Genome-Wide Epistatic Interaction between DEF1B and APOL1 High-Risk Genotypes for Chronic Kidney Disease


CKD disproportionately affects Black and Hispanic/Latino individuals. APOL1 risk variants are common in individuals of recent African ancestry and explain some disparities. However, not all individuals with APOL1 high-risk genotypes develop CKD, suggesting gene-environment and gene-gene (epistatic) modifiers. Prior genetic studies studying single nucleotide polymorphism (SNP) interactions with APOL1 risk genotypes were limited by small sample sizes and did not identify genome-wide significant interactions (1). We conducted a genome-wide SNP-APOL1 interaction analysis using two large datasets with genetic and phenotypic data.

The Population Architecture Using Genomics and Epidemiology (PAGE) study is a consortium of cohorts of diverse populations (2) composed of the Atherosclerosis Risk in Communities study, the Coronary Artery Risk Development in Young Adults study, the Hispanic Community Health Study/Study of Latinos, the Multiethnic Cohort study, the Multi-Ethnic Study of Atherosclerosis, the Women’s Health Initiative, and BioMe. BioMe Biobank is an electronic health record biobank from across the Mount Sinai Health System. Because BioMe contributed part of its data to PAGE, we excluded all BioMe participants from PAGE. PAGE used a multiethnic genotyping array, and BioMe used the global screening array. Both datasets were imputed using the TOPMed freeze 8 imputation reference panel. APOL1 risk variants are covered by both arrays with high accuracy (3). We extracted individuals who self-identified as either “African American/Black” and “Hispanic” and grouped them into four cohorts. After removing related individuals up to second-degree genetic relatedness (KING kinship >0.88), we had data on 10,733 Black and 13,726 Hispanic/Latino individuals from PAGE and 6456 Black and 9389 Hispanic/Latino individuals from BioMe. CKD stage 3 or higher was defined using a validated electronic phenotyping algorithm combining diagnosis codes, laboratory values, and text searches with over 95% positive and negative predictive values. Proteinuria was not included in the definition due to the relative lack of electronic health record measurements (4). APOL1 high-risk and low-risk were defined on the basis of G1/G2 risk alleles. CKD proportions varied from 4% and 10% in PAGE to 16% and 20% in BioMe; this was likely due to differences in the cohort ascertainment, with PAGE being community-based recruitment and BioMe being hospital-based recruitment. In the PAGE and BioMe cohorts, the proportions of APOL1 high-risk genotypes were 0.5% and 2% for Hispanic/Latino individuals, respectively, and 12% and 14% in Black individuals, respectively. Baseline and outcome characteristics of the population are shown in Figure 1A. We fitted a logistic regression to test for the interaction effect of each SNP with APOL1 on CKD, adjusted for age, sex, and the first ten principal components. We performed a logistic regression test genome wide for each of the four cohorts separately, restricting to variants with minor allele frequency >1%, and performed a meta-analysis with METAL using effective sample size weighting.

We obtained summary statistics of approximately $3 \times 10^5$ SNPs from the meta-analysis with acceptable genomic inflation factor (AGC=0.91) and identified seven genome-wide significant SNPs ($P<0.001$) interacting with APOL1 high-risk genotypes. However, only one significant SNP was found within an annotated functional genetic region. This SNP, rs73188225 (chr8:6866830, G→A), is in the downstream region of the Defensin 1 (DEF1B) gene on chromosome 8. To explore how this SNP interacts with APOL1, we divided participants into two groups on the basis of APOL1 risk and tested for the association of CKD within each group. Meta-analysis showed that the SNP is associated with a four times higher odds of CKD (odds ratio, 4.4; 95% confidence interval, 2.1 to 8.9; $P<0.001$) among individuals with APOL1 high risk but did not have a significant association in individuals with low risk (Figure 1B).

Our results imply that this risk variant at the DEF1B locus is a genome-wide significant epistatic...
modifier of the association between APOL1 risk genotypes and CKD. *DEFB1* encodes β-defensin-1, an antimicrobial peptide that protects mucosal surfaces against infections. When correlating with public gene expression data (GTEx Analysis Release V8), *DEFB1* is mostly expressed in the salivary gland, kidney cortex, and medulla tissue. Enrichment testing using human kidney single-cell RNA sequencing data shows that *DEFB1* is highly expressed in the thick ascending limb of the loop of Henle and connecting tubule cells. Underexpression of β-defensin-1 is associated with acute kidney allograft rejection (5). The mechanisms by which the *DEFB1* variant interacts with APOL1 risk genotypes is unclear and needs future studies. However, the antimicrobial defense function of *DEFB1* and the association of *APOL1* risk variants with sepsis suggest that endothelial function/innate immunity may be involved. Finally, differing effect sizes and risk allele frequencies between Black and Hispanic/Latino individuals warrant future studies into population-specific epistatic interactions.

In summary, an SNP downstream of the *DEFB1* locus is the first genome-wide association that modifies the APOL1-CKD association. This provides supportive evidence for the epistatic hypothesis and implicates tubular pathophysiology in individuals with high-risk APOL1 genotypes. This also provides the impetus for future work to understand the pathophysiologic and mechanistic bases of this gene-gene interaction for understanding disparities in CKD.

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**Author Contributions**
G.N. Nadkarni conceptualized the study; S. Buyske, R. Cooper, J. Coresh, R. Do, N. Franceschini, M. Graff, F.F. Gulamali, O.M. Gutierrez, C. Haiman, C. Horowitz, T. Johns, C. Kooperberg, R. Loos, T.C. Matise, G.N. Nadkarni, R. Nassir, A. Reiner, P.F. Schnatz, and F. Thomas were responsible for data curation; K.N. Campbell, B.M. Lin, and T.H.T. Vy were responsible for data curation; K.N. Campbell, M. Graff, C. Haiman, C. Kooperberg, T.C. Matise, and J. Wong were responsible for software; R. Do, N. Franceschini, and F.F. Gulamali were responsible for validation; O.M. Gutierrez was responsible for visualization; T.C. Matise and G.N. Nadkarni were responsible for funding acquisition; R. Do and R. Loos provided supervision; G.N. Nadkarni and T.H.T. Vy wrote the original draft; and S. Buyske, K.N. Campbell, R. Cooper, J. Coresh, R. Do, N. Franceschini, M. Graff, F.F. Gulamali, O.M. Gutierrez, C. Haiman, C.R. Horowitz, T. Johns, C. Kooperberg, B.M. Lin, R. Loos, T.C. Matise, G.N. Nadkarni, R. Nassir, A. Reiner, P.F. Schnatz, F. Thomas, T.H.T. Vy, and J. Wong reviewed and edited the manuscript.

Data Sharing Statement
All data used in this study are available in this article.

**References**


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