review

NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: A HuGE review

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Nephrotic syndrome, characterized by edema, proteinuria, hyperlipidemia and low serum albumin, is a manifestation of kidney disease involving the glomeruli. Nephrotic syndrome may be caused by primary kidney disease such as focal segmental glomerulosclerosis. Mutations in the podocin gene, *NPHS2*, have been shown in familial and sporadic forms of steroid-resistant nephrotic syndrome, including focal segmental glomerulosclerosis. Podocin is an integral membrane protein located at the slit diaphragm of the glomerular permeability barrier. Complete information is lacking for the population frequency of some *NPHS2* variants for all racial and ethnic groups. The most frequently reported variant, R229Q, is more common among European-derived populations than African-derived populations. We calculated crude odds ratios and 95% confidence intervals of childhood nephrotic syndrome and focal segmental glomerulosclerosis associated with R229Q heterozygosity using data from five studies. The R229Q variant is not associated with focal segmental glomerulosclerosis in the US population of African descent. In contrast, the R229Q variant is associated with a trend toward increased focal segmental glomerulosclerosis risk in European-derived populations, with an estimated increased risk of 20–40%. Our insight into the association between *NPHS2* variants and nephrotic disease is hampered by the limitations of the existing studies, including small numbers of affected individuals and suboptimal control groups. Nevertheless, the available data suggest that large epidemiological case-control studies to examine the association between *NPHS2* variants and nephrotic syndrome are warranted. ***Genet Med* 2006:8(2):63–75.**

Key Words: *FSGS, podocin, NPHS2, prevalence, nephrotic syndrome*

GENE

The *NPHS2* gene (OMIM number 604766) is located at chromosome 1q25-q31 and was first mapped by linkage analysis in families with autosomal recessive steroid-resistant nephrotic syndrome.[1] The *NPHS2* gene was subsequently identified by positional cloning.[2] The *NPHS2* coding region is 1,149 bp in length and is followed by a 635-bp 3′ UTR containing atypical polyadenylation signals (AATTAAA) situated 13 nt upstream of the poly (A) tail. The gene has 8 exons and encodes the 42 kD integral membrane protein podocin which is expressed in both fetal and mature kidney. Podocin is homologous to stomatins (band-7 proteins), sharing 46% amino acid identity to human stomatin and 40% identity to *MEC-2* and Unc1.[3] Stomatin is an integral membrane protein of unknown function. Defects in stomatin trafficking leading to its absence in red blood cell membranes cause hereditary stomatocytosis, a rare form of hemolytic anemia associated with transmembrane leak of sodium and potassium.[4] *MEC-2* mutations cause peripheral touch sensation dysfunction by interfering with a neuronal transduction mechanism associated with an ion channel.[4] Sequence analysis of podocin indicates that it consists of 383 amino acids, with cytosolic C- and N-terminal domains and one short transmembrane domain, forming a hairpin-like structure.[5] Similar to stomatins, podocin forms homo-oligomeric complexes that directly interact with cholesterol in specialized microdomains of the plasma membrane called lipid rafts.[6]

By in situ hybridization, podocin has been localized exclusively in glomeruli, with transcript expression restricted to the podocytes.[2] Podocin is located at the foot process of the podocytes in the slit diaphragm, the site responsible for size and charge selectivity of filtration.[7,8] It localizes to the lipid rafts which have high concentration of signal transduction molecules.[8,9] There, podocin interacts with nephrin and CD2AP, among other proteins in the slit diaphragm complex. Mutations of the nephrin gene, *NPHS1*, cause congenital nephrotic syndrome of the Finnish type, a form of severe nephrotic syndrome first manifested in utero and characterized by immature glomeruli and rapid progression to kidney

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failure.[10] Podocin is necessary for recruitment of nephrin into the lipid rafts.[9] In fact, some podocin mutations result in changes in the distribution of nephrin and other proteins in podocytes.[11] Homozygous mutations of podocin have been implicated in few cases of congenital nephrotic syndrome.[9,12,13] In addition, podocin-deficient *NPHS2* knock-out mice present with massive proteinuria at birth.[14] Thus, it is clear that podocin plays an essential role in the maintenance of the glomerular permeability barrier.

GENE VARIANTS

We searched Medline and PubMed using the combination of the keywords "*NPHS2*," "podocin," "genetics" and "epidemiology" to identify studies for the estimation of the prevalence of *NPHS2* polymorphisms in different populations. In addition, we searched the combination of terms "podocin," "*NPHS2*" and "nephrotic syndrome," "focal segmental glomerulosclerosis" or "steroid-resistant nephrotic syndrome" to identify population-based studies describing the association of the *NPHS2* gene and kidney disease. Papers published in English between 2000 and June, 2005 were reviewed since the gene was first identified in 2000. Reference lists from published articles were also reviewed. We also searched for relevant abstracts published by the American Society of Nephrology, International Society of Nephrology, European Renal Association and European Dialysis and Transplant Association, and the International Genetic Epidemiology Society from 2002 to November, 2004. Additional information regarding podocin variant frequencies in control subjects was obtained by direct contact with study authors. Unpublished data of the National Institutes of Health (NIH) FSGS Genetic Study were provided by three of the coauthors (JK, LM, and CW).

We selected for this review only studies that described the allele or genotype frequency of the *NPHS2* polymorphisms in a minimum of 40 control participants. Allele frequencies were obtained from published studies and the 95% confidence intervals (CI) were calculated using standard methods and using Excel software (Microsoft, Bellevue, WA).[15] Pooled allele frequencies and their 95% confidence intervals (CI) were calculated on the control sample stratified by ethnicity using standard methods[15] and SAS statistical sofware (SAS Institute, NC). When available, allele frequencies were used to calculate expected genotypic proportions according to Hardy-Weinberg equilibrium (HWE). Chi-square statistics were used to determine if the expected number of persons with a given genotype deviated from expectation. For the association analysis, all studies displaying deviations from HWE controls were excluded.

Most published studies included cases of individuals with steroid-resistant nephrotic syndrome (lacking a kidney biopsy) and biopsy-proven focal segmental glomerulosclerosis (FSGS). They described new mutations of the *NPHS2* gene in families or in individual cases (sporadic). Studies usually included ethnic-matched controls consisting of blood donors, spouses of affected individuals or other unrelated individuals defined as healthy controls or blood donors (Table 1). Since FSGS is uncommon and often symptomatic, it is likely that these control groups did not have FSGS. In general, studies described the allele frequency of polymorphisms and less often genotype frequencies.

Table 1 displays the allele frequencies of non-synonymous and synonymous variants in exons and introns observed in control subjects. Limited data are available on race and geographic distribution for many of the podocin alleles (Table 1). The majority of the non-synonymous minor alleles have allele frequencies $< 1\%$. Figure 1 shows the gene location of the polymorphisms. Four non-synonymous variants are present more frequently: R229Q (range: 0.5% to 7%), G34E (2% in Japanese), A61V (1.6% in African Americans (AA)) and A242V (4% African descent in Europe and 6 to 8.7% in AA). For those studies reporting HWE expectations, only one non-synonymous polymorphism was out of HWE. This study observed one 34E/E in 44 normal donors, but no heterozygotes were observed.[16] Among the six synonymous polymorphisms, one each is observed in exons 1, 2, and 7 and three occur in exon 8. The allele frequencies vary among racial/ethnic groups (range 0.03 to 50%) but tend to be more frequent than the non-synonymous polymorphisms. Polymorphisms in non-coding regions of the *NPHS2* gene have been reported in selected populations (Table 1).

R229Q in one of the most commonly reported *NPHS2* polymorphism (Table 1 and Table 2). A G to A nucleotide exchange at position 686 in exon 5 results in an arginine to glutamine substitution at codon 229. The allele frequency was reported in nine published studies and one large unpublished ongoing study at the National Institutes of Health (NIH). One study had controls recruited from a population-based source.[17] In general, the polymorphism is more common in people of European descent (allele frequency varies from 2 to 7%) and is less common in people of African descent from the US and Brazil (0.5 to 2.5%) (Table 1). The allele frequency in Africa and Asia is unknown. Based on available data, we estimated the R229Q minor allele frequency and 95% CI in controls by combining the number of alleles of individuals with similar ethnic background from different studies. Figure 2 shows the R229Q minor allele frequency in individual studies and the results of the pooled analysis of the allele frequency by ethnicity using the whole sample. These results confirm that the 'A' allele frequency is more common in populations European descent (0.03, 95% CI, 0.02–0.04) compared to African descent (0.016, 95% CI, 0.00–0.02).

Eight studies described the genotype frequency distribution of R229Q polymorphism (Table 2). All but one of the studies reporting genotype frequency for this polymorphism had more than 100 individuals tested. The genotype distribution of R229Q conformed to HWE in control populations, although three studies had a mixed ethnic population (Table 2). The estimated frequency of heterozygotes varied from 0.03 to 0.13 in Caucasians and it was 0.025 in a single study of African Americans (Table 2).

Table 1

Allele frequency of non-synonymous and synonymous *NPHS2* variants[a] and intronic variants in normal controls by geographic area and by study population

Amino acid exchange (nucleotide position)	Country	Race or ethnicity	Control source[e]	Number of individuals	Minor allele frequency proportion (95% confidence interval)	Reference
Non-synonymous changes						
Exon 1						
P20L (59C>T)	USA	European descent	Blood donors	282	0.004 (0.00–0.009)[c]	NIH[g], unpublished
	USA	African descent	Blood donors	634	Not observed	NIH, unpublished
	USA	African descent	Individuals without renal disease	96	0.005 (0.00–0.01)[b]	Dusel et al., 2005[18]
	France	French and African descent	Control cohort	160	Not observed	Weber et al., 2004[13]
	Italy	Italians	Blood donors	260	Not observed	Caridi et al., 2003[33] and personal communication
	Germany	Central Europeans, Turkish and Indians	Healthy control subjects	80	0.025 (0.00–0.05)[d]	Ruf et al., 2004[35]
G34E (101G>A)	Japan	Japanese	Unrelated healthy volunteers	44	0.02 (0.00 to 0.05)[d]	Maruyama et al., 2003[16]
G42R	USA	European descent	Blood donors	281	Not observed	NIH, unpublished data
	USA	African descent	Blood donors	634	0.014 (0.01 to 0.02)[c]	NIH, unpublished data
A44E (176C>A)	USA	African descent	Individuals without renal disease	96	0.005 (0.00 to 0.01)[b]	Dusel et al., 2005[18]
A61V (182C>T)	USA	African descent	Individuals without renal disease	96	0.016 (0.00 to 0.03)[b]	Dusel et al., 2005[18]
	France	French and African descent	Control cohort	160	Not observed	Weber et al.[13]
Exon 3						
R138Q (413G>A)	USA	European descent	Blood donors	272	0.002 (0.00 to 0.005)[c]	NIH, unpublished data
	USA	African descent	Blood donors	75	0.002 (0.00 to 0.009)[c]	NIH, unpublished data
	Germany	Northern Europeans	Healthy controls	100	Not observed	Karle et al., 2002[21]
Exon 5						
R229Q (686G>A)	USA	Africans and African descent	Controls	32	0.016 (0.01 to 0.05)[b]	Tsukaguchi et al., 2002[3]
	Brazil	Not reported	Controls from Brazil	49	0.031 (0.00 to 0.07)[b]	Tsukaguchi et al., 2002[3]
	USA/West Europe	Majority European	Individuals without kidney disease (spouses of FSGS patients)	124	0.036 (0.01 to 0.06)[b]	Tsukaguchi et al., 2002[3]
	USA	African descent	Blood donors, IV drugs abusers from the ALIVE cohort[h]	634	0.013 (0.007 to 0.02)[c]	NIH, unpublished data
	USA	European descent	Blood donors and healthy volunteers	272	0.039 (0.02 to 0.06)[c]	NIH, unpublished data
	USA	African descent	Individuals without renal disease	96	0.005 (0.00 to 0.01)[b]	Dusel et al., 2005[18]

Table 1
Continued.

Amino acid exchange (nucleotide position)	Country	Race or ethnicity	Control source[e]	Number of individuals	Minor allele frequency proportion (95% confidence interval)	Reference
	Brazil	African descent	Participants of the WHO- MONICA cohort study[f]	NR	0.025[b]	Pereira et al, 2004[17]
	Brazil	Mulatto		NR	0.049[b]	Pereira et al, 2004[17]
	Brazil	European descent		NR	0.069[b]	Pereira et al., 2004[17]
	Brazil	All races		1755	0.0276 (0.02 to 0.03)[c]	Pereira et al., 2004[17]
	Germany	Germans	Healthy controls	100	0.03 (0.006 to 0.05)[c]	Karle et al., 2002[21]
	Germany	Central Europeans, Turkish and Indians	Healthy control individuals	80	0.056 (0.02 to 0.09)[b]	Ruf et al., 2004[35]
	France	French and African descent	Control cohort	160	0.038 (0.02 to 0.06)[c]	Weber et al., 2004[13]
	Italy	Italians	Blood donors	260	0.02 (0.008 to 0.03)[c]	Caridi et al., 2003[33] and personal communication
	Italy	Italians	Blood donors	124	0.028 (0.007 to 0.05)[c]	Aucella et al., 2005[34]
	Sweden	Europeans, Asians, Maltese	Normal controls	60	Not observed	Koziell et al., 2002[12]
E237Q (709G>C)	USA	Europeans descent	Individuals without renal disease	96	Not observed	Dusel et al., 2005[18]
	France	French and African descent	Control cohort	160	0.003 (0.00 to 0.009)[c]	Weber et al., 2004[13]
A242V (725C>T)	USA	European descent	Blood donors	282	0.002 (0.00 to 0.006)	NIH, unpublished data
	USA	African descent	Blood donors	603	0.062 (0.05 to 0.08)[c]	NIH, unpublished data
	USA	African descent	Individuals without renal disease	278	0.087 (0.06 to 0.11)[b]	Dusel et al., 2005[18]
	France	French and African descent	Control cohort	160	0.006 (0.00 to 0.01)[c]	Weber et al., 2004[13]
	France	African descent	Control cohort	75	0.04 (0.008 to 0.07)[c]	Weber et al., 2004[13]
Synonymous changes						
Exon 1						
G34G (102G>A)	USA	African descent	Individuals without renal disease	96	0.15 (0.10 to 0.20)[b]	Dusel et al., 2005[18]
	Germany	Europeans	Healthy controls	100	Not reported[b]	Karle et al., 2002[21]
Exon 2						
S96S (288C>T)	USA	African descent	Individuals without renal disease	96	0.098 (0.06 to 0.14)[b]	Dusel et al., 2005[18]
	China	Han Chinese	Healthy controls	50	0.06 (0.01 to 0.11)[c]	Wu et al., 2001[19]
	China	Chinese	Unrelated volunteers	53	0.04 (0.003 to 0.08)[c]	Yu et al., 2005[39]
	Italy	Europeans	Blood donors	50–70	0.06[c]	Caridi et al., 2001[22]
Exon 7						
A297A	USA	African descent	Individuals without renal disease	96	0.016 (0.00 to 0.03)[b]	Dusel et al., 2005[18]

Table 1
Continued.

Amino acid exchange (nucleotide position)	Country	Race or ethnicity	Control source[e]	Number of individuals	Minor allele frequency proportion (95% confidence interval)	Reference
Exon 8						
A317A (951T>C)	Italy	Europeans	Blood donors	50–70	0.42[c]	Caridi et al., 2001[22]
A318A (954T>C)	USA	African descent	Individuals without renal disease	278	0.40 (0.36 to 0.44)[b]	Dusel et al., 2005[18]
	China	Han Chinese	Healthy controls	50	0.51 (0.44 to 0.58)[d]	Wu et al., 2001[20]
	Japan	Japanese	Unrelated healthy volunteers	44	0.43 (0.33 to 0.53)[c]	Maruyama et al., 2003[16]
	China	Chinese	Unrelated volunteers	53	0.42 (0.33 to 0.51)[c]	Yu et al., 2005[39]
	Germany	Europeans	Healthy controls	100	Not reported	Karle et al., 2002[21]
L346L (1038A>G)	USA	African descent	Individuals without renal disease	96	0.095 (0.05 to 0.14)[b]	Dusel et al., 2005[18]
	China	Han Chinese	Healthy controls	50	0.06 (0.01 to 0.11)[c]	Wu et al., 2001[20]
	Japan	Japanese	Unrelated healthy volunteers	44	0.00 (0.00 to 0.00)[c]	Maruyama et al., 2003[16]
	China	Chinese	Unrelated volunteers	53	0.04 (0.003 to 0.08)[c]	Yu et al., 2005[39]
	Italy	Italians/Europeans	Blood donors	50–70	0.03[c]	Caridi et al., 2001[22]
A1023G/	Israel	Arab-descent	Healthy unrelated individuals	40	Unable to calculate from study	Frishberg et al., 2002[40]
	Israel	Jewish-descent	Healthy unrelated individuals	40		Frishberg et al., 2002[40]
Untranslated regions#						
Promoter						
−2169A>T	USA	African descent	Individuals without renal disease	96	0.021 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
−1842C>G	USA	African descent	Individuals without renal disease	96	0.026 (0.00 to 0.05)[b]	Dusel et al., 2005[18]
−1709G>A	USA	African descent	Individuals without renal disease	96	0.108 (0.06 to 0.15)[b]	Dusel et al., 2005[18]
−1707delCT	USA	African descent	Individuals without renal disease	96	0.01 (0.00 to 0.02)[b]	Dusel et al., 2005[18]
−1628G>C	USA	African descent	Individuals without renal disease	96	0.069 (0.03 to 0.10)[b]	Dusel et al., 2005[18]
−1441G>A	USA	African descent	Individuals without renal disease	96	0.01 (0.00 to 0.02)[b]	Dusel et al., 2005[18]
−1376G>A	USA	African descent	Individuals without renal disease	96	0.01 (0.00 to 0.02)[b]	Dusel et al., 2005[18]
−1147delATCT	USA	African descent	Individuals without renal disease	96	0.021 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
−999T>A	USA	African descent	Individuals without renal disease	96	0.148 (0.10 to 0.20)[b]	Dusel et al., 2005[18]
−748C>T	USA	African descent	Individuals without renal disease	96	0.088 (0.05 to 0.13)[b]	Dusel et al., 2005[18]
−704G>A	USA	African descent	Individuals without renal disease	96	0.019 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
−670C>T	USA	African descent	Individuals without renal disease	96	0.236 (0.18 to 0.30)[b]	Dusel et al., 2005[18]

Table 1
Continued.

Amino acid exchange (nucleotide position)	Country	Race or ethnicity	Control source[e]	Number of individuals	Minor allele frequency proportion (95% confidence interval)	Reference
−556delCTTTTTT	USA	African descent	Individuals without renal disease	96	0.135 (0.09 to 0.18)[b]	Dusel et al., 2005[18]
−494G>A	USA	African descent	Individuals without renal disease	96	0.019 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
−486insA	USA	African descent	Individuals without renal disease	96	0.156 (0.10 to 0.21)[b]	Dusel et al., 2005[18]
−440T>C	USA	African descent	Individuals without renal disease	96	0.019 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
−364C>T	USA	African descent	Individuals without renal disease	96	0.021 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
−185T>C	USA	African descent	Individuals without renal disease	96	0.085 (0.05 to 0.12)[b]	Dusel et al., 2005[18]
−116C>T	USA	African descent	Individuals without renal disease	96	0.109 (0.06 to 0.15)[b]	Dusel et al., 2005[18]
5′ untranslated region						
−51G>T	China	Chinese	Unrelated volunteers	53	0.15 (0.08 to 0.22)[c]	Yu et al., 2005[39]
	USA	African descent	Individuals without renal disease	96	0.179 (0.12 to 0.23)[b]	Dusel et al., 2005[18]
−52C>G	USA	African descent	Individuals without renal disease	96	0.168 (0.12 to 0.22)[b]	Dusel et al., 2005[18]
Intron 1						
Sequence:	Italy	Italians	Blood donors	50–70	5 alleles	Caridi et al., 2001[22]
CACGCATGTTT					1=0.01	
ATAGCAGCACT					2=0.29	
CCTCTTCATGG					3=0.27	
CTGAGTAGC					4=0.34	
(301 to 309)					5=0.09	
Intron 3						
IVS3-46C>T	China	Chinese	Unrelated volunteers	53	0.06 (0.01 to 0.11)[c]	Yu et al., 2005[39]
	USA	African descent	Individuals without renal disease	96	0.06 (0.03 to 0.09)[b]	Dusel et al., 2005[18]
IVS3-21C>T	China	Chinese	Unrelated volunteers	53	0.06 (0.01 to 0.11)[c]	Yu et al., 2005[39]
	USA	African descent	Individuals without renal disease	96	0.06 (0.03 to 0.09)[b]	Dusel et al., 2005[18]
IVS3+9insA	USA	African descent	Individuals without renal disease	278	0.002 (0.00 to 0.006)[c]	Dusel et al., 2005[18]
IVS3-144C>T	USA	African descent	Individuals without renal disease	96	0.033 (0.01 to 0.06)[b]	Dusel et al., 2005[18]
IVS3-31T>C	USA	African descent	Individuals without renal disease	96	0.136 (0.09 to 0.18)[b]	Dusel et al., 2005[18]
Intron 4						
IVS4-98C>T	USA	African descent	Individuals without renal disease	96	0.021 (0.00 to 0.04)[b]	Dusel et al., 2005[18]
Intron 5						
IVS5+110T>A	USA	African descent	Individuals without renal disease	96	0.163 (0.11 to 0.22)[b]	Dusel et al., 2005[18]

Table 1
Continued.

Amino acid exchange (nucleotide position)	Country	Race or ethnicity	Control source[e]	Number of individuals	Minor allele frequency proportion (95% confidence interval)	Reference
Intron 7						
EX7+7A>G	Italy	Italians	Blood donors	50–70	0.04[c]	Caridi et al., 2001[22]
IVS7+7A>G	USA	African descent	Individuals without renal disease	96	0.01 (0.00 to 0.02)[b]	Dusel et al., 2005[18]
IVS7+132A>G	USA	African descent	Individuals without renal disease	96	0.016 (0.00 to 0.03)[b]	Dusel et al., 2005[18]
IVS7+261C>T	USA	African descent	Individuals without renal disease	96	0.01 (0.00 to 0.02)[b]	Dusel et al., 2005[18]
IVS7+304T>C	USA	African descent	Individuals without renal disease	96	0.01 (0.00 to 0.02)[b]	Dusel et al., 2005[18]
IVS7-74G>C	China	Chinese	Unrelated volunteers	53	0.04 (0.003 to 0.08)[c]	Yu et al., 2005[39]
	USA	African descent	Individuals without renal disease	96	0.095 (0.05 to 0.14)[b]	Dusel et al., 2005[18]
3′ untranslated region						
1206C>G	USA	African descent	Individuals without renal disease	96	0.40 (0.36 to 0.44)[b]	Dusel et al., 2005[18]
1309A>G	USA	African descent	Individuals without renal disease	96	0.116 (0.07 to 0.16)[b]	Dusel et al., 2005[18]
1352G>A	USA	African descent	Individuals without renal disease	96	0.058 (0.02 to 0.09)[b]	Dusel et al., 2005[18]
1410A>G	USA	African descent	Individuals without renal disease	96	0.20 (0.17 to 0.23)[b]	Dusel et al., 2005[18]
1580G>A	USA	African descent	Individuals without renal disease	96	0.144 (0.09 to 0.19)[b]	Dusel et al., 2005[18]
1589G>T	USA	African descent	Individuals without renal disease	96	0.013 (0.00 to 0.03)[b]	Dusel et al., 2005[18]

[a]A polymorphism was defined as an allele frequency equal or greater than 1% in at least one studied population.
[b]Reports Hardy Weinberg Equilibrium (HWE) but genotype frequency distribution not published.
[c]Can not reject null hypothesis of HWE based on published genotype frequency distribution, $P = 0.27–0.96$.
[d]Reject null hypothesis of HWE, $P < 0.001$ for P20L, $P = 0.002$ for G34E and $P = 0.02$ for A318A.
[e]Controls were ethnic matched unless stated otherwise.
[f]World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease, a cross-sectional study of risk factors for cardiovascular disease performed in Vitoria, Brazil.
[g]NIH, National Institutes of Health.
[h]ALIVE, AIDS Link to Intravenous Experience cohort study, Baltimore, MD.

Another non-synonymous polymorphism in exon 5 is A242V which results from a change of C to T at the nucleotide 725. A242V is more common in subjects of African descent in the US and Europe, with an allele frequency varying from 4 to 8.7%, and is uncommon in populations of European descent (Table 1). The genotype frequency of this polymorphism was described in only one European study.[13] Among 160 control subjects of French descent and African descent, 1% carried A242V C/T heterogosity, and 0% had the T/T genotype. In the 75 subjects of African descent, 8% had the C/T genotype and 0% had the T/T genotype.[13]

The G34E polymorphism results from a G to A nucleotide substitution at position 101 of exon 1 and has only been reported in one study from Japan.[16] The A61V polymorphism in exon 1 was described in a US study of African descent controls but was not observed in controls of African descent in France.[18,13]

The synonymous polymorphisms A318A, S96S and L346L were initially described in China[19,20] and later by others.[16,18,21] A138A results from a T to C nucleotide exchange at position 954 in exon 8. The minor allele frequency is similar among African-American and Asian populations. The S96S polymorphism is a result of a nucleotide change from C to T at position 288 on exon 2. This polymorphism has similar frequency among Europeans and Asians and higher allele frequency among subjects of African descent in the US. The L346L polymorphism is a product of an A to G nucleotide substitution at position 1038 in exon 8. The allele frequency is higher in those of African descent compared to Asians or Europeans.

The A44E, G34G and A297A polymorphisms have been described in a study of non-diabetic African-American patients with end-stage renal disease (ESRD).[18] A317A has only been described in an Italian study.[22]

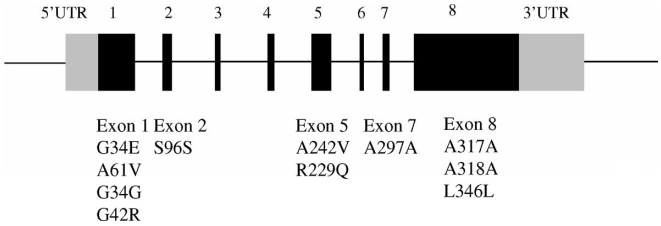

Fig. 1. Podocin polymorphisms (1% or more minor allele frequency in control populations) by gene exon. Exons are represented by boxes with corresponding numbers above and introns by lines between the exons. Non-synonymous and synonymous changes in podocin protein are shown below the exons. Exons are proportional to their lenght but introns and UTRs are not. For polymorphisms involving introns and untranslated regions refer to Table 1. Adapted from Dusel et al.[18]

A small number of studies have described polymorphism in untranslated regions including intronic positions (Table 1). Further studies are necessary to determine the allele and genotype frequencies across different population.

DISEASE

Idiopathic nephrotic syndrome and focal segmental glomerulosclerosis (FSGS)

Idiopathic nephrotic syndrome is characterized by edema, increased urinary protein excretion, hyperlipidemia and low serum albumin. Nephrotic syndrome results from a group of heterogeneous diseases affecting the glomeruli (the filtration units of the kidney). Nephrotic syndrome can be due to systemic diseases, such as diabetic mellitus and lupus, and to primary renal diseases, including minimal change nephropathy and focal segmental glomerulosclerosis (sporadic FSGS). In adults, sporadic FSGS is the most common forms of primary nephrotic syndrome in the US.[23] In children, who frequently do not undergo renal biopsy, nephrotic syndrome is classified based on response to therapy in steroid-sensitive (SSNS) and steroid-resistant nephrotic syndrome (SRNS). Most SSNS is due to minimal change nephropathy and most SRNS is due to FSGS.

Acquired forms of FSGS include post-adaptive FSGS (following renal adaptation consisting of glomerulomegaly and hyperfiltration), medication-associated FSGS (pamidronate, interferon-alpha, cyclosporine, tacrolimus, lithium) and idiopathic FSGS (unknown cause). Patients with FSGS may present with asymptomatic proteinuria or with edema, and may manifest nephrotic proteinuria (>3.5 g/day) or subnephrotic proteinuria.[24–26] Collapsing glomerulopathy is often considered to be a subset of FSGS, although its distinctive histology features argue for a separate classification. Compared to patients with FSGS, patients with collapsing glomerulopathy tend to have heavier proteinuria, more steroid-resistance, and worse prognosis. Acquired forms of collapsing glomerulopathy include HIV-associated collapsing glomerulopathy (also termed HIV-associated nephropathy), idiopathic collapsing glomerulopathy, and medication-associated collapsing glomerulopathy. Most papers have not distinguished between FSGS and collapsing glomerulopathy, and so in the present manuscript FSGS will often stand for both syndromes.

Individuals of African descent are at increased risk for FSGS (4-fold), HIV-associated collapsing glomerulopathy (14-fold), and idiopathic collapsing glomerulopathy (risk not determined) compared to European-Americans.[27] African-Americans with HIV-associated collapsing glomerulopathy are 5.4 times more likely to have a family history of ESRD compared to HIV controls without renal dysfunction (95% CI, 1.6, 18.4), suggesting a genetic risk for progressive renal injury in response to a range of renal insults.[28]

The diagnosis of FSGS is made by a renal biopsy. Because renal biopsy is rarely performed among patients presenting with low levels of proteinuria and in those with advanced kidney failure, the incidence and prevalence of FSGS are likely underestimated by studying renal biopsy data.[29] The annual

Table 2
Genotype frequencies for R229Q polymorphism in *NPHS2* by geographic area and by study population

Country	Race/ethnicity	Study sample	Genotype frequency			HWE[b] *P*-value	Frequency of heterozygotes	Reference
			GG	AG	AA			
USA	African descent	634	618	16	0	0.65	0.03	NIH[a], unpublished
USA	European descent	272	251	21	0	0.36	0.07	NIH, unpublished
USA	Western individuals	124	115	9	0	0.67	0.07	Tsukaguchi et al., 2002[3]
Brazil	European, African descent and mixed	1577	1491	85	1	0.99	0.05	Pereira et al., 2004[17]
Germany	Central Europeans, Turkish and Indians	80	71	9	0	0.18	0.13	Ruf et al., 2004[35]
Germany	Europeans	100	94	6	0	0.76	0.06	Karle et al., 2002[21]
France	French/African descent	160	148	12	0	0.62	0.08	Weber et al., 2004[13]
Italy	Italian Caucasians	260	252	8	0	0.80	0.03	Caridi et al., 2003[33] and personal communication
Italy	Italian Caucasians	124	117	7	0	0.75	0.05	Aucella et al., 2005[34]

[a] NIH, National Institutes of Health.
[b]HWE, Hardy-Weinberg Equilibrium.

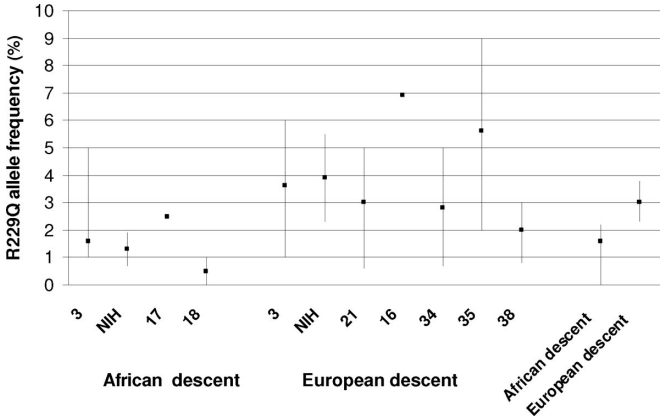

Fig. 2. Allele frequency of R229Q polymorphism in controls, by ethnic group. Results are the estimates and 95% confidence interval from individual studies and the pooled estimates and 95% confidence interval by ethnicity using combined data from the studies. Numbers under the x-axis are the studies' references. Studies included in the pooled analysis include patients of African descent (Tsukaguchi et al.[3](N = 32), National Institutes of Health cohort study (unpublished, N = 634), Pereira et al.[17] (N = not reported), Dusel et al.[18] (N = 96)), and European descent (Tsukaguchi et al.[3] (N = 124), National Institutes of Health cohort study (N = 272), Pereira et al.[17] (N = not reported), Karle et al.[21] (N = 100), Aucella et al.[34] (N = 124), Ruf et al.[35] (N = 80), Caridi et al.[38] (N = 260)). We were unable to calculate allele frequency confidence interval for the study by Pereira et al. because the number of screened individuals by ethnicity was not reported.

incidence of FSGS based on biopsy records is estimated to be 1.4 per million in Asia (Singapore), 2.3 to 12 per million in Europe and 21 per million in Oceania (Australia).[29] The incidence of the disease in the US is unknown.

FSGS has poor renal outcome, with approximately 50% kidney survival over 10 years.[30,31] The disease is currently the most common cause of incident ESRD due to primary glomerulonephritis. Glomerulonephritis are the third leading cause of ESRD in the US after diabetes and hypertension. FSGS compromises 2–3% of overall cases of incident ESRD in the US.[29] African-Americans may be at increased risk of progression to renal failure and are over-represented in ESRD groups. During the period 1995 to 1999, the annual incidence rate of ESRD due to FSGS was 7 cases/million for the general US population, with five cases per million in whites and 20 cases/million in African-Americans.[29] In addition, the highest annual incident rates of ESRD in African-Americans occur at younger ages compared to other races (ages 40–49 years for African-Americans and ages 70–79 years for other races), contributing for the increased burden of the disease in this ethnic group.[29] Risk factors associated with progression to ESRD are nephrotic syndrome and elevated serum creatinine at biopsy, lack of remission of the nephrotic syndrome (or resistance to therapy), and possible African descent.[32] Genetic factors associated with progression of FSGS have not been investigated.

GENOTYPE-PHENOTYPE CORRELATIONS

Association studies

NPHS2 polymorphisms associated with nephrotic syndrome and FSGS

Studies that report genotype frequency among affected individuals and controls were systematically identified (see section Gene Variants). The studies were designed to identify mutations of the *NPHS2* gene in affected families or individuals presenting with steroid-resistant nephrotic syndrome and/or focal segmental glomerulosclerosis (FSGS). Controls were selected from the same ethnic population of affected individuals in order to determine allele frequency of polymorphisms in the population. For the association analysis, we excluded studies that described multiple affected individuals in the same family (familial disease). Some studies have pooled data from individuals with familial and sporadic nephrotic syndrome. We included these studies in the analysis if the available data differentiated familial from sporadic cases. We calculated the crude odds ratio of disease and 95% CI by ethnicity according to Mantel-Haenszel method using SAS software.

Most studies have only described the R229Q polymorphism; thus insufficient data were available for consideration of other *NPHS2* polymorphisms in subsequent analyses. This polymorphism has been described in familial and sporadic SRNS and in FSGS (Table 3). Affected individuals are usually homozygous, compound heterozygous or heterozygous for the R229Q polymorphism.[3,13,21,33–35] Compound heterozygous individuals have a second mutation in the *NPHS2* gene. However, heterozygous and homozygous R229Q genotypes have also been described in unaffected individuals.[13,17] The arginine residue at position 229 is highly conserved in sequence analyses from *NPHS2* orthologs. In addition, in vitro studies have shown that the R229Q polymorphism may cause reduced binding to nephrin.[3] Therefore, this polymorphism is a likely candidate for genetic susceptibility to diseases presenting with proteinuria.

Seven studies met the selection criteria (Table 3). Most of these studies included primarily Caucasian individuals, with exception of the unpublished NIH study that included individuals of both African and European descent. Four European studies included children with SRNS, of which most had FSGS at renal biopsy. Three studies included adults with FSGS. Because few individuals had the A/A genotype among cases and controls, we estimated the risk of disease for individuals heterozygous (G/A) or compound heterozygous for the R229Q variant and some other *NPHS2* variant.

Table 3 shows the study characteristics and the risk estimates of disease for R229Q heterozygosity. The study by Karle et al. did not find any R229Q heterozygous among 25 children with nonfamilial SRNS from Europe.[21] The authors expanded the analysis to 165 families with SRNS and 120 families with SSNS, mostly of European descent.[35] Controls were healthy individuals. Combined results of homozygous, heterozygous and compound heterozygous were reported and, therefore, we were unable to calculate an estimate of the disease risk from this study.

Five of the above studies had complete data or at least one individual with the G/A genotype in cases and controls for association analysis. The number of individuals with the G/A genotype was small among the cases and controls and estimates were imprecise as reflected by wide confidence intervals. Caridi et al. studied 120 Italian children with sporadic SRNS and 59 with sporadic SSNS, most of them with FSGS.[33] Controls were blood donors. Weber et al. studied 172 children with

Table 3
Findings from studies of the relation between *NPHS2* polymorphism R229Q (686G/A) and nephrotic syndrome or focal segmental glomerulosclerosis

		Cases		Controls		Crude odds ratio		
Country/ethnicity	Phenotype	G/G *n*	G/A *n*	G/G *n*	G/A *n*	Disease	OR[a] (95% CI)	References
Europe								
Germany/mostly Germans, other Europeans	SRNS[b]; median age 3 years (range 0.1 to 16.6 years)	25	0	94	6	SRNS	Unable to calculate	Karle et al., 2002[21]
Italy/Caucasian Italians	SRNS and SSNS[b]; age of 70 months (range 1 to 216); 71% were FSGS	113 54	7 5	95	5	SRNS SSNS Combined	1.2 (0.4 to 3.8) 1.8 (0.5 to 6.4) 1.4 (0.5 to 4.0)	Caridi et al., 2003[33]
France/Europe (mainly France) and North Africa	SRNS; mean age was 103 months; 66% were FSGS	158[d]	11	148	12	SRNS	0.9 (0.4 to 2.0)	Weber et al., 2004[13]
Germany/ Central Europeans, Turkish and Indians	SRNS from 165 families (median age 3.5 years); SSNS from 120 families (median age 4.4 years)	177 118	13[e] 6[e]	71	9	SRNS SSNS	[c]	Ruf et al., 2004[35]
Italy/ Italians	Adult-onset FSGS[b], mean age 36 years (range 12–57)	30	3	117	7	FSGS	1.7 (0.8 to 6.9)	Aucella et al., 2005[34]
United States of America								
USA/Northeast US	Adults with FSGS (63 Caucasians, 17 African American, 11 Hispanics)	80	911[g]	115	9[h]	FSGS FSGS[g]	1.4 (0.5 to 3.8)[f] 1.8 (0.7 to 4.4)[f]	Tsukaguchi et al., 2002[3]
USA	Adult FSGS African descent	243	5	618	16	FSGS	0.8 (0.3 to 2.2)	NIH, unpublished
USA	Adult FSGS European descent	118	12	251	21	FSGS	1.2 (0.6 to 2.5)	NIH, unpublished

[a]OR, odds ratio; CI, confidence interval. Estimates were calculated using published data.
[b]SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; FSGS, focal segmental glomerulosclerosis.
[c]Unable to calculate due to unknown frequency of heterozygous by family.
[d]Two individuals with A/A alleles and one compound heterozygote were excluded from the case strata.
[e]Combined homozygous, heterozygous or compound heterozygous individuals.
[f]Results may be confounded by population admixture.
[g]Two of 11 were compound heterozygous.
[h]Controls from the Western panel of DNA samples.

sporadic SRNS mainly from France but also included a few children from North Africa.[13] Controls were individuals of French and African descent. The odds of sporadic SRNS were 0.9 (95% CI 0.4–0.9) and 1.2 (95% CI 0.4–3.8), respectively, compared to controls in the two above described European studies but 1.8 (95% CI 0.5–6.4) for children with SSNS.[33,13]

One European study and two US studies described podocin variants among adult patients with sporadic FSGS. In the Italian study, blood donor controls were screened for the R229Q polymorphism.[34] The study by Tsukaguchi et al. had ethnic matched controls screened for podocin variants. The NIH cohort study recruited patients with biopsy-proven FSGS from 22 medical centers throughout the US, in two subcohorts of European and African descent (unpublished). Cases of HIV-1 associated collapsing FSGS were included in the African American cohort. Control subjects included African American HIV positive subjects without renal disease after at least eight years of exposure recruited from the AIDS Link to Intravenous Experience cohort (ALIVE) and randomly recruited blood donors from the NIH. The European descent controls were blood donors from the NIH and healthy volunteers.

FSGS disease risk estimates for R229Q heterozygosity are shown in Table 3. In adults with sporadic FSGS, the risk of disease was increased by 20 to 70% in studies that included mainly Caucasian patients, although the results were not statistically significant (Table 3). The highest risk was found in the Italian study that had a small number of cases (OR 1.7). Larger studies of Caucasians from the US showed a smaller risk although the estimated risk of one of the studies could be confounded by population admixture.[3] Estimates including compound heterozygous individuals were higher compared to those including individuals without a second *NPHS2* mutation (Table 3). R229Q heterozygosity was not associated with increased risk of FSGS among those of African descent in the NIH study, the only one to include this population.

The results reported here were from unadjusted analysis and should be interpreted with caution. Several important potential biases should be considered including population admixture, selection bias in control participants and lack of important data to evaluate confounding. For example, some studies included persons of different ethnicity.[3,13,35] Moreover, although populations of African descent are at higher risk of

developing FSGS, the majority of the studies included mainly Caucasian populations.[3,34] In addition, studies were not designed to address the effect of the polymorphism on disease and therefore, controls were not always selected from population-based sources.[3,13,21,33,34] Data on potential environmental confounders were also not available.

Overall, the available studies were not designed to answer the question of the role of *NPHS2* gene polymorphisms on genetic susceptibility to disease. Based on the available data, it is likely that R229Q polymorphism in isolation is not associated with FSGS in US populations of African descent. In contrast, European descent populations appear to have a non-significant increased risk of FSGS by 20 to 70%. Further studies with larger number of patients are required to answer this question. Small number of affected individuals and inadequate controls are some of the problems that need to be addressed in future case-control studies of this variant and the disease. Clearly, more large case-control studies are warranted and will be required to properly evaluate the association between *NPHS2* gene variants and FSGS and other podocyte diseases.

Other related traits

One study has investigated the association of the polymorphism R229Q with microalbuminuria.[17] Microalbuminuria, defined as the daily urine albumin excretion of 30–300 mg, affects 5% of the general population and is a marker of increased glomerular capillary permeability. In diabetic subjects microalbuminuria is associated with the subsequent development of overt renal disease, but microalbuminuria does not precede FSGS. Microalbuminuria also correlates with extra-renal endothelial dysfunction and is a risk factor for cardiovascular disease. Subjects, aged 25 to 64, were recruited from a cross-sectional study of risk factors for cardiovascular disease in Vitoria, Brazil.[17] Eighty-five of 1577 individuals were heterozygous for R229Q and one was homozygous for R229Q. Eighty-five heterozygous individuals were compared to 1491 subjects without the polymorphism. Individuals with R229Q had similar age and gender distribution to those without it. Only 1027 individuals had information regarding microalbuminuria, defined as a urine albumin higher than 2 mg/dL. The odds ratio of microalbuminuria was 2.8 (95% CI 1.2, 6.3) for those with the allele R229Q compared to those without it, adjusting for age, ethnicity, hypertension, obesity, and diabetes.[17] The increased risk of microalbuminuria was present in obese subjects but not in non-obese patients. This study failed to consider other *NPHS2* polymorphisms in the analysis. In addition, although they have reported differences in the allele frequency among different ethnic groups, the study used pooled data from subjects of different racial backgrounds in their analysis.

A recent published case-control study compared *NPHS2* variants among African-American non-diabetic ESRD patients to population-based controls.[18] The R229Q polymorphism was not identified among 96 cases. The majority of the variants were not associated with ESRD but an intronic variant, IVS3 + 9insA, present in 10 of 288 cases and 1 of 278 controls (allele frequency in controls of 0.002), was significant associated with ESRD ($P = 0.02$).[18] Haplotype analysis was conducted. The haplotype of 5 SNPs tagging this intronic variant was associated with non-diabetic ESRD ($P = 0.012$). The most common polymorphism was the A242V variant (allele frequency of 0.087 in controls) and it was not associated with ESRD. Given the low allele frequency of IVS3 + 9insA in African-American controls, it is unlikely that this variant is a major cause of ESRD. Nonetheless, these data represent interesting preliminary data and demonstrate the importance of conducting much larger case-control studies so that rare variants may be considered with adequate power to detect genetic effects.

GENE-GENE INTERACTIONS

There are no population-based studies of gene-gene interaction including *NPHS2* polymorphisms and nephrotic syndrome. However, four studies described combined mutations or polymorphisms of the *NPHS2* and *NPHS1* genes. The *NPHS1* gene, mapped at chromosome 19q13.1, encodes nephrin, a podocyte protein. Nephrin and podocin may interact directly or indirectly and are important for maintenance of the glomerular capillary permeability barrier. Koziel et al. examined the genotype/phenotype correlation of *NPHS1* and *NPHS2* gene mutations in patients with congenital nephrotic syndrome.[12] Four patients were found to have *NPHS1* and *NPHS2* mutations. Patients had either homozygous mutations in *NPHS1* and heterozygous mutation in *NPHS2* or homozygous mutations in *NPHS2* and heterozygous mutation in *NPHS1*. The two *NPHS2* homozygous mutations were 436delA frameshift and R138Q. One patient had R229Q heterozygous *NPHS2* polymorphism in association with a homozygous aberrant splicing mutation of nephrin. Parents that were compound heterozygous carriers of the *NPHS1* and *NPHS2* mutations were phenotypically normal.[12] Others have also described SRNS associated with homozygous *NPHS2* R138Q mutations and a heterozygous splice mutation of *NPHS1*[13] and compound heterozygous A297V/R229Q of *NPHS2* and heterozygous mutations of *NPHS1*.[33] However, one study has described a lack of genotype/phenotype correlation among five patients with *NPHS1* and *NPHS2* mutations; although most of the patients had the R229Q variant.[36] Therefore, triallelic mutations of podocin and nephrin may exhibit a phenotype that resembles the phenotype of nephrin homozygotes, although this remains controversial.

There are no population studies of gene-environment interaction including the *NPHS2* gene. A study of genetic polymorphism in subjects of African descent with FSGS and collapsing glomerulopathy is currently ongoing in the US (Kopp et al., unpublished).

LABORATORY TESTS

The extraction of genomic DNA from peripheral blood cells was completed using standard techniques.[2] Mutational analysis was usually performed by direct resequencing of the two

strands of all eight exons of the *NPHS2* gene. A few studies have also performed sequence analysis of the intronic and promoter regions of the gene.[13,18,22] For analysis of the polymorphisms, DNA from healthy controls, blood donors and volunteers were also resequenced.

POPULATION TESTING

Currently, genotype analysis of *NPHS1* and *NPHS2* is commercially available (Athena Diagnostics, Worcester, MA). Bidirectional resequencing of coding region of *NPHS1* and *NPHS2* can be ordered individually or in combination. In addition, these tests are also available in research facilities at Harvard University, University of Michigan, and the National Institutes of Health at no cost. Because some *NPHS2* mutations are associated with steroid-resistance, some have advocated the screening of patients with familial nephrotic syndrome and childhood sporadic FSGS for podocin mutations in order to spare drug toxicity or prevent delay of more effective therapy.[37] Current available data are insufficient to provide recommendations. In addition, there are insufficient data to determine whether *NPHS2* polymorphisms such as R229Q cause FSGS. Given the complexity of FSGS, it is unlikely that a single nucleotide polymorphism (SNP) in a gene will be sufficiently implicated to warrant genetic testing.

COMMENTARY

Studies to date have focused primarily on identifying gene loci for familial and idiopathic nephrotic syndrome and in characterizing mutations of known causal genes such as *NPHS1* and *NPHS2*. Although several podocin polymorphisms have been described, the allele and genotype frequencies are often not reported, which limits their use in genetic association studies. In some cases, published studies have relied on descriptive analysis of allele frequency among affected and controls without considering gene-gene interaction and environmental risk factors.

Several questions should be considered in future work. For example, do *NPHS2* variants 1) increase the risk for sporadic FSGS or collapsing glomerulopathy, including idiopathic, postadaptive, virus-associated, and medication-associated forms, 2) increase the risk to develop other renal diseases, including diabetic nephropathy, 3) increase the risk for progression to ESRD with any renal disease and 4) interact with other podocyte gene variants in any of these foregoing renal conditions. For evaluation of these hypotheses, large well-characterized patient populations, with uniform assessment of renal histology and clinical findings, are required to establish standardize phenotypic criteria. These patient populations must be ethnically-matched to control subjects. Sophisticated analytic techniques will be required to test multiple disease subgroups while maintaining statistical power. Increasingly, it is apparent that such studies will require thousands of affected subjects and control subjects, and this will require of collaboration among investigators.

In summary, we have reviewed the *NPHS2* gene variants and its association with nephrotic syndrome and FSGS across different ethnic populations. We have found that R229Q is one of the best characterized polymorphism, although with low frequencies across multiple ethnic groups. There is insufficient evidence implicating R229Q in the etiology of FSGS among Caucasians and our unadjusted analysis suggests small increased risk among this population. Clearly, large case-control studies will be required to evaluate the role of this polymorphism in disease. The R229Q variant is noticeably rare in populations of African descent, and was not associated with FSGS in African American subjects. Nonetheless, given the disparity of prevalence of FSGS among African American patients and the lack of studies evaluating NPHS2 variants with FSGS, further evaluation of this gene in the etiology of FSGS is warranted.

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