ashman[d] Ruth Jennifer Pepper[a] Sean Mason[e] Jill Norman[a] Detlef Bockenhauer[a] Horia Constantin Stanescu[a] Robert Kleta[a] Daniel Philip Gale[a]

[a]UCL Department of Renal Medicine, University College London, London, UK; [b]Institute of Cardiovascular Sciences, University of Manchester, Manchester, UK; [c]Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [d]Department of Renal Medicine, Barts Health, London, UK; [e]UCB Pharma, Slough, UK; [f]Research Department of Pathology, University College London, London, UK

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Genetic risk score · Membranous nephropathy · Autoantibody · Phospholipase A2 receptor 1 · Thrombospondin type-1 domain containing 7A

Abstract
Introduction: Membranous nephropathy (MN) is the leading cause of nephrotic syndrome in adults and is characterized by detectable autoantibodies against glomerular antigens, most commonly phospholipase A2 receptor 1 (PLA2R1) and thrombospondin type-1 domain containing 7A (THSD7A). In Europeans, genetic variation in at least five loci, *PLA2R1*, *HLA-DRB1*, *HLA-DQA1, IRF4, and NFKB1,* affects the risk of disease. Here, we investigated the genetic risk differences between different autoantibody states. ***Methods:*** 1,409 MN individuals were genotyped genome-wide with a dense SNV array. The genetic risk score (GRS) was calculated utilizing the previously identified European MN loci, and results were compared with 4,929 healthy controls and 422 individuals with steroid-sensitive nephrotic syndrome. ***Results:*** GRS was calculated in the 759 MN individuals in whom antibody status was known. The GRS for MN was elevated in the anti-PLA2R1 antibody-positive ($N = 372$) compared with both the unaffected control ($N = 4{,}929$) and anti-THSD7A-positive ($N = 31$) groups ($p < 0.0001$ for both comparisons), suggesting that this GRS reflects anti-PLA2R1 MN. Among PLA2R1-positive patients, GRS was inversely correlated with age of disease onset ($p = 0.009$). Further, the GRS in the dual antibody-negative group ($N = 355$) was intermediate between controls and the PLA2R1-positive group ($p < 0.0001$). ***Conclusion:*** We demonstrate that the genetic risk factors for PLA2R1- and THSD7A-antibody-associated MN are different. A higher GRS is associated with younger age of onset of disease. Further, a proportion of antibody-negative MN cases have an elevated GRS similar to PLA2R1-positive disease. This suggests that in some individuals with negative serology the disease is driven by autoimmunity against PLA2R1.

karger@karger.com
www.karger.com/gdz

Correspondence to:
Daniel Philip Gale, d.gale@ucl.ac.uk

Introduction

Membranous nephropathy (MN) is a rare kidney disease with an annual incidence of 10 per million per population [1]. It is the most common cause of nephrotic syndrome in European adults and is associated with considerable morbidity as 30–40% of cases progress to end-stage kidney disease [2]. The peak age of onset is 40–50 years of age [2], with a strong male preponderance [3]. MN occurs either as a primary (or idiopathic) disorder (75% of cases) or secondary to an identified underlying disorder, such as systemic autoimmune disease (e.g., systemic lupus erythematosus and rheumatoid arthritis), malignancy, medication, or viral infection [2]. In primary MN, to date a number of different autoantibodies have been identified including those specific for M-type phospholipase A2 receptor 1 (PLA2R1) [4], thrombospondin type-1 domain containing 7A (THSD7A) [5], neutral endopeptidase [6], neural epidermal growth factor-like 1 protein [7], serine protease high-temperature requirement A serine peptidase 1 [8], protocadherin 7 [9], semaphorin 3B [10], and contactin-1 [11]). Of these, PLA2R1 antibodies are most common, identified either in serum samples or via glomerular staining in approximately 75% of primary MN cases [12]. Titres of anti-PLA2R1 antibodies correlate with clinical outcomes such as end-stage kidney disease and remission status [13, 14].

There have been a variety of genetic studies in MN (reviewed elsewhere [15]). Although almost never inherited as a Mendelian trait, genome-wide association studies in primary MN in three European cohorts initially identified two loci, spanning *HLA-DQA1* and *PLA2R1*, at which common variants are strongly associated with disease [16]. These results have been replicated in both European and non-European cohorts [17–19]. Most recently, these results have been replicated including the finding of an additional HLA locus; *HLA-DRB1* and two non-HLA loci that contribute a smaller risk to disease; *NFKB1* and *IRF4* [20]. In Europeans, the strongest association is with the *HLA-DQA1* region with an odds ratio (OR) for the risk allele of 3.71 followed by *PLA2R1* with an OR of 1.98 [20]. Weaker effects were observed in European individuals at *NFKB1* and *IRF4,* with ORs for the risk alleles of 1.14 and 1.2, respectively [20]. The HLA risk haplotype interacts with the *PLA2R1* risk alleles when both are present in homozygosity and increases the risk of MN by 14-fold in Europeans [20]. This is biologically plausible as the associated HLA allomorph may directly drive an autoimmune response by interaction with the PLA2R1 self-peptide; further, it may exacerbate autoimmune response by displaying the PLA2R1 self-peptide [21].

An association of the *PLA2R1* genotype with PLA2R1 antibody positivity was previously demonstrated: 76 percent of individuals with the high-risk *PLA2R1* genotypes had detectable PLA2R1 antibodies in renal biopsies compared to only four percent with the low-risk genotype [19]. However, this has not been replicated and two other cohorts found no association between anti-PLA2R1 antibody titres and *PLA2R1* risk alleles, instead finding an association with the *HLA-DQA1* risk alleles [13, 22]. We sought to understand how these established genetic risk factors for MN vary according to serological findings in a previously unreported cohort.

Methods

Case Selection

DNA from 1,409 individuals with biopsy-proven idiopathic MN was available from patients recruited across three European centres: North East and Central London, UK (96 patients); Manchester, UK (893 patients); and Hamburg, Germany (420 patients). Written informed consent was obtained by each collaborator at each site in accordance with local guidelines. Genomic DNA was extracted from peripheral blood using a standard protocol. Serum taken within 6 months of diagnosis of MN was available in only a subset of these cases. Anti-PLA2R1 and anti-THSD7A antibody titres were measured at a single laboratory within one of our centres. Anti-PLA2R1 antibody titres were measured using the commercially available EUROIMMUN ELISA and were positive if they were over the threshold of ≥12 Kunits/L. Anti-THSD7A antibody positivity was measured by an indirect immunofluorescence test; the serum is considered positive if immunofluorescence is present after a serum dilution of 1:10. There was only one individual with dual positivity for both antibodies, and they were excluded from statistical analysis. Detailed phenotype data were available in a subset of 224 anti-PLA2R1 antibody-positive cases; see Table 1.

Control Selection

Ethnically matched self-reported white or European controls were obtained from publicly available sources that contained datasets genotyped on an Illumina platform.

1. A dataset containing 432 healthy individuals was obtained through the European Genome Archive (EGAD00010000144 and EGAD00010000520) [23, 24]. This dataset was genotyped on a HumanOmniExpress-12 v1_J microarray chip.
2. Ninety Illumina European ethnicity controls were downloaded from the Illumina website (http://www.illumina.com) [25]. This dataset was genotyped using the HumanOmniExpress-12 v1_C microarray chip.
3. The Wellcome Trust Case Control Consortium 2 (WTCC2) 5,604 controls are a combined dataset of the 1959 UK birth cohort controls and the UK blood service control group and are available through the WTCC2 webpage (https://www.wtcc.org.

Table 1. Clinical parameters of the 224 anti-PLA2R1 antibody-positive cohort

Clinical parameter	Mean/median	Standard deviation/interquartile range
Age, years	55	±15
Gender	male 0.71: female 0.39	
Immunosuppression	received 0.76: none 0.34	
eGFR at diagnosis, mL/min/1.73 m^2	81	49–98
eGFR decline, mL/min/1.73 m^2/year	−4.32	−7.7–0.41
Urinary protein-creatinine ratio at diagnosis, mg/mmol	6,450	4,083–10,160
Anti-PLA2R1 antibody, Kunits/L	112	45–294

eGFR, estimated glomerular filtration rate; PLA2R1, phospholipase A2 receptor 1.

uk) [26, 27]. This dataset was genotyped using the Illumina 1.2M Duo Custom BeadChip.

Individuals with steroid-sensitive nephrotic syndrome (SSNS) (n = 722, from our previous study [28]) were used as renal disease controls. These individuals met the standard international criteria for nephrotic syndrome, and steroid sensitivity was defined as per the international guidelines of standard response to steroid treatment within 4 weeks of treatment [28].

Genotyping

Genotyping of MN and SSNS cases was undertaken at UCL Genomics (UCL Great Ormond Street, Institute of Child Health, London) on the Illumina Infinium Multi-Ethnic Global BeadChip with 1,779,818 markers. Sample processing for Illumina was done in accordance with the Infinium HD Ultra Assay protocol (Part #15023140 Rev. A) (Illumina Inc., San Diego, USA).

Post-Genotyping Processing and Quality Control

In-house software was utilized to re-encode genotypes to the genomic forward encoding scheme from the genotyping microarray chip manifest [29]. Standard quality control procedures were applied using PLINK v1.9 [30] to the 1,409 MN cases, 722 SSNS, and 6,126 controls, initially separately and then on the combined case-control dataset [31]. Quality control filtering included per individual filtering and per single-nucleotide variant (SNV) steps. In brief, per individual filtering criteria for exclusion were genotype call rate <98%, heterozygosity rate >3 standard deviations above or below the mean of all samples, and identity by descent value >0.1875. Per SNV filtering criteria for exclusion were genotype call rate <98%, minor allele frequency <5%, and deviation from Hardy-Weinberg equilibrium $p < 0.001$ on the control dataset. Finally, principal component analysis was performed on the combined dataset for European ancestry selection with the 1,000 Genome project ancestry controls [32]. Outliers with a principal component standard deviation > 2.5 from the European controls were removed. Completion of quality control left 1,038 MN cases (of which serum results were available in 759), 4,929 controls, and 422 SSNS cases.

Imputation

As the lead SNVs were not present in the post-QC genotyped data, whole genome imputation was performed using Beagle 5.0 [33]. The 1,000 Genomes Project Phase 3 data (version 5a) were utilized as the reference panel [32]. The post-quality control combined dataset was used as the input for imputation. Post-imputation filtering exclusion criteria were multi-allelic variants, insertions and deletions, minor allele frequency <5%, genotyping rate <98%, deviation from Hardy-Weinberg equilibrium $p < 0.0001$ in controls, imputation quality score DR^2 <80%. From this complete filtered dataset, the six variants as identified and code-labelled from the previous study, rs17831251 (*PLA2R1*), rs9405192 (*IRF4*), rs230520 (*NFKB1*), rs9271541 (HLA1), rs9265949 (HLA2), and rs2858309 (HLA3), were extracted [20].

Genetic Risk Score

A genetic risk score (GRS) was calculated using the previously reported mutually adjusted effect size in the above listed 6 SNVs. The European effect size was utilized for these SNVs as per Xie *et al.* [20] (see online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000529959). In each individual, for each SNV the effect size was multiplied by the number of risk alleles and the totals for each SNV were summed. The effect for the added epistatic interaction between *PLA2R1* and *HLA-DRB1* was accounted for – if all 4 risk alleles

were present in these 2 SNVs, then the interaction effect was added to the previous sum. The overall result was divided by 6 (to correct for the total number of loci) [34].

$$GRS = \sum \frac{Number\ of\ risk\ alleles_{at\ each\ SNP} \times mutually\ adjusted\ effect_{at\ that\ SNP}}{Number\ of\ loci}$$

SNV Association Analysis

A SNV association analysis was conducted using PLINK v1.9 and 2.0 [30, 35]. Logistic regression was done for categorical phenotypes and linear regression for quantitative phenotypes. The 10 lead principal components were used as a covariate to correct for ancestry stratification.

Simulation Analysis

A simulation analysis was undertaken to predict the proportions of anti-PLA2R1 antibody MN cases or controls based on the GRS calculations in the dual negative antibody group. The median, interquartile range (IQR), and mean for the dual antibody-negative group GRS were calculated. These statistics were then best simulated and matched by combining different proportions of the GRS from both anti-PLA2R1 antibody cases and controls. The random selection tool (RANDBETWEEN) in Microsoft Excel was used to extract random samples from the anti-PLA2R1 antibody and control groups. The median, IQR, and mean were calculated, and the best matching proportion model was selected.

Statistics and Data Representation

R version 3.6.1 was used to undertake regression analyses, and figures were produced using the packages ggplot2 and qqman [36–38]. Normality of the GRS was assessed using the Shapiro-Wilks method, and this demonstrated significant deviation from a normal distribution in all antibody groups apart from the anti-THSD7A group. Hence, medians and IQR are reported and non-parametric statistical tests were utilized for comparison. Kruskal-Wallis and Dunn's multiple comparison test was performed to assess differences with a stringent Bonferroni correction to take into account pairwise comparisons of 5 different groups using a p value threshold for significance of <0.005 (0.05/10). For the SNV association analysis, a p value < 0.003 (0.05/15) was used to account for the multiple analyses of 6 SNVs. For linear regression, a standard p value < 0.05 was used.

Table 2 . Composition of cohort

Healthy controls	4,929
Steroid-sensitive nephrotic syndrome	422
MN: Anti-PLA2R1 antibody positive	372
MN: Anti-THSD7A antibody positive	31
MN: Dual antibody (PLA2R1 and THSD7A) positive	1
MN: Dual antibody (PLA2R1 and THSD7A) negative	355
Total membranous nephropathy	759

MN, membranous nephropathy; PLA2R1, phospholipase A2 receptor 1; THSD7A, thrombospondin type-1 domain containing 7A.

Results

Antibody Group and Genetic Risk

The composition of the cohort is detailed in Table 2. The GRS was calculated in the 759 MN cases with European genetic ancestry that passed genotyping quality control and had serum available for antibody testing. The GRS significantly deviated from a normal distribution in all groups apart from the anti-THSD7A group. Compared with the GRS in 4,929 healthy controls (median = 0.2, IQR = 0.14–0.28) and 422 SSNS controls (median = 0.2, IQR = 0.15–0.34), GRS was significantly elevated in the anti-PLA2R1 antibody group (N = 372; median = 0.34, IQR = 0.26–0.42; $p < 0.0001$) (Fig. 1). GRS in the 355 individuals with dual antibody-negative MN was intermediate (median = 0.29, IQR = 0.2–0.4) and statistically significantly different from, the control ($p < 0.0001$) and PLA2R1-positive groups ($p < 0.0001$), consistent with them comprising a mixture of PLA2R1-positive and PLA2R1-negative individuals. The median GRS among the 372 with PLA2R1 antibodies was significantly higher than among the 31 with THSD7A antibodies (median = 0.12, range = 0.15–0.27, $p < 0.0001$).

SNV Association Analysis

To determine the individual contribution to the genetic risk of MN, we undertook a SNV association test with logistic regression for each of the lead 6 SNVs.

PLA2R1 SNV

Corroborating previous studies [19], our data demonstrated that the lead *PLA2R1* SNV frequency was significantly different between healthy subjects and the anti-PLA2R1 antibody cases (OR = 2.17, $p = 1.36 \times 10^{-19}$). Healthy subjects were also significantly different from the dual antibody negative (OR = 1.42, $p = 1.66 \times$

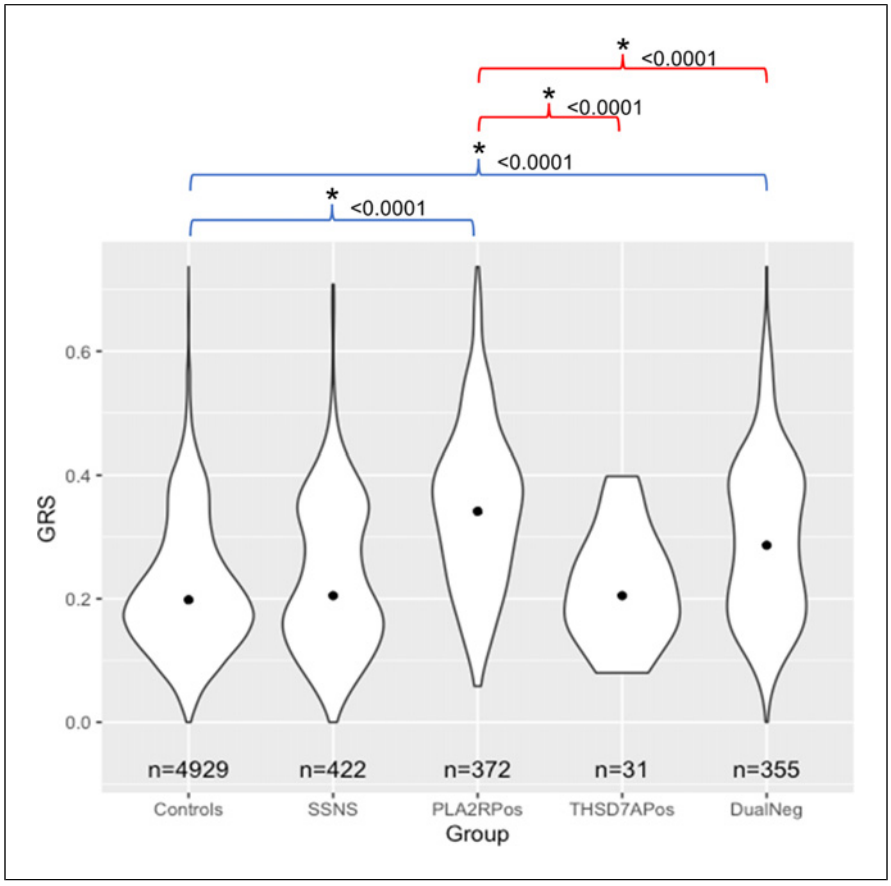

Fig. 1. The genetic risk score (GRS) is associated with a diagnosis of MN and distinguishes between the different antibody types. The number of individuals in each group is shown. The black dot represents the median. Statistically significant differences (using a Kruskal-Wallis test) from Controls are highlighted by blue braces ($p < 0.0001$). Statistically significant differences from PLA2RPos are highlighted by red braces ($p < 0.0001$). SSNS, steroid-sensitive nephrotic syndrome; PLA2RPos, anti-PLA2R1 antibody-positive MN; THSD7APos, anti-THSD7A antibody-positive MN; DualNeg, PLA2R1 and THSD7A antibody-negative MN.

10^{-5}) but not statistically different to the anti-THSD7A antibody negative or SSNS groups. Comparing the anti-PLA2R1 antibody group with the anti-THSD7A demonstrated a statistically significant difference (OR = 2.54, $p = 0.0008$), whereas there was no statistical difference compared to the dual antibody-negative group.

HLA SNVs

The lead HLA SNVs demonstrated similar results. The SNV for *HLA-DRB1* was statistically different between controls versus the anti-PLA2R1 antibody cases (OR = 4.62, $p = 2.83 \times 10^{-72}$) and controls versus the dual antibody-negative cases (OR = 2.34, $p = 1.18 \times 10^{-26}$). No statistically significant difference was found with controls versus anti-THSD7A cases. Comparing the antibody groups, the anti-PLA2R1 antibody group was statistically different to the anti-THSD7A antibody group (OR = 4.54, $p = 1.36 \times 10^{-5}$) but not the dual antibody-negative group (OR = 1.18, $p = 0.16$). The same statistically different findings applied for the *HLA-DQA1* SNV in all comparisons are summarized in Table 3.

Table 3. Results of the *HLA-DQA1* SNV logistic regression association analysis between different antibody status groups

Comparison groups	Odds ratio	*p* value
PLA2RPos versus Ctrls	3.94	5.3×10^{-47}
DualNeg versus Ctrls	2.78	3.9×10^{-26}
THSD7APos versus Ctrls	1.03	0.93
PLA2RPos versus THSD7APos	4.15	0.00054
PLA2RPos versus DualNeg	0.75	0.027

PLA2RPos, anti-PLA2R1 antibody-positive MN; Ctrls, healthy controls; DualNeg, PLA2R1 and THSD7A antibody-negative MN; THSD7APos, anti-THSD7A antibody-positive MN.

Other SNVs

The SNVs at *NFKB1* and *IRF4* did not exhibit any statistically significant differences in frequency between any of the groups.

Simulation Analysis

Different proportions of controls and anti-PLA2R1 antibody GRS scores were simulated and combined to

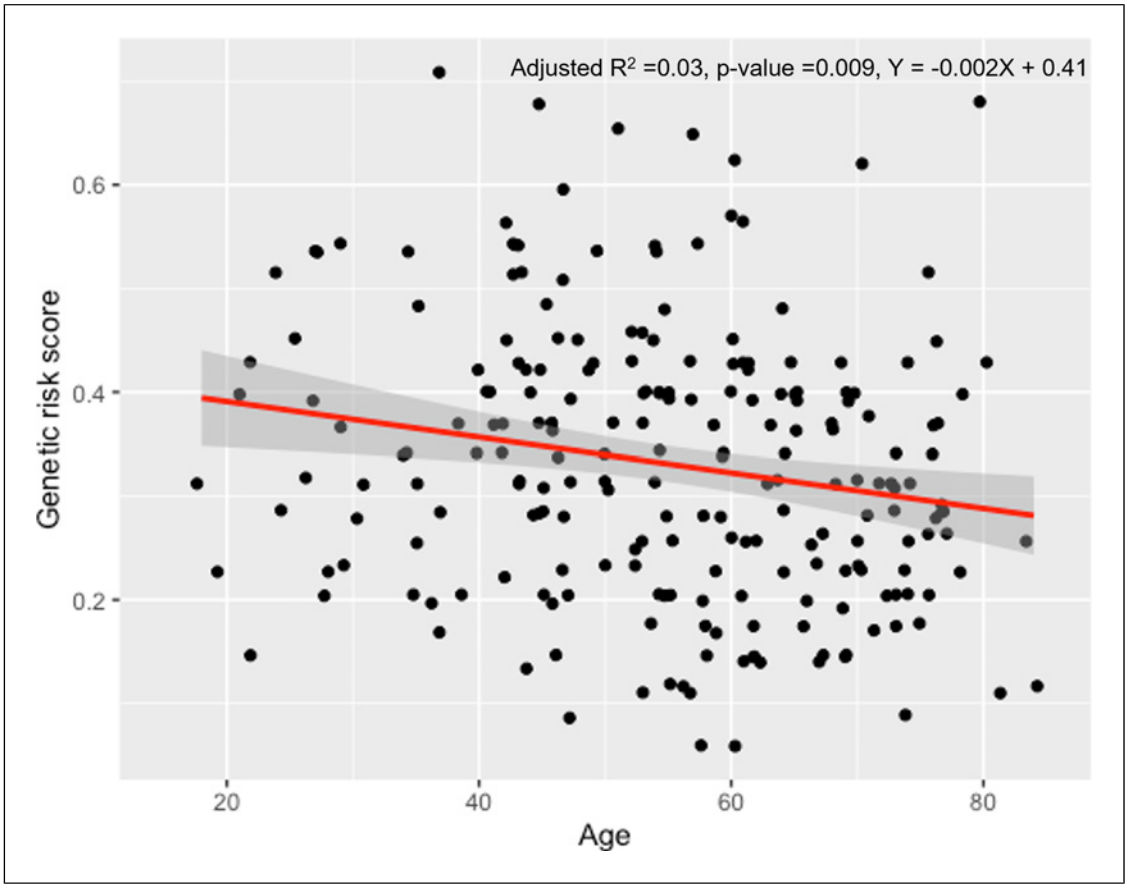

Fig. 2. The genetic risk score is associated with age of onset in anti-PLA2R1 antibody membranous nephropathy. Linear regression of genetic risk score and the age of onset of anti-PLA2R1 antibody membranous nephropathy in a German European cohort, $n = 224$, $p = 0.009$. The red line demonstrates the line of best fit for the model; $Y = -0.002 \times +0.41$, and the shaded grey area demonstrates the standard error of the estimate (+/− 0.03).

best match the median (0.29), IQR (0.2), and mean (0.3) for the dual antibody-negative group. The best matching model was 67–70% controls and 30–33% anti-PLA2R1 antibody cases; see online supplementary Table 2.

Age and Genetic Risk

Analysis of a subset of 224 anti-PLA2R1-positive cases with demographic data was performed. GRS and age of onset of disease were found to be weakly but statistically significantly inversely associated in the anti-PLA2R1 antibody-positive cases ($R^2 = 0.003$; $p = 0.009$, Fig. 2). A SNV linear regression association analysis with age and the lead 6 SNVs did not demonstrate any statistically significant associations. Additional analysis with multiple confounders (urinary protein-creatinine ratio [at diagnosis], glomerular filtration rate [at diagnosis], anti-PLA2R1 antibody titre [at diagnosis], and sex) remained statistically significant ($p = 0.026$) but weakened the strength of the association ($R^2 = 0.02$).

Other Clinical Parameters

Among the 224 German subjects for whom uniformly collected phenotype data were available, linear regression analyses of GRS with urinary protein-creatinine ratio [at diagnosis], glomerular filtration rate decline per year [minimum 5-year follow-up data], anti-PLA2R1 antibody titre [at diagnosis], and sex revealed no statistically significant associations; see online supplementary Figures 1–3. The lead SNVs association analysis also did not demonstrate any significant associations.

Discussion

Antibodies and Genetic Risk

This study confirms there are genetic differences between different autoantigen-defined subgroups of autoimmune MN. Despite sharing a clinical and pathological phenotype, the anti-PLA2R1 antibody group is genetically distinguishable from the anti-THSD7A group at the *PLA2R1* and *HLA* risk loci. It may be expected that the two antibody groups would differ most significantly at the *PLA2R1* locus because it has been suggested that the variation in the *PLA2R1* sequence alters PLA2R1 expression and increases the risk of being presented as an autoantigen [39]. We observed that the difference in the magnitude of effect with both *HLA* loci was almost double that of the *PLA2R1* risk SNV. The risk effect of HLA for anti-PLA2R1-positive MN could be explained by HLA antigen specificity, whereby the associated allomorph is more likely than others to present self-PLA2R1 peptide(s), but not more likely to present self-THSD7A peptide(s). This phenomenon is recognized in other autoimmune diseases such as ANCA-associated vasculitis, in which the different specificities of autoantibodies are associated with distinct subsets of HLA class II allomorphs [40].

One explanation for the elevated GRS in the dual antibody-negative group is that this group actually comprises two populations: one in which PLA2R1 autoimmunity was not driving the disease (in whom the GRS would be expected to be similar to the other unaffected cohorts studied) and another group in which PLA2R1 autoimmunity was driving the disease (despite lack of detectable autoantibody) in whom the GRS would be expected to approximate that of the anti-PLA2R1 antibody group. This explanation is supported by the bimodal distribution of the GRS among dual negative cases (Fig. 1). This contrasts with previous work that only found the *PLA2R1* risk allele association in PLA2R1 antibody-positive cases [41], although PLA2R1 positivity was detected with biopsy immunofluorescence in that study. This may be explained by the sensitivity of the assay to detect anti-PLA2R1 antibodies in some patients, loss of antibody due to prolonged storage of serum samples or to patients becoming seronegative between onset of disease and sampling of their serum [42]. Some antibody-negative cases might be associated with the rarer antigens for which we did not test, such as neutral endopeptidase, high-temperature requirement A serine peptidase 1, protocadherin 7, semaphorin 3B, and neural epidermal growth factor-like 1 protein. Nonetheless, one possible explanation for the elevation in MN GRS among this antibody-negative group is that a significant proportion of the dual antibody-negative patients have disease driven by PLA2R1 immunoreactivity. This is supported by previous data showing that 11% of individuals serologically negative for anti-PLA2R1 antibodies show immunoreactivity histologically [43, 44], although in our cohort of dual antibody-negative group ($N = 355$) this proportion on its own would not account for the similarity in GRS we observed between PLA2R1-positive and antibody-negative groups.

The sensitivity of serological testing is variable, and previous estimates range between 50 and 80% [45]. Simulation analyses indicated that the GRS observed in the antibody-negative group could be explained by this group comprising between 30 and 33% PLA2R1-positive individuals. Since we did not observe a correlation between anti-PLA2R1 antibody titre and GRS, we regard this as the best estimate for the proportion of apparently antibody-negative individuals who actually have disease driven by autoimmunity to PLA2R1. This equates to 117 individuals and suggests (assuming a specificity of ≥97% as previously reported [46]) that the sensitivity of the PLA2R1 assay used to detect PLA2R1 autoimmunity was 76% (117 out of 489 proven and inferred PLA2R1-positive cases not detected). This figure is likely to be slightly higher (closer to 80% in keeping with other studies) due to the low number of individuals that may have become serologically negative by the time the serum sample was collected within the first 6 months. PLA2R1 antibodies can be exclusively deposited in the kidney and therefore not detectable serologically. We reviewed a small proportion of the biopsies available from the dual negative cases and confirmed that the biopsy PLA2R1 immunofluorescence was negative.

Age and HLA

We observed that the GRS is associated with a younger age of onset of disease in anti-PLA2R1 antibody-positive cases, with an inverse correlation between age and GRS observed in our European cohort. None of the SNVs alone were associated with age; however, when the burden of all the 6 risk variants was combined and interactions were accounted for, then this became significant. In a Chinese cohort of 100 anti-PLA2R1 antibody-positive cases, the HLA type (HLA *DRB1*1,501* positive and *DRB3*0202* negative) was associated with a younger age of onset [47]. However, no association with GRS and age was found in 1,752 unselected primary MN cases [20]. With the inclusion of only PLA2R1-positive cases, we were able to identify this association. The phenomenon of HLA risk alleles being associated with age of onset has been described in paediatric cases of SSNS where increased number of risk alleles in *HLA-DRB1* and *DQB1* was associated with a younger age of disease onset in SSNS [48]. In systemic lupus

erythematosus, this phenomenon has been described; individuals in the high quartile GRS group had onset of disease 6 years prior to those in the lower quartiles [49]. Furthermore, a recent examination of 24 common diseases in the UK Biobank found the predominant pattern to be an increased risk of early onset disease with greater genetic risk [50]. The increased genetic risk increases the likelihood that the sum of risk factors exceeds the threshold for disease manifestations: the disease is more likely to happen and thus tends to occur earlier in life.

Limitations

One limitation of our study was the lack of detailed phenotype on the clinical parameters for a large proportion of our cohort which limited power to detect associations. A second limitation is that we only considered the effect on disease risk of the independent, significantly associated alleles identified by previous genome-wide association studies. Owing to the large magnitude of the effect on disease risk of the two leading loci (*HLA* and *PLA2R1*), we consider it unlikely that the findings would have been substantially different if alternative analysis strategies, such as by summating far smaller (and individually undetected) potential risk effects across the genome, had been employed. Finally, while potentially providing insight into the biological differences between PLA2R1- and non-PLA2R1-mediated MN, our study did not address the clinical question of whether knowledge of the GRS in an individual might assist in their clinical management or investigation.

Conclusion

We observed genetic differences between anti-PLA2R1 antibody and anti-THSD7A antibody positivity MN in European populations. We conclude that the GRS presented in this paper is specific for anti-PLA2R1 antibody-mediated MN. The elevated GRS in the dual antibody-negative group suggests a greater similarity to the anti-PLA2R1 antibody group in a subgroup of approximately one-third of these dual negative cases. This suggests that the sensitivity of the serological assay used to detect PLA2R1-driven autoimmunity was 76% in our cohort due to various reasons. On a wider scale, this implies that a negative anti-PLA2R1 antibody assay alone should not be used to determine diagnosis or treatment as, even where the analytical sensitivity of an assay is perfect, antibodies can disappear and/or be entirely deposited in the kidney. We demonstrate that application of the GRS in MN however can distinguish different antibody states even in the presence of potentially false-negative serological testing. Individuals with a higher GRS have a younger age of onset. Most recently, the clinical utility of GRS in MN has been demonstrated to help establish the diagnosis in 20–37% of apparent dual antibody-negative cases and we provide further evidence to support this.

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Statement of Ethics

This study protocol was reviewed and approved by Bloomsbury Ethics Committee, approval number 05/Q0508/6. Written informed consent was obtained from participants in all three studies.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Daniel Philip Gale: project conception. Sanjana Gupta, Daniel Philip Gale, Detlef Bockenhauer, Horia Constantin Stanescu, and

Robert Kleta: project protocol development. Sanjana Gupta: data analysis and draft manuscript preparation. Mallory Lorraine Downie, Dr. Chris Cheshire, Dr. Stephanie Dufek-Kamperis, Dr. Adam Paul Levine, Prof. Paul Brenchley, Dr. Elion Hoxha, Dr. Rolf Stahl, Dr. Neil Ashman, Dr. Ruth Jennifer Pepper, Dr. Sean Mason, and Prof. Jill Norman undertook manuscript review.

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author DPG upon reasonable request.

References

1 McGrogan A, Franssen CF, de Vries CS. The incidence of primary glomerulonephritis worldwide: a systematic review of the literature. Nephrol Dial Transplant. 2011;26(2):414–30.
2 Lai WL, Yeh TH, Chen PM, Chan CK, Chiang WC, Chen YM, et al. Membranous nephropathy: a review on the pathogenesis, diagnosis, and treatment. J Formos Med Assoc. 2015;114(2):102–11.
3 Pierides AM, Malasit P, Morley AR, Willkinson R, Uldall PR, Kerr DN. Idiopathic membranous nephropathy. Q J Med. 1977;46(182):163–77.
4 Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11–21.
5 Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371(24):2277–87.
6 Debiec H, Guigonis V, Mougenot B, Decobert F, Haymann JP, Bensman A, et al. Antenatal membranous glomerulonephritis due to anti–neutral endopeptidase antibodies. N Engl J Med. 2002;346(26):2053–60.
7 Sethi S, Debiec H, Madden B, Charlesworth MC, Morelle J, Gross L, et al. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. Kidney Int. 2020;97(1):163–74.
8 Al-Rabadi LF, Caza T, Trivin-Avillach C, Rodan AR, Andeen N, Hayashi N, et al. Serine protease HTRA1 as a novel target antigen in primary membranous nephropathy. J Am Soc Nephrol. 2021;32(7):1666–81.
9 Sethi S, Madden B, Debiec H, Morelle J, Charlesworth MC, Gross L, et al. Protocadherin 7–associated membranous nephropathy. J Am Soc Nephrol. 2021;32(5):1249–61.
10 Sethi S, Debiec H, Madden B, Vivarelli M, Charlesworth MC, Ravindran A, et al. Semaphorin 3B–associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. Kidney Int. 2020;98(5):1253–64.
11 Le Quintrec M, Teisseyre M, Bec N, Delmont E, Szwarc I, Perrochia H, et al. Contactin-1 is a novel target antigen in membranous nephropathy associated with chronic inflammatory demyelinating polyneuropathy. Kidney Int. 2021;100(6):1240–9.
12 van de Logt AE, Fresquet M, Wetzels JF, Brenchley P. The anti-PLA2R antibody in membranous nephropathy: what we know and what remains a decade after its discovery. Kidney Int. 2019;96(6):1292–302.
13 Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M, et al. Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. Kidney Int. 2013;83(5):940–8.
14 Radice A, Trezzi B, Maggiore U, Pregnolato F, Stellato T, Napodano P, et al. Clinical usefulness of autoantibodies to M-type Phospholipase A2 Receptor (PLA2R) for monitoring disease activity in Idiopathic Membranous Nephropathy (IMN). Autoimmun Rev. 2016;15(2):146–54.
15 Gupta S, Köttgen A, Hoxha E, Brenchley P, Bockenhauer D, Stanescu HC, et al. Genetics of membranous nephropathy. Nephrol Dial Transplant. 2017;33(9):1493–502.
16 Stanescu HC, Arcos-Burgos M, Medlar A, Bockenhauer D, Kottgen A, Dragomirescu L, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med. 2011;364(7):616–26.
17 Bullich G, Ballarin J, Oliver A, Ayasreh N, Silva I, Santin S, et al. HLA-DQA1 and PLA2R1 polymorphisms and risk of idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2014;9(2):335–43.
18 Cui G, Zhang L, Xu Y, Cianflone K, Ding H, Wang DW. Development of a high resolution melting method for genotyping of risk HLA-DQA1 and PLA2R1 alleles and ethnic distribution of these risk alleles. Gene. 2013;514(2):125–30.
19 Lv J, Hou W, Zhou X, Liu G, Zhou F, Zhao N, et al. Interaction between PLA2R1 and HLA-DQA1 variants associates with anti-PLA2R antibodies and membranous nephropathy. J Am Soc Nephrol. 2013;24(8):1323–9.
20 Xie J, Liu L, Mladkova N, Li Y, Ren H, Wang W, et al. The genetic architecture of membranous nephropathy and its potential to improve non-invasive diagnosis. Nat Commun. 2020;11(1):1600.
21 Trowsdale J. The MHC, disease and selection. Immunol Lett. 2011;137(1–2):1–8.
22 Ramachandran R, Kumar V, Nada R, Jha V. Serial monitoring of anti-PLA_2R in initial PLA_2R-negative patients with primary membranous nephropathy. Kidney Int. 2015;88(5):1198–9.
23 Fairfax BP, Humburg P, Makino S, Naranbhai V, Wong D, Lau E, et al. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science. 2014;343(6175):1246949.
24 Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dilthey A, et al. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nat Genet. 2012;44(5):502–10.
25 Illumina genotyping control database. 2010. Available from: https://www.illumina.com/documents/icontroldb/document_purpose.pdf.
26 Wellcome Trust Case Control Consortium 2. 2008. Available from: https://www.wtccc.org.uk/ccc2/.
27 Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661–78.
28 Dufek S, Cheshire C, Levine AP, Trompeter RS, Issler N, Stubbs M, et al. Genetic identification of two novel loci associated with steroid-sensitive nephrotic syndrome. J Am Soc Nephrol. 2019;30(8):1375–84.
29 Cheshire C. Bioinformatic investigations into the genetic architecture of renal disorders. London: University College London; 2019.
30 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559–75.
31 Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nat Protoc. 2010;5(9):1564–73.
32 1000 Genome Project. 1000. Genome Project File transfer protocol Webpage. Available from: ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502.

33 Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am J Hum Genet. 2007;81(5):1084–97.
34 Downie ML, Gupta S, Tekman MC, Cheshire C, Arora S, Licht C, et al. Identification of a locus on the X chromosome linked to familial membranous nephropathy. Kidney Int Rep. 2021;6(6):1669–76.
35 Chang C. PLINK v2.0. 2018.
36 R Core Team.R. A language and environment for statistical computing. In: R foundation for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
37 Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag; 2016.
38 D Turner S. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. J Open Source Softw. 2018;3(25):731.
39 Coenen MJ, Hofstra JM, Debiec H, Stanescu HC, Medlar AJ, Stengel B, et al. Phospholipase A2 Receptor (PLA2R1) sequence variants in idiopathic membranous nephropathy. J Am Soc Nephrol. 2013;24(4):677–83.
40 Lyons PA, Rayner TF, Trivedi S, Holle JU, Watts RA, Jayne DR, et al. Genetically distinct subsets within ANCA-associated vasculitis. N Engl J Med. 2012;367(3):214–23.
41 Saeed M, Beggs ML, Walker PD, Larsen CP. PLA2R-associated membranous glomerulopathy is modulated by common variants in PLA2R1 and HLA-DQA1 genes. Genes Immun. 2014;15(8):556–61.
42 Akiyama S, Akiyama M, Imai E, Ozaki T, Matsuo S, Maruyama S. Prevalence of antiphospholipase A2 receptor antibodies in Japanese patients with membranous nephropathy. Clin Exp Nephrol. 2015;19(4):653–60.
43 Dai H, Zhang H, He Y. Diagnostic accuracy of PLA2R autoantibodies and glomerular staining for the differentiation of idiopathic and secondary membranous nephropathy: an updated meta-analysis. Sci Rep. 2015;5:8803.
44 Pourcine F, Dahan K, Mihout F, Cachanado M, Brocheriou I, Debiec H, et al. Prognostic value of PLA2R autoimmunity detected by measurement of anti-PLA2R antibodies combined with detection of PLA2R antigen in membranous nephropathy: a single-centre study over 14 years. PLoS One. 2017;12(3):e0173201.
45 Francis JM, Beck LH Jr, Salant DJ. Membranous nephropathy: a journey from bench to bedside. Am J Kidney Dis. 2016;68(1):138–47.
46 Timmermans SA, Damoiseaux JG, Heerings-Rewinkel PT, Ayalon R, Beck LH Jr, Schlumberger W, et al. Evaluation of anti-PLA2R1 as measured by a novel ELISA in patients with idiopathic membranous nephropathy: a cohort study. Am J Clin Pathol. 2014;142(1):29–34.
47 Le WB, Shi JS, Zhang T, Liu L, Qin HZ, Liang S, et al. HLA-DRB1*15:01 and HLA-drb3*02:02 in pla2r-related membranous nephropathy. J Am Soc Nephrol. 2017;28(5):1642–50.
48 Debiec H, Dossier C, Letouze E, Gillies CE, Vivarelli M, Putler RK, et al. Transethnic, genome-wide analysis reveals immune-related risk alleles and phenotypic correlates in pediatric steroid-sensitive nephrotic syndrome. J Am Soc Nephrol. 2018;29(7):2000–13.
49 Reid S, Alexsson A, Frodlund M, Morris D, Sandling JK, Bolin K, et al. High genetic risk score is associated with early disease onset, damage accrual and decreased survival in systemic lupus erythematosus. Ann Rheum Dis. 2020;79(3):363–9.
50 Jiang X, Sheng H, Li J, Xun P, Cheng Y, Huang J, et al. Association between renin-angiotensin system gene polymorphism and essential hypertension: a community-based study. J Hum Hypertens. 2009;23(3):176–81.