Genetic Risk Variants for Membranous Nephropathy: Extension and Association with Other CKD Etiologies

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Abstract

Background: Membranous Nephropathy (MN) is a common cause of nephrotic syndrome in adults. Previous genome-wide association studies (GWAS) of 300,000 genotyped variants identified MN-associated loci at *HLA-DQA1* and *PLA2R1*.

Methods: We used a combined approach of genotype imputation, GWAS, HLA imputation and extension to other etiologies of chronic kidney disease (CKD) to investigate genetic MN risk variants more comprehensively.

GWAS using 9 million high-quality imputed genotypes and classical HLA alleles were conducted for 323 MN European-ancestry cases and 345 controls. Additionally, 4,960 patients with different CKD etiologies in the German Chronic Kidney Disease (GCKD) study were genotyped for risk variants at *HLA-DQA1* and *PLA2R1*.

Results: In GWAS, known variants (rs9272729, *HLA-DQA1*, OR=7.3 per risk allele, $p=6.7*10^{-27}$ and rs17830558, *PLA2R1*, OR=2.2, $p=1.9*10^{-8}$) were significantly associated with MN**.** No novel signals emerged in GWAS of X-chromosomal variants or in sex-specific analyses. Classical HLA alleles (DRB1*0301-DQA1*0501-DQB1*0201 haplotype) were associated with MN but provided little additional information beyond rs9272729.

Associations replicated in 137 GCKD patients with MN (*HLA-DQA1*: $p=6.4*10^{-24}$; *PLA2R1*: $p=5.0*10^{-4}$). MN risk increased steeply for patients with high-risk genotype combinations (OR>79). While genetic variation in *PLA2R1* exclusively associated with MN across 19 CKD etiologies, the *HLA-DQA1* risk allele was also associated with lupus nephritis ($p=2.8*10^{-6}$), type I diabetic nephropathy ($p=6.9*10^{-5}$) and focal segmental glomerulosclerosis ($p=5.1*10^{-5}$), but not with IgA nephropathy.

Conclusions: *PLA2R1* and *HLA-DQA1* are the predominant risk loci for MN detected by GWAS. While *HLA-DQA1* risk variants show association with other CKD etiologies, *PLA2R1* variants are specific to MN.

Keywords: chronic kidney disease, genome-wide association study, membranous nephropathy

Summary

PLA2R1 and *HLA-DQA1* are the predominant risk loci for MN. Common variants in these two loci give rise to rare high-risk genotype combinations; the associated MN risk is of a magnitude otherwise only observed for single-gene disorders. The risk allele at *HLA-DQA1*, but not in *PLA2R1*, is also associated with CKD resulting from type I diabetes, SLE and FSGS, but not from IgA nephropathy, another auto-immune kidney disease.

Introduction

Membranous Nephropathy (MN) is one of the most common causes of the nephrotic syndrome in adults.[1, 2] It is considered an immune complex-mediated disease, with sub-epithelial deposits of immune complexes detectable using immunofluorescence or electron microscopy. In some patients, MN is secondary and consequential to tumors, infections or exposure to environmental factors. The majority of patients, however, suffer from so-called primary MN, termed "idiopathic" (IMN) until the recent recognition as an autoimmune disease.

A genetic component to MN has long been suspected based on familial clustering.[3] The involvement of a risk allele at the HLA locus, and more specifically of DQA1, has already been proposed in MN pathogenesis in the late 1980's.[4] Additional insights into MN pathophysiology from animal experiments showed that immune complex formation can result from interactions of antibodies with a local antigen on podocytes.[1] In humans, the M-type phospholipase A2 receptor protein at the podocyte surface was identified as the major autoantigen in patients with MN.[5] More recently thrombospondin type-1 domain-containing 7A (THSD7A) was identified as another autoantigen in phospholipase A2 receptor antibody negative patients with MN.[6]

Genome-wide association studies (GWAS) can be used to evaluate the association between genome-wide genetic markers and a disease in a comprehensive and unbiased way. The first meta-analysis of GWAS of MN identified strong associations between MN and single nucleotide polymorphisms (SNPs) at the *HLA-DQA1* locus and in *PLA2R1*, encoding the M-type phospholipase A2 receptor.[7] This study did not only provide evidence for the influence of an individual's genetic make-up in shaping the interaction between adaptive immune system and auto-antigens, but also provided striking evidence that the combination of risk alleles at only

two genetic loci was associated with large increases in disease risk of a magnitude that is typically only observed for single gene disorders.

However, previous GWAS of MN have only evaluated a limited set of approximately 300,000 genotyped genetic variants and have not used imputation methods[8, 9] to increase marker coverage or to obtain information on classical HLA alleles.[10] Genotype imputation can increase statistical power to detect genetic associations.[9] Imputation to the 1000 Genomes reference panel in previous studies has led to the identification of additional risk loci that were missed in previous association studies of at least equal size, for example for body mass index, fasting glucose or coronary artery disease.[11, 12] In addition, GWAS conditioning on the associated index variants can lead to the identification of secondary independent signals in associated regions.[13] Moreover, although MN shows a male predilection,[1] previous studies have combined both sexes to evaluate genetic associations and only studied autosomes. Finally, it has not been studied systematically whether the previously identified genetic risk variants in the HLA locus and *PLA2R1* confer risk that is specific to MN, or if they also increase risk for other etiologies of chronic kidney disease (CKD). The present study therefore aimed to address these important gaps.

Subjects and Methods

For the GWAS of MN, DNA samples from 336 British MN cases that provided written informed consent were collected by the MRC/Kidney Research UK National DNA Bank for Glomerulonephritis and racially matched to samples from 349 control subjects of the 1958 UK Birth Control study (http://www.b58cgene.sgul.ac.uk). Study protocol and sample characteristics have been described previously.[7] On average, MN patients were of 52.5 (±13.3) years old at the time of diagnosis, and 69% of them were men.

For replication and characterization, we examined patients taking part in the German Chronic Kidney Disease (GCKD) study, a prospective observational study of patients with CKD with either eGFR between 30 and 60 mL/min/1.73m² or overt proteinuria (albumin-to-creatinine ratio > 300mg/g or protein-to-creatinine ratio > 500 mg/g) upon study inclusion.[14] The study was approved by local review boards at each participating academic institution and is described in detail elsewhere.[15] Briefly, 5,217 patients who gave their written informed consent were enrolled into the study between 2010 and 2012. The patients' long-term treating nephrologists were asked to select the leading i.e. primary cause of CKD for each patient. For disease categories in which biopsy was not performed for the majority of patients, such as diabetic or hypertensive kidney disease, diagnosis was established by the nephrologists based on clinical grounds. The assignment of these causes was consistent with comorbidities reported by the patients as well as with the presence of elevated biomarkers at the study visit (elevated hemoglobin A1c and blood pressure) and the intake of anti-diabetic and anti-hypertensive medication, respectively. All patients were of Caucasian ancestry, and 60% were men. At the time of entry, patients were on average 60.0 (±12.0) years old and had CKD of various etiologies.[16] MN as the leading cause of CKD was identified in 151 of 5,217 patients, 144 of

whom had a biopsy to confirm the diagnosis (80% men). In addition, other specific, leading causes of CKD that comprised at least 40 patients were evaluated as well as one smaller group of patients with rapidly progressive glomerulonephritis (pauci-immune; n=28). Analyses were restricted to patients with a biopsy-proven diagnosis when the proportion of patients with biopsies in a given group was >70%. Information on steroid sensitivity of FSGS was not available. As a control group, genotypes from 379 individuals (47% men) comprising the European subgroup of the 1000 Genomes project were used (http://www.1000genomes.org/home; phase 1, release v3, panel EUR). Data were downloaded from http://www.sph.umich.edu/csg/abecasis/MaCH/download/1000G.2012-03-14.html, and genotypes for selected SNPs were subsequently extracted.

Because of the complexity, detailed information on genotyping, quality control and imputation as well as on statistical analyses can be found in **Complete Methods (Supplementary Text 1 and 2)**.

In brief, GWAS study samples were genotyped using HumanCNV370-Quad SNP chip or the HumanHap300 SNP chip. After stringent quality control using Plink and Eigensoft, the cleaned data set comprised 282,462 SNPs of 323 cases and 345 controls.[17, 18] Subsequently, genotype imputation was carried out using Shapeit and Impute2.[19, 20] Data from the 1000 Genomes Project (phase 1, release v3, panel ALL) served as reference panel. The final data set contains 8.9 million SNPs of high quality. In the GCKD study, genotyping of index variants was carried out using an Agena (formerly Sequenom) iPLEX assay (*PLA2R1*) and using a Life Technologies TaqMan assay (*HLA-DQA1*) with call rates of 99% and genotype concordance of 100% among duplicate samples.

Association tests relating MN case status to autosomal genetic variants were carried out using genotype probabilities (dosages) and an additive genetic model with the software SNPtest version 2.4.1.[21] The first five principle components were included as covariates. Statistical significance was set to the standard threshold of $5.0*10^{-8}$. Independent replication was defined as a direction-consistent 2-sided p-value <0.05 (equivalent to 1-sided test). Inverse-variance weighted, fixed effect meta-analysis was conducted to pool results.

In GCKD, genotype information was also evaluated for association with CKD of 17 additional specific etiologies as well as using all GCKD participants as a CKD case group, all in comparison to the 1000 Genomes control group. The statistical significance cutoff for these analyses was set to $2.6*10^{-3}$.

Results

Genome-Wide Association Studies of MN

We carried out a GWAS among 323 MN cases and 345 controls of European ancestry using imputed genotypes as illustrated in the detailed analysis plan in **Supplementary Figure 1**. GWAS results did not show any evidence for inflation of the test statistics, indicating no systematic biases (Quantile-Quantile plot, **Supplementary Figure 2**). The two previously reported genetic regions, *PLA2R1* and *HLA-DQA1*,[7] contained SNPs associated with MN at genome-wide significance (**Figure 1**). The index SNPs at the two loci used in subsequent analyses were rs9272729 in *HLA-DQA1* (p-value=$6.7*10^{-27}$) and rs17830558 in *PLA2R1* (p-value=$1.9*10^{-8}$, **Table 1**)**.** In both instances, the index SNPs were imputed with high imputation quality and were correlated with the previously published SNPs (**Table 1**). **Supplementary Figures 3a** and **3b** display the two associated regions in more detail. The previously described extensive linkage disequilibrium (LD) in the HLA region is evident, extending several hundred kilobases around *HLA-DQA1* and including other HLA genes such as *HLA-DQB1*, *HLA-DRB1*, *HLA-DRB6*, and *HLA-DRB5*.

No additional loci on the autosomes or the X chromosome contained SNPs associated with MN at genome-wide significance. **Supplementary Table 1** contains all SNPs on autosomes and chromosome X with minor allele frequency (MAF) >5% and corrected p-value<10^{-5}.

Secondary Analyses

To further examine the genetic architecture of MN, several secondary analyses were carried out: first, ~~because of the observed male predilection,~~ additional GWAS were conducted separately for men (222 cases, 106 controls) and women (101 cases, 239 controls). These analyses were carried out because of the strong observed male predilection for MN, although

the smaller sample sizes limit statistical power. No additional signals of genome-wide significance were identified in either men or women (data not shown). Second, we evaluated whether a recessive model instead of an additive model, which is typically used in discovery GWAS, better fit the data. Consistent with lower statistical power of a recessive model, p-values were larger than those obtained from the additive model for both *PLA2R1* and *HLA-DQA1*. No other genomic regions contained significantly associated markers (data not shown).

We evaluated the identified regions containing *PLA2R1* and *HLA-DQA1* more closely in order to assess the presence of more than one independent signal of genome-wide significance. After conditioning on the index SNP of the corresponding region, no additional SNP across the respective chromosome was associated with MN at genome-wide significance. Thus, based on the variants assessed in this study, there was no evidence of multiple common independent variants in the known regions that contributed to the observed associations at genome-wide significance.

Finally, a very recent publication by Gbadegesin and colleagues reported on the association of a *HLA-DQA1* risk allele and steroid-sensitive nephrotic syndrome (SSNS) in children.[22] We therefore assessed whether the reported index variant, rs1129740, was also associated with MN in our genome-wide data. The reported risk allele for SSNS, the major A allele, was significantly associated with MN risk in our data (OR=2.48, 95% CI 1.81-3.40, p-value=$4.45*10^{-9}$). The OR for MN was similar to the one published for SSNS. The SSNS index variant rs1129740 was correlated with the MN index SNP evaluated in this study, rs9272729 (r^2=0.19, D'=1, based on the MN GWAS data). Besides MN, the SSNS risk allele at rs1129740 was also nominally associated with minimal change disease (OR=1.6, 95% CI 1.1-2.5, p-value=0.014) and FSGS (OR=1.5, 95% CI 1.2-2.0, p-value=0.002) in the GCKD study.

Replication and Interactions

Replication of SNP Associations and Meta-Analysis

We assessed evidence for replication of the association at the index variants in *PLA2R1* and *HLA-DQA1* in 137 participants of the GCKD study with biopsy-proven MN and 379 ethnically matched controls (see Concise Methods). Both index SNPs were associated with MN after correction for multiple testing in this replication sample (**Table 1**, p-value=$5.0*10^{-4}$ for *PLA2R1* and $6.4*10^{-24}$ for *HLA-DQA1*). The odds ratios for MN after meta-analysis of the discovery and replication samples were 1.87 (95% CI 1.54-2.28) for *PLA2R1* and 7.07 (95% CI 5.28-9.47) for *HLA-DQA1* for each additional copy of a risk allele.

Combined Allelic Effects and Interactions

Figure 2 presents the association with MN for the combination of risk alleles at *PLA2R1* and *HLA-DQA1*. The risk for MN increased dramatically with a higher number of risk alleles: almost all persons carrying two risk alleles at *HLA-DQA1* were affected with MN (14 of 16, 88%), and all three individuals who carried two risk alleles at both loci were affected with MN (so that no risk estimates could be provided, see arrow in Figure). The second highest risk of MN was observed for individuals carrying two risk alleles at *HLA-DQA1* and one risk allele at *PLA2R1*. Compared to individuals who did not carry any risk alleles, their MN risk was increased almost 80-fold (OR 79.4, p-value=$7.1*10^{-5}$). **Supplementary Table 2** displays case and control numbers for all risk categories, and - to obtain more stable numerical estimates - the corresponding results when combining individuals carrying one or two risk alleles at the *HLA-DQA1* locus into a single category.

Evidence for statistical interaction between the two index variants at *PLA2R1* and *HLA-DQA1* was assessed by simultaneous inclusion of the variants as well as of an interaction term

into the regression model (see Concise Methods). Compared to a model containing both variants but no interaction term, the inclusion of the interaction term lead to a decrease of Akaike's information criterion (AIC) from 491.9 to 487.2, which was statistically significant (LR-test p-value: 0.009). The odds ratio of the interaction term in this model was 2.20 (p-value=0.01), which together with the lower AIC supports that the combination of risk alleles increases MN risk to a stronger degree than expected based on the effect of the individual risk alleles at the two loci by themselves.

Characterization across CKD Etiologies Reveals Shared Associations at HLA-DQA1

Subsequently, we evaluated whether the association at *PLA2R1* and *HLA-DQA1* is specific to MN, or whether it extends to additional etiologies of CKD in the large GCKD study, which collected information on the underlying cause of CKD (see Concise Methods). **Table 2** shows the association of the two index variants with CKD defined based on estimated glomerular filtration rate (eGFR) and/or albuminuria, as well as with 17 specific etiologies of CKD. *PLA2R1* was only associated with MN but no other CKD etiology after correction for multiple testing. Conversely, a significant association of the *HLA-DQA1* risk variant was observed not only with MN, but also with lupus nephritis (p-value=$2.8*10^{-6}$), CKD in type 1 diabetes mellitus (p-value=$6.9*10^{-5}$), and focal segmental glomerulosclerosis (FSGS, p-value=$5.1*10^{-5}$). The ~~latter~~ association with FSGS remained significant even after the exclusion of 8 patients with HIV and hepatitis, potential causes of ~~and thus potentially suffering from~~ secondary FSGS (p-value=$2.3*10^{-5}$). The observed association with CKD (p-value=$1.3*10^{-3}$) was mainly due to the inclusion of patients with MN, lupus nephritis, type 1 diabetes mellitus and FSGS.

Associations with Classical HLA Alleles

Lastly, we attempted to assess whether MN showed stronger associations with carrier status of classical HLA alleles than with the index variant identified in the GWAS, and thus imputed classical HLA alleles from the genotyped SNPs in the discovery sample (see Concise Methods). MN was significantly associated with seven of the classical HLA alleles at genome-wide significance (**Table 3**). Whereas the lowest p-value was observed for HLA-DRB1*0301 carrier status (OR 6.15, p-value=$1.6*10^{-28}$), HLA-DQA1*0501 conferred the largest risk (OR 6.23, p-value=$1.4*10^{-27}$). Carrier status at these two HLA alleles was almost perfectly correlated (Spearman r=0.995), indicating a long shared risk haplotype, to which also HLA-DQB1*0201 belongs (Spearman correlation with HLA-DQA1*0501 r=1). **Supplementary Table 3** presents the associations of all imputed common HLA alleles (MAF>5%) with MN.

Using the classical HLA allele HLA-DQA1*0501 as a proxy for this risk haplotype, we assessed whether the index SNP at *HLA-DQA1* in the discovery GWAS, rs9272729, provided independent information. Individually, the association of rs9272729 with MN was of similar magnitude as the association of HLA-DQA1*0501 with MN: OR 6.51 (p-value=$7.16*10^{-19}$) for the SNP vs. OR 5.87 (p-value=$1.25*10^{-18}$) for the HLA allele among individuals with both types of information available. Upon simultaneous inclusion of rs9272729 and HLA-DQA1*0501 into the model, the effects of both variables were attenuated but still significant. There was a significant improvement of model fit (likelihood ratio test p-value=0.02), suggesting that the SNP captures most of the information but other variants in the genomic region and/or classical HLA alleles may still add some additional information.

Discussion

In this first GWAS of MN using imputed genotypes, we confirm the predominant importance of risk alleles at *PLA2R1* and *HLA-DQA1* in modulating MN risk. With the available number of cases, we did not find evidence for additional or independent common risk alleles or of sex-specific risk loci. While we found that genetic variation in *PLA2R1* exclusively associated with MN across a wide range of CKD etiologies studied, the risk allele at *HLA-DQA1* was also associated with higher odds of a number of other auto-immunity associated kidney diseases – lupus nephritis, type I diabetic nephropathy and FSGS - but not with IgA nephropathy.

In accordance with other studies,[23] our results from the GCKD study confirmed the association between MN and previously reported risk variants or their proxies in *PLA2R1* and *HLA-DQA1*. In addition, in our GWAS of high coverage, we did not identify additional common risk variants of similar importance. While imputation to the 1000 Genomes reference panel has led to the identification of additional risk loci missed in earlier studies of similar size for a variety of traits and diseases,[11, 12] this was not the case for MN. Our study therefore highlights the oligo-genic architecture of MN, informs future studies to detect genes encoding for additional antigens, and confirms *PLA2R1* as a risk locus of major and specific importance. This is consistent with the presence of anti-PLA2R1 antibodies in about 70-80% of MN patients.[1] In the independent GCKD study, we also confirmed the large increase in MN risk conferred by the combination of risk variants at *PLA2R1* and *HLA-DQA1*, as well as a statistical interaction between the risk variants at both loci.[24] In fact, all GCKD patients who were homozygous for the risk alleles at both loci were affected with MN, as were the great majority of persons homozygous for the risk allele at *HLA-DQA1* and heterozygous at *PLA2R1*. There are several potential explanations for the higher proportion of MN patients among carriers of high-risk

genotype combinations in the GCKD study compared to the initial study,[7] including random fluctuations due to small numbers, differences in the prevalence of contributing environmental triggers, and recruitment of patients under nephrological care with reduced eGFR into the GCKD study, which may enrich for MN patients with advanced disease and high-risk genotypes. Only long-term prospective studies, if not lifetime studies, will be able to answer how many individuals carrying high-risk genotype combinations will not develop MN over their life course. Likewise, conclusive answers relating to long-term outcomes require standardized observational studies that follow patients for at least 10 years and/or until death while collecting information on immunosuppressive treatments and other comorbidities.

Our imputation of classical HLA alleles revealed the strongest associations with HLA-DQA1*0501, HLA-DRB1*0301, and HLA-DQB1*0201. The almost perfect correlation between these alleles implicates one long shared MN risk haplotype on which these alleles reside, DRB1*0301-DQA1*0501-DQB1*0201. This is supported by the reported finding that circulating anti-PLA2R1 antibody levels in MN cases are linked to the number of alleles of DQA1*0501 and DQB1*0201.[25] Therefore, although we refer to the HLA risk allele as the *HLA-DQA1* locus, the underlying causal variant could also be situated in *HLA-DQB1* or *HLA-DRB1*. Given the large inter-individual genetic variability of the HLA region, it is noteworthy that our analyses suggest that most of the strong association with MN at this locus can be captured by knowledge of the genotype at a single common SNP in the region, which may have clinical implications for risk prediction. Our observations are consistent with a relatively common genetic predisposition which controls antigen processing and/or presentation, that only together with additional factors such as the presence of specific auto-antigens processed peptides – from, for example, specific forms of PLA2R1 for MN - and/or certain environmental triggers gives rise to a specific

autoantibody to cause disease.[7] In support, the DRB1*0301-DQA1*0501-DQB1*0201 haplotype has also been described to increase the risk for other common auto-immune diseases including type I diabetes,[26] lupus erythematosus (SLE),[27] celiac disease,[28] and thyroid disease.[29]

Our data indicate that this observation may extend to SSNS. As SSNS is commonly observed in children, it was not among the etiologies that could be evaluated in the GCKD study. However, the association of the reported SSNS risk allele with MN risk in our GWAS and the high correlation of the risk allele of the reported SSNS variant and the risk allele of the MN index variant in our data suggests that the genetic predisposition at the *HLA-DQA1* locus shares at least some degree of overlap between SSNS and MN. The results from the GCKD study further suggest that the shared susceptibility between the SSNS variant extends to an association with minimal change disease and FSGS in adulthood. In contrast, there was no statistically significant association between the *HLA-DQA1* risk allele for MN and IgA nephropathy, another auto-immune kidney disease. This is consistent with the fact that previous GWAS of IgA nephropathy have highlighted other associated alleles in the *HLA-DQA1* region.[30]

While genetic variants in the *HLA-DQA1* locus were associated with a variety of underlying causes of CKD, those in *PLA2R1* uniquely associated with MN across almost 20 different etiologies of CKD. The genetic associations between HLA risk alleles and nephropathy resulting from SLE or type I diabetes are biologically plausible, given the shared genetic susceptibility to these auto-immune diseases. Because of the case-only design of the GCKD study, we could not specifically assess whether the HLA risk allele increases risk for nephropathy in the setting of type I diabetes and SLE, or whether the observed associations are due to an association with the primary cause of CKD. The mechanisms underlying the observed association with FSGS need further study to delineate whether they are indicative of a common

mechanism resulting in FSGS-defining features in the biopsies, or whether some of the cases in whom the primary CKD etiology was assigned as FSGS may in fact have additional specific causes contributing to their nephropathy such as SLE. ~~Moreover, we cannot exclude the possibility that – although intended – not all patients with FSGS may have the primary disease form.~~ Although not significant after correction for multiple testing, the risk allele at *HLA-DQA1* also more than doubled the odds of membranoproliferative GN. ~~If this association could be shown in a larger cohort of patients, it~~This could be indicative of an auto-immune component specific to ~~rapidly progressing~~membranoproliferative GN, or represent cases of secondary membranoproliferative GN, such as those observed in SLE.

Our approach highlights the power of collecting detailed information on kidney disease phenotypes, as the specific evaluation of associations across disease subtypes can reveal significant associations that would be missed in genome-wide association studies of these CKD etiologies. A similar observation has been made for the *APOL1* risk alleles, where associations with FSGS and nondiabetic end-stage renal disease - originally identified using genome-wide approaches - were shown to extend to HIV-associated nephropathy without conducting a separate genome-wide genetic screen.[31, 32]

Strengths of our study include the availability of high-coverage genome-wide data as well as the variety of CKD etiologies in the GCKD study that allowed us to evaluate the presence of novel associations, carefully characterize known associations and identify a shared genetic predisposition to several auto-immune kidney diseases. Our study also has potential limitations: genotypes were generated on different platforms, which we addressed through careful data cleaning and combined imputation based on a common SNP set. Although – for a rare disease such as MN - we studied a sizeable number of cases, our study may still be underpowered to

identify associations with rare alleles or alleles conferring moderate or small increases in risk. For example, we did not find evidence for association between *THSD7A* variant and MN, which may not be surprising given the fact that this autoantigen has only been described in a minority of patients. In addition, because we evaluated chip-based and imputed genotypes, there may be rare risk variants that can only be discovered through sequencing-based approaches. At least for *PLA2R1*, however, targeted re-sequencing of the exons and canonical splice sites did not yield rare variants that could explain the observed association.[33] In our study of prevalent MN cases, we did not have information on PLA2R1-antibody concentrations and could therefore not investigate antibody-negative patients separately. In any case, statistical power to detect novel associated loci among antibody-negative patients in the current study, assuming 20% antibody-negative patients among the cases, would have been <30% even for very common alleles. The assembly of larger collectives of MN patients with antibody-status and genome-wide genotypes is therefore an important future endeavor. Likewise, larger collectives of patients with other primary forms of specific CKD etiologies such as FSGS are needed to confirm our findings or to establish statistical significance such as with membranoproliferative GN. Although in the GCKD study the patients' treating nephrologists were asked to identify the leading cause of CKD, misclassification can occur especially for leading causes of CKD that are typically not confirmed by kidney biopsy or for which no specific diagnostic criteria are available. Finally, as in all association studies, we cannot directly pinpoint causal variants, but associated variants may still be valuable for risk stratification.

In conclusion, *PLA2R1* and *HLA-DQA1* are the predominant risk loci for MN. Common variants in these two loci give rise to rare high-risk genotype combinations, which associate with disease risk of a magnitude otherwise observed for single gene disorders. The risk allele at *HLA-*

DQA1, but not in *PLA2R1*, is also associated with nephropathy resulting from type I diabetes, SLE and FSGS, but not with IgA nephropathy, another auto-immune kidney disease. Future studies including those in PLA2R1-antibody negative patients and sequencing-based association studies should evaluate the presence of additional rare genetic risk variants for MN.

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Transparency Declarations

None to declare.

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Table 1: Replicated SNPs Associated with MN at Genome-Wide Significance

gene	*PLA2R1*	*HLA-DQA1*
SNP-ID	rs17830558†	rs9272729‡
chr:pos (hg19)	chr2:160878364	chr6:32609594
function	intronic	intronic
imputed (quality)	yes (0.938)	yes (0.901)
A_1/A_2	T/G	A/G
Discovery analysis		
Allele frequency A_1	0.54	0.24
OR	2.16	7.29
95% CI*	1.65-2.82	4.81-11.04
p-value*	$1.9*10^{-8}$	$6.7*10^{-27}$
Replication analysis		
Allele frequency A_1	0.49	0.16
OR	1.63	6.86
95% CI	1.23-2.15	4.55-10.34
p-value	$5.0*10^{-4}$	$6.4*10^{-24}$
Meta-analysis		
Allele frequency A_1	0.52	0.20
OR	1.87	7.07
95% CI	1.54-2.28	5.28-9.47
p-value	$3.7*10^{-10}$	$2.1*10^{-39}$

OR: odds ratio; CI: confidence interval; * GC-corrected values

† SNP rs17830558 was used in all subsequent analyses of replication and meta-analysis as proxy for rs17830307 that showed the lowest p-value in the association analysis (results can be found in Supplementary Table 1)

‡ In GCKD, instead the proxy rs2187668 was genotyped

Pairwise correlations (within our data of 668 subjects) of rs9272729 and previously reported rs2187668: $r^2=0.87$; of rs17830558 and previously reported rs4664308, $r^2=0.40$.

Table 2: Association of Replicated SNPs with Different CKD Etiologies in the GCKD Study

CKD etiology (leading cause)	n	% biopsy proven	rs17830558 (*PLA2R1*)			rs9272729 (*HLA-DQA1*)#		
			OR	95% CI	p-value	OR	95% CI	p-value
MN*	137	100	1.63	1.23-2.15	**$5.0*10^{-4}$**	6.86	4.55-10.34	**$6.4*10^{-24}$**
lupus nephritis*	104	100	1.10	0.82-1.47	$5.2*10^{-1}$	3.00	1.90-4.72	**$2.8*10^{-6}$**
diabetes mellitus, type 1	89	3.4	0.76	0.55-1.05	$9.2*10^{-2}$	2.69	1.67-4.34	**$6.9*10^{-5}$**
focal segmental glomerulosclerosis*	139	100	0.87	0.66-1.14	$3.1*10^{-1}$	2.36	1.56-3.57	**$5.1*10^{-5}$**
membranoproliferative GN*	36	100	1.36	0.85-2.19	$1.9*10^{-1}$	2.15	1.10-4.22	$3.5*10^{-2}$
minimal change GN*	53	100	1.30	0.87-1.94	$2.0*10^{-1}$	1.70	0.90-3.22	$1.2*10^{-1}$
interstitial nephritis	135	19.3	0.98	0.75-1.29	$9.1*10^{-1}$	1.56	1.00-2.43	$5.3*10^{-2}$
nephrosclerosis	1087	7.9	0.97	0.82-1.15	$7.4*10^{-1}$	1.47	1.10-1.96	$7.8*10^{-3}$
diabetes mellitus, type 2	649	4.2	0.89	0.74-1.07	$2.0*10^{-1}$	1.38	1.02-1.87	$3.3*10^{-2}$
renal artery stenosis	47	0	1.32	0.87-2.00	$1.9*10^{-1}$	1.29	0.64-2.62	$4.9*10^{-1}$
Wegener's granulomatosis*	77	100	0.93	0.67-1.31	$6.9*10^{-1}$	1.18	0.66-2.13	$5.8*10^{-1}$
IgA nephropathy*	308	100	1.02	0.83-1.25	$8.6*10^{-1}$	1.14	0.79-1.63	$4.8*10^{-1}$
autosomal dominant polycystic kidney disease	170	1.8	1.07	0.83-1.37	$6.2*10^{-1}$	1.13	0.73-1.77	$5.8*10^{-1}$
tumor nephrectomy	61	11.5	0.91	0.63-1.32	$6.2*10^{-1}$	1.07	0.55-2.08	$8.4*10^{-1}$
analgesic nephropathy	52	3.8	1.12	0.76-1.67	$5.6*10^{-1}$	1.03	0.49-2.16	$9.4*10^{-1}$
acute kidney injury	59	10.2	0.90	0.61-1.32	$5.9*10^{-1}$	1.02	0.50-2.08	$9.5*10^{-1}$
micropolyangiitis*	54	100	0.82	0.55-1.22	$3.3*10^{-1}$	0.52	0.20-1.33	$1.4*10^{-1}$
rapidly progressive GN, pauci-immune*	25	100	0.72	0.41-1.29	$2.7*10^{-1}$	0.45	0.11-1.88	$2.2*10^{-1}$
KDIGO-defined CKD (all GCKD participants)	4960	25.8	0.99	0.86-1.15	$9.1*10^{-1}$	1.50	1.16-1.94	**$1.3*10^{-3}$**

OR: odds ratio; CI: confidence interval; MN: membranous nephropathy; GN: glomerulonephritis; CKD: chronic kidney disease

P-values that are statistically significant after correction for multiple testing are bolded (p-value<2.6*10-3=0.05/19).

* Analysis based on patients with biopsy-proven (leading) cause only because proportion of available biopsy results >70%

In GCKD, the proxy SNP rs2187668 was genotyped

Table 3: Association of Imputed Classical HLA Alleles with MN

HLA allele	MAF	OR	95% CI	p-value
DRB1-0301	0.27	6.15	4.27-8.85	$1.6*10^{-28}$
DQA1-0501	0.30	6.23	4.29-9.04	$1.4*10^{-27}$
DQB1-0201	0.27	5.89	4.11-8.45	$1.5*10^{-27}$
B-0801	0.24	4.47	3.16-6.33	$7.5*10^{-20}$
DRB3-0100	0.26	3.98	2.83-5.59	$1.3*10^{-17}$
DRB3-9901	0.47	0.29	0.21-0.39	$1.6*10^{-17}$
C-0701	0.28	2.94	2.14-4.03	$2.4*10^{-12}$

MAF: minor allele frequency; OR: odds ratio; CI: confidence interval

Figure Legends

Figure 1: Results from the GWAS of MN using Imputed Genotype Data (GC-corrected p-values, filtered by MAF>10%).

Figure 2: Combined Effect of Genetic Risk Variants at Replicated Loci on Risk of MN in the GCKD Study.

Figure 1: Results from the GWAS of MN using Imputed Genotype Data (GC-corrected p-values, filtered by MAF>10%).

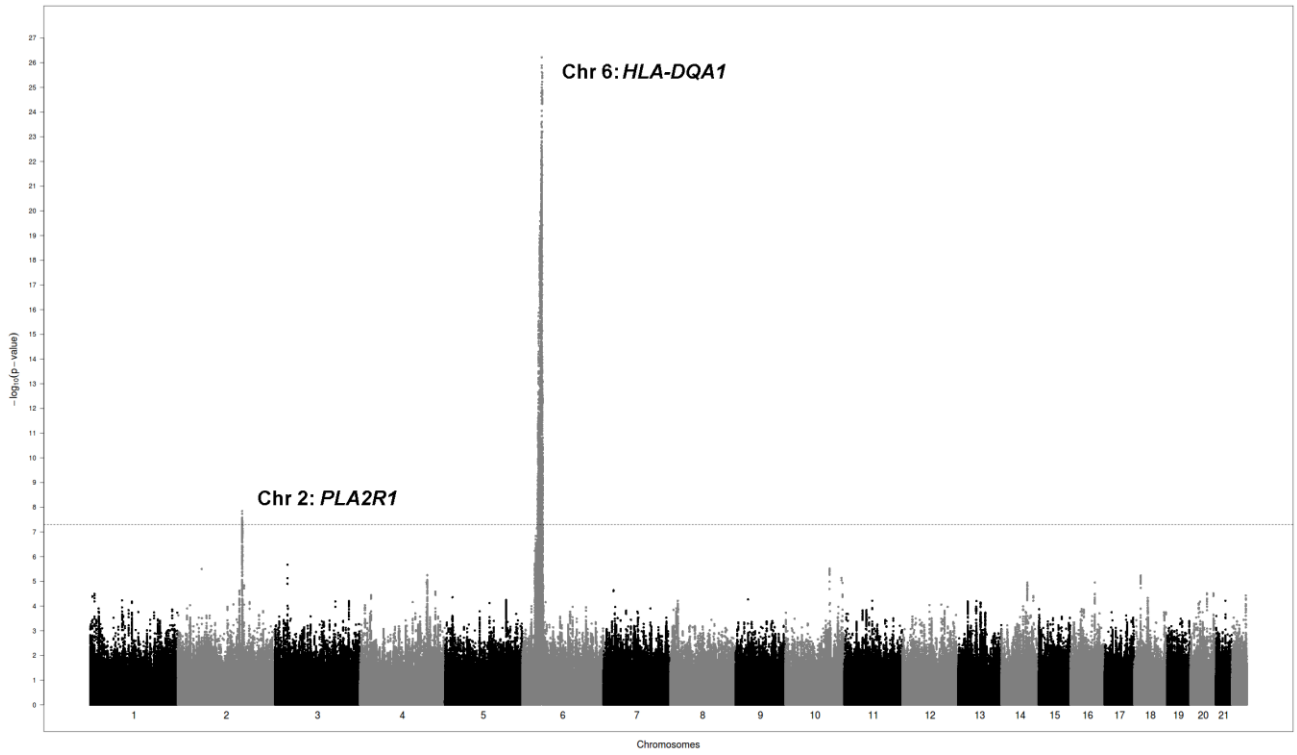
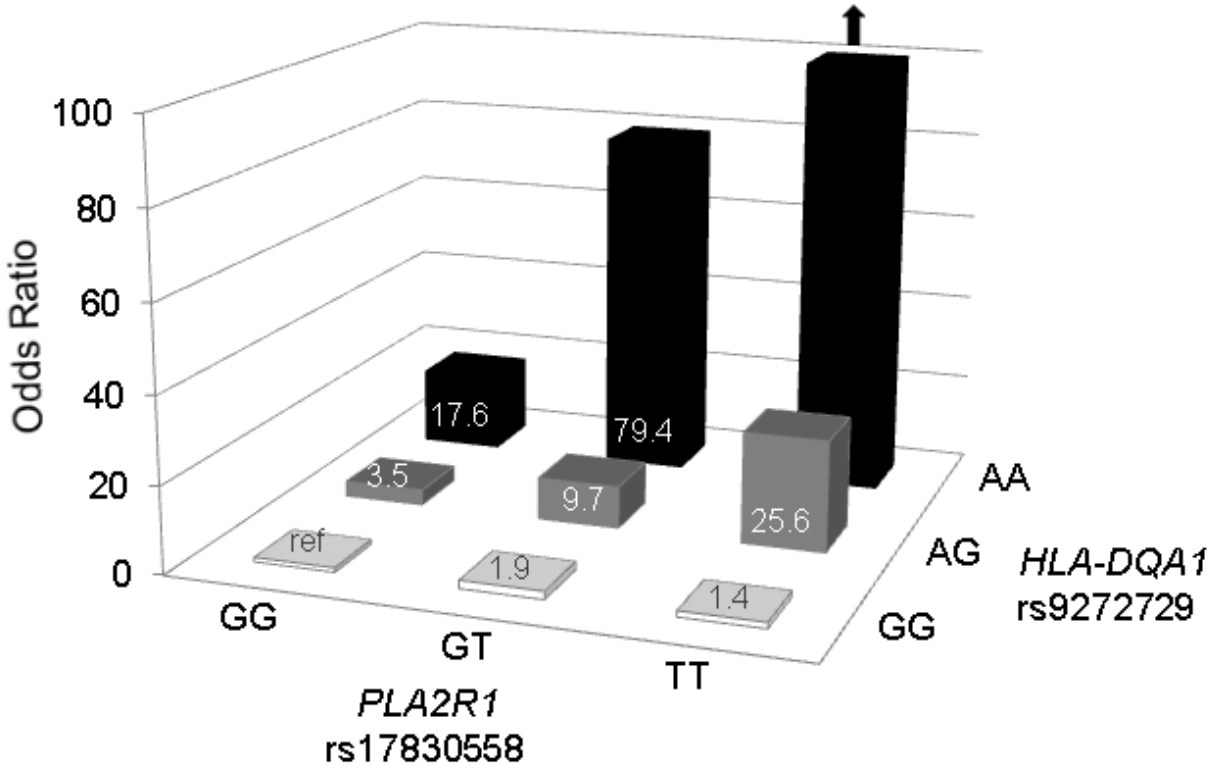


Figure 2: Combined Effect of Genetic Risk Variants at Replicated Loci on Risk of MN in the GCKD Study.



Note: Please see Supplementary Table 2 for tabular presentation of results.