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 >0.088), we had data on 10,733 Black and 13,726 Hispanic/Latino individuals from PAGE and 6,456 Black and 9,389 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 $3 \times 10^7$ 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 (DEFB1) 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 DEFB1 locus is a genome-wide significant epistatic
modifier of the association between APOL1 risk genotypes and CKD. DEF1B encodes β-defensin-1, an antimicrobial peptide that protects mucosal surfaces against infections. When correlating with public gene expression data (GTEx Analysis Release V8), DEF1B is mostly expressed in the salivary gland, kidney cortex, and medulla tissue. Enrichment testing using human kidney single-cell RNA sequencing data shows that DEF1B 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 DEF1B variant interacts with APOL1 risk genotypes is unclear and needs future studies. However, the antimicrobial defense function of DEF1B 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 DEF1B 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.

**Disclosures**

S. Buyske reports consultancy agreements with Advanced Infrastructure Design, Inc. K.N. Campbell reports consultancy agreements with Caliditas, Goldfinch Bio, Mallinckrodt Pharmaceuticals, Trave, and Vertex; research funding from Aurinia, Goldfinch Bio, and Mallinckrodt Pharmaceuticals; an advisory or leadership role for the medical advisory board of the National Kidney Foundation of Greater New York and the Nephcure Foundation; and board memberships for the National Kidney Foundation and the Nephcure Foundation. J. Coresh reports ownership interest in Healthy.io; consultancy agreements with Healthy.io and Somalogic; research funding from the National Institutes of Health and the National Kidney Foundation (which receives industry support); and an advisory or leadership role for Healthy.io and SomaLogic; research funding from the National Institutes of Health and the National Kidney Foundation. R. Do reports consultancy agreements with Pensieve Health and Variant Bio; ownership interest in Pensieve Health; and research funding from AstraZeneca and Goldfinch Bio. N. Franceschini reports serving as consultancy agreements with Advanced Infrastructure Design, Inc. K.N. Campbell reports consultancy agreements with Caliditas, Goldfinch Bio, Mallinckrodt Pharmaceuticals, Trave, and Vertex; research funding from Aurinia, Goldfinch Bio, and Mallinckrodt Pharmaceuticals; an advisory or leadership role for the medical advisory board of the National Kidney Foundation of Greater New York and the Nephcure Foundation; and board memberships for the National Kidney Foundation and the Nephcure Foundation. J. Coresh reports ownership interest in Healthy.io; consultancy agreements with Healthy.io and Somalogic; research funding from the National Institutes of Health and the National Kidney Foundation (which receives industry support); and an advisory or leadership role for Healthy.io and SomaLogic; research funding from AstraZeneca and Goldfinch Bio. N. Franceschini reports serving as
the Women’s Health Initiative Vice-Chair of the Ancillary Committee and a National Heart, Lung, and Blood Institute TOPMed kidney working group convener and serving on the editorial board of *American Journal of Physiology-Renal Physiology*, the editorial board of *Contemporary Clinical Trials*, and the Women’s Health Initiative Publication and Presentation Committee. O.M. Gutierrez reports consultancy agreements with QED; research funding from Amgen and GlaxoSmithKline; honoraria from Akebia, Amgen, Ardelyx, AstraZeneca, and Reata; and serving as an associate editor of *CJASN*. R. Loos reports consultancy agreements with Regenron and serving as a board member of the European Association of the Study of the Diseases. G.N. Nadkarni reports consultancy agreements with AstraZeneca, Daiichi Sankyo, GLG Consulting, Qiming Capital, Reata, Renalityx, Siemens Healthineers, and Variant Bio; equity in Data2Wisdom LLC, Doximity, Nexus iConnect, Renalityx, and Verici; equity in Pensieve Health as a cofounder; research funding from Renalityx; honoraria from Daiichi Sankyo; patents or royalties from Renalityx; an advisory or leadership role for Renalityx; and serving as a speaker for Daiichi Sankyo. P.F. Schnatz reports ownership interest in Evertec Inc., First Interstate Bancsystem Inc., Heico Corp., Mastercard Inc., MBIA Inc., and Block Inc. and an advisory or leadership role for AstraZeneca; an advisory or leadership role for Renalytix; funding from Renalytix; honoraria from Daiichi Sankyo; patents or royalties from Renalityx; an advisory or leadership role for Renalityx; and serving as an associate editor for *Journal of Clinical Oncology* (the flagship journal of the American Society for Clinical Oncology: 5-year term from November 1, 2021 to October 31, 2026; contract is with the American Society for Clinical Oncology: 5-year term from November 1, 2021 to October 31, 2026; contract is with the American Society for Clinical Oncology). All remaining authors have nothing to disclose.

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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 T.H.T. Vy were responsible for data curation; K.N. Campbell, B.M. Lin, and T.H.T. Vy were responsible for visualization; T.C. Matise and G.N. Nadkarni, R. Loos, G.N. Nadkarni, T.H.T. Vy, and J. Wong were responsible for methodology; K.N. Campbell was responsible for project administration; S. Buyske, R. Cooper, J. Coresh, M. Graff, C. Haiman, T. Johns, C. Kooperberg, B.M. Lin, T.C. Matise, G.N. Nadkarni, R. Nassir, A. Reiner, P.F. Schnatz, F. Thomas, and T.H.T. Vy were responsible for resources; 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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