3-1-2018

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Recommended Citation

Adeyemo A, Esezobor C, Solarin A, et al. HLA-DQA1 and APOL1 as Risk Loci for Childhood-Onset Steroid-Sensitive and Steroid-Resistant Nephrotic Syndrome. Am J Kidney Dis. 2018;71(3):399-406. doi:10.1053/j.ajkd.2017.10.013

HHS Public Access

Author manuscript

Am J Kidney Dis. Author manuscript; available in PMC 2019 March 01.

Published in final edited form as:

Am J Kidney Dis. 2018 March ; 71(3): 399–406. doi:10.1053/j.ajkd.2017.10.013.

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

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Authors' Contributions: Research idea and study design: all authors; data acquisition: all authors; data analysis/interpretation: AAd, RG; statistical analysis: AAd, RG. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Financial Disclosure: The authors declare that they have no other relevant financial interests.

Peer Review: Received June 12, 2017. Evaluated by three external peer reviewers and a statistician, with editorial input from an Associate Editor and the Editor-in-Chief. Accepted in revised form October 5, 2017.

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Abstract

Background—Few data exist on the genetic variants underlying the risk of 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 classical HLA 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 classical HLA alleles and amino acids was done in 363 South Asian children.

Results—The two *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 classical 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 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 classical HLA types and amino acids of the *HLA-DQα1* molecule.

Keywords

nephrotic syndrome; genetics; childhood; African American; corticosteroids; steroid sensitivity; SSNS, SRNS, risk loci; *HLA-DQA1*; *APOL1*; renal disease; nonmodifiable risk factor; ethnic disparities; pediatric kidney disease

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) while 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 (ESKD)[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]. Epidemiological 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 other ethnicities; African-American (AA) and Hispanic children tend to have a more protracted course[4–6]. However, it is unclear if 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 four 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 classical 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 three-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.

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). The children were enrolled from major tertiary medical centers. The 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 >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. IRB approval was obtained from all participating centers. The parents and children provided informed consent and assent, respectively. Data collection included demographic information (age, gender, 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 enrolment.

Genotyping for *HLA-DQA1* C34Y (corresponding to reference single-nucleotide polymorphism identification number [rs]1129740) and F41S (rs1071630) was done by direct sequencing (see primers in Table S1). For controls, we used data from the NHLBI GO Exome Sequencing Project (ESP) African-American sample (n=2303) accessed through the Exome Variant Server (http://evs.gs.washington.edu/EVS/).[19] While the 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 as it is unlikely that there is more than one case in the control dataset. 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. The samples were genotyped for the *APOL1* G1 and G2 alleles by direct sequencing. Control data was 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 that 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 (i.e. G1/G1, G1/G2 or G2/G2). Since G1 and G2 are derived from two 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, 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 Midwest Pediatric Nephrology

Consortium (MWPNC). Ascertainment, enrolment and sample collection were done the same way as with the children with SSNS. The DNA samples were sequenced for the two polymorphisms as for the samples with SSNS and association tests were done using the same methods.

Imputation and Association Tests With Classical HLA Alleles and Amino Acids

Imputation of classical four-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 four-digit (rather than two-digit) HLA alleles because four-digit HLA alleles correspond to specific HLA molecules whereas two-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 number of 115 four-digit HLA alleles, 76 two-digit HLA-alleles and 896 amino acid positions were successfully imputed. Imputed HLA alleles and amino acid positions were filtered for frequency ≥ 1% and $r^2 \geq 0.9$ before analysis. Logistic regression was used to test association between the imputed dosages of the variants and SSNS. For testing association at amino acid positions with three or more possible states, we used a multi-*df* omnibus test constructed by testing a model with all amino acid alleles of a given position, fitting individual effects of each allele and evaluating significance by testing the deviance of the alternative model compared to the null model. Therefore, the omnibus test of association tests all residues at the amino acid position simultaneously instead of the usual "one-vs-others" tests. Conditional analyses were conducted by including the most significant variant in the regression model and testing for residual association in the other variants.

Evaluation of Significant Amino Acids on Three-Dimensional Structure of HLA-DQA1

The entry with identifier 4OZI (S2 protein complex consisting of HLA-DQ-α1 chain, HLA-DQ-β1 chain, T-cell receptor S2 α chain, T-cell receptor S2 α chain and deaminated α1-gliadin peptide) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank and used as the reference three-dimensional structure of HLA-DQα1. The structure was visualized and manipulated using UCSF Chimera [23–24].

Results

Clinical Characteristics of Study Population

We identified 115 African-American patients with childhood-onset nephrotic syndrome: 65 with SSNS and 50 with SRNS. Children with SSNS were younger at presentation compared with children with SRNS (SSNS: mean age, of 5.4 ± 3.6 [standard deviation] years; SRNS: mean age, 10.4 ± 5.0 years). There was male preponderance in both groups (SSNS: 68.3%; SRNS: 56.5%). Kidney biopsy was performed in 20 of 65 patients with SSNS. The majority (12 of 20) had minimal change disease (MCD) while the remaining 8 children with SSNS had focal segmental glomerulosclerosis (FSGS; n=3), IgM nephropathy (n=1), membranoproliferative glomerulonephritis (MPGN; n=1), C1Q nephropathy (n=1) or focal glomerular scarring (n=1). We have information about kidney biopsy for 37 of 50 of children

with SRNS. The majority (32 of 37 [85%]) of the SRNS patients had FSGS while the histological diagnosis in the others was MCD in three children and membranous nephropathy in two children.

HLA-DQA1 Associations With SSNS in African-American Children

The two HLA-DQA1 variants are significantly associated with SSNS in African-American children (rs1129740 [C34Y]: $p=5.7 \times 10^{-11}$; rs1071630 [F41S]: $p=1.2 \times 10^{-13}$) (Table 1). Carriers of the risk variant of C34Y had 3.5 times the risk of SSNS (OR, 3.53; 95% CI, 2.33–5.42) while carriers of the risk variant for F41S had approximately four-fold the risk of SSNS (OR, 4.08; 95% CI, 2.70–6.28) compared to non-carriers.

HLA-DQA1 Associations With SRNS in African-American Children

Neither of the two HLA-DQA1 variants showed significant association with SRNS in African-American children (rs1129740 [C34Y]: p=0.6; rs1071630 [F41S]: p=0.233) (Table 1).

Association of APOL1 High-Risk Alleles With SSNS and SRNS in African-American Children

To determine the role of *APOL1* locus as a risk factor for SSNS, we tested for association between the *APOL1* high risk genotype (G1/G1, G1/G2, G2/G2) with SSNS under a recessive model. There was no significant association with SSNS (frequency 16.9% versus 13.8% in controls) (Table 2). Conversely, there was a significant association between the *APOL1* high-risk alleles and SRNS (frequency 40% versus 13.8% in controls; $p = 1.0 \times 10^{-7}$) with a four-fold difference in risk. This indicates that *APOL1* is a risk locus for SRNS but not for SSNS. The distribution of *APOL1* high risk alleles is found in Table S2.

Classical HLA Allele and Amino Acid Association With SSNS in Asian Children

Association tests with classical HLA alleles in 363 South Asian children (214 with SSNS, 149 controls) showed that the most significantly associated four-digit HLA alleles were HLA-DRB1*07:01 ($p=4.6 \times 10^{-6}$), HLA-DQA1*02:01 ($p=5.5 \times 10^{-6}$), HLA-DQB1*02:01 ($p=1.0 \times 10^{-5}$) and HLA-DQA1*01:01 ($p=3.3 \times 10^{-4}$) (Table 3). A longer list of top-scoring 2-digit and 4-digit alleles are shown in tables *a* and *b* of Item S1. However, the strongest associations in a test of all variants (single-nucleotide polymorphisms [SNPs], classical HLA types, amino acid positions) were at four *HLA-DQA1* amino acid positions (Table 3), including *HLA-DQA1* position 56 (omnibus $p=2.0 \times 10^{-7}$), position 76 (omnibus $p=2.0 \times 10^{-7}$) and position 69 (omnibus $p=8.1 \times 10^{-7}$). The most significant amino acid residues at each of these positions are shown in Table 4 (a more comprehensive list is presented in Table S5). The amino acids (at positions 34 and 41) coded for by the original discovery hits (rs1129740 and rs1071630, respectively) showed significance as expected (logistic model $p=2.5 \times 10^{-6}$ [they have the same p value because they are completely correlated]). However, conditioning on these two amino acid positions left residual association on *HLA-DQA1* positions 56 ($p=8.5 \times 10^{-3}$) and 76 ($p=8.5 \times 10^{-3}$). Conversely, conditioning on *HLA-DQA1* position 56 and position 76 left no residual association in the other remaining markers, including C34Y and F41S (p = 0.9 for both amino acid positions).

The location of the amino acid positions of interest on the three-dimensional model of *HLA-DQA1* are shown in Figure 1. The two *HLA-DQA1* positions 56 and 76 are in the extracellular topological domain of the DQ-α1 chain. An examination of the S2 protein complex's pentameric structure (consisting of HLA-DQ-α1 chain, HLA-DQ-β1 chain, T-cell receptor S2 α chain, T-cell receptor S2 α chain and deaminated α1-gliadin peptide) (Molecular Modeling Database [MMDB] ID:119261) showed that the HLA-DQα1 residues in amino positions 56 and 76 are in the antigen-binding pocket of the protein and come into close contact with bound peptides (for example, they are within 3 Angstroms of the bound deaminated α1-gliadin peptide in the specified structure). Since binding of peptides derived from exogenous antigens is a key process in antigen presentation by class II major histocompatibility complex proteins, variation in the amino acid residues at these positions are likely to have important functional consequences as has indeed been previously reported.

Relationship With Reported Loci for Celiac Disease/Gluten Sensitivity

We noted the overlap of some of our findings with reported HLA associations with celiac disease and/or non-celiac gluten sensitivity[25–27]. Therefore, we examined this issue further in our dataset. The most commonly reported SNP and one of the strongest associations with celiac disease is HLA-DQA1 SNP rs2187668[25–26]. This SNP, which is not in strong linkage disequilibrium with the lead SNP for SSNS (rs1129740; r^2=0.05), does not show strong association with SSNS in our dataset (allelic p = 0.08). For classical HLA alleles, some of the alleles that have been associated with celiac disease[27–28] also show association with SSNS in this study. Most patients with celiac disease carry *DQA1*05* and *DQB1*02* alleles (HLA-DQ2.5 heterodimers); these classical alleles show significant association with SSNS in this study: *DQA1*05* ($p = 2.6 \times 10^{-3}$) and *DQB1*02* ($p=1.0 \times 10^{-5}$). One of the specific 4-digit HLA-alleles implicated in celiac disease – (*DQB1*0201)* is one of the most strongly associated classical HLA-alleles in this study (Table 2)[27]. HLA-DQ2.5 (i.e. co-carriage of *DQA1*05* and *DQB1*02*) was associated with a roughly two-fold increase in risk of SSNS (frequency in SSNS: 10.1% versus 5.5% in controls; OR, 2.1; $p=2.3 \times 10^{-2}$). On the other hand, *DQB1*03:02* (the most important allele encoding DQ8 heterodimers found in most DQ2.5-negative celiac patients)[29] does not show significant association with SSNS (p=0.3) (Table S6). The frequency of DQ8 did not differ significantly between SSNS cases and controls (frequency in cases 9.4% versus 11.1% in controls; p=0.4).

Discussion

The *HLA-DQA1* association was the first locus for SSNS reported in a population-based study of the sporadic form of SSNS[7]. The discovery was made in children of South Asian ancestry and was replicated in children of European ancestry[7]. In the present study, we extended these findings to children of African-American ancestry and confirmed a strong association of the two *HLA-DQA1* variants with SSNS. Our findings imply that *HLA-DQA1*-SSNS association is present in several populations and not population-specific. Our findings of the more severe manifestations of nephrotic syndrome in AA children, namely SRNS[5,30], showed that the two *HLA-DQA1* SSNS-associated variants are not associated with SRNS.

For individuals of African ancestry, the *APOL1* G1 and G2 risk alleles are of special importance with regards to several renal phenotypes associated with this locus, including but not limited to end-stage kidney disease, HIV nephropathy and hypertension-attributable nephropathy[8–16]. This association has been consistently demonstrated in populations of African ancestry and with effect sizes that are large and comparable to what is often seen in HLA associations. Given the African specificity of this genetic risk factor, we tested a cohort of African-American children with SSNS in the present study for known *APOL1* risk variants and demonstrated that *APOL1* G1 and G2 alleles show limited evidence of association with childhood SSNS although more data is needed given the limited sample size in this study. Conversely, the *APOL1* high risk alleles show significant association with SRNS, with an estimated four-fold increased risk. This is an important finding in several respects. The finding is consistent with the reported association of *APOL1* with FSGS[8,17] given that FSGS is the predominant histological type associated with clinical SRNS in this study. Taken together with the findings on *HLA-DQA1* association, the finding provides genetic evidence to support the notion that SSNS and SRNS are distinct entities and not likely to be on the same continuum of clinical kidney disease in children. While the differences in age, histological types, and clinical course are well-established, there are little or no data on differences at the biomarker and genetic level. This study demonstrates that genetic variation also differentiates SSNS and SRNS, with SSNS displaying increased risk with *HLA-DQA1* but no association with *APOL1* whereas SRNS shows the opposite pattern of genetic association. If the findings from the present study is confirmed in a larger cohort of African-American children, genotyping for both *HLA-DQA1* and *APOL1* risk alleles may help in predicting pattern of therapy response and therefore tailoring of immunosuppression.

Recently, the German Chronic Kidney Disease (GCKD) study—a study primarily of adults—reported that the SSNS risk rs1129740 was nominally associated with MCD (OR, 1.64; p = 0.01) in a genome-wide association study (GWAS)[31]. Given that MCD is often the most common histological type associated with SSNS, the GCKD findings provides additional support for the role of this variant in SSNS. Interestingly, the GCKD also found significant association between this SNP and membranous nephropathy with similar effect sizes to what was found with SSNS in the discovery report and with FSGS (OR, 1.52; p = 0.002)[7]. In summary, these findings indicate a complex role for *HLA-DQA1* rs1129740 in the risk of kidney disorders: in children it is associated with SSNS risk but not risk of SRNS; in adults it is associated with multiple kidney disorders, including membranous nephropathy and MCD.

We found that the most important classical HLA alleles associated with SSNS risk are *HLA-DQA1*0201, HLA-DQB1*0201*, and *HLA-DRB1*0701.* This is consistent with the findings of previous studies, despite some limitations with the ability to do direct comparisons, given that most previous studies of SSNS typed the HLA loci at two-digit resolution. For example, a recent study of children with nephrotic syndrome from South India showed a significant association between SSNS and both DRB1*07 and DQB1*02, as well as with the haplotype DRB1*07-DQB1*02[32]. Interestingly, some of the classic HLA alleles found to be significantly associated with SSNS in the present study have previously been reported to be associated with celiac disease and non-celiac gluten sensitivity[25–29].

This is intriguing because some difficult-to-manage cases of childhood nephrotic syndrome are known to be responsive to a gluten free or hypoallergenic diet[33–38]. While there are no clinical or epidemiological studies that studied the association between the two conditions, we find no evidence from the literature that this is a commonly observed association among patients. This may not be unexpected in view of the low population frequency of each of these disorders. However, the HLA findings lead us to speculate that the same HLA alleles may be associated with risk of both gluten sensitivity and SSNS and/or that gluten may be one of the potential environmental triggers for SSNS. Future studies that explicitly evaluate gluten sensitivity and SSNS would provide data on this hypothesis. Since not all children with SSNS respond to a gluten-free diet, it will be interesting to explore in future studies if classical HLA type can predict the subset of children who are likely to respond to dietary modification to induce remission of SSNS or control the frequency of relapse in frequently-relapsing/steroid-dependent SSNS. Such a biomarker would be immensely useful for risk stratification in children at initial presentation of nephrotic syndrome. Other opportunities for clinical and epidemiological research in SSNS that would be beneficial include evaluating the association between SSNS and gluten sensitivity as well as better estimates of the incidence and prevalence of SSNS in various populations beyond the mostly small and outdated studies that currently exist.[40,41]

In the present study, specific HLA-DQα1 amino acid positions yielded the strongest association with SSNS when all markers (SNPs, classical HLA alleles, amino acids) were considered together. In particular, HLA-DQα1 positions 76, 56, 69, and -16 (the last in the signal peptide) showed strong association with SSNS, with the first two seemingly the most critical since conditioning on them yielded no residual association in other markers including the originally reported SNPs. They are also involved in peptide binding, providing further support for a functional role for these amino acids. There have been no previous studies of specific HLA amino acids being associated with SSNS risk, so there are currently no studies with which we can compare these findings. Interestingly, amino acids at positions 76 and 56 show strong association with giant cell arteritis, an autoimmune form of vasculitis[39]. While the omnibus tests for the two positions were highly significant in the two studies, the direction of effect for each specific amino acid residue was inconsistent. This indicates that this may just be a coincidental finding.

The present study has some limitations. The limited sample sizes mean that the study is only powered to detect large effect sizes (ORs of 3 to 4 and higher) but is underpowered to detect small-to-moderate effect sizes (OR < 2). Future studies with larger sample sizes would detect these smaller effect sizes while providing better precision around the estimates. Also, the sample of African-American children studied may not be representative of all African-American children with SSNS or SRNS, especially since we did not have a large panel of SNPs to evaluate admixture and population stratification.

In summary, we confirmed in African-American children the *HLA-DQA1* association with SSNS previously reported in children of South Asian and European ancestry[7]. We found that *APOL1* G1 and G2 alleles show strong association with SRNS but not with SSNS in African-American children. With the aid of imputation, we refined the *HLA-DQA1* locus further by identifying the classical HLA alleles and amino acid positions that are most

critical in the observed association. The classical HLA alleles showed some overlap with reported alleles for celiac disease and non-celiac gluten sensitivity. The findings of the present study extend current knowledge about the genetic architecture of SSNS in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the family members of the Duke Genetics of Kidney Disease study and the personnel of the Genomic Core of the Duke Molecular Physiology Institute (DMPI), Duke University. We also acknowledge the Nephrotic Syndrome Group and participating centers in the MWPNC. Molecular graphics and analyses were performed with the UCSF Chimera package. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by National Institute of General Medical Sciences P41-GM103311).

Support: Dr Gbadegesin received funding from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) (5R01DK098135 and 5R01DK094987). This study was supported in part by the Doris Duke Charitable Foundation DDCSD award to Dr Gbadegesin. We acknowledge support from the Renal Genomics Core (Core B) of the Duke O'Brien Center for Kidney Research supported by theNIDDK/NIH (award P30DK096493). Dr Adeyemo is supported by the Intramural Research Program of the Center for Research on Genomics and Global Health (CRGGH). The CRGGH is supported by funds from the Office of the Director, NIDDK, and National Human Genome Research Institute at the NIH (Z01HG200362). The funding agencies had no role in study design, data collection and analysis, interpretation, and writing of the manuscript, or the decision to submit it for publication.

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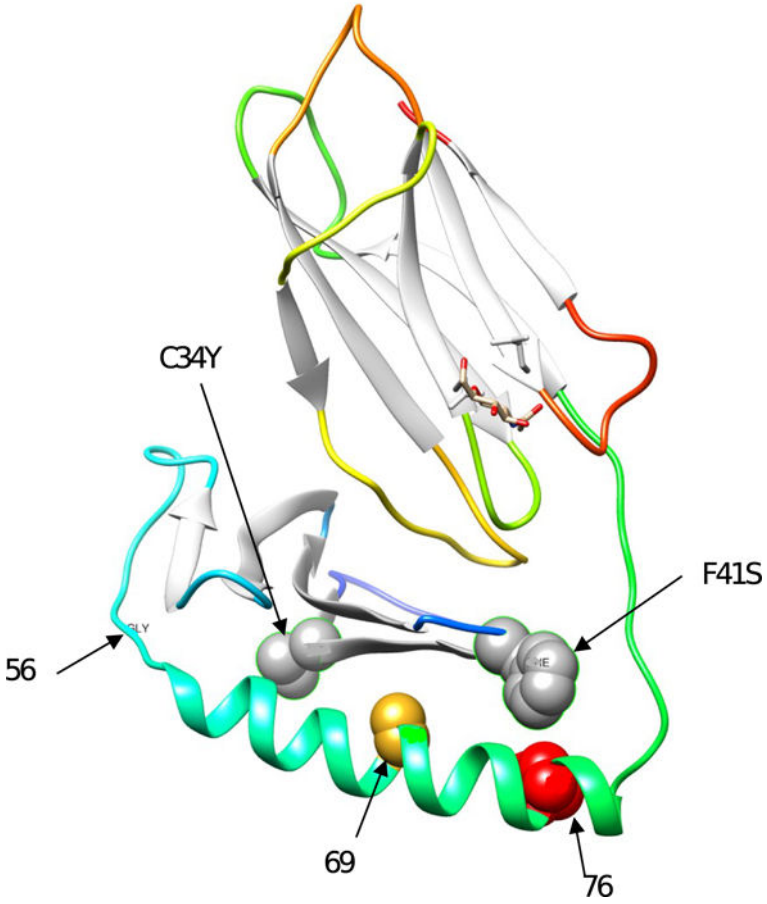

Figure 1. Amino acid associations of HLA-DQA1 with SSNS (model based on Protein Data Bank [PDB] entry 4OZI)

Figure showing model of HLA-DQA1 molecule and indicating the most significant amino acid positions in this study as well as the amino acid variants (C34Y, F41S) encoded by the original SNP association study.

Table 1

Association of *HLA-DQA1* risk variants with SSNS compared with SRNS in African-American children

	rs1129740 (A)	**rs1071630 (G)**
SSNS		
Frequency risk allele in cases (n=65)	0.721	0.720
Frequency risk allele in controls (n=2203)	0.424	0.388
Association p value	5.7×10^{-11}	1.2×10^{-13}
OR (95% CI)	3.53 (2.33–5.42)	4.08 (2.70–6.28)
SRNS		
Frequency risk allele in cases (n=50)	0.452	0.452
Frequency risk allele in controls (n=2203)	0.424	0.388
Association p value	0.6	0.2
OR (95% CI)	1.12 (0.71–1.77)	1.30 (0.82–2.05)

OR = Odds ratio; CI = confidence interval; rs, reference single-nucleotide polymorphism identification number; SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; A, adenine; G, guanine

Table 2

Frequency of *APOL1* risk alleles in SSNS and SRNS in African-American children

	High risk-*APOL1* genotype: G1/G1 or G1/G2 or G2/G2	**Low-risk *APOL1* genotype: G1 or G2 or G0/G0**	**Fisher exact p**	**OR (95% CI)**
SSNS	11/65 (16.9%)	54/65 (83.1%)	0.5	1.27 (0.60–2.8)
SRNS	20/50 (40.0%)	30/50 (60.0%)	1.04×10^{-7}	4.17 (2.23–7.64)
Population controls*	751/5453 (13.8%)	4702/5453 (86.2%)	–	–

*Derived from Zhang et al[19] and 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.

OR = odds ratio; CI = confidence interval; rs, reference single-nucleotide polymorphism identification number; SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome

Table 3

Most significant 4-digit HLA alleles associated with SSNS in South Asian children

Classical HLA 4-digit allele	Frequency in cases	Frequency in controls	INFO	OR	SE	P
HLA-DRB1*0701	0.3406	0.1915	0.8779	2.510	0.201	4.6×10^{-6}
HLA-DQA1*0201	0.3383	0.1907	0.8789	2.492	0.201	5.5×10^{-6}
HLA-DQB1*0201	0.3644	0.2120	0.9493	2.282	0.187	1.0×10^{-6}
HLA-DQA1*0101	0.1167	0.2173	0.9910	0.465	0.213	3.3×10^{-4}

OR, odds ratio; SE, standard error; SSNS, steroid-sensitive nephrotic syndrome; INFO, _____

Table 4

Most significant association tests of HLA amino acid positions with SSNS in South Asian children

			Frequency				
Protein (AA position)	**Omnibus P**	**Most significant AA residue at position**	**SSNS cases**	**Controls**	**OR**	**SE**	**P**
DQA1 (56)	2.8×10^{-7}	deletion	0.5098	0.3091	2.376	0.166	2.0×10^{-7}
DQA1 (76)	2.8×10^{-7}	L	0.5098	0.3091	2.376	0.166	2.0×10^{-7}
DQA1 (−16)	3.0×10^{-7}	M	0.6543	0.4529	2.267	0.160	3.0×10^{-7}
DQA1 (69)	8.1×10^{-7}	L	0.6543	0.4529	2.267	0.160	3.0×10^{-7}
DRB1 (73)	1.3×10^{-6}	G	0.4245	0.2444	2.333	0.175	1.3×10^{-6}
DQA1 (129)	1.3×10^{-6}	Q	0.2285	0.3967	0.423	0.178	1.3×10^{-6}
DQA1 (218)	2.5×10^{-6}	Q	0.3273	0.5099	0.473	0.159	2.5×10^{-6}

Note: AA, amino acid; OR = Odds ratio; SE = Standard error; SSNS, steroid-sensitive nephrotic syndrome