HLA-DQA1 and *APOL1* as Risk Loci for Childhood-Onset Steroid-Sensitive and Steroid-Resistant Nephrotic Syndrome

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Background: Few data exist for the genetic variants underlying the risk for steroid-sensitive nephrotic syndrome (SSNS) in children. The objectives of this study were to evaluate *HLA-DQA1* and *APOL1* variants as risk factors for SSNS in African American children and use classic HLA antigen types and amino acid inference to refine the *HLA-DQA1* association.

Study Design: Case-control study.

Setting & Participants: African American children with SSNS or steroid-resistant nephrotic syndrome (SRNS) were enrolled from Duke University and centers participating in the Midwest Pediatric Nephrology Consortium.

Factor: Genetic variants in *HLA-DQA1* (C34Y [rs1129740]; F41S [rs1071630]) and *APOL1* high-risk alleles.

Outcomes: SSNS and SRNS.

Measurements: Direct sequencing for the *HLA-DQA1* and *APOL1* variants in 115 African American children (65 with SSNS and 50 with SRNS). Imputation of classic HLA alleles and amino acids was done in 363 South Asian children.

Results: The 2 *HLA-DQA1* variants were significantly associated with SSNS in African American children (C34Y: $P = 5.7 \times 10^{-11}$; OR, 3.53; 95% CI, 2.33-5.42; F41S: $P = 1.2 \times 10^{-13}$; OR, 4.08; 95% CI, 2.70-6.28), but not with SRNS (C34Y: $P = 0.6$; F41S: $P = 0.2$). *APOL1* high-risk variants were not associated with SSNS ($P = 0.5$) but showed significant associations with SRNS ($P = 1.04 \times 10^{-7}$; OR, 4.17; 95% CI, 2.23-7.64). *HLA-DQA1*0201, HLA-DQB1*0201,* and *HLA-DRB1*0701* were the classic HLA alleles with the most significant associations with SSNS risk. The most significantly associated amino acid positions were HLA-DQα1 56 and 76 (both $P = 2.8 \times 10^{-7}$). Conditional analysis revealed that these variants most likely account for the observed association.

Limitations: Modest sample size and limited statistical power to detect small to moderate effect sizes. Children studied may not be representative of all African American children in the United States.

Conclusions: *HLA-DQA1* is a risk locus for SSNS, but not SRNS, in African American children, consistent with its role in SSNS risk in children of European, Asian, and African ancestries. There is little evidence of a significant role for the *APOL1* high-risk alleles in childhood SSNS in African American children. Refinement of the *HLA-DQA1* association identified the critical classic HLA antigen types and amino acids of the HLA-DQ α1 molecule.

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Nephrotic syndrome is an important cause of kidney disease in the pediatric population.[1] Most cases of nephrotic syndrome in children are responsive to corticosteroid therapy and are therefore referred to as steroid-sensitive nephrotic syndrome (SSNS), whereas a small proportion (<20%) are steroid resistant and referred to as steroid-resistant nephrotic syndrome (SRNS).[2] The pattern of response to corticosteroids is the single most important predictor of outcome; the majority of children with SRNS will progress to end-stage kidney disease.[2]

The pathogenesis of SSNS is not completely known; however, there are clinical and epidemiologic data to suggest that the disease may be due to dysregulation of the immune system, leading to effects on the podocyte and other components of the glomerular filtration barrier.[3] Epidemiologic studies have established that there is significant ethnic disparity in the prevalence and clinical course of SSNS.[4-6] The incidence is higher in Asian children than for other ethnicities; African American and Hispanic children tend to have a more protracted course.[4-6] However, it is unclear whether these observations are due to differences in genetic risk factors, environmental factors, or gene-environment interactions.

A recent study used an extreme phenotype, exome array association approach to identify genetic risk factors for SSNS.[7] Starting from a discovery sample of South East Asian children, the study identified 4 exome-wide significant variants in or around *HLA-DQA1* and *HLA-DQB1*.[7] Two of these variants (*HLA-DQA1* C34Y and F41S [a substitution of cysteine by tyrosine at amino acid 34 and of phenylalanine by serine at amino acid 41, respectively]) were replicated in children of European ancestry, establishing a robust genetic association for SSNS.[7] The role of this risk locus in other populations is unknown.

In the present study, we aimed to further replicate and refine the SSNS *HLA-DQA1* locus. We test the association between the *HLA-DQA1* locus and SSNS in African American children and confirm the original association. To fine map the associated loci and identify potentially functional variants, we conduct imputation of classic HLA alleles and amino acids using a population-appropriate reference and test their association with SSNS. Moreover, we undertake a set of conditional analyses and refinement of amino acid associations and deduce their impact on the 3-dimensional structure of *HLA-DQA1*.

APOL1 variants are associated with a variety of chronic kidney diseases in populations of African ancestry.[8-18] Hence, we examine whether high-risk *APOL1* variants are associated with SSNS in populations of African ancestry.

Methods

Study Participants

For the genetic association studies of *HLA-DQA1* and *APOL1*, we enrolled 65 African American children with SSNS and 50 African American children with SRNS, making a total of 115 African American patients. Age at onset of disease in the study population was 2 to 10 years.

Replication Study in African American Children With SSNS

The sample of 65 African American children with SSNS was enrolled as part of an ongoing study of nephrotic syndrome at Duke University and the Midwest Pediatric Nephrology Consortium (MWPNC). Children were enrolled from major tertiary medical centers. Inclusion criteria were African American ethnicity by report, age at onset of disease of 2 to 10 years, diagnosis of nephrotic syndrome (defined as proteinuria with protein excretion > 40 mg/m^2/h, hypoalbuminemia, and edema), and complete remission following 8 to 12 weeks of corticosteroid treatment. Children with secondary nephrotic syndrome were excluded. Institutional review board approval was obtained from all participating centers. Parents and children provided informed consent and assent, respectively. Data collection included demographic information (age, sex, race, and ethnicity), family history of nephrotic syndrome or other kidney disease, age at onset of nephrotic syndrome, therapies, and clinical outcome. DNA was extracted from blood or saliva samples collected on enrollment.

Genotyping for *HLA-DQA1* C34Y (corresponding to reference single-nucleotide polymorphism [SNP] identification number [rs]1129740) and F41S (rs1071630) was done by direct sequencing (see primers in Table S1). For controls, we used data from the National Heart, Lung and Blood Institute GO Exome Sequencing Project (ESP) African American sample (n = 2,303) accessed through the Exome Variant Server (http://evs.gs.washington.edu/EVS/).[19] Although samples in the ESP were ascertained for various phenotypes, the population frequency of SSNS (1 in 16,000) means that the chances of misclassification are negligible because it is unlikely that there is more than 1 case in the control data set. Association tests with SSNS were done under an additive genetic model.

In view of the role of *APOL1* variants in a range of kidney disorders in populations of African ancestry,[8-18] in this sample of 115 African American children, we tested the hypothesis that the known *APOL1* risk variants are associated with SSNS or SRNS. Samples were genotyped for the *APOL1* G1 and G2 alleles by direct sequencing. Control data were obtained from published figures based on 5,543 African Americans from the BioMe biobank of the Institute of Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York.[20] This is one of the largest samples of African Americans who were genotyped for the *APOL1* G1 and G2 high-risk alleles. High risk for *APOL1* alleles is defined by having the genotype G1 or G2 in the recessive state (ie, G1/G1, G1/G2, or G2/G2). Because G1 and G2 are derived from 2 genomic positions (G1 is rs73885319 [or rs60910145, with which it is usually in complete linkage disequilibrium]); G2 is a 6-bp [base pair] indel [insertion-deletion], rs71785313), they are better described as diplotypes. However, we have retained their description as "alleles" to be consistent with the literature. Association tests were conducted to test the association between *APOL1* high-risk alleles (G1/G1, G1/G2, and G2/G2) and SSNS, as well as between the *APOL1* high-risk alleles and SRNS.

Evaluating the Role of *HLA-DQA1* in SRNS

To test the hypothesis that *HLA-DQA1* variants are also associated with SRNS, we identified 50 African American children who had a diagnosis of SRNS. They were enrolled at Duke University and participating centers in the MWPNC. Ascertainment, enrollment, and sample collection were done the same way as with the children with SSNS. DNA samples were sequenced for the 2 polymorphisms as for the samples with SSNS, and association tests were done using the same methods.

Imputation and Association Tests With Classic HLA Alleles and Amino Acids

Imputation of classic 4-digit HLA alleles and amino acids was done using exome array data generated on 214 South Asian children with SSNS and 149 controls, as previously described.[7] We focus on 4-digit (rather than 2-digit) HLA alleles because 4-digit HLA alleles correspond to specific HLA molecules, whereas 2-digit HLA alleles represent allele groups or groups of similar HLA molecules. Therefore, the higher specificity and resolution of 4-digit alleles facilitates further analyses in terms of amino acid residues and functional domains of the protein. Imputation was done with *SNP2HLA*[21] using the Pan Asian reference panel,[22] which was developed for South East Asian and South Asian populations. A total of 115 four-digit HLA alleles, 76 two-digit HLA alleles, and 896 amino acid