

CUBN Is a Gene Locus for Albuminuria

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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.

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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 States¹ and 6% of adults from Germany.² 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.^{3–6} Moreover, the presence of albuminuria offers key prognostic information at each stage of decline in GFR.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.⁸ Heritability of albuminuria ranges from 0.16 to 0.49 in families enriched with hypertension or diabetes.^{9,10} Rare genetic variants are known to cause monogenic diseases featuring severe, nephrotic range proteinuria.¹¹ However, linkage or candidate gene studies have not reproducibly identified common genetic variants in association with lower levels of albuminuria.^{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,^{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 array¹⁴ 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 1.1×10^{-7} to 5.7×10^{-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 4.0×10^{-8} for UACR in the combined stage 1 and stage 2 analysis (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,¹⁵ FastSNP,¹⁶ and PolyPhen¹⁷). 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 ($P = 0.33$).¹⁸

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.¹⁹

CARE IBC SNP Array Stage 1

Concurrently, the CARE Consortium carried out a large densely tagged candidate gene screen using the IBC SNP ar-

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Table 1. Study sample characteristics in the CKDGen and CARE Consortia

Study	n ^a	Women %	Age, years	UACR ^b	MA %	CKD %	HTN %	DM %
Stage 1: GWAS								
Amish	744	45.0	42.8	3.8 (2.2, 6.1)	NA	1.2	11.6	0.7
ARIC	6525	53.1	63.1	3.9 (2.0, 7.7)	9.4	9.1	42.9	13.6
BLSA	354	46.9	69.8	7 (4.4, 11.0)	NA	15.5	53.7	14.4
CHS	1865	60.8	71.2	9.3 (5.3, 19.9)	23.0	16.0	61.2	13.7
Colaus	5311	53.2	53.4	5.1 (3.4, 9.1)	9.5	3.8	36.1	9.6
EPIC	2371	53.3	59.2	3.6 (1.5, 8.3)	8.1	NA	49.3	3.0
Fenland	1398	56.2	44.9	4.5 (3.2, 7.1)	5.5	0.9	18.9	1.4
Framingham Heart Study	6523	53.2	47.6	4.6 (2.6, 9.9)	9.6	3.4	27.4	5.3
KORA F3	1530	50.5	62.5	4.9 (2.1, 11.1)	11.7	10.8	41.1	10.3
KORA F4	1803	51.3	60.9	10.7 (7.0, 18.4)	20.9	7.0	20.9	9.2
Micros	503	56.3	46.0	NA	5.4	3.6	40.6	3.7
SHIP	2653	48.9	55.3	8.9 (5.0, 20.6)	25.3	8.5	54.2	12.4
Stage 2								
AGES	3196	57.9	76.4	2.66 (1.2, 7.0)	11.9	24.2	80.6	11.6
Amish	695	54.4	47.2	7 (4.3, 13.5)	NA	NA	21.0	11.5
ARIC	759	56.0	63.0	3.9 (2.1, 7.5)	7.6	7.7	39.3	13.1
GENOA	987	43.0	59.0	3.1 (1.7, 5.9)	6.4	10.7	73.5	15.8
Health ABC	211	27.0	73.9	13.9 (6.1, 49.6)	42.2	25.0	64.1	100.0
HUNT	2386	54.5	51.4	6.1 (4.4, 9.2)	8.6	4.7	43.4	4.0
KORA F3	1392	52.4	50.8	4.4 (1.9, 9.6)	10.9	5.5	28.6	4.5
KORA F4	1197	52.3	49.2	10.1 (6.6, 20.1)	23.7	5.9	13.4	4.0
KORCULA	863	63.9	56.3	4.6 (2.5, 10.4)	12.4	7.5	57.3	10.4
Nurses Health Study	4138	100.0	58.2	2.8 (1.7, 4.5)	3.6	10.4	36.1	4.4
PREVEND	7803	50.5	49.5	7.1 (4.8, 13.3)	16.7	6.4	33.5	3.7
SAPHIR Study	1707	37.3	51.4	3.8 (2.3, 8.2)	9.8	1.4	56.5	3.2
SORBS	877	58.7	48.0	6.8 (4.6, 13.5)	13.6	4.0	52.1	9.2
SPLIT	472	56.5	49.3	2.5 (1.3, 5.8)	7.8	3.6	39.4	5.1
ULSAM	1063	0.0	71.0	7.5 (4.9, 16.1)	15.9	15.2	74.0	11.0
CARE Consortium								
European Americans ^c								
CARDIA	1242	50.4	40.7	4.4 (3.3, 6.9)	4.5	0.53	9.3	5.0
MESA	2289	52.1	62.7	4.7 (3.1, 8.6)	9.6	9.7	38.7	6.3
African Americans								
ARIC	2256	64.5	61.7	2.7 (0.9, 9.6)	16.3	7.3	70.9	27.7
CARDIA	1089	56.3	39.5	4.3 (3.1, 7.4)	9.6	0.9	24.7	6.4
CHS	426	64.3	72.1	10.9 (5.0, 26.9)	31.9	18.4	68.2	24.4
Jackson Heart Study	1530	58.5	51.8	6 (4.0, 13.0)	17.8	5.9	58.8	16.1
MESA	1680	54.6	62.2	5.5 (3.1, 13.2)	16.6	8.5	59.8	17.8

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

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

^bMedian (interquartile range: 25th percentile, 75th percentile).

^cRefers to studies that are nonoverlapping with CKDGen; the sample size of ARIC, CHS, and FHS in CARE was 7687, 2073, and 6208, respectively.

ray¹⁴ 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 $P < 2.2 \times 10^{-6}$ 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 1.1×10^{-11} ($n = 63,153$; Table 2), with 0.15% of the UACR variance explained by rs1801239. The

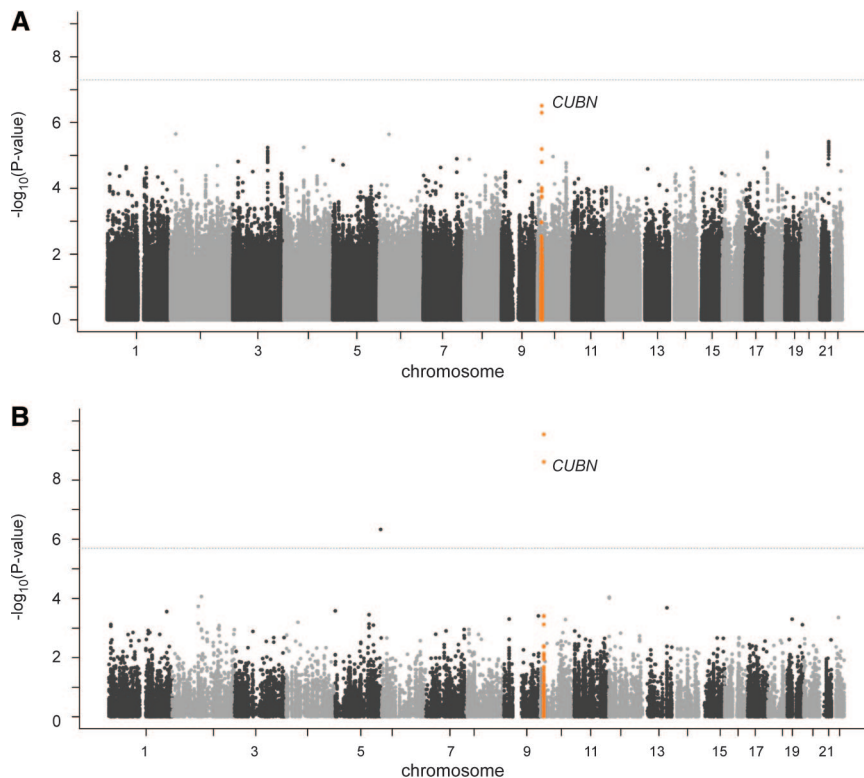


Figure 1. Genome-wide \log_{10} P value plot from stage 1 analyses of UACR. Participants of European ancestry in the CKDGen Consortium (A) and the CARE Consortium [IBC chip analyses; (B)].

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

Table 2. Results for CUBN SNP rs1801239 on chromosome 10 in the CKDGen and CARE Consortia^a and DCCT/EDIC

	UACR P Value ^b	Microalbuminuria P value ^c	Odds Ratio (95% CI) for Clinical Outcomes per Copy of Minor C Allele
Overall samples			Microalbuminuria (MA)
CKDGen Stage 1 discovery	3.0×10^{-7}	8.7×10^{-7}	1.25 (1.15 to 1.37)
CARE IBC discovery in European Americans	2.9×10^{-10}	2.4×10^{-7}	1.31 (1.18 to 1.45)
CKDGen Stage 2 follow-up	0.02	0.43	1.01 (0.98 to 1.05)
Combined populations of European ancestry ^d	1.1×10^{-11}	0.001	1.06 (1.02 to 1.09)
CARE African Americans	0.005	0.008	1.42 (1.10 to 1.84)
Diabetes stratified ^d			
no diabetes	3.2×10^{-8}	0.06	1.03 (1.00 to 1.07)
diabetes	0.006	4.7×10^{-4}	1.27 (1.11 to 1.45)
Hypertension stratified ^e			
no hypertension	1.3×10^{-6}	6.0×10^{-4}	1.23 (1.09 to 1.38)
hypertension	7.5×10^{-8}	1.4×10^{-7}	1.34 (1.20 to 1.49)
DCCT/EDIC ^f			
			Persistent MA
simple model		0.02	1.42 (1.08 to 1.88)
extended model		0.02	1.41 (1.06 to 1.87)
			Severe Nephropathy
simple model		0.53	1.14 (0.76 to 1.69)
extended model		0.67	1.10 (0.72 to 1.67)

^aSample sizes are as follows (microalbuminuria case numbers in parentheses): CKDGen Stage 1: 31580 (3698); CARE European Americans: 19,499 (2100); CKDGen Stage 2: 27,746 (3313); CARE African Americans: 6981 (1159).

^bP 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.

^cP values from inverse variance weighted fixed effects model.

^dCombined 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).

^eCombined 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).

^fModeled as hazard ratio; n = 1304 including 318 cases of persistent microalbuminuria and 116 cases of severe nephropathy.

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.²⁰ In the kidney, cubilin is expressed predominantly in the apical brush border of proximal tubular cells.²¹ We queried publicly available expression databases^{22–24} 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. *In vitro*, CUB domains 22 through 27 demonstrated Ca²⁺-dependent binding to megalin.²⁵ 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.²⁶ 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.²⁶ Yet urine in its final composition is nearly devoid of pro-

teins in healthy humans, highlighting the effectiveness of the tubular reabsorptive process to prevent significant protein loss.

The essential role of the cubilin-megalín complex in the reuptake of albumin by the proximal tubule has been demonstrated in both animal and human studies.^{26,27} For example, dogs with a functional defect in cubilin and mice lacking cubilin and/or megalin excrete large amounts of urinary albumin.^{27,28} 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 proteinuria²⁹ as a result of a molecular defect leading to inefficient proximal tubular protein reabsorption.

Dysfunction of the megalín-cubilin system has also been implicated in the pathogenesis of diabetic kidney disease. In rat models, renal tubular expression of cubilin is decreased³⁰ and reabsorption of filtered albumin by the proximal tubule has been shown to be altered.^{31,32} In humans, shedding of megalín and cubilin in urine is increased in individuals with type 1 diabetes and microalbuminuria compared with nonalbuminuric controls.³³ These studies suggest that reduced expression or loss of the cubilin-megalín complex may contribute to the albuminuria of early diabetic kidney disease via reduced tubular reuptake of filtered albumin, which is supported by our

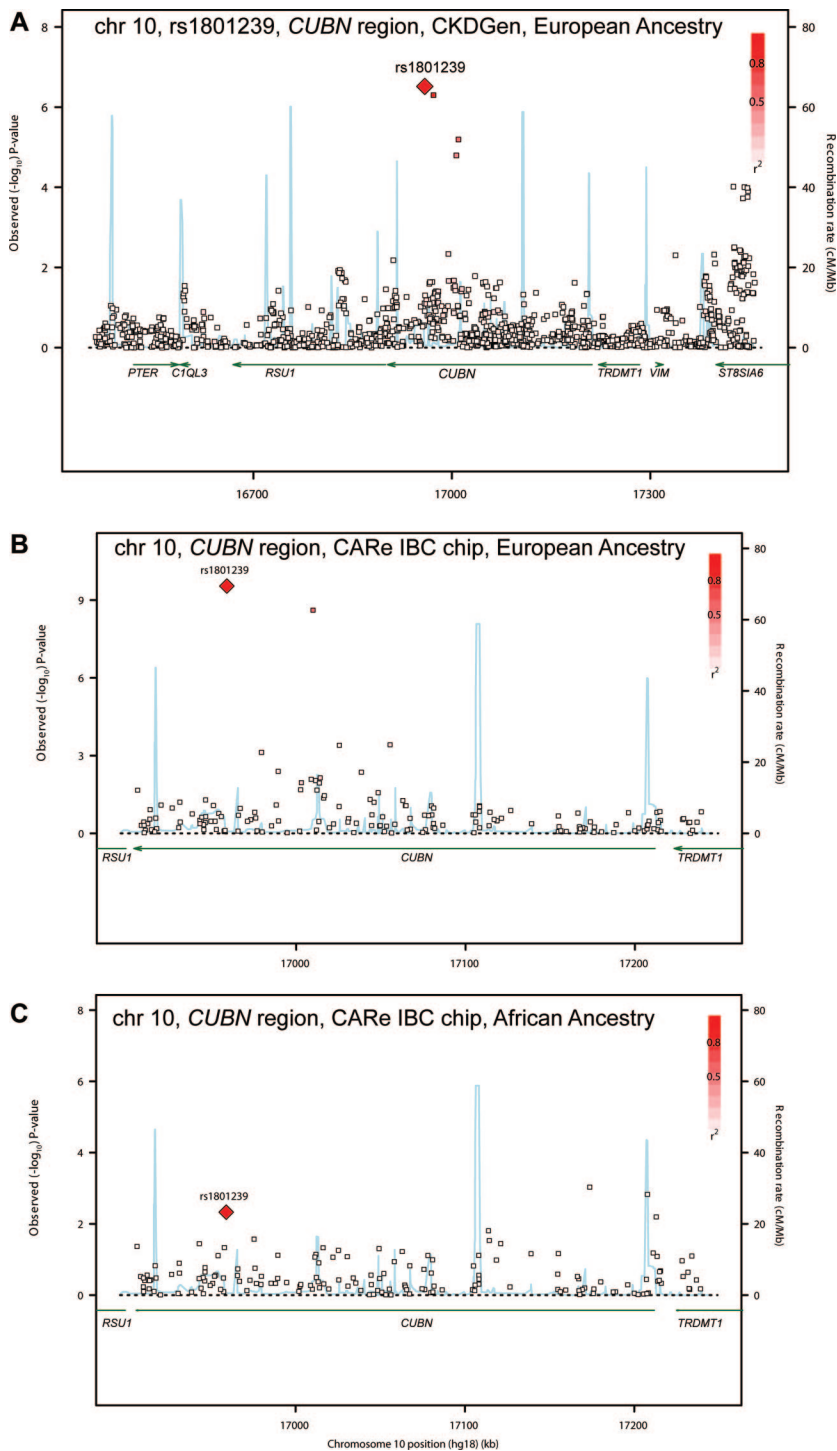


Figure 2. Regional Association Plot for the *CUBN* gene region. CKDGen Consortium stage 1 analyses (A), CARE IBC SNP chip results in participants of European Ancestry (B) and African ancestry (C). \log_{10} *P* values are plotted versus genomic position (build 36). The lead SNP in each region is labeled. 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). Gene 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.

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.^{34–36} 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.^{37–39}

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.¹⁴ 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^{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 previously¹⁸ to determine the prevalence of chronic kidney disease (defined as eGFR < 60 ml/min per 1.73 m²) 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 μ g/min), and (2) severe nephropathy, defined as the time to AER > 300 mg/24 h (> 208 μ g/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,¹⁴ a gene-centric array containing 50,000 SNPs tagging genes across a range of cardiovascular, met-

abolic, 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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