



Published in final edited form as:

Am J Kidney Dis. 2008 November ; 52(5): 868–875. doi:10.1053/j.ajkd.2008.02.306.

The association of podocin R229Q polymorphism with increased albuminuria or reduced estimated GFR in a large population-based sample of U.S. adults

Anna Köttgen, MD, MPH¹, Charles C. Hsu, PhD¹, Josef Coresh, MD, PhD¹, Alan R. Shuldiner, MD², Yvette Berthier-Schaad, PhD^{1,3}, Tejal Rami Gambhir, MPH¹, Michael W. Smith, PhD^{3,4}, Eric Boerwinkle, PhD⁵, and W. H. Linda Kao, PhD, MHS¹

¹Johns Hopkins University, Baltimore, MD

²University of Maryland, Baltimore, MD

³Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD

⁴Basic Research Program. SAIC- Frederick, NCI-Frederick, Frederick, MD

⁵University of Texas, Houston, TX

Abstract

Background—Rare mutations in nephrosis 2 (*NPHS2*), encoding podocin, are found in patients with familial and sporadic steroid resistant nephrotic syndrome and focal segmental glomerular sclerosis. The objective of this study was to assess the contribution of the commonly reported functional podocin polymorphism R229Q to kidney disease in the population-at-large and to replicate a prior study of an association of R229Q and albuminuria in the general population.

Study design—Large sample of the Atherosclerosis Risk in Communities (ARIC) Study, a population-based prospective study.

Setting and Participants—4424 white and 3746 black middle-aged adults

Predictor—Genotype at the R229Q polymorphism in podocin

Outcomes—Urinary albumin-to-creatinine ratio (ACR) and decreased estimated glomerular filtration rate (eGFR) as measures of kidney damage/dysfunction

Measurements—Crude and multivariable adjusted linear and logistic regression models

Results—The R229Q allele frequency was 3.7% in 4424 white and 0.6% in 3746 black individuals. No significant association of R229Q with increased ACR or decreased eGFR was observed (adjusted odds ratio of ACR ≥ 30 mg/g in RQ/QQ vs. RR carriers 1.18 (95% CI 0.76–1.84; adjusted odds ratio of eGFR < 60 ml/min/1.73m² in RQ/QQ vs. RR carriers 1.18 (95% CI 0.76–1.83). As expected, the established kidney disease risk factors hypertension and diabetes mellitus were strongly associated

Corresponding author: Linda Kao, PhD, MHS, Address: 615 N. Wolfe St., Room W6513, Baltimore, MD, 21205, USA, Phone: +1 (410) 614-0945, Fax: +1 (410) 955-0863, Email: wkao@jhsph.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Competing financial interest statement:

No financial conflicts of interest exist.

with measures of kidney damage/dysfunction, but the R229Q polymorphism was not associated with a further increase of kidney disease measures.

Limitations—Single measurement of ACR, sample of all ARIC participants

Conclusion—No significant association of the relatively rare R229Q variant and ACR or eGFR was found in either white or black individuals. The phenotypic effect of a variant such as R229Q would have to be of great magnitude in order to meaningfully contribute to the risk of kidney disease on a population level. The importance of such variants in the general population as well as replication studies can best be evaluated in large community-based studies that allow for accounting of established disease risk factors.

Index words

NPHS2; podocin; albuminuria; association study; functional variant; population-based sample

Introduction

The integral membrane protein podocin is located exclusively at the podocyte slit diaphragm, part of the glomerular filtration barrier.¹ Podocin is encoded by *NPHS2* on chromosome 1q25–31. Originally identified in families with an autosomal recessive form of early-onset steroid resistant nephrotic syndrome (SRNS),² mutations in *NPHS2* were also found in sporadic SRNS, familial and sporadic late-onset focal segmental glomerulosclerosis (FSGS), and non-diabetic end-stage renal disease.^{3–9} The polymorphism R229Q is one of the most commonly reported podocin sequence variations, and has repeatedly been found with slightly increased frequency in SRNS and FSGS cases compared to healthy controls.^{5, 8, 10} The arginine (R) residue at protein position 229 is highly conserved across species, and the arginine-to-glutamine substitution R229Q (p.229Arg>Glu, corresponding to the nucleotide substitution g.686G>A) has been reported to alter functional properties of podocin *in vitro*⁵ and possibly *in vivo*.¹¹

To assess the importance of the R229Q polymorphism for kidney disease on a population level, it is necessary to obtain estimates of the allele frequency in a large sample of the general population along with the assessment of the effect size and other factors possibly influencing this effect. Our study had 3 objectives: first, to estimate the frequency of the R229Q variant in a large sample from a population-based cohort of self-identified black and white U.S. individuals. Second, to evaluate the R229Q variant for crude and adjusted association with albuminuria, as a hallmark of SRNS and FSGS, and reduced estimated glomerular filtration rate (eGFR), as a measure of impaired kidney function. Third, to examine whether any association of the R229Q variant and a renal phenotype is modified by hypertension or diabetes mellitus (DM), two established major risk factors for kidney damage/dysfunction.

Methods

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective, population-based, ongoing study. From 1987–89, 15,792 adults aged 45 to 64 years were recruited from four U.S. communities (Forsyth County, NC; suburban Minneapolis, MN; Washington County, MD; and Jackson, MS). Participants underwent 4 standardized examinations approximately every 3 years, with the 4th examination from 1996–98. Further details of the study design have been reported previously.¹² In this study, a subsample of all ARIC participants was studied, consisting of all black participants (n = 4,095) and an equal number of white participants who had and had not developed DM by the 4th examination (n = 2,432 each). Persons not consenting to genetic research as well as those who reported race other than black or white were excluded from this study. Racial affiliation was reported as “black” or “white” and will be referred to as

such throughout our study. Resting, seated systolic (SBP) and diastolic (DBP) blood pressure measurements were recorded by certified technicians using a random-zero sphygmomanometer, and the average of the second and third readings was used. Body mass index (BMI) was calculated as measured weight (kg)/height (m²). Medication use was self-reported and verified by the inspection of medication bottles. For laboratory measurements, fasting blood samples were drawn, centrifuged, frozen, and shipped to ARIC study laboratories for analysis.¹³ Measurement of serum glucose, plasma high-density lipoprotein cholesterol (HDL), and triglycerides followed standard ARIC protocols.^{14, 15} Participants were considered as having DM if they reported a physician diagnosis of diabetes, the current intake of diabetes medication, or if they had a fasting glucose of ≥ 126 mg/dl (≥ 7 mmol/l) or non-fasting glucose of ≥ 200 mg/dl (≥ 11.1 mmol/l). Hypertension was defined as SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or use of antihypertensive medication. Prevalent coronary heart disease (CHD) was defined as evidence of previous myocardial infarction by electrocardiogram, history of physician-diagnosed myocardial infarction, and previous coronary reperfusion procedure.

Urinary albumin and creatinine levels were measured in the University of Minnesota Physicians Outreach Laboratories (Minneapolis, MN) from an untimed urine sample collected at the 4th examination (1996–98). Aliquots were frozen and stored within 12 hours at -70°C until they were thawed and albumin and creatinine were measured in 2003–04. Albumin levels were measured by a nephelometric method either on the Dade Behring BN100 (assay sensitivity, 2.0 mg/l) or on the Beckman Immage Nephelometer, and creatinine using the Jaffe method. The albumin-to-creatinine ratio (ACR) as a measure of albuminuria was calculated, and albuminuria was categorized as normoalbuminuria (ACR < 30 mg/g), microalbuminuria (ACR 30–299 mg/g), and macroalbuminuria (ACR ≥ 300 mg/g).¹⁶ Blinded samples (n=516) analyzed for quality assurance showed a correlation coefficient (r) of the log_e-transformed ACR as r=0.95. The estimated glomerular filtration rate (eGFR) was calculated using the four-variable Modification of Diet in Renal Disease (MDRD) Study equation¹⁷: $\text{eGFR (ml/min per } 1.73 \text{ m}^2) = 186.3 * \text{serum creatinine (mg/dl)}^{-1.154} * \text{age}^{-0.203} * 0.742 \text{ (if female)} * 1.21 \text{ (if black)}$. Serum creatinine was measured using a modified kinetic Jaffe reaction from plasma samples at the 4th examination. Creatinine values were calibrated using regression to the Cleveland Clinic laboratory, where the MDRD Study equation was developed.^{18, 19} Information on both genotype and ACR (eGFR) was available for 5765 (5807) of the ARIC participants that were genotyped in this study.

DNA isolation in the ARIC Study was performed by the central ARIC DNA laboratory using a standard extraction protocol with phenol chloroform, and stored at -80°C until use for genotyping. Genotyping was performed in two separate batches that were random samples of our overall study sample (n = 8,703). The first batch (n = 6,656) was genotyped using the Beckman UHT system, a single base extension assay, with a call rate of 90.4% and agreement between replicate pairs of 99.2%. Primers used were 5' - TGCAATTCCTTGTGCAAAC (5' at position g.710) and 5' - ATCTTGGGCGATGCTCTT (5' at position g.801). The polymerase chain reaction (PCR) step was performed using 2 ng of input DNA and 0.5 nM of each primer under standard cycling conditions, and single base extension step was performed using 10 μM of primer. The second batch (n = 2,213, including 166 replicate samples from batch 1) was genotyped using Taqman assay with a call rate of 93.4% and 100% agreement in the 166 replicate samples. Taqman assays were performed with the primers of 5'-GGCGATGCTCTTCCTCTCTAGAA and 5'-GCAATTCCTTGTGCAAACCACTATG which were used with the probes R allele = Vic- 5'-CCTAGCACATCGATCC and Q allele = Fam-5'-CTAGCACATCAATCC. The TaqMan reactions were performed in 384 well plates using 5 ng of input DNA, 1 μM of each primer and 0.5 μM of probe, with 50 cycles of polymerase chain reaction (PCR) at 59 $^{\circ}\text{C}$ annealing temperature.

All statistical analyses were conducted stratified by race. A dominant genetic model was assumed (RR vs. RQ/QQ) since only 4 white and no black participants were homozygous for the minor allele (QQ). Chi-squared- and t-tests were used to test differences of study characteristics by genotype. Because of the non-normal distribution, continuous ACR was analyzed as $\ln(\text{ACR})$. The crude association of genotype and ACR / eGFR was evaluated using simple regression analysis. In addition, multiple logistic (linear) regression models were used to evaluate the association of genotype and ACR ≥ 30 mg/g or eGFR < 60 ml/min/1.73m² (continuous ACR or eGFR) while controlling for demographic and cardiovascular risk factors for impaired kidney function that were identified a priori. Three regression models were explored: the crude model (model 1), a model adjusting for age, gender, and study center (model 2), and a multivariable model adjusting for age, gender, study center, hypertension, DM, BMI per 5 units increase, current smoking, prevalent CHD, HDL cholesterol per 10 mg/dl (0.26 mmol/l) increase, and $\ln(\text{triglyceride})$ levels (model 3). Models 2 and 3 were evaluated adjusted for hypertension and DM, as well as stratified on hypertension, DM, and the presence or absence of both. Calculations to assess the power our study would have to detect an association of the same magnitude than reported by a previous study²⁰ were performed *a priori* among white ARIC participants assuming the allelic frequencies and effect sizes reported from this prior study, an alpha of 0.05 and using Fisher's exact test. All analyses were conducted using Stata version 9.2 software.²¹

Results

Overall, genotyping was successful in 8170 of 8869 samples (92.1%). The distribution of genotypes within each race conformed to Hardy-Weinberg expectations in white and black individuals with normoalbuminuria (ACR < 30 mg/g) and those with eGFR ≥ 60 ml/min/1.73m² (eGFR ≥ 1.02 ml/s). Missingness for both ACR and eGFR was non-differential with respect to genotype distribution, and measures of kidney function were similar for persons in whom genotyping was or was not successful. In 4,424 white and 3,746 black ARIC Study participants, the minor allele frequency for the R229Q variant was 3.7% and 0.6%, respectively. The distribution of genotypes is presented in table 1.

Table 2 shows study characteristics by genotype at the 4th ARIC study visit, at which ACR was measured, for 3517 white study participants only because of the low frequency of the Q allele in black participants. None of the demographic and major risk factors for renal dysfunction evaluated differed significantly between carriers and non-carriers of the risk genotypes (RQ/QQ). In particular, both mean and categorical ACR and eGFR did not differ significantly between the groups (table 2). In black participants, neither ACR nor eGFR differed significantly between carriers and non-carriers of the risk genotype (p-value 0.9 for mean $\ln(\text{ACR})$ and 0.5 for categorical ACR, and 0.9 and 0.9 for mean eGFR and categorical eGFR, data not shown).

Supplementary table 1 shows the crude genotype distribution for black and white persons stratified for the presence of hypertension, DM, neither, and both. The prevalence of microalbuminuria was consistently higher in black compared to white persons, except in the group without hypertension and DM. In all subgroups containing individuals with the risk genotype and microalbuminuria, we observed a slight but insignificantly higher percentage of carriers of the risk genotype RQ/QQ with microalbuminuria compared to those with the reference genotype RR, except in the group of whites with both hypertension and DM (Suppl. table 1). On the other hand, the presence of hypertension, DM, or both was strongly associated with a higher proportion of individuals with micro- and macroalbuminuria for both RQ/QQ and RR carriers compared to those without hypertension and/or DM (p < 0.001 for each and in both races).

Table 3 summarizes results from crude and multivariable adjusted logistic regression of the presence of micro- or macroalbuminuria, $\text{ACR} \geq 30$ mg/g, and moderately or severely reduced kidney function, $\text{eGFR} < 60$ ml/min/1.73m² ($\text{eGFR} < 1.02$ ml/s), in 3,413 white individuals. Overall, the crude and adjusted odds of $\text{ACR} \geq 30$ mg/g were increased for carriers of the risk genotype, albeit not statistically significant (table 3). Results from crude and multivariable adjusted linear regression of $\ln(\text{ACR})$ considering the same covariates did not show a significant association with the risk genotype overall nor in any of the hypertension/DM subgroups. Carriers of the risk genotype also did not have significantly increased odds of $\text{eGFR} < 60$ ml/min/1.73m² overall or in any of the subgroups, and the risk genotype was not significantly associated with continuous eGFR in linear regression models. Sensitivity analyses that were stratified by study center were conducted to assess the adjusted odds ratios of $\text{ACR} \geq 30$ mg/g in risk genotype carriers (RQ/QQ) compared to reference genotype carriers (RR). In these analyses, the OR of $\text{ACR} \geq 30$ mg/g comparing carriers to non-carriers was 1.83 (95% CI 0.85–3.94, $p=0.12$) in Forsyth County, NC, 0.95 (95% CI 0.41–2.23, $p=0.91$) in Minneapolis, MN, and 0.95 (95% CI 0.45–1.98, $p=0.88$) in Washington County, MD. These odds ratios did not show statistical evidence for heterogeneity ($p=0.4$), however, the number of risk genotype carriers with $\text{ACR} \geq 30$ mg/g per study site was small ($n \sim 10$). Genotype counts and minor allele frequencies did not show a significant difference by study center among white ARIC participants ($p=0.9$). Figure 1 shows the odds ratios (ORs) of $\text{ACR} \geq 30$ mg/g and $\text{eGFR} < 60$ ml/min/1.73m² for white carriers of the risk genotype overall and stratified by hypertension and DM, neither, and both. The highest multivariable adjusted ORs of $\text{ACR} \geq 30$ mg/g were observed in the groups without evidence of hypertension and those with neither hypertension nor DM (1.47 (95% confidence interval (CI) 0.7–3.09) and 1.74 (95% CI 0.66–4.62), respectively).

Four white individuals in our study carried the QQ genotype, and two of them had information on ACR and eGFR at visit 4 available. In these persons, ACR was 2.1 and 26.1 mg/g and the corresponding eGFR was 78.7 ml/min/1.73m² (1.31 ml/s) and 110.0 ml/min/1.73m² (1.83 ml/s). These individuals would not be classified as having chronic kidney disease according to National guidelines.²²

Discussion

In our study of 8170 ARIC participants, the allele frequency of the podocin R229Q variant was 3.7% in white and 0.6% in black individuals. These are the first estimates from a large, population-based U.S. study. A prior study in the general Brazilian population reported Q allele frequencies of 2.8% in 1576 individuals.²⁰ Allele frequencies from smaller numbers of control individuals in prior SRNS and FSGS case-control studies ranged from 2–6% for persons of European and 0.5–2% for those of African descent.¹⁰ Our estimates therefore confirm and refine earlier reports of the frequency of the R229Q polymorphism, a relatively rare variant that is currently not included in the HapMap or dbSNP databases.^{23, 24}

We did not observe a significant crude or multivariable adjusted association of the R229Q risk genotype (RQ/QQ) with either continuous or categorical ACR, or continuous or categorical eGFR. However, the crude prevalence of microalbuminuria among black and white RQ/QQ carriers compared to RR carriers was consistently higher across most subgroups studied, albeit small and not statistically significant. This is the first large population-based study of the association of the podocin R229Q variant with kidney disease in black and white U.S. individuals. Our results differ from the study by Pereira *et al.* that found a significant association of microalbuminuria and the RQ genotype in the general Brazilian population.²⁰ A possible explanation for the differing results between our study and the Brazilian study could lie in the age of the individuals studied. The mean age of the ARIC participants at study visit 4, when ACR was measured, was 63 years, whereas the individuals in the study by Pereira *et al.* were

on average 19 years younger. The contribution of genetic effects would be expected to be stronger at younger ages, when less other risk factors for albuminuria are present, and this could potentially have influenced our results. Another possible explanation for the differences between the two studies could lie in undetected population substructure, which can be a problem particularly for rare alleles and in highly admixed populations such as the Brazilian population. The authors in the study of Pereira *et al.* did adjust for ethnicity (classified as “European descent, African descent, or mulatto,”²⁰ number of individuals within each group not provided) in their regression analyses, but we were unable to assess the possible contribution of undetected population structure on their results from the single, non-significant point estimate provided for the contribution of ethnicity to the association of microalbuminuria and the R299Q variant.

Power calculations performed *a priori* showed that our study has >95% power to detect an odds ratio of microalbuminuria in white RQ carriers compared to RR carriers of 2.77 as described by Pereira *et al.* However, as the initial description of a genetic association of weak or moderate hypothesized effect size most likely overestimates the strength of the real association,²⁵ we performed a retrospective power calculation to detect an OR of 1.21 for the association of genotype and albuminuria at the $\alpha=0.05$ level using Fisher’s exact test and the allele frequencies and number of individuals with albuminuria we found in white ARIC participants. The OR of 1.21 was selected as a realistic estimate for a weak genetic effect, and because it was the lower limit of the large 95% CI provided in the study by Pereira *et al.* These calculations showed that, despite the large sample size of 3486 white individuals who had information on albuminuria and genotype available, our study would have only had 15% power to detect such a weak genetic effect.

The occurrence of different variants within a gene, some of which rare and disease-causing and others more common and merely altering disease risk in the general population, has been observed for other heritable diseases such as DM or hypercholesterolemia.^{26, 27} Since our study was comprised of individuals aged 45–64 years at enrollment, we cannot exclude the possibility that an association might have existed in younger individuals, and carriers of the risk genotype did not survive to be enrolled in our study. Because of the low expected number of QQ carriers, our observation that RQ carriers on average did not differ from RR homozygotes, and similar allele frequency estimates from studies of younger individuals,¹⁰ this is unlikely to have influenced our results. On the other hand, our findings in older individuals make it unlikely that R229Q carriers develop disease susceptibility with increasing age.²⁸

Small effect sizes (OR <1.5) have been reported from several recent genome-wide association studies of common genetic variants and complex diseases.^{29, 30} While we cannot exclude such a weak association of the R229Q variant and microalbuminuria in the general population, supplementary table 1 shows that any such effect is very small compared to the large effect the presence of hypertension and/or DM has on the prevalence of micro- and macroalbuminuria regardless of R229Q genotype. Even with a highly prevalent condition such as microalbuminuria, the genetic effect of a relatively rare variant such as R229Q would clearly have to be larger in order to obtain a meaningful contribution to the risk of kidney disease on a population level. From our multivariable adjusted models, we have no evidence that the presence of either hypertension or DM strengthens the susceptibility to microalbuminuria in carriers of the risk genotype.

Our study has several limitations that deserve discussion: we assumed a dominant genetic model by grouping carriers of the RQ and QQ genotypes in our analyses. While this was done because of the low Q allele frequency, the information from the two QQ carriers does not suggest that an additive or recessive model would have been more appropriate. Previous studies

of FSGS and SRNS found cases homozygous, compound heterozygous, and heterozygous for the R229Q variant.^{5, 28} The white individuals in our study sample were selected as an equal number of those with DM by study visit 4 and those surviving to visit 4 without DM, which could potentially introduce survival bias. We have, however, no evidence for differential survival to study visit 4 by genotype. Urinary ACR values were only obtained at visit 4, limiting our study to be cross-sectional in nature. The single measurement of ACR could have introduced misclassification into our study, but analyses of continuous ACR yielded similar results. Due to the low Q allele frequency in black individuals, only crude distributions of the R229Q genotype by categories of albuminuria could be provided. Finally, we could not assess the importance of other polymorphisms in podocin to distinguish between R229Q heterozygous and compound heterozygous individuals, nor did we have information about mutations in genes coding for other slit diaphragm proteins possibly contributing to albuminuria via the same pathway.³¹

In summary, the frequency of the functional podocin R229Q variant in 8170 individuals from a population-based sample was 3.7% in white and 0.6% in black individuals. We found no evidence of a contribution of R229Q to kidney disease measured as increased albuminuria or reduced eGFR on a population level. Large community-based studies such as this are required to investigate the effect of relatively rare variants such as R229Q in the general population as well as to set any observed genetic associations with disease in relation to established disease risk factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abstract presented at the American Heart Association 47th Cardiovascular Disease Epidemiology and Prevention Annual Conference, Orlando, FL, February 2007. The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022. A. K. was supported by a Fellowship from the German Research Foundation. W.H.L.K. was supported by K01-DK-067207. This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400. This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The authors thank the staff and participants of the ARIC Study for their important contributions.

References

1. Roselli S, Gribouval O, Boute N, et al. Podocin localizes in the kidney to the slit diaphragm area. *Am J Pathol* 2002;160:131–139. [PubMed: 11786407]
2. Boute N, Gribouval O, Roselli S, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 2000;24:349–354. [PubMed: 10742096]
3. Caridi G, Bertelli R, Carrea A, et al. Prevalence, genetics, and clinical features of patients carrying podocin mutations in steroid-resistant nonfamilial focal segmental glomerulosclerosis. *J Am Soc Nephrol* 2001;12:2742–2746. [PubMed: 11729243]
4. Karle SM, Uetz B, Ronner V, Glaeser L, Hildebrandt F, Fuchshuber A. Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 2002;13:388–393. [PubMed: 11805166]
5. Tsukaguchi H, Sudhakar A, Le TC, et al. NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease-associated allele. *J Clin Invest* 2002;110:1659–1666. [PubMed: 12464671]

6. Caridi G, Bertelli R, Scolari F, Sanna-Cherchi S, Di Duca M, Ghiggeri GM. Podocin mutations in sporadic focal-segmental glomerulosclerosis occurring in adulthood. *Kidney Int* 2003;64:365. [PubMed: 12787432]
7. Caridi G, Bertelli R, Di Duca M, et al. Broadening the spectrum of diseases related to podocin mutations. *J Am Soc Nephrol* 2003;14:1278–1286. [PubMed: 12707396]
8. Weber S, Gribouval O, Esquivel EL, et al. NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. *Kidney Int* 2004;66:571–579. [PubMed: 15253708]
9. Dusel JA, Burdon KP, Hicks PJ, Hawkins GA, Bowden DW, Freedman BI. Identification of podocin (NPHS2) gene mutations in african americans with nondiabetic end-stage renal disease. *Kidney Int* 2005;68:256–262. [PubMed: 15954915]
10. Franceschini N, North KE, Kopp JB, McKenzie L, Winkler C. NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: A HuGE review. *Genet Med* 2006;8:63–75. [PubMed: 16481888]
11. Zhang SY, Marlier A, Gribouval O, et al. In vivo expression of podocyte slit diaphragm-associated proteins in nephrotic patients with NPHS2 mutation. *Kidney Int* 2004;66:945–954. [PubMed: 15327385]
12. The atherosclerosis risk in communities (ARIC) study: Design and objectives. the ARIC investigators. *Am J Epidemiol* 1989;129:687–702. [PubMed: 2646917]
13. National Heart. Operations manual 7: Blood collecting and processing. Bethesda, MD: National Heart, Lung and Blood Institute; 1988. Lung and Blood Institute Atherosclerosis Risk in Communities (ARIC) Study.
14. National Heart. Operations manual 8: Lipid and lipoprotein determinations. Bethesda, MD: National Heart, Lung and Blood Institute; 1987. Lung and Blood Institute Atherosclerosis Risk in Communities (ARIC) Study.
15. National Heart. Operations manual 10: Clinical chemistry determinations. Bethesda, MD: National Heart, Lung and Blood Institute; 1987. Lung and Blood Institute Atherosclerosis Risk in Communities (ARIC) Study.
16. Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: A position statement from kidney disease: Improving global outcomes (KDIGO). *Kidney Int* 2005;67:2089–2100. [PubMed: 15882252]
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. modification of diet in renal disease study group. *Ann Intern Med* 1999;130:461–470. [PubMed: 10075613]
18. Coresh J, Astor BC, McQuillan G, et al. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 2002;39:920–929. [PubMed: 11979335]
19. Manjunath G, Tighiouart H, Ibrahim H, et al. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol* 2003;41:47–55. [PubMed: 12570944]
20. Pereira AC, Pereira AB, Mota GF, et al. NPHS2 R229Q functional variant is associated with microalbuminuria in the general population. *Kidney Int* 2004;65:1026–1030. [PubMed: 14871423]
21. StataCorp. Stata statistical software: Release 9.0. College Station, TX: StataCorp.; 2005.
22. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39:S1–S266. [PubMed: 11904577]
23. Thorisson GA, Smith AV, Krishnan L, Stein LD. The international HapMap project web site. *Genome Res* 2005;15:1592–1593. [PubMed: 16251469]
24. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: The NCBI database of genetic variation. *Nucleic Acids Res* 2001;29:308–311. [PubMed: 11125122]
25. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med* 2002;4:45–61. [PubMed: 11882781]
26. Gudmundsson J, Sulem P, Steinthorsdottir V, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. *Nat Genet.* 2007

27. Kotowski IK, Pertsemlidis A, Luke A, et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am J Hum Genet* 2006;78:410–422. [PubMed: 16465619]
28. Caridi G, Perfumo F, Ghiggeri GM. NPHS2 (podocin) mutations in nephrotic syndrome. clinical spectrum and fine mechanisms. *Pediatr Res* 2005;57:54R–61R.
29. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 2007;445:881–885. [PubMed: 17293876]
30. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–678. [PubMed: 17554300]
31. Tryggvason K, Patrakka J, Wartiovaara J. Hereditary proteinuria syndromes and mechanisms of proteinuria. *N Engl J Med* 2006;354:1387–1401. [PubMed: 16571882]

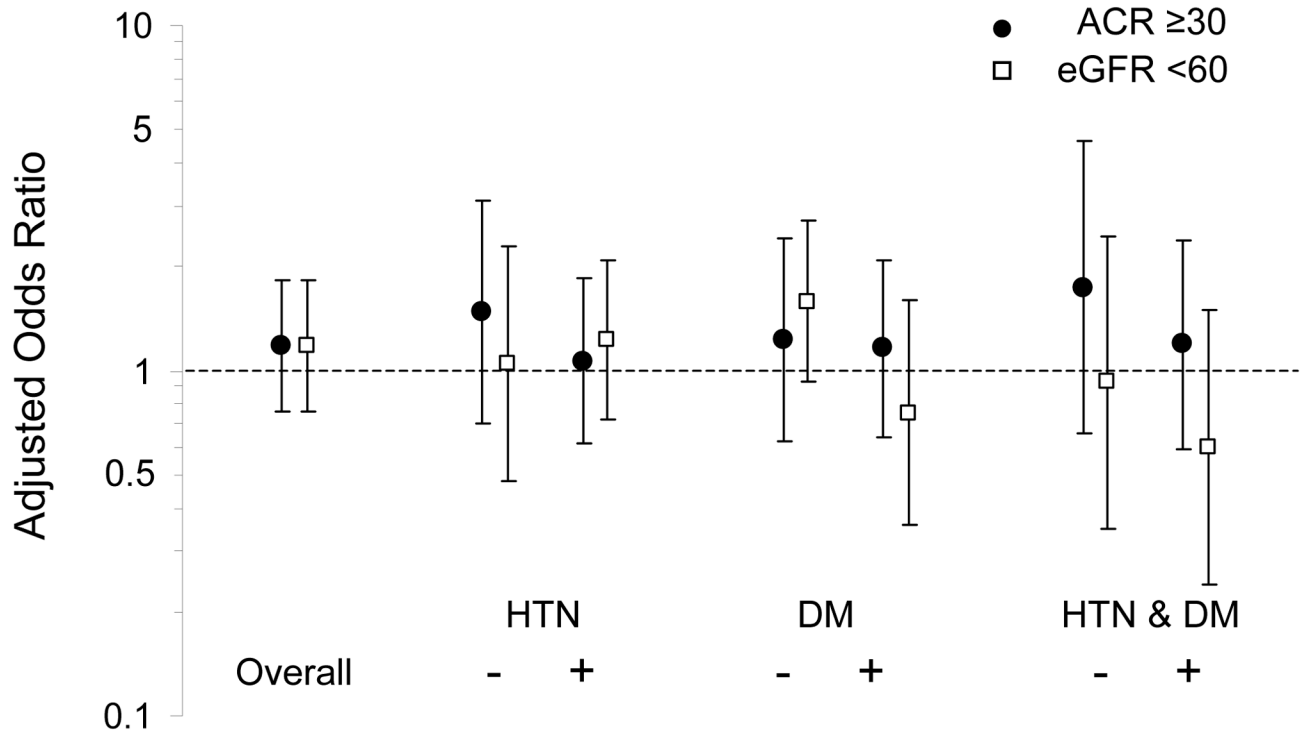


Figure 1. Odds ratios of ACR ≥30 mg/g (dots) and eGFR <60 ml/min/1.73m² (<1.02 ml/s) (squares) for white carriers of the risk genotype (RQ/QQ) overall and stratified by hypertension and DM, neither, and both

Numbers of individuals in each of the seven categories are, from left to right: for ACR (3385, 1767, 1618, 2341, 1044, 1345, 622), eGFR (3413, 1776, 1637, 2353, 1060, 1351, 635).

Abbreviations: ACR: albumin-to-creatinine ratio, eGFR: estimated glomerular filtration rate, HTN: hypertension, DM: diabetes mellitus

Table 1

Distribution of the podocin R229Q variant in 4,424 white and 3,746 black ARIC Study participants

	White	Black
	%(n)	%(n)
Genotypes		
RR	92.6% (4096)	98.8% (3703)
RO	7.3% (324)	1.2% (43)
OO	0.1% (4)	0% (0)
Alleles		
R	96.3%	99.4%
O	3.7%	0.6%

Exact p-values for testing Hardy-Weinberg equilibrium were 0.3 for white and 1.0 for black individuals with eGFR \geq 60 ml/min/1.73m² or ACR \geq 30 mg/g.

Table 2
Distribution of study characteristics in 3,517 white ARIC Study participants at visit 4 by genotype

	RR		RO/OO		
	mean (SD) / %	n	mean (SD) / %	n	p-value
Age (years)	63.5 (5.6)	3249	63.3 (5.9)	268	0.6
Male, %	48.6%	1580	46.6%	125	0.5
Hypertension, %	48.4%	1565	47.2%	126	0.7
Diabetes mellitus, %	30.7%	993	34.7%	93	0.2
BMI, kg/m ²	29.1 (5.5)	3245	28.9 (5.3)	268	0.6
Current smoking, %	13.3%	430	10.8%	29	0.3
Prevalent CHD, %	9.7%	308	11.5%	30	0.3
HDL, mg/dl	46.8 (15.6)	3243	46.7 (15.9)	267	0.9
Triglycerides, mg/dl	163.5 (99.4)	3243	159.1 (100.2)	267	0.5
ln(ACR), mg/g	1.6 (1.4)	3219	1.6 (1.5)	267	0.7
ACR <30	90.9%	2926	88.4%	236	
30-299	7.0%	225	9.4%	25	
>300	2.1%	68	2.3%	6	0.3
eGFR, ml/min/1.73m ²	80.9 (17.6)	3243	79.9 (17.6)	267	0.4
>90	33.2%	1078	30.0%	80	
60-89	57.5%	1863	59.6%	159	
<60	9.3%	302	10.5%	28	0.5

Data presented as mean and standard deviation (SD) for continuous and percentages for categorical variables. Sample size (n) is the total n for continuous and the number of affected individuals for categorical variables. Abbreviations: BMI: body mass index, CHD: coronary heart disease, HDL: high-density lipoprotein cholesterol, ACR: albumin-to-creatinine ratio, eGFR: estimated glomerular filtration rate, NC: Covariates missing: hypertension (13), diabetes (14), BMI (4), smoking (7), HDL (72), HDL (7), ACR (31), eGFR (7). To convert HDL in mg/dl to mmol/l, multiply by 0.0259, triglycerides in mg/dl to mmol/l, multiply by 0.0113; eGFR in ml/min/1.73m² to ml/s, multiply by 0.0167.

Table 3 Crude and adjusted logistic regression of ACR ≥ 30 mg/g and eGFR < 60 ml/min/1.73m² in white ARIC participants

	Model 1			Model 2			Model 3		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
<i>ACR ≥ 30 mg/g</i>									
RR (3128)	1.0 (Ref)			1.0 (Ref)			1.0 (Ref)		
RO/QO (257)	1.19	0.78–1.80	0.4	1.21	0.79–1.84	0.4	1.18	0.76–1.84	0.5
<i>eGFR < 60 ml/min/1.73m²</i>									
RR (3155)	1.0 (Ref)			1.0 (Ref)			1.0 (Ref)		
RO/QO (258)	1.12	0.74–1.71	0.6	1.14	0.74–1.76	0.5	1.18	0.76–1.83	0.5

Model 1: crude; Model 2: adjusted for age, gender, and study center; Model 3: adjusted for age, gender, study center, hypertension, diabetes mellitus, smoking, body mass index, prevalent coronary heart disease, HDL cholesterol, and triglycerides. Number of individuals provided with each genotype. eGFR < 60 ml/min/1.73m² corresponds to eGFR < 1.02 ml/s in SI units.