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Abstract

Identification of genetic risk factors for albuminuria may alter strategies for early prevention of CKD progression, particularly among patients with diabetes. Little is known about the influence of common genetic variants on albuminuria in both general and diabetic populations. We performed a meta-analysis of data from 63,153 individuals of European ancestry with genotype information from genome-wide association studies (CKDGen Consortium) and from a large candidate gene study (CARe Consortium) to identify susceptibility loci for the quantitative trait urinary albumin-to-creatinine ratio (UACR) and the clinical diagnosis microalbuminuria. We identified an association between a missense variant (I2984V) in the CUBN gene, which encodes cubilin, and both UACR \((P = 1.1 \times 10^{-11})\) and microalbuminuria \((P = 0.001)\). We observed similar associations among 6981 African Americans in the CARe Consortium. The associations between this variant and both UACR and microalbuminuria were significant in individuals of European ancestry regardless of diabetes status. Finally, this variant associated with a 41% increased risk for the development of persistent microalbuminuria during 20 years of follow-up among 1304 participants with type 1 diabetes in the prospective DCCT/EDIC Study. In summary, we identified a missense CUBN variant that associates with levels of albuminuria in both the general population and in individuals with diabetes.

Elevated levels of urinary albumin (albuminuria) are a cardinal manifestation of chronic kidney disease (CKD) and affect as many as 8% of adults from the United States and 6% of adults from Germany.
Higher levels of albuminuria, even within the low normal range, are associated with not only increased risks of ESRD but also cardiovascular disease and mortality.\textsuperscript{4–6} Moreover, the presence of albuminuria offers key prognostic information at each stage of decline in GFR.\textsuperscript{7} However, the pathophysiologic basis of albuminuria remains incompletely understood, and as a result, interventions for the prevention and treatment of albuminuria are limited.

Diabetes mellitus and hypertension are key risk factors for albuminuria, but neither of these factors fully account for the high prevalence of albuminuria nor its association with adverse health outcomes.\textsuperscript{8} Heritability of albuminuria ranges from 0.16 to 0.49 in families enriched with hypertension or diabetes.\textsuperscript{9,10} Rare genetic variants are known to cause monogenic diseases featuring severe, nephrotic range proteinuria.\textsuperscript{11} However, linkage or candidate gene studies have not reproducibly identified common genetic variants in association with lower levels of albuminuria.\textsuperscript{9,10} Given recent successes in the use of genome-wide association studies (GWAS) of quantitative traits that can lead to the identification of relevant variants for a disease phenotype,\textsuperscript{12,13} we conducted a genome-wide association (GWA) analysis of albuminuria in 31,580 participants of European ancestry from the CKDGen Consortium, with follow-up in 27,746 additional participants. Albuminuria was analyzed as the quantitative trait urinary albumin-to-creatinine ratio (UACR) and as the dichotomous trait microalbuminuria (MA). Concurrently, we performed an analysis of albuminuria in the CARE Consortium using the ITMAT/Broad/CARE Vascular Disease 50k (IBC) single-nucleotide polymorphism (SNP) chip array\textsuperscript{14} in 19,499 Europeans and 6981 African Americans. Here, we report the results of our combined findings.

**RESULTS**

**Study Samples**

Basic characteristics of the participants from the studies in CKDGen and CARE are shown in Table 1. Studies in these consortia are primarily population-based, with mean age ranging from 42 to 74 years. Details regarding study-specific genotyping information can be found in Supplemental Table 1, A and B, and in the Supplemental Text (Methods).

**CKDGen Stage 1**

Figure 1A and Supplemental Figure 1A show the Manhattan plots of the meta-analysis P values for UACR and microalbuminuria (UACR >25 mg/g [women], >17 mg/g [men]), respectively. Meta-analysis of GWAs from CKDGen stage 1 showed that no locus achieved genome-wide significance ($P < 5.0 \times 10^{-8}$) for either UACR or microalbuminuria in both the overall and the nondiabetic analyses. Supplemental Figure 2, A and B, shows the quantile-quantile plot of the UACR and microalbuminuria meta-analysis results.

**CKDGen Stage 2 Follow-up**

In CKDGen, the 16 top independent SNPs ($P$ value range $1.1 \times 10^{-7}$ to $5.7 \times 10^{-6}$) were moved into stage 2 follow-up in 15 additional studies ($n = 27,746$ individuals of European descent). These SNPs and their study-specific imputation scores are displayed in Supplemental Tables 2 and 3, respectively. Supplemental Table 4 shows the results of these 16 SNPs for all analyzed traits.

Overall, rs1801239, a missense SNP (T→C) located in CUBN on chromosome 10 (minor allele frequency = 0.10), demonstrated direction-consistent association in stage 2 ($P = 0.02$, Table 2), with a genome-wide significant $P$ value of $4.0 \times 10^{-8}$ for UACR in the combined stage 1 and stage 2 analysis.
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(Supplemental Tables 2 and 4). The regional association plot for CUBN is shown in Figure 2A. The minor C allele of rs1801239 in CUBN leads to an isoleucine-to-valine substitution (I2984V) in the encoded protein cubilin, which is predicted to be “probably benign” (SIFT, FastSNP, and PolyPhen). No additional nonsynonymous coding variants in high LD to rs1801239 ($r^2 > 0.2$) were observed in dbSNP. Because albuminuria is a risk factor for progressive CKD, we assessed whether rs1801239 is associated with estimated GFR (eGFR) and CKD in the CKDGen eGFR data set. Among 67,093 individuals with available data, we observed no association with eGFR ($P = 0.53$) or CKD ($P = 0.33$).

The second highest ranking SNP for UACR in the combined stage 1 and stage 2 analysis was rs17319721, an intronic SNP in SHROOM3 (Supplemental Table 4). The minor allele (A) of rs17319721 is associated with lower albuminuria levels, and we have previously identified this same allele in association with lower eGFR.

**CARe IBC SNP Array Stage 1**

Concurrently, the CARe Consortium carried out a large densely tagged candidate gene screen using the IBC SNP array and identified the same SNP (rs1801239) in CUBN in association with UACR ($P = 2.9 \times 10^{-10}$; Table 2 and Figure 1B) and microalbuminuria (2100 cases and $P = 2.4 \times 10^{-7}$; Table 2) in 19,499 European Americans. The regional association plot for the CUBN tagging SNPs on the IBC array in the CARe European Americans is shown in Figure 2B. The only other significant SNP (significance threshold defined as $P < 2.2 \times 10^{-7}$ for the IBC array) in the CARe IBC analysis for UACR was rs13177732 ($P = 4.7 \times 10^{-7}$), but this SNP did not replicate in CKDGen stage 1 (discovery) studies after excluding overlapping studies ($n = 16,667$, $P = 0.71$ for UACR).

**Joint CKDGen/CARe Analyses in Samples of European Ancestry**

Because three of the studies from CARe (ARIC, CHS, and FHS) were also part of CKDGen, we removed them from CKDGen to avoid duplication. The overall $P$ value for CKDGen stage 1 (after removing ARIC, CHS, and FHS), CKDGen stage 2 follow-up (after removing the ARIC in silico replication samples), and the CARe stage 1 analysis in participants of European ancestry for the association between rs1801239 and UACR was $1.1 \times 10^{-11}$ ($n = 63,153$; Table 2), with 0.15% of the UACR variance explained by rs1801239. The study-specific association results are shown in Supplemental Figure 3A; there was no significant heterogeneity across studies ($I^2 = 19.6\%$, $P$ value for heterogeneity = 0.18). The odds ratio for microalbuminuria per copy of the minor C allele at rs1801239 was 1.06 ($P = 0.001$; Table 2).

**CARe African-American IBC SNP Array Results**

Because albuminuria is an important manifestation of CKD across ethnicities, we examined rs1801239 in 6981 African-American CARe participants (1159 microalbuminuria cases) and found that the minor C allele (frequency = 0.03) was consistently associated with higher UACR ($P = 0.005$, explaining 0.06% of the UACR variance) and the presence of microalbuminuria. Each copy of the C allele at rs1801239 was associated with an odds ratio of 1.42 for microalbuminuria ($P = 0.008$; Table 2). The study-specific association results for UACR are shown in Supplemental Figure 3B. Given the potential for allelic heterogeneity across ethnic groups and the availability of densely genotyped IBC chip data, we further investigated the CUBN region (Figure 2C) to determine if there were any other SNPs that showed stronger association with UACR and microalbuminuria in African Americans. We uncovered rs1996316 in the CUBN gene region (minor allele frequency = 0.33, $P$ value = 0.0009). The SNP rs1996316 was not correlated with rs1801239 ($r^2 = 0.01$); however, this SNP did not meet statistical significance after
correcting for multiple testing of 158 SNPs in the region \(0.05/158 = 0.0003\), nor was it associated with UACR in individuals of European descent from CKDGen \((P = 0.65)\) or CARe \((P = 0.36)\).

### Stratified Analysis

Because hypertension and diabetes are key risk factors for albuminuria, we performed stratified analyses for rs1801239 in the joint CKDGen/CARe analysis of populations of European descent. For UACR, we observed significant association among individuals with \((n = 4915; P = 0.006)\) and without \((P = 3.2 \times 10^{-8})\) diabetes as well as among individuals with \((n = 13,097, P = 7.5 \times 10^{-8})\) and without \((P = 1.3 \times 10^{-6})\) hypertension. Similar findings across these groups were also observed for microalbuminuria (Table 2). In particular, among individuals with diabetes, each copy of the C allele at rs1801239 was associated with an odds ratio of 1.27 for microalbuminuria (95% CI: 1.11 to 1.45).

To investigate if CKD modifies the association between rs1801239 and UACR, we performed an analysis of rs1801239 and UACR in 6 of the largest cohorts in CKDGen stratified by CKD status. We found that the association of the \(CUBN\) C allele and UACR was of similar magnitude among participants with CKD \((n = 1808, \beta = 0.09, P = 0.26)\) as compared with those without CKD \((n = 21299, \beta = 0.10, P = 9.6 \times 10^{-11})\), although the power was low in the CKD stratum because of its smaller sample size.

### Independent Replication in the Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC)

To understand the potential impact of rs1801239 on microalbuminuria in a high-risk population, we tested the association of rs1801239 with time to persistent microalbuminuria over 20 years of follow-up among 1304 participants of European ancestry with type 1 diabetes from the DCCT/EDIC Study (mean baseline age 26.7 years). With use of survival analysis, the minor C allele was associated with an increased risk of incident persistent microalbuminuria. Individuals with diabetes with one copy of the C allele had a 42% greater risk of developing persistent microalbuminuria than their counterparts with zero copies of the C allele (hazard ratio 1.42 per copy of the C allele, \(P = 0.02\); Table 2). This association was essentially unchanged with further multivariable adjustment (Table 2).

### DISCUSSION

We have identified and validated a missense SNP in the \(CUBN\) gene that is associated with albuminuria. This association is robust across subgroups defined by diabetes and hypertension, two major risk factors for albuminuria, and among populations of both European and African ancestry. Finally, we have validated this finding in association with persistent microalbuminuria among patients with type 1 diabetes from the DCCT/EDIC Study.

Cubilin was first identified as the intrinsic factor/vitamin B12 complex receptor in the ileal mucosa. \(^2^0\) In the kidney, cubilin is expressed predominantly in the apical brush border of proximal tubular cells. \(^2^1\) We queried publicly available expression databases \(^2^2-^2^4\) but did not find evidence for altered gene expression associated with the conservative amino acid substitution in cubilin encoded by rs1801239 (I2984V). On the basis of UniProt, the amino acid position 2984 is part of the 22nd, out of a total of 27, CUB domains. \(^2^5\) In \textit{vivo}, CUB domains 22 through 27 demonstrated Ca\(^{2+}\)-dependent binding to megalin. \(^2^6\) Together with megalin (\(LRP2\)) and amnionless (\(AMN\)), cubilin plays a key role in the receptor-mediated endocytotic reabsorption of albumin and other low-molecular-weight proteins. \(^2^6\) Interrogation of these respective genomic regions in our data did not reveal any significant findings.

The process of endocytotic reabsorption of albumin is of importance because an estimated 3 g of albumin per day are not retained by the glomerular filter and enter the primary urine. \(^2^0\) Yet urine in its
final composition is nearly devoid of proteins in healthy humans, highlighting the effectiveness of the tubular reabsorptive process to prevent significant protein loss.

The essential role of the cubilin-megalin complex in the reuptake of albumin by the proximal tubule has been demonstrated in both animal and human studies. For example, dogs with a functional defect in cubilin and mice lacking cubilin and/or megalin excrete large amounts of urinary albumin. Similarly, humans with Imerslund-Gräsbeck disease (OMIM #261100), a rare autosomal-recessive condition caused by a variety of mutations in the CUBN gene or the cubilin-associated AMN gene, typically have anemia with varying degrees of proteinuria as a result of a molecular defect leading to inefficient proximal tubular protein reabsorption.

Dysfunction of the megalin-cubilin system has also been implicated in the pathogenesis of diabetic kidney disease. In rat models, renal tubular expression of cubilin is decreased and reabsorption of filtered albumin by the proximal tubule has been shown to be altered. In humans, shedding of megalin and cubilin in urine is increased in individuals with type 1 diabetes and microalbuminuria compared with nonalbuminuric controls. These studies suggest that reduced expression or loss of the cubilin-megalin complex may contribute to the albuminuria of early diabetic kidney disease via reduced tubular reuptake of filtered albumin, which is supported by our observed association of rs1801239 with incident persistent microalbuminuria in the DCCT/EDIC Study.

Our results suggest that levels of albuminuria in the general population are determined in part by tubular reabsorption, and not only by glomerular filtration. Although the prognostic implications of tubular as compared with glomerular albuminuria remain to be determined, a pathogenic role for tubular albuminuria, in addition to glomerular, has been demonstrated in experimental data. The identification of a SNP that is associated with albuminuria among individuals irrespective of diabetes or hypertension status suggests some common pathophysiology that is independent of these known albuminuria risk factors. The CUBN SNP we identified is specifically associated with albuminuria, and not with eGFR. Finally, results from the DCCT/EDIC Study underscore the relative strength of the CUBN SNP in association with persistent microalbuminuria that is comparable in magnitude to other albuminuria risk factors in patients with diabetes, including diabetes duration, BP, hemoglobin A1c, and obesity.

Important strengths of this study include consistency of association across populations of European and African descent and across groups defined by diabetes and hypertension, as well as the known role of cubilin in tubular albumin reabsorption. The exploration of genetic determinants for albuminuria in predominantly population-based cohorts reduces confounding by disease progression, which may be related to important nongenetic factors. Nonetheless, some limitations warrant mention. First, the causal nature of the missense SNP in CUBN is unclear. Second, urine albumin and creatinine were assessed with different assays and at one point in time in most studies, which may lead to misclassification of the outcome and bias our results toward the null hypothesis. However, this is unlikely to yield a true positive finding.

In summary, through a series of genetic association analyses, we have identified a missense SNP in the CUBN gene that is associated with higher levels of albuminuria among individuals of European and African descent with and without diabetes or hypertension. These findings highlight a novel genetic susceptibility for albuminuria that is consistent across multiple study populations and shared across diverse clinical settings.

**CONCISE METHODS**
Overall Study Design

Genetic association testing for urinary albumin-to-creatinine ratio (UACR) and MA was performed in the CKDGen and CARe cohorts of European ancestry, with further follow-up genetic analysis of significant SNPs in CARe cohorts of African-American ancestry and in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. A graphical overview is given in Supplemental Figure 4.

CKDGen Stage 1 Discovery Meta-analysis

In the stage 1 (discovery) meta-analysis we searched for SNPs associated with UACR or MA in 12 CKDGen population-based cohorts totaling \( n = 31,580 \) patients of European descent. Individual GWA analyses encompassing approximately 2.5 million imputed SNPs were performed within each of the 12 stage 1 (discovery) CKDGen population-based cohorts. In each cohort, these analyses were performed in the overall group and separately in patients without diabetes. Next, we conducted four meta-analyses combining the study-specific UACR or MA GWA analysis results (i) including all patients and (ii) separately in patients without diabetes. From these four sets of meta-analysis results, we selected a list of independent SNPs (pairwise \( r^2 < 0.2 \)) with a \( P \) value \(< 1 \times 10^{-6} \) and minor allele frequencies (MAF) \(>5\%). The 16 highest ranking SNPs from this list were then selected for CKDGen stage 2 follow-up to replicate our findings.

CKDGen Stage 2 Follow-up Meta-analysis

Association testing for each of the 16 SNPs for UACR and MA was performed in each of the 15 independent CKDGen stage 2 cohorts totaling \( n = 27,746 \) individuals of European descent, again, including all patients and separately in patients without diabetes. Study-specific association results of the 16 SNPs were then meta-analyzed across stage 2 studies.

SNPs showing evidence of replication in CKDGen stage 2 were further evaluated for their effects both in the presence and absence of diabetes and hypertension as major risk factors of albuminuria.

CARe Discovery Association Analysis

The CARe Consortium consists of nine studies. For the present analysis, we included five studies in European Americans (19,499 patients in total) and five studies in African Americans (6981 patients in total) with the IBC SNP chip. Study-specific genetic association analysis of UACR and MA were performed in the same manner as in CKDGen. CARe study-specific results were then meta-analyzed within each ethnic group for both UACR and MA.

Joint CKDGen and CARe Meta-analysis

For SNPs that reached genome-wide significance in the combined CKDGen stage 1 and stage 2 meta-analysis and were also significant in the CARe meta-analysis, we conducted meta-analyses for UACR and MA across a total of 28 nonoverlapping cohorts: in 9 CKDGen stage 1 studies with 16,667 patients (excluding ARIC, CHS, and FHS since they were also members of the CARe Consortium), 14 CKDGen stage 2 follow-up studies with 26,987 patients (excluding ARIC in silico results), and 5 CARe European American cohorts with 19,499 patients, involving a total of 63,153 patients of European descent.

Follow-up Analysis in DCCT/EDIC

Significant SNPs from the joint CKGen and CARe meta-analysis were replicated in the DCCT/EDIC Study, which currently consists of 1304 Caucasian participants who underwent genotyping on the
Illumina 1M SNP chip. This is a longitudinal study using Cox proportional hazards models to analyze time to renal events (see definition of the outcome below).

Study-Specific Information and Statistical Analysis

In all studies, all participants gave informed consent. All studies were approved by their appropriate Research Ethics Committees.

A list of all contributing studies is given in Table 1 and more study-specific information including genotyping and imputation methods are given in Supplemental Table 1, A and B, as well as in the Study-Specific Methods Section of the Supplemental material. Details on statistical analyses on the study-specific level as well as for meta-analyses are given in the Supplemental material.

Outcomes and Covariates

In each of the CKDGen and CARe studies, the continuous outcome urinary albumin-to-creatinine ratio (mg/g, measured as described in the Study-Specific Methods section in the Supplemental material) as well as the dichotomous outcome microalbuminuria (MA, defined as urinary albumin-to-creatinine ratio >17 mg/g for men and >25 mg/g for women\(^\text{40, 41}\)) were analyzed. For creating the continuous trait UACR used in the analysis, urinary albumin-to-creatinine ratio was log-transformed, and sex-specific residuals were computed by regression on age and, in multicenter studies, on study center.

Hypertension was defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or treatment; and diabetes was defined as fasting plasma glucose of at least 126 mg/dl or treatment if not stated otherwise in the study-specific methods. We estimated GFR (eGFR) using the four-variable MDRD formula as described previously\(^\text{18}\) to determine the prevalence of chronic kidney disease (defined as eGFR <60 ml/min per 1.73 m\(^2\)) in each cohort.

In DCCT/EDIC, renal outcomes were (1) time from DCCT baseline until persistent microalbuminuria, defined as time to two consecutive albumin excretion rates (AERs) >30 mg/24 h (>20.8 μg/min), and (2) severe nephropathy, defined as the time to AER >300 mg/24 h (>208 μg/min) with prior persistent microalbuminuria or end-stage renal disease).

Genotypes

All the studies in CKDGen stage 1 had genotype data from genome-wide SNP arrays available, whereas the studies in the CARe Discovery studies genotyped the IBC SNP array,\(^\text{14}\) a gene-centric array containing 50,000 SNPs tagging genes across a range of cardiovascular, metabolic, and inflammatory syndromes. In CKDGen, the genotyped SNPs were imputed to approximately 2.5 million HapMap SNPS based on HAPMAP CEU samples. Imputation provides a common SNP panel across all studies to facilitate a meta-analysis across all contributing SNPs. Information on study-specific genotyping platforms and imputation procedures are presented in Supplemental Table 1, A and B. Information on genotyping in CKDGen stage 2 cohorts are given in the Study-Specific Methods section of the Supplemental material.

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Supplementary Material

Supplemental Data:
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**KORA:** The genetic epidemiologic work was funded by the NIH subcontract from Children's Hospital, Boston, (H.E.W. and I.M.H., prime grant 1 R01 DK075787-01A1), the German National Genome Research Net NGFN2 and NGFNplus (H.E.W. 01GS0823; WK project A3, number 01GS0834), the Munich Center of Health Sciences (MC Health) as part of LMIinnovativ, and by the Else Kröner-Fresenius-Stiftung (P48/08//A11/08; C.A.B. and B.K.K.). The kidney parameter measurements in F3 were funded by the Else Kröner-Fresenius-Stiftung (C.A.B. and B.K.K.) and the Regensburg University Medical Center, Germany; in F4 by the University of Ulm, Germany (W.K.). Genome-wide genotyping costs in F3 and F4 were in part funded by the Else Kröner-Fresenius-Stiftung (C.A.B. and B.K.K.). *De novo* genotyping in F3 and F4 were funded by the Else Kröner-Fresenius-Stiftung (C.A.B. and B.K.K.). The KORA research platform and the MONICA Augsburg studies were initiated and financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, by the German Federal Ministry of Education and Research and by the State of Bavaria. Genotyping was performed in the Genome Analysis Center (GAC) of the Helmholtz Zentrum München. The LINUX platform for computation was funded by the University of Regensburg for the Department of Epidemiology and Preventive Medicine at the Regensburg University Medical Center.

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Institute of Technology-Broad Institute (N01-HC-65226) to create this genotype/phenotype database for wide dissemination to the biomedical research community:

**Atherosclerotic Risk in Communities (ARIC):** University of North Carolina at Chapel Hill (N01-HC-55015), Baylor Medical College (N01-HC-55016), University of Mississippi Medical Center (N01-HC-55021), University of Minnesota (N01-HC-55019), Johns Hopkins University (N01-HC-55020), University of Texas, Houston (N01-HC-55022), University of North Carolina, Forsyth County (N01-HC-55018).

**Cardiovascular Health Study (CHS):** University of Washington (N01-HC-85079), Wake Forest University (N01-HC-85080), Johns Hopkins University (N01-HC-85081), University of Pittsburgh (N01-HC-85082), University of California, Davis (N01-HC-85083), University of California, Irvine (N01-HC-85084), New England Medical Center (N01-HC-85085), University of Vermont (N01-HC-85086), Georgetown University (N01-HC-35129), Johns Hopkins University (N01-HC-15103), University of Wisconsin (N01-HC-75150), Geisinger Clinic (N01-HC-45133), University of Washington (N01-HC-55222, U01 HL080295).

**Cleveland Family Study (CFS):** Case Western Reserve University (NIH HL 46380, M01 RR00080).

**Cooperative Study of Sickle Cell Disease (CSSCD):** University of Illinois (N01-HB-72982, N01-HB-97062), Howard University (N01-HB-72991, N01-HB-97061), University of Michigan (N01-HB-72992, N01-HB-97064), Duke University (N01-HB-72993), George Washington University (N01-HB-72994), University of Tennessee (N01-HB-72995, N01-HB-97070), Yale University (N01-HB-72996, N01-HB-97072), Children's Hospital-Philadelphia (N01-HB-72997, N01-HB-97056), University of Chicago (N01-HB-72998, N01-HB-97053), Medical College of Georgia (N01-HB-73000, N01-HB-97060), WA University (N01-HB-73001, N01-HB-97071), Jewish Hospital and Medical Center of Brooklyn (N01-HB-73002), Trustees of Health and Hospitals of the City of Boston, Inc. (N01-HB-73003), Children's Hospital-Oakland (N01-HB-73004, N01-HB-97054), University of Mississippi (N01-HB-73005), St. Luke's Hospital-New York (N01-HB-73006), Alta Bates-Herrick Hospital (N01-HB-97051), Columbia University (N01-HB-97058), St. Jude's Children's Research Hospital (N01-HB-97066), Research Foundation, State University of New York-Albany (N01-HB-97068, N01-HB-97069), New England Research Institute (N01-HB-97073), Interfaith Medical Center-Brooklyn (N01-HB-97085).

**Coronary Artery Risk in Young Adults (CARDIA):** University of Alabama at Birmingham (N01-HC-48047), University of Minnesota (N01-HC-48048), Northwestern University (N01-HC-48049), Kaiser Foundation Research Institute (N01-HC-48050), University of Alabama at Birmingham (N01-HC-95095), Tufts-New England Medical Center (N01-HC-45204), Wake Forest University (N01-HC-45205), Harbor-UCLA Research and Education Institute (N01-HC-05187), University of California, Irvine (N01-HC-45134, N01-HC-95100).

**Framingham Heart Study (FHS):** Boston University (N01-HC-25105, R01 HL092577-01A1, R01 HL076784, R01 AG028321).

**Jackson Heart Study (JHS):** Jackson State University (N01-HC-95170), University of Mississippi (N01-HC-95171), Tougaloo College (N01-HC-95172).

**Multi-Ethnic Study of Atherosclerosis (MESA):** University of Washington (N01-HC-95159), Regents of the University of California (N01-HC-95160), Columbia University (N01-HC-95161), Johns Hopkins University (N01-HC-95162, N01-HC-95168), University of Minnesota (N01-HC-95163), Northwestern University (N01-HC-95164), Wake Forest University (N01-HC-95165), University of Vermont (N01-HC-95166), New England Medical Center (N01-HC-95167), Harbor-UCLA Research and Education Institute (N01-HC-95169), Cedars-Sinai Medical Center (R01-HL-071205), University of Virginia (subcontract to R01-HL-071205).

**Sleep Heart Health Study (SHHS):** Johns Hopkins University (U01 HL064360), Case Western University (U01 HL063463), University of California, Davis (U01 HL053916), University of Arizona (U01 HL053938), University of Minnesota (relocating in 2006 to Univ Arizona) (U01 HL053934), University of Pittsburgh (U01 HL077813), Boston University (U01 HL053941), MedStar Research Institute (U01 HL063429), Johns Hopkins University (U01 HL053937).
**Stage 2 Cohorts:** AGES: The Age, Gene/Environment Susceptibility—Reykjavik Study has been funded by NIH contract N01-AG-12100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The researchers are indebted to the participants for their willingness to participate in the study.

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DCCT/EDIC: The DCCT/EDIC Research Group is sponsored through research contracts from the National Institute of Diabetes, Endocrinology and Metabolic Diseases of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institutes of Health. The authors are grateful to the patients in the DCCT/EDIC cohort for their long-term participation. A.D.P. holds a Canada Research Chair in the Genetics of Complex Diseases. This work has received support from National Institute of Diabetes and Digestive and Kidney Diseases Contract N01-DK-6–2204, National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-077510 and support from the Canadian Network of Centres of Excellence in Mathematics and Genome Canada through the Ontario Genomics Institute. Clinical data and DNA from the DCCT/EDIC study will be made available through the National Institute of Diabetes and Digestive and Kidney Diseases repository at https://www.niddkrepository.org/niddk/home.do. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases or the National Institutes of Health.

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KORCULA: This study was supported through the grants from the Medical Research Council UK; and Ministry of Science, Education and Sport of the Republic of Croatia (no. 108-1080315-0302) and the European Union framework program 6 EUROSPAN project (contract LSHG-CT-2006-018947). We would like to acknowledge the invaluable contributions of the recruitment team in Korcula, the administrative teams in Croatia and Edinburgh, and the people of Korcula. The albuminuria measurements were supported by grants from the Belgian agencies FNRS and FRSM (3.4.592.06F), the “Fondation Alphonse & Jean Forton”, the Programme d’excellence “Marshall” DIANE convention from the Region Walonne, the Interuniversity Attraction Pole (IUAP P6/05), and the Genecure (FP6) and EUNEFRON (FP7, GA#201590) programs of the European Community. We would also like to thank S. Druart and N. Amraoui for excellent technical assistance.

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**PREVEND**: PREVEND study is supported by the Dutch Kidney Foundation (Grant E033), The Netherlands Heart Foundation (Grant 2006B140, 2006T003), and the EU project grant GENECURE (FP-6 LSHM CT 2006 037697). P. van der Harst is supported by NWO VENI grant 916.76.170 and the ICIN.

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**SPLIT**: This study was supported through the grants from the Medical Research Council UK and Ministry of Science, Education and Sport of the Republic of Croatia (no. 108-1080315-0302). We would like to acknowledge the invaluable contributions of the recruitment team from the Croatian Centre for Global Health, University of Split, the administrative teams in Croatia and Edinburgh (Rosa Bisset), and the people of Split. The albuminuria measurements were supported by grants from the Belgian agencies FNRS and FRSM (3.4.592.06F), the “Fondation Alphonse & Jean Forton”, the Programme d’excellence “Marshall” DIANE convention from the Region Walonne, the Interuniversity Attraction Pole (IUAP P6/05), and the Geneucore (FP6) and EUNEFRON (FP7, GA#201590) programs of the European Community. We would also like to thank S. Druart and N. Amraoui for excellent technical assistance.

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CUBN Is a Gene Locus for Albuminuria


Footnotes

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REFERENCES


http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3060449/ [02/08/2013 15:07:32]
CUBN Is a Gene Locus for Albuminuria


**Figures and Tables**

**Table 1.**

Study sample characteristics in the CKDGen and CARe Consortia

<table>
<thead>
<tr>
<th>Study</th>
<th>n\textsuperscript{a}</th>
<th>Women % Age, years</th>
<th>UACR\textsuperscript{b}</th>
<th>MA % CKD % HTN % DM %</th>
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### Table

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<th>Study</th>
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<td>16.6</td>
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</table>

Data presented as means except where otherwise indicated. DM, diabetes; HTN, hypertension.

\(^a\) Refers to the successfully genotyped and analyzed sample with UACR or MA data, which may differ from the recruited sample described in Supplemental online Methods.

\(^b\) Median (interquartile range: 25th percentile, 75th percentile).

\(^c\) Refers to studies that are nonoverlapping with CKDGen; the sample size of ARIC, CHS, and FHS in CARe was 7687, 2073, and 6208, respectively.

**Figure 1.**

A

![Graph A](image)

B

![Graph B](image)
CUBN Is a Gene Locus for Albuminuria

Genome-wide log$_{10}$ $P$ value plot from stage 1 analyses of UACR. Participants of European ancestry in the CKDGen Consortium (A) and the CARe Consortium [IBC chip analyses; (B)].

Table 2.

Results for CUBN SNP rs1801239 on chromosome 10 in the CKDGen and CARe Consortia\(^a\) and DCCT/EDIC

<table>
<thead>
<tr>
<th></th>
<th>UACR $P$ Value(^b)</th>
<th>Microalbuminuria $P$ value(^c)</th>
<th>Odds Ratio (95% CI) for Clinical Outcomes per Copy of Minor C Allele</th>
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<td>Overall samples</td>
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<tr>
<td>CKDGen Stage 1 discovery</td>
<td>$3.0 \times 10^{-7}$</td>
<td>$8.7 \times 10^{-7}$</td>
<td>1.25 (1.15 to 1.37)</td>
</tr>
<tr>
<td>CARe IBC discovery in European Americans</td>
<td>$2.9 \times 10^{-10}$</td>
<td>$2.4 \times 10^{-7}$</td>
<td>1.31 (1.18 to 1.45)</td>
</tr>
<tr>
<td>CKDGen Stage 2 follow-up</td>
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<td>0.43</td>
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<tr>
<td>CARe African Americans</td>
<td>0.005</td>
<td>0.008</td>
<td>1.42 (1.10 to 1.84)</td>
</tr>
<tr>
<td>Diabetes stratified(^d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no diabetes</td>
<td>$3.2 \times 10^{-8}$</td>
<td>0.06</td>
<td>1.03 (1.00 to 1.07)</td>
</tr>
<tr>
<td>diabetes</td>
<td>0.006</td>
<td>$4.7 \times 10^{-4}$</td>
<td>1.27 (1.11 to 1.45)</td>
</tr>
<tr>
<td>Hypertension stratified(^e)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no hypertension</td>
<td>$1.3 \times 10^{-6}$</td>
<td>$6.0 \times 10^{-4}$</td>
<td>1.23 (1.09 to 1.38)</td>
</tr>
<tr>
<td>hypertension</td>
<td>$7.5 \times 10^{-8}$</td>
<td>$1.4 \times 10^{-7}$</td>
<td>1.34 (1.20 to 1.49)</td>
</tr>
<tr>
<td>DCCT/EDIC(^f)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simple model</td>
<td>0.02</td>
<td></td>
<td>1.42 (1.08 to 1.88)</td>
</tr>
<tr>
<td>extended model</td>
<td>0.02</td>
<td></td>
<td>1.41 (1.06 to 1.87)</td>
</tr>
<tr>
<td>Persistent MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simple model</td>
<td>0.53</td>
<td></td>
<td>1.14 (0.76 to 1.69)</td>
</tr>
<tr>
<td>extended model</td>
<td>0.67</td>
<td></td>
<td>1.10 (0.72 to 1.67)</td>
</tr>
<tr>
<td>Severe Nephropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simple model</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>extended model</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Sample sizes are as follows (microalbuminuria case numbers in parentheses): CKDGen Stage 1: 31,580 (3698); CARe European Americans: 19,499 (2100); CKDGen Stage 2: 27,746 (3313); CARe African Americans: 6981 (1159).

\(^b\)P values from CKDGen and CARe European and African American Stage 1 analyses are from the inverse variance weighted fixed effects method, and $P$ values from the CKDGen Stage 2 analyses, the combined analyses, and the hypertension- and diabetes-stratified analyses are from the sample size weighted $Z$ score method. In all analyses, the minor allele C showed an increase in UACR, direction consistent with the odds ratio for MA.

\(^c\)P values from inverse variance weighted fixed effects model.

\(^d\)Combined populations of European ancestry, includes CKDGen Stages 1 and 2 (after removal of ARIC, CHS, and FHS) and all five cohorts of CARe European Americans: 63,153 (7383 microalbuminuria cases).

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3060449/
Combined populations of European ancestry, includes CKDGen Stage 1 (after removal of ARIC, CHS, and FHS) and all five cohorts of CARe European Americans: 36,166 (4128 microalbuminuria cases).

Modeled as hazard ratio; \( n = 1304 \) including 318 cases of persistent microalbuminuria and 116 cases of severe nephropathy.

**Figure 2.**

Regional Association Plot for the \( CUBN \) gene region. CKDGen Consortium stage 1 analyses (A), CARe IBC SNP chip results in participants of European Ancestry (B) and African ancestry (C). \( \log_{10} P \) values are plotted versus genomic position (build 36). The lead SNP in each region is labeled. Other SNPs in each region are color-coded based on their LD to the lead SNP (LD based on the HapMap CEU and YRI for the participants of African ancestry; see color legend).
annotations are based on UCSC Genome Browser (RefSeq Genes, b36) and arrows indicate direction of transcription. Graphs were generated using the software SNAP (http://www.broadinstitute.org/mpg/snap/index.php). Regions vary between panels because there was no coverage beyond the CUBN gene on the IBC chip used to generate panels B and C.