Apolipoprotein L1 Risk Variants Associate with Systemic Lupus Erythematosus-Associated Collapsing Glomerulopathy

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ABSTRACT
Collapsing glomerulopathy is a devastating renal disease that primarily affects African Americans and associates with numerous etiologies, such as HIV and autoimmune disease. The presence of APOL1 risk alleles associates with HIV-associated collapsing glomerulopathy, but it is unknown whether these risk alleles also associate with systemic lupus erythematosus (SLE)-associated collapsing glomerulopathy. Here, re-examination of 546 renal biopsies from African-American patients with SLE identified 26 cases of collapsing glomerulopathy, which we genotyped for APOL1 risk alleles using DNA extracted from archived biopsy tissue. APOL1 strongly associated with SLE-associated collapsing glomerulopathy ($P$, 0.001). In a recessive model, two APOL1 risk alleles conferred 5.4-fold (95% CI=2.4 to 12.1) higher odds of developing SLE-associated collapsing glomerulopathy ($P$, 0.001). In conclusion, APOL1 genotyping of African-American patients with SLE might help identify patients at risk for collapsing glomerulopathy, an entity with a poor prognosis that is often resistant to treatment.


Collapsing glomerulopathy (CG) is an aggressive form of glomerular injury primarily affecting African Americans that frequently has a poor prognosis.1,2 It is best described in the setting of HIV, in which case it is known as HIV-associated nephropathy (HIVAN). However, it can be seen in many other settings, including autoimmune disease, other viral illnesses, malignancy, drug exposure, and idiopathic disease.3 The association with systemic lupus erythematosus (SLE) was the subject of a recent large case series detailing the findings in 19 patients with SLE.4 This series found that patients with CG in the setting of SLE share similar clinical, pathologic, and demographic features with the better-defined forms of CG such as HIVAN.4 There has been significant progress in our understanding of HIVAN in recent years with the discovery of two independent risk alleles in the gene encoding apolipoprotein L1 (APOL1).5,6 Kopp et al.7 have shown that the presence of two APOL1 risk alleles is associated with an odds ratio of 29 for the development of HIVAN. These risk alleles consist of two distinct haplotypes, G1 and G2, that are mutually exclusive and very common, with an allele frequency greater than 30% in African Americans.5 The high allele frequency of these kidney disease variants results from their ability to provide protection against trypanosomal infection. Thus, these gene variants have been selected because of the heterozygous advantage that they provide.5 This model is similar to a model of sickle cell disease, in which the protection afforded heterozygotes against parasite comes at the expense of significant disease in homozygotes.

Given the clinical and pathologic similarities between SLE-associated CG and HIVAN, we hypothesized that SLE-associated CG is also associated with the presence of APOL1 risk variants. We sought to determine this hypothesis through the genotyping for these risk alleles in a large cohort of African-American patients with lupus nephritis. We also evaluated the biopsies to determine if there were morphologic findings associated with the presence of APOL1 risk alleles.

RESULTS
A total of 546 renal biopsies of African Americans were identified from African-American patients with SLE and genotyped for APOL1 polymorphisms. Overall, there were 188 cases with zero risk alleles, 264 cases with one risk allele, and 94

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cases with two risk alleles. Morphologic evaluation of these biopsies by two pathologists revealed 26 cases with CG. The breakdown of CG according to the number of risk alleles is detailed in Table 1 along with the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification. The collapsing cases were also given an ISN/RPS classification as follows: two class 1 cases; five class 2 cases; two class 3 cases; three class 4 cases; nine class 5 cases; two class 6 cases; and three class 4/5 cases.

Table 2 details the morphologic features according to the number of APOL1 risk alleles. The presence of two APOL1 risk alleles was found to be associated with non-necrotizing crescents ($P=0.008$, odds ratio [OR]=1.6, 95% confidence interval [95% CI]=1.2 to 2.3) and microcystic dilatation ($P=0.001$, OR=2.2, 95% CI=1.4 to 3.3). These risk alleles remained significant even after all CG cases were removed from analysis, with the non-necrotizing crescents association essentially unchanged and the strength of the microcystic dilatation association decreasing ($P=0.02$, OR=1.9, 95% CI=1.1 to 3.3). There was also a trend to higher chronicity scores for cases with two APOL1 risk alleles. Other morphologic features, including glomerulosclerosis, fibrous crescent formation, wire loops, endocapillary proliferation, necrotizing lesions, interstitial inflammation, tubular atrophy, interstitial fibrosis, and presence of thrombotic microangiopathy, did not associate with APOL1.

APOL1 was strongly associated with SLE-associated CG ($P<0.001$). In a recessive model, two APOL1 risk alleles conferred 5.4-fold higher odds of developing CG in the setting of SLE ($P<0.001$, 95% CI=2.4 to 12.1).

### DISCUSSION

We present the largest series of SLE-associated CG to date and show, for the first time, that it is strongly associated with the presence of APOL1 risk alleles. In addition to HIV, SLE is now the second disease in which the phenotype of CG is shown to be associated with these risk alleles. The pathogenesis of CG and the role of APOL1 in the development of this distinctive morphologic pattern are currently a mystery. However, a two-hit hypothesis seems likely, in which the presence of APOL1 risk alleles combined with an altered inflammatory milieu, such as occurs in HIV infection or lupus, leads to disease.

It is curious that HIV and SLE, two pathogenically dissimilar processes, can have very similar glomerular pathology. We have already detailed the fact these two diseases can result in CG. However, it is also well described that patients with HIV can have a proliferative GN, which is morphologically indistinguishable from the GN of proliferative lupus nephritis to the extent that this form of HIV-associated disease is referred to as lupus-like GN. Furthermore, the ultrastructural finding of tubuloreticular inclusions in the glomerular endothelial cytoplasm is often regarded as being relatively unique to HIV and SLE. Normal cells develop these inclusions after exposure to elevated levels of IFN, such that tubuloreticular inclusions are referred to as the footprints of IFN. Perhaps this morphologic overlap is a clue to the pathogenesis of CG and the type of inflammatory milieu that incites disease, specifically elevated IFN. This hypothesis that IFN is the pathogenic link between these diseases in inducing CG is also supported by evidence that pharmacologic treatment with IFN is associated with CG.

We found two morphologic features to be significantly correlated with the presence of two APOL1 risk alleles in African Americans with lupus nephritis of all sorts, microcystic tubular dilatation, and non-necrotizing crescents. The correlation with microcystic tubular dilatation is not surprising, because this lesion is commonly found as a part of the spectrum of CG, regardless of the associated disease. The fact that it remained significant after all collapsing cases were removed from analysis suggests that this pathologic finding is correlated with APOL1 risk alleles and not pathogenically dependent on the presence of CG. The correlation of non-necrotizing crescents with the presence of APOL1 risk alleles is likely a result of the morphologic similarity between a florid collapsing lesion and cellular crescent formation. Given this overlap and the higher incidence of crescents in biopsies from patients with two APOL1 risk alleles, it is plausible that some of these proliferative cases also had a component of undiagnosed CG. If so, then the results of this study would actually under-represent the strength of association between APOL1 risk alleles and CG in SLE.

Approximately 17% of patients in our series carried two copies of the APOL1 risk allele. This result is higher than the previously published frequency in the African-American population of approximately 13%. Lin et al. recently reported that APOL1 risk alleles had only nominal effect in SLE and that the neighboring MYH9 gene showed no association. Therefore, it is unlikely that...
this increased frequency is a result of APOL1 being a risk allele for lupus nephritis. This increased frequency is more likely a reflection of the fact that the cohort in this series represents a subset of patients who had kidney disease sufficient to prompt a kidney biopsy.

CG from whatever cause has been shown to have a poor prognosis, with rapid progression to renal failure. There is currently no evidence-based treatment with a proven therapeutic effect for this disease. Still, distinguishing between CG and proliferative lupus nephritis is important given the likely very different response to therapy. Additionally, it could potentially spare the

Table 2. Lupus nephritis morphologic features according by APOL1 genotype

<table>
<thead>
<tr>
<th>APOL1 Risk Allele Status</th>
<th>Number (%) Total FSGS NOS</th>
<th>Number (%) Cases with Crescents</th>
<th>Number (%) Non-Necrotizing Crescents</th>
<th>Number (%) TMA</th>
<th>Number (%) MCTD</th>
<th>Mean Activity/Chronicity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero risk alleles (n=188)</td>
<td>105 (56%)</td>
<td>84 (45%)</td>
<td>67 (36%)</td>
<td>35 (19%)</td>
<td>13 (7%)</td>
<td>20 (11%)</td>
</tr>
<tr>
<td>One risk allele (n=264)</td>
<td>156 (59%)</td>
<td>114 (43%)</td>
<td>84 (32%)</td>
<td>53 (20%)</td>
<td>11 (4%)</td>
<td>33 (13%)</td>
</tr>
<tr>
<td>Two risk alleles (n=94)</td>
<td>53 (56%)</td>
<td>52 (55%)</td>
<td>42 (45%)</td>
<td>30 (32%)</td>
<td>8 (9%)</td>
<td>24 (26%)</td>
</tr>
</tbody>
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NOS, not otherwise specified; TMA, thrombotic microangiopathy; MCTD, microcystic tubular dilatation.

Figure 1. Morphology of SLE-associated collapsing glomerulopathy. (A) Glomerular tuft collapse with overlying epithelial hypertrophy and hyperplasia in Bowman’s space (Jones methenamine silver). Original magnification, ×400. (B) Characteristic tubulointerstitial changes are evident from lower power, including focal tubular dilatation with large intraluminal hyaline casts and proximal tubules stuffed with protein droplets (periodic acid–Schiff). Original magnification, ×100. (C) Positive staining for IgG is present in glomeruli and tubular nuclei (direct immunofluorescence). Original magnification, ×200. (D) Transmission electron photomicrograph showing diffuse foot process effacement as well as mesangial and subepithelial deposits (unstained). Original magnification, ×3000.
patient from unnecessary exposure to the numerous adverse effects of cytotoxic agents that are frequently used to treat proliferative lupus nephritis. Additional studies evaluating the relationship between SLE disease activity and the onset of CG might prove helpful in determining if this form is a preventable form of glomerulopathy. There is some evidence that the incidence of HIVAN has decreased with the advent of highly active antiretroviral therapy.\textsuperscript{16,17} A similar proactive preventive strategy of systemic infammation and SLE flares could potentially be efficacious in preventing the onset of SLE-associated CG, particularly if the patient is known to be at risk for this form of glomerulopathy.

We investigated a large cohort of biopsies from African-American patients with lupus nephritis and for the first time, show that the presence of two APOL1 risk alleles is associated with SLE-associated CG. This finding raises interesting questions about the pathogenesis of CG and suggests that a common inflammatory milieu in HIV and SLE acts as a second hit, resulting in disease. Future studies are warranted that aim to determine whether APOL1 genotyping of African-American patients with SLE and prophylactic immunosuppression of those patients at risk for CG can improve outcomes in this patient population.

**CONCISE METHODS**

A total of 546 renal biopsies was identified in the case file of our institution from African-American patients with SLE. Two renal pathologists confirmed the diagnosis of CG based on the previously published definition of the disease (Figure 1).\textsuperscript{18} HIV-positive patients were excluded from the study. Additional features were noted in each biopsy, including the ISN/RPS Classification,\textsuperscript{19} activity score,\textsuperscript{20} chronicity score,\textsuperscript{21} presence of thrombotic microangiopathy, global and segmental glomerulosclerosis, crescent formation, and presence of microcystic tubular dilatation. DNA was extracted from archived biopsy tissue and genotyped for APOL1 risk alleles using TaqMan assays (details in Supplemental Material). As previously suggested, only the G1S42G and G2 insertion/deletion were typed to assess kidney disease risk.\textsuperscript{7} The pathologists were blinded to the APOL1 genotype at the time of morphologic evaluation. This study was approved by the Institutional Review Board of the University of Arkansas for Medical Sciences.

**DISCLOSURES**

None.

**REFERENCES**


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