The Association of Podocin R229Q Polymorphism With Increased Albuminuria or Reduced Estimated GFR in a Large Population-Based Sample of US Adults

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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 is to assess the contribution of the commonly reported functional podocin polymorphism R229Q to kidney disease in the population at large and 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 & Participants: 4,424 white and 3,746 black middle-aged adults.

Predictor: Genotype at the R229Q polymorphism in podocin.

Outcomes: Urinary albumin-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: R229Q allele frequencies were 3.7% in 4,424 white and 0.6% in 3,746 black individuals. No significant association of R229Q with increased ACR or decreased eGFR was observed (adjusted odds ratio of ACR $\geq$ 30 mg/g in RQ/QQ versus RR carriers, 1.18; 95% confidence interval, 0.76 to 1.84; adjusted odds ratio of eGFR $<$ 60 mL/min/1.73 m^2 in RQ/QQ versus RR carriers, 1.18; 95% confidence interval, 0.76 to 1.83). As expected, the established kidney disease risk factors hypertension and diabetes mellitus were associated strongly with measures of kidney damage/dysfunction, but the R229Q polymorphism was not associated with an additional increase in kidney disease measures.

Limitations: Single measurement of ACR, subsample 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 as R229Q would have to be of great magnitude 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 be evaluated best in large community-based studies that allow for accounting of established disease risk factors.
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INDEX WORDS: NPHS2; podocin; albuminuria; association study; functional variant; population-based sample.

The integral membrane protein podocin is located exclusively at the podocyte slit diaphragm, part of the glomerular filtration barrier.[1] Podocin is encoded by nephrosis 2 (*NPHS2*) on chromosome 1q25.2. Originally identified in families with an autosomal recessive form of early-onset steroid-resistant nephrotic syndrome (SRNS),[2] mutations in *NPHS2* also were found in patients with sporadic SRNS, familial and sporadic late-onset focal segmental glomerulosclerosis (FSGS), and nondiabetic end-stage renal disease.[3-9] The polymorphism R229Q is one of the most commonly reported podocin sequence variations and repeatedly was found with slightly increased frequency in patients with SRNS and FSGS compared with healthy controls.[5,8,10] The arginine (R) residue at amino acid 229 is highly conserved across species, and the arginine-to-glutamine substitution R229Q (p.229Arg→Gln, corresponding to the nucleotide substitution c.686G→A [nucleotide numbering based on the translation initiation codon at

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position +1]), was 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 assessment of effect size and other factors possibly influencing this effect. Our study has 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 US individuals; second, to evaluate the R229Q variant for crude and adjusted association with albuminuria as a hallmark of SRNS and FSGS and decreased estimated glomerular filtration rate (eGFR) as a measure of impaired kidney function; and third, to examine whether any association of the R229Q variant and a renal phenotype is modified by hypertension or diabetes mellitus (DM), 2 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 to 1989, a total of 15,792 adults aged 45 to 64 years were recruited from 4 US communities (Forsyth County, NC; suburban Minneapolis, MN; Washington County, MD; and Jackson, MS). Participants underwent 4 standardized examinations approximately every 3 years, with the fourth examination from 1996 to 1998. Additional details of the study design were 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 fourth 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 is referred to as such throughout our study. Seated systolic and diastolic blood pressure measurements at rest were recorded by certified technicians using a random-zero sphygmomanometer, and the average of the second and third readings was used. Body mass index was calculated as measured weight (kg)/height (m^2). Medication use was self-reported and verified by 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, and triglycerides followed standard ARIC protocols.[14,15] Participants were considered to have DM if they reported a physician diagnosis of DM, current intake of DM medication, or had a fasting glucose level of 126 mg/dL or greater (≥7 mmol/L) or nonfasting glucose level of 200 mg/dL or greater (≥11.1 mmol/L). Hypertension was defined as systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, or use of antihypertensive medication. Prevalent coronary heart disease was defined as evidence of previous myocardial infarction by means of electrocardiogram, history of physician-diagnosed myocardial infarction, and previous coronary reperfusion procedure.

Urinary albumin and creatinine were measured in the University of Minnesota Physicians Outreach Laboratories (Minneapolis, MN) from an untimed urine sample collected at the fourth examination (1996 to 1998). Aliquots were frozen and stored within 12 hours at −70°C until they were thawed and albumin and creatinine were measured in 2003 to 2004. Albumin levels were measured by using a nephelometric method either on the Dade Behring BN100 (Dade Behring, Inc, Deerfield, IL; assay sensitivity, 2.0 mg/L) or Beckman Immage Nephelometer (Beckman Coulter, Inc, Fullerton, CA), and creatinine, by using the Jaffé method. Albumin-creatinine ratio (ACR) as a measure of albuminuria was calculated, and albuminuria was categorized as normoalbuminuria (ACR < 30 mg/g), microalbuminuria (ACR, 30 to 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 $r = 0.95$. eGFR was calculated using the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation[17]: eGFR (mL/min/1.73 m^2) = 186.3 × serum creatinine (mg/dL)$^{-1.154}$ × age$^{-0.203}$ × 0.742 (if female) × 1.21 (if black). Serum creatinine was measured by using a modified kinetic Jaffé reaction from plasma samples at the fourth examination. Creatinine values were calibrated using regression to the Cleveland Clinic Laboratory, where the MDRD Study equation was developed.[18,19] Information for both genotype and ACR (eGFR) was available for 5,765 (5,807) ARIC participants who 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 2 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-3′ (5′ at position c.641) and 5′-ATCTTGGGCGATGCTCTT-3′ (5′ at position c.732). The polymerase chain reaction step was performed using 2 ng of input DNA and primers in a concentration of 50 nmol/L under standard cycling conditions, and a single-base extension step was performed using 10 μmol/L of primer. The second batch (n = 2,213, including 166 replicate samples from batch 1) was genotyped using TaqMan assay (Applied Biosystems, Foster City, CA) with a call rate of 93.4% and 100% agreement in the 166 replicate samples. TaqMan assays were performed with the primers 5′-GGCGATGCTCTTCCTCTCTAGAA-3′ and 5′-GCAATTC-CTTGTGCAAACCACTATG-3′, which were used with the probes Vic-5′-CCTAGCACATC**G**ATCC-3′ (R allele) and Fam-5′-CTAGCACATC**A**ATCC-3′ (Q allele). TaqMan reac-