findings in this study. If the effect of FGF23 resistance on mortality was simply mediated by FGF23 levels, then elevated FGF23 should have a consistent effect on mortality independent of FePi, but this result is not the case. Is phosphate the culprit? Dominguez et al. did not specifically examine the relationship of phosphate with outcomes or control for phosphate levels in multivariable models. Prior studies, however, show the associations of FGF23 with mortality to be independent of phosphate. Although phosphate levels were highest in the group with high FGF23 and low FePi, mean levels were within the normal range in all groups. It remains possible, however, that the combination of FePi and FGF23 reflects a chronic phosphate burden not captured by isolated plasma measurements.

FGF23 may itself be toxic at high levels, which was suggested by a recent study showing a direct klotho-independent effect of FGF23 on cardiac myocytes. One explanation to tie these results together is that FGF23 resistance takes time to develop and that the combination of high FGF23 and FePi reflects sustained exposure to high FGF23 levels. Alternatively, it may be that estimated GFR is an incomplete measure of kidney disease and that low FePi identifies subjects at risk for future progression. It is possible that FePi does, indeed, reflect klotho expression and that decreased availability of klotho sites allows for increased action of FGF23 on klotho-independent pathways, such as those pathways described in cardiac myocytes. Lastly, it remains possible that FGF23 is not a culprit itself but rather, a marker of underlying disease. Indeed, a recent study found that administration of FGF23 neutralizing antibodies in an animal model of CKD led to increased rather than decreased mortality. Although this of FGF23 neutralizing antibodies in an animal model of CKD led to increased rather than decreased mortality,12 although this is not the case. Is phosphate the culprit? Dominguez et al. did not specifically examine the relationship of phosphate with outcomes or control for phosphate levels in multivariable models. Prior studies, however, show the associations of FGF23 with mortality to be independent of phosphate. Although phosphate levels were highest in the group with high FGF23 and low FePi, mean levels were within the normal range in all groups. It remains possible, however, that the combination of FePi and FGF23 reflects a chronic phosphate burden not captured by isolated plasma measurements.

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DISCLOSURES
None.

REFERENCES
7. Renal Section and Department of Medicine, Boston University Medical Center, Boston, Massachusetts


Genetic Variants in Membranous Nephropathy: Perhaps a Perfect Storm Rather than a Straightforward Conformeropathy?

David J. Salant

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Primary (or idiopathic) membranous nephropathy is an organ-specific autoimmune disease in which circulating antibodies to a conformation-dependent epitope in the target antigen, the M-type receptor for phospholipase A2 (PLA2R), are detectable in up to 80% of patients from various ethnic groups.\(^1\)\(^2\) It mainly affects older adults and is unusual in that the immune deposits and circulating anti-PLA2R antibodies are predominantly IgG4. Primary membranous nephropathy is the most common cause of nephrotic syndrome in white populations.\(^3\) It mainly affects older adults and is unusual in that the immune deposits and circulating anti-PLA2R antibodies are predominantly IgG4. Primary membranous nephropathy is the most common cause of nephrotic syndrome in white populations or the Rare Diseases Clinical Research Network (200,000 prevalent cases in the United States).

Apart from rare instances in which more than one family member is affected, membranous nephropathy is not a typical hereditary disease in Mendelian terms. On the other hand, it has long been known that primary membranous nephropathy is associated with certain HLA class II immune response genes.\(^3\)\(^-\)\(^7\) Further evidence of a genetic association in primary membranous nephropathy was documented in studies from Korea and Taiwan shortly after researchers discovered that PLA2R is a major target antigen.\(^8\)\(^9\) Both studies found a significant association with a nonsynonymous single nucleotide polymorphism (SNP; rs35771982) in exon 5, which encoded an amino acid substitution (His300Asp) in the first C-type lectin-like domain of PLA2R.\(^1\) This finding raised the intriguing possibility that genetic variations in the amino acid sequence of PLA2R might explain the conformation dependence of the PLA2R1 autoantigen in membranous nephropathy.

This is especially noteworthy because other members of the mannose receptor family, to which PLA2R belongs, are known to exist in bent or extended configurations, and the SNP identified in the Asian studies is located in the N-terminal region of PLA2R, the part that undergoes conformational change in other mannose receptor family members.\(^10\)

In 2011, researchers reported a remarkable discovery. A genome-wide association study (GWAS) in primary membranous nephropathy conducted by three European consortia revealed strong associations with a noncoding SNP in PLA2R1 (rs4664308) and another in HLA-DQA1 (rs2187668), a member of HLA class II.\(^11\) Although each of these two associated gene polymorphisms was significant alone, the odds ratio of membranous nephropathy increased substantially to almost 80 in individuals who were homozygous for both HLA-DQA1 and PLA2R1 variants. This result strongly suggests an interaction (direct or indirect) between the two. Furthermore, the intronic SNP in PLA2R1 is in linkage disequilibrium with a coding SNP in exon 5 (rs3749117, Val292Met) in the N-terminal region that is predicted to induce a structural change in the protein, and is close to one of the SNPs (rs35771982, His300Asp) identified in the studies from Korea and Taiwan. The GWAS simply demonstrated an association between primary membranous nephropathy and a genetic variation in PLA2R1. However, it raised the possibility that a significant proportion of such patients have one or more private variants in the coding sequence that are not present in unaffected persons and might produce structural or functional changes in PLA2R and account for the conformation dependent nature of the target epitope.

In a follow-up study published in this issue of JASN, Coenen and colleagues from the European Membranous Nephropathy consortium sequenced the PLA2R1 exons and exon-intron boundaries of 90 patients with membranous nephropathy from the Dutch and French cohorts, a subset of whom were known to be positive for anti-PLA2R. The authors sought to determine whether they could identify rare or private variants to explain the association identified in their GWAS.\(^12\) Using the dbSNP and 1000 Genomes project databases for comparison, Coenen and colleagues found only two novel and seven rare variants in PLA2R1 distributed among the patients and concluded that rare coding variants or splice-site mutations are unlikely to explain the pathogenesis of membranous nephropathy. Moreover, of the 9 patients who had private or rare variants, only 4 were among the 60 patients with PLA2R2-associated membranous nephropathy. Thus, contrary to expectations, the investigators did not discover unique coding variants that would account for the fact that the antibodies in all patients with anti-PLA2R–associated membranous nephropathy react with a conformation-dependent epitope. Instead, what they did find was a significant association with six known common variants, two of which are nonsynonymous SNPs previously identified by the genotyping studies from Taiwan and Korea.

How might one explain the fact that the most strongly associated PLA2R1 variants in primary membranous nephropathy are common in the general population and yet membranous nephropathy is a rare disease? The interaction between PLA2R1 variants and specific HLA class II antigen receptors might be a clue. It is well known that there is a strong association between certain HLA class II genotypes and various autoimmune diseases. In particular, for example, HLA-DQA1*0501 is strongly associated with celiac disease, in which a specific deamidated gliadin peptide binds preferentially to the antigen-binding pocket created by HLA-DQA1*0501 and HLA-DQB1*0201 from where the antigen is most efficiently presented to T cells.\(^13\) Thus, the rarity of membranous nephropathy might be the result not of rare PLA2R1 polymorphisms but of the infrequent concurrence of common PLA2R1 variants in individuals predisposed to autoimmunity and positive for an HLA-DQ2 haplotype that includes particular alleles of HLA-DQA1 and HLA-DRB1 or HLA-DQB1.

As suggested by Coenen and colleagues, it is also possible that a combination of common variants of PLA2R1 may make up one or more rare haplotype blocks. Another possibility is that the genetic susceptibility may play out in the severity rather than the initiation of the disease. This was suggested by another genetic association study finding that patients with higher anti-PLA2R levels at the time of diagnosis had higher levels of proteinuria and were more likely to have progressive disease.\(^14\) Moreover, the levels of anti-PLA2R were
significantly correlated with the gene dose of HLA-DQA1*0501. Although the correlation between anti-PLA2R levels and the nonsynonymous SNP in exon 5 (rs3739117, Val292Met) did not quite reach statistical significance, this may have been due to the relatively small sample size. It would be of interest to know whether anti-PLA2R levels correlate with other significantly associated common polymorphisms in PLA2R1 identified in the sequencing study of Coenen and colleagues.

Much work has yet to be done to elucidate the nature of the genetic interaction between HLA class II and PLA2R and the development of membranous nephropathy. Is this a direct interaction between a specific HLA class II receptor defined by the HLA-DQA1*0501 α chain and its β-chain partner and specific PLA2R peptides whose conformation or amino acid sequence is genetically defined? Alternatively, could it be an indirect interaction involving molecular mimicry in which patients immunologically predisposed to autoimmunity by carrying HLA-DQA1*0501 are exposed to a microbe or other environmental antigen that resembles a common PLA2R variant? Perhaps the genetically determined structure of PLA2R renders it susceptible to endogenous or exogenous inflammatory stimuli, such as binding of soluble PLA2 to the receptor, that alter its expression and expose the nephritogenic epitope. Might direct sequencing of the whole PLA2R gene reveal variations in noncoding regulatory regions, or might there be epigenetic events that alter expression levels of PLA2R, as previously suggested? We should also be alert to other possible genetic interactions, such as complement regulatory proteins, or environmental factors that lead to the development of the disease in genetically susceptible individuals.

One such factor might be the nature of the anti-PLA2R autoantibodies that are produced in primary membranous nephropathy. Although IgG4 does not activate the classic complement pathway, there are usually abundant deposits of complement, including C4 (but not C1q) in the immune deposits in primary membranous nephropathy. This, together with reports of mannann-binding lectin (MBL) in the deposits and preliminary studies showing that anti-PLA2R IgG4 can bind MBL, suggests the possibility that the lectin pathway of complement might be activated in the glomerular immune deposits. Because MBL has been shown to bind N-linked sugars on IgG that lack terminal galactose and degalactosylation of IgG occurs with aging, it is possible that the production of anti-PLA2R IgG4 antibodies that lack galactose accounts in part for the predominant occurrence of membranous nephropathy in older patients.

In summary, the development of membranous nephropathy and its rarity in the general population may be the result not of rare genetic mutations in PLA2R but of the rare confluence of three relatively common conditions: HLA-DQA1 that confers susceptibility to autoimmunity, polymorphisms in PLA2R1 that create a unique conformation identified by HLA class II on antigen presenting cells and that serve as a target for the resulting autoantibodies, and production of hypogalactosylated IgG4 anti-PLA2R antibodies that activate the lectin pathway of complement and cause podocyte injury and proteinuria.

Thus, although the study by Coenen and colleagues has not identified unique variants to explain the genetic association between PLA2R1 variants and primary membranous nephropathy, it has quite convincingly excluded the straightforward explanation that one or more rare coding variants in white patients causes a unique structural effect on PLA2R conformation in all or most patients with the disease. It also raises interesting questions about alternative mechanisms. As has been true for several decades, membranous nephropathy continues to raise new questions as we learn more about this intriguing disease.

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See related article, “Phospholipase A2 Receptor (PLA2R1) Sequence Variants in Idiopathic Membranous Nephropathy,” on pages 677–683.