

Genes That Link Nephritis to Autoantibodies and Innate Immunity

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The rapid evolution of array techniques and the completion of the map of the human genome have led to a host of genomewide association studies over the past few years. Yet many of these studies have been disappointing; they identify genes that confer only a moderate increase in risk and explain only a small fraction of the known hereditary characteristics of the diseases.¹ However, the results of genomewide association studies for idiopathic membranous nephropathy conducted by Stanescu and coworkers, reported in this issue of the *Journal*, are more striking than usual.² The authors report highly significant associations between membranous nephropathy and single-nucleotide polymorphisms (SNPs) in genes that encode HLA-DQA1 and the M-type phospholipase A₂ receptor (PLA₂R).

The relative risks for the two implicated alleles are remarkably similar in three independent French, Dutch, and British cohorts; for the HLA-DQA1 SNP (rs2187668), the odds ratios were calculated as 4.48, 3.76, and 5.33, respectively, and the corresponding odds ratios for the receptor SNP (rs4664308) were 1.87, 2.27, and 2.10. Even more striking is the multiplicative increase in the odds ratio for each risk allele added. Among persons carrying one risk allele at the rs4664308 locus, the odds ratio increases from 2.22 to 8.49 with the addition of one risk allele and to 31.03 with the addition of two risk alleles at the rs2187668 locus. Furthermore, the odds ratio for persons who are doubly homozygous is 78.46. Even though membranous nephropathy is rare, such an increase in risk has substantial implications on the individual level.

The annual incidence of membranous nephropathy is approximately 1 case per 100,000 population — representing a lifelong risk of about 1 per 1000 for a person in a Western country who reaches average life expectancy. This implies a lifelong increased risk for persons with this specific genotype — at a level where screening and preventive measures would be meaningful, if effective interventions become available.

The results reported by Stanescu et al. confirm the findings of two recent studies from Asia (Taiwan and Korea) that used a candidate gene ap-

proach.^{3,4} Both those studies examined only SNPs in the PLA₂R gene that resulted in amino acid changes, and a significant association was found for one SNP (rs35771982). Stanescu et al. do not report on rs35771982, but for a more C-terminally oriented SNP, rs3828323, both the European and Korean studies show weak but significant associations with the C allele. Taken together, these findings suggest that similar genetic mechanisms confer a predisposition to membranous nephropathy in different human populations.

Although a relation between the HLA system and membranous nephropathy has been recognized for some time,⁵ the knowledge of an association between this disease and PLA₂R is recent. In 2009, Beck and colleagues reported autoantibodies with specificity for PLA₂R that were present in 70% of their patients with idiopathic membranous nephropathy but not in controls or patients with secondary membranous nephropathy.⁶ These results are now confirmed and extended in a letter by Debiec and Ronco in this issue of the *Journal*.

The results of the genomewide association study by Stanescu et al. strongly suggest a direct interaction between HLA-DQ and PLA₂R in the pathogenesis of membranous nephropathy. How would this occur? The most straightforward interaction would be that certain genetic variants of PLA₂R yielded peptides with strong affinity for specific HLA-DQA1 variants that subsequently would confer a predisposition to autoantibody generation. However, the SNP in the PLA₂R gene with the strongest association does not alter the amino acid sequence. The authors provide two possible explanations for this observation. First, the true association might not be with rs4664308 but rather with rs3749117, which is a nonsynonymous SNP reported to be in linkage disequilibrium with rs4664308. If so, the higher odds ratios in all three cohorts would have occurred at random.

The second potential explanation is that rs4664308 might be a marker for a rare variation in the protein sequence, below the threshold required to be considered a SNP. This putative variant would then confer a very high risk of disease for its carriers.

A more likely explanation for the findings reported by Stanescu et al. is that rs4664308 (or a closely linked variant) affects a regulatory element, such as a transcription-factor binding site or a microRNA gene, that controls production of the autoantigen. If so, this would be analogous to a finding in another disease, vitiligo, in which a SNP with a substantial effect on the expression of the autoantigen tyrosinase associates with the disease.⁷ Increased exposure of an autoantigen to the immune system may be a general phenomenon; for example, a deficiency in circulating DNase seems to confer a predisposition to anti-DNA antibodies and systemic lupus erythematosus.⁸ If increased synthesis of PLA₂R is essential to confer an increased risk of membranous nephropathy, other genetic variants, such as gene copy number or epigenetic events that affect the transcription of PLA₂R, may be important.

Although the findings of Stanescu et al. strengthen the link between PLA₂R and membranous nephropathy, many new questions arise. Why are there receptors for a phospholipase on glomerular epithelial cells? The ligand, secretory phospholipase A₂ (sPLA₂), is an important effector molecule against gram-positive bacteria, which can extensively degrade the bacterial plasma membrane.⁹ The molecular size of sPLA₂ (14 kD), which is produced by neutrophils and other inflammatory cells and released in large quantities into biologic fluids on activation, suggests that it is freely filtered through the glomerular basement membrane. Once activated, PLA₂R releases cytokines and metalloproteinases and generates nitric oxide.¹⁰ Although sPLA₂ is believed to play a major role in asthma, its role in nephritis is largely unknown. Autoantibodies with specificity for molecules of the innate immune system are present in other nephropathies. For example, autoantibodies to complement factor C3bBb, the alternative pathway C3 convertase, are common in membranoproliferative nephritis, and antibodies to factor H can induce the hemolytic-uremic syndrome. The two best-characterized au-

toantigens in rapidly progressive glomerulonephritis, proteinase 3 and myeloperoxidase, are potent neutrophil antimicrobial proteins. Thus, the role of sPLA₂ requires further elucidation.

In summary, this new genomewide association study highlights the interaction between the HLA system and the receptor for sPLA₂ and provides substantial credence for a pathogenic role for the recently discovered autoantibodies in membranous nephropathy. It may redirect research toward finding a remedy for this troublesome disease.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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