

Specific Podocin Mutations Correlate with Age of Onset in Steroid-Resistant Nephrotic Syndrome

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ABSTRACT

Mutations in the gene encoding podocin (*NPHS2*) cause autosomal recessive steroid-resistant nephrotic syndrome (SRNS). For addressing the possibility of a genotype–phenotype correlation between podocin mutations and age of onset, a worldwide cohort of 430 patients from 404 different families with SRNS were screened by direct sequencing. Recessive podocin mutations were present in 18.1% (73 of 404) of families with SRNS, and 69.9% of these mutations were nonsense, frameshift, or homozygous R138Q. Patients with these mutations manifested symptoms at a significantly earlier age (mean onset <1.75 yr) than any other patient group, with or without podocin mutations, in this study (mean onset >4.17 yr). All but one patient affected by truncating or homozygous R138Q mutations developed SRNS before 6 yr of age. Patient groups with other recessive podocin mutations, with single heterozygous podocin mutations, with sequence variants, and with no podocin changes could not be distinguished from each other on the basis of age of onset. In conclusion, nephrotic syndrome in children with truncating or homozygous R138Q mutations manifests predominantly before 6 yr of life, and the onset of disease is significantly earlier than for any other podocin mutations. Because the age of onset can vary by several years among those with identical mutations, additional factors may modify the phenotype.

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Nephrotic syndrome (NS) is a common childhood kidney disease caused by impaired glomerular function, which results in proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Although underlying causes of NS are numerous, standard steroid treatment regimens are used to classify idiopathic forms of NS clinically. On the basis of the patients' response to steroid treatment, NS is descriptively classified as steroid-sensitive NS (SSNS) or steroid-resistant NS (SRNS).

Positional cloning of various genes as mutated in patients with SRNS in recent years has led to the surprising finding that up to 28% of sporadic cases of SRNS are caused by recessive mutations of *NPHS2*.¹ Mutations causing recessive NS in children were found in nephrin (*NPHS1*),² podocin (*NPHS2*),³ laminin- β -2 (*LAMB2*), and phospho-

lipase C- ϵ (*PLCE1*).^{4–6} Dominant mutations of exons 8 and 9 of the Wilms' tumor 1 gene (*WT1*) also cause childhood NS.⁷ In adult patients with SRNS, dominant disease causing mutations in *ACTN4* and *TRPC6* were found.^{8–10} Conjointly, these findings have shown that podocyte dysfunction affecting the glomerular slit diaphragm is a key feature of SRNS.¹¹

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Among these SRNS genes identified so far, mutations in *NPHS2* encoding podocin represent the most frequent cause.^{1,12} Patients with *NPHS2* mutations show histology of FSGS in approximately 80% of cases and progress to ESRD. Extensive studies have documented that the detection of two *NPHS2* mutations is clinically relevant, because affected patients do not respond to standard steroid treatment.¹ Thus, adverse effects of such treatment attempts can be avoided in children with recessive *NPHS2* mutations. In addition, patients with *NPHS2* mutations have a reduced risk (8 versus 33%) for recurrence of FSGS in a kidney transplant.^{1,13}

NPHS2 mutations have been found in patients presenting at a wide range of ages, in contrast to *NPHS1* (nephrin) mutations, which lead to congenital NS with onset within the first 3 mo of life. The reason for this phenotypic variability of patients with *NPHS2* mutations remains elusive. In our recent study on NS manifesting in the first year of life (NSFL), we identified *NPHS2* mutations as the most frequent cause of both congenital (0 to 3 mo) and infantile (3 to 12 mo) NS among 89 European children with NSFL.¹⁴ In 94.1% of patients with NSFL caused by *NPHS2* mutations, we found nonsense, frameshift, or homozygous R138Q mutations. These findings and the suggestive data by Weber *et al.*¹³ led to the question of whether specific *NPHS2* mutations determine the age of initial onset of SRNS.

To evaluate this genotype–phenotype correlation among patients with *NPHS2* mutations, we examined 430 patients from 404 families from a worldwide cohort who presented with SRNS between 0.0 and 21.0 yr of age for mutations in *NPHS2* by direct sequencing of all eight exons. In this study, we demonstrate that nonsense, frameshift, or homozygous R138Q mutations are frequent recessive mutations of *NPHS2*. We show that these *NPHS2* mutations are found in patients

with a significantly earlier onset than in patients with other recessive mutations, single mutations, or sequence variants of *NPHS2*. We observe that identical mutations lead to onset of SRNS within a range of several years and speculate that additional factors modify the *NPHS2* phenotype. These findings will be important for the prognostic evaluation and management of renal replacement therapy in children with SRNS.

RESULTS

Frequency of *NPHS2* Mutations

A total of 430 patients with SRNS from 404 families were analyzed for mutations of *NPHS2* by direct DNA sequencing. Mutations in *NPHS2* were present in 18.1% (73 of 404) of all families and affected 82 patients (Table 1). In families with more than one affected member, mutations were present in 39.1% (nine of 23 families). In families with one affected child, mutations were present in 16.8% (64 of 381 families; data not shown). The R138Q mutation that has been described as a European founder mutation³ was found in 57.5% (42 of 73) of families with the presence of two disease-causing mutations (Table 1, groups A through D; Supplementary Table 1). In 5.1% (21 of 404) of all families, only one single heterozygous mutation was detected (Table 1, groups E and F; Supplementary Table 1).

The distribution of the four groups with two recessive mutations (A through D) among 73 families with *NPHS2* mutations and clinical data were as follows (Table 1): A, 39.7% (29 of 73) with one truncating mutation (nonsense or frameshift) in combination with any other mutation; B, 30.1% (22 of 72) with a homozygous R138Q mutation; C, 12.3% (nine of 73) with one R138Q mutation in combination with one missense

Table 1. A total of 430 patients from 404 families with SRNS analyzed for mutations and sequence changes in *NPHS2*^a

Group	<i>NPHS2</i> Mutation	Affected Individuals		Affected Families		Mean Onset (yr)	Onset Range (yr)
		Total	Onset known	Total	Onset known		
A	Truncating × any ^b	32	31	29	28	1.75 ^c	0.0 to 9.1
B	R138Q × R138Q	27	26	22	21	1.77 ^c	0.0 to 5.4
C	R138Q × missense	10	9	9	8	5.95	0.0 to 14.3
D	Missense × missense	13	12	13	12	4.17	0.0 to 16.6
		82	78	73	69	2.61	0.0 to 16.6
E	Single × R229Q	14	13	13	12	6.74	0.8 to 16.3
F	Single × ?	9	9	8	8	8.12	1.6 to 14.7
G	R229Q × R229Q	2	2	2	2	6.48	0.0 to 13.0
		24	23	24	23		
		49	47	47	45	6.87	0.0 to 16.3
H	No mutation	299	267	284	253	6.4	0.0 to 21.0
Total		430	392	404	367	5.71	0.0 to 21.0

^aA total of 430 patients from 404 families with SRNS were analyzed by direct exon sequencing for sequence changes in *NPHS2*. Clinical data on 392 patients from 367 families allowed for correlation on *NPHS2* sequence variants with age of onset. Patients were categorized into patients with two disease-causing *NPHS2* mutations (A through D), patients with a single *NPHS2* mutations and/or sequence variant R229Q (E through G), and patients without *NPHS2* variants (H).

^bGroup A includes 14 individuals from 11 families with a single R138Q mutation accompanying the truncating mutation. The total number of families/individuals with R138Q mutations is 42/5.

^cAge of onset for both groups A and B was significantly lower than for all other groups ($P < 0.01$).

mutation; and D, 17.9% (13 of 73) with two missense mutations other than R138Q (Table 1). Six previously unpublished *NPHS2* mutations were found (Supplementary Table 1).

In 47 families, we detected only one single *NPHS2* mutation or the sequence variant R229Q of unknown significance (Table 1, groups E through G). In these 47 families, one heterozygous mutation in combination with the R229Q sequence variant of unknown significance was present in 27.7% (13 of 47; E). In 17.0% (eight of 47) of families, one heterozygous *NPHS2* mutation only was present (F), and in 55.3% (26 of 47) of families, the R229Q sequence variant only was present heterozygously (24 of 47) or homozygously (G; Table 1). The frequency of R229Q variants among all 404 families with SRNS in this study was 9.2% (37 of 404) in the heterozygous state and 0.5% (two of 404) in the homozygous state.

Correlation of *NPHS2* Mutations and Age of Onset

Age at onset of SRNS was documented for 392 patients from 367 families and ranged from 0.0 to 21.0 yr. Two disease-causing *NPHS2* mutations were found in 19.9% (78 of 392) of these patients. The relative frequency with which any two disease-causing *NPHS2* mutations were detected declined with increasing age at manifestation.

We then evaluated genotype–phenotype correlations between type of mutation and age of onset (Table 1). This correlation showed significantly earlier onset of SRNS in children with two disease-causing *NPHS2* mutations (A through D; mean 2.61 yr; range 0.0 to 16.6 yr) than in children with single heterozygous *NPHS2* mutations and sequence variant R229Q (E through G; mean 6.87 yr; range 0.0 to 16.3 yr; $P < 0.0001$). Children with two recessive *NPHS2* mutations (A through D) also manifested significantly earlier when compared with children without *NPHS2* sequence variants (H; mean 6.4 yr; range 0.0 to 21.0 yr; $P < 0.0001$). In particular, the presence of two truncating mutations (nonsense or frameshift) in *NPHS2* (A) was associated with much earlier onset (mean 1.75 yr; range 0.0 to 9.1 yr). Likewise, the homozygous presence of the *NPHS2* founder mutation R138Q (B) was associated with early onset (1.77 yr; range 0.0 to 5.4 yr). These two groups (A and B) were responsible for the earlier onset of patients with the presence of two *NPHS2* mutations (groups A through D; Table 1). Figure 1A illustrates the significant difference in age at onset of disease between mutation groups A and C ($P = 0.006$). Age of onset for patients with the presence of two other disease-causing *NPHS2* mutations was 5.95 yr (range 0.0 to 14.3 yr) for children with one R138Q and one missense mutation (C). It was 4.17 yr (range 0.0 to 16.6 yr) for children with two missense mutations other than R138Q (D). Both groups (C and D) showed no significant difference compared with patients without disease-causing mutations (E through G; Table 1). The mean age at onset of SRNS was 6.87 yr (range 0.0 to 16.3 yr) for patients with no disease-causing *NPHS2* mutation (E through G) and 6.40 yr (range 0.0 to 21.0 yr) for children free of *NPHS2* changes (H; Table 1). Patients with at least one truncating mutation (nonsense or frameshift) or with homozygous R138Q

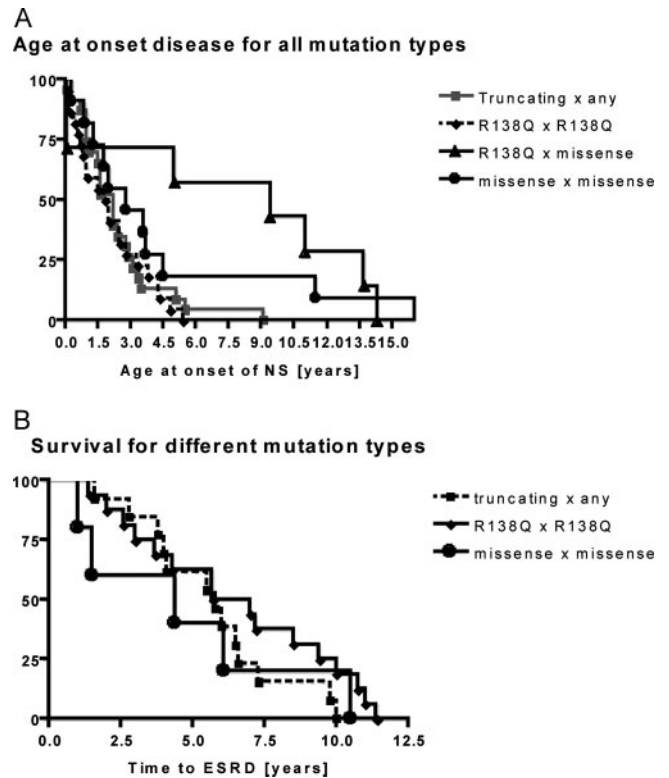


Figure 1. Kaplan Meier survival analysis of time intervals between *NPHS2* mutation groups A (truncating × any), group B (R138Q × R138Q), group C (R138Q × missense), and group D (missense × missense). (A) Age at onset of disease for all four groups. Each mutation group was compared with group A (earliest age of onset). The age at onset of disease between groups A and C was significantly different ($P = 0.006$). (B) The time interval from onset of disease to development of ESRD. Data on development of ESRD were present in one of nine patients in group C; therefore, this group was excluded from the calculation. Each mutation group was compared with group D (shortest time interval). The time interval was not significantly different between the groups.

mutations therefore showed a significantly earlier onset of SRNS than any other ESRD group of *NPHS2* changes. Survival analysis shows that there is no significant difference in the time interval from onset of disease to development of ESRD between different mutation types (Figure 1B).

The three patient groups with a single mutation or the sequence variant R229Q of unknown significance manifested later; however, there was no significant difference of age of onset neither among groups E, F, or G nor when they were compared with 267 individuals without *NPHS2* changes (H). Later onset of SRNS in children with single *NPHS2* mutations could thus not be observed in this cohort of patients manifesting between 0.0 and 21.0 yr of age. The mean age at manifestation for individuals in these groups was as follows (Table 1): Group E, 6.74 yr (range 0.8 to 16.3 yr) for individuals with single mutation and sequence variant R229Q; group F, 8.12 yr (range 1.6 to 14.7 yr) for patients with single mutations only;

and group G, 6.48 yr (range 0.0 to 13.0 yr) for children with one single heterozygous or a homozygous R229Q sequence variant (Table 1).

Seventy-one (91.0%) of 78 patients with two *NPHS2* mutations and clinical data manifested before their ninth birthday. Six of the seven children who had two mutations and were older than 9 yr at diagnosis carried two missense mutations other than R138Q only.

DISCUSSION

We report here an *NPHS2* genotype–phenotype correlation for age of onset of SRNS in the largest cohort of children who had SRNS and were examined for *NPHS2* mutations published to date. Of 404 families with SRNS included in this study, 73 (18.1%) of 404 carried two disease-causing *NPHS2* mutations, and 5.1% (21 of 404) carried only one single heterozygous mutation. The R229Q sequence variant of unknown significance was present in 9.2% (37 of 404) of families heterozygously and in 0.5% (two of 404) of families homozygously.

The R138Q mutation was found as the most frequent mutation in 42 (57.5%) of 73 families with two disease-causing mutations. This confirms in our large cohort the role of R138Q as a founder mutation as had been stated by Boute *et al.*³ in the initial description of *NPHS2*. The proportion of children who had SRNS and carried two recessive *NPHS2* mutations was particularly high among children who were younger than 6 yr. Notably, the presence of at least one truncation mutation or of a homozygous R138Q mutation led to strikingly earlier onset (<1.77 yr) compared with all other groups of *NPHS2* mutations (<4.17 yr). These findings will be important for the counseling of affected families and the prognostic evaluation and management of renal replacement therapy in children with SRNS.

Our analysis shows a genotype–phenotype correlation between specific *NPHS2* mutations and age at onset. Nonsense, frameshift (“truncating mutations”), or homozygous R138Q resulted in significantly earlier presentation of SRNS than all other *NPHS2* changes. In patients with these severe mutations, SRNS had started before 6 yr of age in 98.2% of cases. These findings are consistent with a study by Weber *et al.*¹³ Our study further shows that children with one truncating mutation (nonsense or frameshift) and one additional R138Q mutation manifest particularly early (mean 1.0 yr; range 0.0 to 5.5 yr; Supplementary Table 1). This finding further supports the idea that R138Q acts as a severe mutation leading to early onset of SRNS. Functional data have shown that podocin with the R138Q mutation is retained in the endoplasmic reticulum and that this condition results in disrupted targeting of nephrin (*NPHS1*) to lipid raft microdomains.^{15–17}

Patients with only one R138Q mutation in combination with one missense mutation (group C) and patients with two missense mutations other than R138Q (group D) manifested significantly later than patients with two severe mutations

(groups A and B; Table 1). Only seven children with two *NPHS2* mutations in this study manifested at >9 yr of age. Six of these seven children carried the missense mutation V290M or V180M.¹² The latter had been reported in association with late onset of SRNS by Weber *et al.*¹³ Therefore recessive *NPHS2* mutations as a rule do predominantly result in childhood SRNS before the age of 9 yr.

Our data also show that identical mutations may result in onset of SRNS over a spectrum of several years. This was seen best in patients who had homozygous R138Q mutations and presented between 0.0 and 5.4 yr of age. We therefore conclude that specific mutations have predictive value for age at onset of SRNS, but additional modifying factors are involved. One could speculate that single heterozygous mutations in *NPHS1* could act as a modifier of the NS phenotype in children, but this has been described only extremely rarely (three cases,¹⁸ one case,¹³ and no cases¹⁴); therefore, we did not examine patients for *NPHS1* mutations. We investigated the influence of gender on age of presentation in all 73 patients with disease-causing mutations. The mean age at onset of disease in male patients was 2.74 yr; the mean age at onset of disease in female patients was 2.42 yr. These differences were not statistically significant (Supplementary Table 2).

To determine whether ethnic background had an influence on the age of presentation in patients with disease-causing mutations, we calculated mean age at onset of disease for four different ethnic groups: European patients, Central Slavic patients, Turkish patients, and others. The differences in ages among the groups are small, and statistical analysis was not done because most groups were too small to reach significant conclusions.

Given that *NPHS2* is a recessive disease, only the presence of two disease-causing mutations explains the phenotype. It is not unusual, however, that the second mutation may not be detected in mutational analysis in recessive diseases. The clinical significance of single mutations and the *NPHS2* sequence variant R229Q have been discussed in various studies.^{18–22} We therefore included single mutations and the sequence variant of unknown significance R229Q in our analysis as separate groups. The mean age at presentation for patients in whom one single *NPHS2* mutation or R229Q was detected (groups E and F) was significantly different neither from patients with two missense mutations other than R138Q (group D) nor from patients without *NPHS2* changes (group H). Our data therefore neither support the idea that single heterozygous mutations result in late onset SRNS nor eliminate this option.

Specifically, the allele frequency of the R229Q sequence variant of unknown significance has been reported as high as 3.9% in healthy individuals.²² We found this change in 9.2% (37 of 404) of our families in the heterozygous state and in only 0.5% (two of 404) of all families in the homozygous state. R229Q has been discussed as a modifier of SRNS^{18–20}; however, its involvement in the development of FSGS seemed inconclusive to other authors.²² Our study does not allow a conclusion that R229Q should be considered a disease-causing mutation.

Ardiles *et al.*²¹ suggested a correlation between *NPHS2* mutations and age of onset of SRNS. Our data put their suggestion on a statistically solid ground with some modification. We conclude that not homozygosity *versus* heterozygosity of *NPHS2* mutations but rather the allelic type of mutations determines the phenotype by onset of NS. We think that, in addition to *NPHS2* mutations, modifying factors would explain the finding that identical mutations lead to presentation at different ages.

CONCLUSIONS

The presence of two recessive *NPHS2* mutations caused SRNS in 18.1% (73 of 404) families in this study. We demonstrate for the first time statistical significance for early onset of SRNS in children with truncating or homozygous R138Q mutations of *NPHS2* (<1.77 yr) *versus* children with all other sequence variants (>4.17 yr), including recessive *NPHS2* mutations, with single detected *NPHS2* mutations, with isolated R229Q sequence variants, or without *NPHS2* mutations. This finding will be important for the prognostic evaluation and management of renal replacement therapy in children with SRNS. The vast majority (56 [98.2%] of 57) of children with two truncating or homozygous R138Q mutations presented at <6 yr of age. Truncating or homozygous R138Q mutations are frequent pathogenic *NPHS2* mutations. In this study, they were detected in 71.9% (59 of 82) of all families. Our correlation for age of onset in SRNS does not allow a conclusion on a pathogenic significance of the frequent *NPHS2* sequence variant R229Q.

CONCISE METHODS

Patient and Data Recruitment

Human subject research was approved by the University of Michigan institutional review board and the ethics commission of the University of Freiburg, Germany. The diagnosis of NS was made by pediatric nephrologists in specialized centers on the basis of published criteria.²³ Patient recruitment for this study was worldwide with predominance of Central European individuals. After informed consent, clinical data were provided by enrolling specialists using a standardized questionnaire (<http://www.renalgenes.org>), which we have described previously.¹ Blood samples of patients with NS were acquired and used for DNA extraction by standard methods.

To evaluate *NPHS2* genotype–phenotype correlations, we analyzed children affected by SRNS only. SRNS was defined according to published criteria by Arbeitsgemeinschaft für Pädiatrische Nephrologie and the International Study of Kidney Disease in Children (ISKDC).^{23,24} Families in which affected individuals had shown any response to standard steroid treatment were excluded from this study. All patients were examined for mutations in *WT1* by direct sequencing of exons 8 and 9.

Of 446 families with SRNS, we excluded from our analysis 42 fam-

ilies in which we identified mutations in *NPHS1*, *LAMB2*, or *WT1*. The cohort analyzed for mutations in *NPHS2* thus included 404 families with 430 patients with SRNS (Table 1).

To compare age of onset between different *NPHS2* sequence changes, we defined onset of SRNS as time of first detection of nephrotic-range proteinuria (>40 mg/m² per h) or diagnosis of persistent low-grade proteinuria (>4 mg/m² per h). Age at onset of SRNS was documented for 392 patients from 367 families and ranged from 0 to 21.0 yr.

When evaluating frequency of mutations, we considered numbers of families, because siblings have identical mutations. When evaluating clinical data, we considered numbers of patients, because siblings may differ in their clinical characteristics (see Table 1). A subgroup of 202 patients (181 families) in this study had been included in the analysis by Ruf *et al.*,¹ in which it was studied under the aspect of steroid responsiveness among children with *NPHS2* mutations, and 89 patients (80 families) have also been studied in an analysis of genetic causes of NSFL.¹⁴

Categories of *NPHS2* Mutations

Patients with two recessive mutations (groups A through D) were classified on the basis of the suggestive data by Weber *et al.*¹³ as patients with at least one nonsense or frameshift mutation in combination with any second mutation (A), patients with the homozygous founder mutation R138Q (B), patients with a single heterozygous R138Q mutation in combination with one different missense mutation (C), and patients with two missense mutations other than R138Q (D; Table 1).

The phenotypic relevance of single *NPHS2* mutations and sequence variants of unknown significance in general and the frequent sequence variant R229Q in particular have been discussed by several authors as being involved in the pathogenesis of SRNS.^{18–22} Because the pathogenic relevance of these findings has not yet been demonstrated conclusively, we also included single detected mutations and the R229Q sequence variant of unknown significance as separate categories (E through G). We subclassified patients with one heterozygous *NPHS2* mutation in combination with sequence variant R229Q (E), patients with one heterozygous *NPHS2* mutation only (F), and patients with one heterozygous or a homozygous sequence variant R229Q only (G; Table 1). Patients without *NPHS2* mutations or sequence variant R229Q were defined as group H.

Mutation Analysis of *NPHS2* by Direct Sequencing

Genomic DNA was isolated from blood samples using the Puregene DNA purification kit (Gentra, Minneapolis, MN) following the manufacturer's guidelines. Mutation analysis was performed by exon-flanking direct sequencing of all eight exons of *NPHS2*.¹ Exon primers are available from the authors. For sequence analysis, the software SEQUENCHER (Gene Codes, Ann Arbor, MI) was used. For all detected mutations and other sequence variants, sequencing of both strands was performed. Segregation of these changes was confirmed by direct sequencing of parental samples when available. The absence of previously unpublished mutations was shown in 160 control chromosomes from healthy individuals of matched ethnic origin.

Statistical Analysis

We tested for differences of mean values of age of onset using a *t* test and considered $P < 0.05$ significant. Range of age of onset is given in years.

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DISCLOSURES

None.

REFERENCES

- Ruf RG, Lichtenberger A, Karle SM, Haas JP, Anacleto FE, Schultheiss M, Zalewski I, Imm A, Ruf EM, Mucha B, Bagga A, Neuhaus T, Fuchshuber A, Bakkaloglu A, Hildebrandt APN: Patients with mutations in NPHS2 (Podocin) do not respond to standard steroid treatment of nephrotic syndrome. *J Am Soc Nephrol* 15: 722–732, 2004
- Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 4: 575–582, 1998
- Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C: NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000
- Hasselbacher K, Wiggins RC, Matejas V, Hinkes B, Mucha B, Hoskins BE, Ozaltin, Nürnberg G, Becker C, Hangan D, Pohl M, Kuwertz-Bröcking E, Griebel M, Schumacher V, Royer-Pokora B, Bakkaloglu A, Nürnberg P, Zenker M, Hildebrandt F: Recessive missense mutations in LAMB2 as a cause of isolated and non-Pierson type congenital nephrotic syndrome. *Kidney Int* 70: 1008–1012, 2006
- Zenker M, Aigner T, Wendler O, Tralau T, Muntefering H, Fenski R, Pitz S, Schumacher V, Royer-Pokora B, Wuhl E, Cochat P, Bouvier R, Kraus C, Mark K, Dötsch J, Rascher W, Maruniak-Chudek I, Lennert T, Neumann LM, Reis A: Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet* 13: 2625–2632, 2004
- Hinkes B, Wiggins RC, Gbadegesin R, Vlangos CN, Seelow D, Nürnberg G, Garg P, Verma R, Chaib H, Hoskins BE, Ashraf S, Becker C, Hennies HC, Goyal M, Wharram BL, Schachter AD, Mudumana S, Drummond I, Kerjaschki D, Waldherr R, Dietrich A, Ozaltin F, Bakkaloglu A, Cleper R, Basel-Vanagaite L, Pohl M, Griebel M, Tsygin AN, Soylyu A, Muller D, Sorli CS, Bunney TD, Katan M, Liu J, Attanasio M, O'toole JF, Hasselbacher K, Mucha B, Otto EA, Airik R, Kispert A, Kelley GG, Smrcka AV, Gudermann T, Holzman LB, Nürnberg P, Hildebrandt F: Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. *Nat Genet* 38: 1397–1405, 2006
- Mucha B, Ozaltin F, Hinkes BG, Hasselbacher K, Ruf RG, Schultheiss M, Hangan D, Hoskins BE, Everding AS, Bogdanovic R, Seeman T, Hoppe B, Hildebrandt F, Members of the APN Study Group: Mutations in the Wilms' tumor 1 gene cause isolated steroid resistant nephrotic syndrome and occur in exons 8 and 9. *Pediatr Res* 59: 325–331, 2006
- Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR: Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 24: 251–256, 2000
- Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL, Pericak-Vance MA, Howell DN, Vance JM, Rosenberg PB: A mutation in the TRPC6 cation channel causes focal segmental glomerulosclerosis. *Science* 308: 1801–1804, 2005
- Reiser J, Polu KR, Moller CC, Kelan P, Altintas MM, Wei C, Faul C, Herbert S, Villegas I, Avila-Casado C, McGee M, Sugimoto H, Brown D, Kalluri R, Mundel P, Smith PL, Clapham DE, Pollak MR: TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. *Nat Genet* 37: 739–744, 2005
- Somlo S, Mundel P: Getting a foothold in nephrotic syndrome. *Nat Genet* 24: 333–335, 2000
- 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* 13: 577–579, 2002
- Weber S, Gribouval O, Esquivel EL, Moriniere V, Tete MJ, Legendre C, Niaudet P, Antignac C: NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. *Kidney Int* 66: 571–579, 2004
- Hinkes BG, Mucha B, Vlangos CH, Gbadegesin R, Liu J, Hoskins B, Hasselbacher K, Hangan D, Ozaltin F, Bakkaloglu A, Zenker M, Hildebrandt F, Members of the APN Study Group: Nephrotic syndrome manifesting in the first year of life: two-thirds are caused by mutations in four genes (NPHS1, NPHS2, WT1, or LAMB2). *Pediatrics* 119: e907–e919, 2007
- Huber TB, Simons M, Hartleben B, Semetz L, Schmidts M, Grundlach E, Saleem MA, Walz G, Benzing T: Molecular basis of the functional podocin-nephrin complex: Mutations in the NPHS2 gene disrupt

- nephrin targeting to lipid raft microdomains. *Hum Mol Genet* 12: 3397–3405, 2003
16. Ohashi T, Uchida K, Uchida S, Sasaki S, Nihei H: Intracellular mislocalization of mutant podocin and correction by chemical chaperones. *Histochem Cell Biol* 119: 257–264, 2003
 17. Nishibori Y, Liu L, Hosoyamada M, Endou H, Kudo A, Takenaka H, Higashihara E, Bessho F, Takahashi S, Kershaw D, Ruotsalainen V, Tryggvason K, Khoshnoodi J, Yan K: Disease-causing missense mutations in NPHS2 gene alter normal nephrin trafficking to the plasma membrane. *Kidney Int* 66: 1755–1765, 2004
 18. Koziell A, Grech V, Hussain S, Lee G, Lenkkeri U, Tryggvason K, Scambler P: Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional inter-relationship in glomerular filtration. *Hum Mol Genet* 11: 379–388, 2002
 19. Tsukaguchi H, Sudhakar A, Le TC, Nguyen T, Yao J, Schwimmer JA, Schachter AD, Poch E, Abreu PF, Appel GB, Pereira AB, Kalluri R, Pollak MR: NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease-associated allele. *J Clin Invest* 110: 1659–1666, 2002
 20. Pereira AC, Pereira AB, Mota GF, Cunha RS, Herkenhoff FL, Pollak MR, Mill JG, Krieger JE: NPHS2 R229Q functional variant is associated with microalbuminuria in the general population. *Kidney Int* 65: 1026–1030, 2004
 21. Ardiles LG, Carrasco AE, Carpio JD, Mezzano SA: Late onset of familial nephrotic syndrome associated with a compound heterozygous mutation of the podocin-encoding gene. *Nephrology* 10: 553–556, 2005
 22. Franceschini N, North KE, Kopp JB, McKenzie L, Winkler C: NPHS2 gene, nephrotic syndrome and focal segmental glomerulosclerosis: A HuGE review. *Genet Med* 8: 63–75, 2006
 23. APN: Short versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. *Lancet* 1: 380–383, 1988
 24. ISKDC: Primary nephrotic syndrome in children: Clinical significance of histopathologic variants of minimal change and of diffuse mesangial hypercellularity—A Report of the International Study of Kidney Disease in Children. *Kidney Int* 20: 765–771, 1981
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- See related editorial, “Podocyte-Specific Gene Mutations Are Coming of Age,” on pages 190–191.
- Supplemental information for this article is available online at <http://www.jasn.org/>.

inappropriately high compared with plasma osmolality, but vasopressin levels are below the limits of RIA detection in a certain portion of the patients (as much as 10 to 20%).¹² The existence of other antidiuretic substances in plasma has been postulated. Oxytocin may fit this profile nicely. A few studies have shown elevated levels of plasma oxytocin in patients with small-cell lung cancer; however, these increments were usually accompanied by concomitant increases in vasopressin.¹³ At present, no report has suggested that oxytocin alone produces SIADH in clinical cases, but this may be attributable to the lack of a reliable RIA for oxytocin in clinical settings. Related to this topic, the nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is caused by a gain-of-function mutation of V2R. In this disease, endogenous vasopressin is completely suppressed while antidiuresis persists.¹⁴ The symptoms of disease start from childhood, but similar mutations seem to explain some sporadic episodes of SIADH in adults.¹⁵

How should we differentiate oxytocin-induced SIADH from NSIAD? Oxytocin-induced SIADH will respond to V2R antagonists as illustrated by *Li et al.*¹⁰, whereas patients with NSIAD are unable to respond to V2R antagonists.¹⁵ Clinicians would thus be well advised to note that oxytocin has antidiuretic activity and contributes to hyponatremia in certain clinical settings and that V2R antagonists may be useful in the differential diagnosis and treatment of inappropriate antidiuresis.

DISCLOSURES

None.

REFERENCES

1. Sausville E, Carney D, Battey J: The human vasopressin gene is linked to the oxytocin gene and is selectively expressed in a cultured lung cancer cell line. *J Biol Chem* 260: 10236–10241, 1985
2. Chini B, Manning M: Agonist selectivity in the oxytocin/vasopressin receptor family: New insights and challenges. *Biochem Soc Trans* 35: 737–741, 2007
3. Birnbaumer M, Seibold A, Gilbert S, Ishido M, Barberis C, Antaramian A, Brabet P, Rosenthal W: Molecular cloning of the receptor for human antidiuretic hormone. *Nature* 357: 333–335, 1992
4. Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S: Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* 361: 549–552, 1993
5. Ishikawa SE, Schrier RW: Pathophysiological roles of arginine vasopressin and aquaporin-2 in impaired water excretion. *Clin Endocrinol* 58: 1–17, 2003
6. Sasaki S, Noda Y: Aquaporin-2 protein dynamics within the cell. *Curr Opin Nephrol Hypertens* 16: 348–352, 2007
7. Pittman JG: Water intoxication due to oxytocin. *N Engl J Med* 268: 481–482, 1963
8. Potter RR: Water retention due to oxytocin. *Obstet Gynecol* 23: 699–702, 1964
9. Chou CL, DiGiovanni SR, Mejia R, Nielsen S, Knepper MA: Oxytocin as an antidiuretic hormone. I. Concentration dependence of action. *Am J Physiol* 269: F70–F77, 1995
10. Li C, Wang W, Summer SN, Westfall TD, Brooks DP, Falk S, Schrier RW: Molecular mechanisms of antidiuretic effect of oxytocin. *J Am Soc Nephrol* 19: 225–232, 2008
11. Verbalis JG, Goldsmith SR, Greenberg A, Schrier RW, Sterns RH: Hyponatremia treatment guideline 2007: Expert panel recommendations. *Am J Med* 120: S1–S21, 2007
12. Robertson GL: Regulation of arginine vasopressin in the syndrome of inappropriate antidiuresis. *Am J Med* 119: S36–S42, 2006
13. North WG, Friedmann AS, Yu X: Tumor biogenesis of vasopressin and oxytocin. *Ann N Y Acad Sci* 689: 107–121, 1993
14. Feldman BJ, Rosenthal SM, Vargas GA, Fenwick RG, Huang EA, Matsuda-Abedini M, Lustig RH, Mathias RS, Portale AA, Miller WL, Gitelman SE: Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med* 352: 1884–1890, 2005
15. Decaux G, Vanderghyest F, Bouko Y, Parma J, Vassart G, Vilain C: Nephrogenic syndrome of inappropriate antidiuresis in adults: High phenotypic variability in men and women from a large pedigree. *J Am Soc Nephrol* 18: 606–612, 2007

See related article, "Molecular Mechanisms of Antidiuretic Effect of Oxytocin," on pages 225–232.

Podocyte-Specific Gene Mutations Are Coming of Age

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Major leaps have been made recently in the understanding of the cause of proteinuria and hence in the regulation of glomerular permeability in health. Progress has been fueled by the description of single-gene mutations, the majority of which affect genes expressed selectively in the podocyte, resulting in nephrotic syndrome in human and mouse.¹ This has placed the podocyte center stage as a key regulator of normal selective permeability to albumin in the glomerular capillary wall, although we should not forget that single-gene mutations affecting components of the glomerular basement membrane can also result in heavy proteinuria,² or that the third component of the glomerular capillary wall, the glomerular endothelial cell, can also play an important role in regulating glomerular permeability in health and disease.³

The first podocyte-specific gene identified by studying disease-associated mutations was *NPHS1*, encoding nephrin⁴; this was swiftly followed by identification of *NPHS2*, encoding podocin,⁵ also a novel protein important in the structure and function of

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glomeruli.⁶ Since the discovery of nephrin and podocin, several other disease-associated podocyte-specific gene defects have been reported, and, undoubtedly, there will be more to come.

Mutations in different podocyte genes or different mutations in the same gene result in varying phenotypes regarding severity and age of onset of proteinuria, and it is clear that there are likely to be other disease-modifying genes or environmental influences. Moreover, congenital forms of nephrotic syndrome are rare, and a question that intrigues nephrologists and basic scientists alike is whether the more common forms of sporadic, often later onset nephrotic syndrome could also be associated with mutations or polymorphisms in podocyte-specific genes, as predisposing factors or contributors to a complex etiology involving genetic–environmental interactions. If so, then study of these genes could be clinically useful in diagnosis and prognosis, especially concerning the likelihood of corticosteroid responsiveness and the issue of likely recurrence in renal transplants for patients who progress to end-stage renal failure.

The article in this issue of *JASN* by Hinkes *et al.*,⁷ the product of an impressive multinational collaboration, sheds light on these issues. The study amassed 430 patients with steroid-resistant nephrotic syndrome, the vast majority of whom were the only affected family member, although the series did include 23 families with more than one affected member. The patients were screened for mutations in *NPHS2* by direct sequencing of all eight exons of the gene. Eighty-two patients (19% of the total) had mutations in *NPHS2*. In the families with more than one affected member, the proportion with *NPHS2* mutations rose to 39%. In patients with two *NPHS2* mutations, the authors report that approximately 40% had one truncating (frameshift or nonsense) mutation and an additional 30% had homozygous *R1308Q* mutations (the “founder” *NPHS2* mutation identified by Boute *et al.*⁵). These two groups of individuals nearly all developed nephrotic syndrome at an early age (<6 yr, with a mean age of onset <2 yr). The remaining 30% of patients with other mutations or variants in *NPHS2* had later onset disease without any further specific link between any given genotype and age of onset (although the numbers of patients with each genotype were small). Mutation type did not affect rate of deterioration, time from onset to ESRD being the same in all groups.

Although this represents real progress, even within the groups with early presentation there was still a wide range of age of onset. Also, >80% of the collection with steroid-responsive nephrotic syndrome did not have any abnormality of *NPHS2*, so their proteinuria remains unexplained; clearly there is more work to be done.

The power of large multinational studies such as this one will be essential if analyses of genotype–phenotype relationships in nephrotic syndrome are to yield informative conclusions. Ideally, genetic analysis should be more widely available as a diagnostic and prognostic aid in patients presenting with nephrotic syndrome; however, at present, clinicians will need further guidance from geneticists about the interpretation of genotype–phenotype relationships. Hinkes *et al.* are to be congratulated for leading the way.

DISCLOSURES

None.

REFERENCES

1. Tryggvason K, Patrakka J, Wartiovaara J: Hereditary proteinuria syndromes and mechanisms of proteinuria. *N Engl J Med* 354: 1387–1401, 2006
2. Zenker M, Aigner T, Wendler O, Tralau T, Müntefering H, Fenski R, Pitz S, Schumacher V, Royer-Pokora B, Wühl E, Cochat P, Bouvier R, Kraus C, Mark K, Madlon H, Dötsch J, Rascher W, Maruniak-Chudek I, Lennert T, Neumann LM, Reis A: Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet* 13: 2625–2632, 2004
3. Ballermann BJ: Contribution of the endothelium to the glomerular permselectivity barrier in health and disease. *Nephron Physiol* 106: 19–25, 2007
4. Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 4: 575–582, 1998
5. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C: *NPHS2*, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349–354, 2000
6. Huber TB, Schermer B, Benzing T: Podocin organizes ion channel-lipid supercomplexes: Implications for mechanosensation at the slit diaphragm. *Nephron Exp Nephrol* 106: e27–e31, 2007
7. Hinkes B, Vlangos C, Heeringa S, Mucha B, Gbadegesin R, Liu J, Hasselbacher K, Ozaltin F, Hildebrandt F, members of the APN Study Group: Specific podocin mutations correlate with age of onset in steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 19:365–371, 2008

See related article, “Specific Podocin Mutations Correlate with Age of Onset in Steroid-Resistant Nephrotic Syndrome,” on pages 365–371.

The Disadvantage of Being Fat

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Given the epidemic of obesity in the United States, it is not surprising that an increasing fraction of patients who are considered for and receiving kidney transplants are also overweight. Friedman *et al.*¹ found a 41.9% decrease in the fraction

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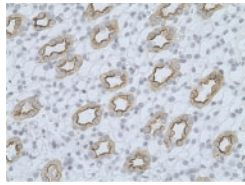
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This Month's Highlights

BASIC RESEARCH

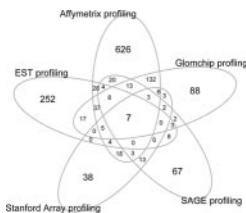
Oxytocin Modulates Expression and Trafficking of Aquaporins

The administration of oxytocin to induce labor in pregnancy is associated with hyponatremia, but the mechanisms underlying the retention of water are incompletely understood. Li *et al.* infused oxytocin for 5 d into vasopressin-deficient rats and observed that oxytocin acts through vasopressin (V2) receptors on the principal cells of the collecting duct to increase the expression and trafficking of aquaporin-2 and aquaporin-3. These results raise the intriguing possibility of treating severe oxytocin-induced hyponatremia with V2 receptor antagonists, which would not affect oxytocin-mediated uterine contractions. See Li *et al.*, pages 225–232.



The Glomerulus Meets Systems Biology

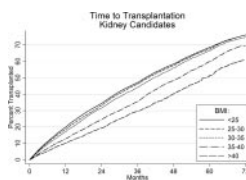
Our knowledge of the roles of glomerular proteins in the pathophysiology of disease is limited. He *et al.* used bioinformatics and an integrative systems biology approach to unravel the complex network of glomerular proteins. By combining published and original data regarding the glomerular transcriptome, the authors created a catalog of glomerulus-enriched genes and constructed a model of predicted protein–protein interactions. This comprehensive analysis of the glomerular transcriptome may facilitate the study of the roles of glomerular proteins in health and disease. See He *et al.*, pages 260–268.



CLINICAL EPIDEMIOLOGY

Obesity Worsens Chance for Transplantation

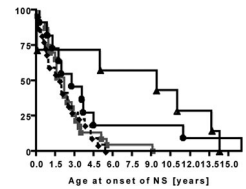
Does obesity hurt one's chances for receiving a kidney transplant? Segev *et al.* analyzed data from a prospective cohort of >130,000 patients who were registered for transplantation in the United States and revealed an independent negative association between body mass index (BMI) and likelihood of transplantation. The morbidly obese are 44% less likely to receive a transplant than those with normal BMI, and when an organ becomes available, providers are more likely to bypass an obese potential recipient in favor of one with a lower BMI. This calls for further scrutiny of the apparent bias against the obese in kidney transplantation. See Segev *et al.*, pages 349–355.



CLINICAL RESEARCH

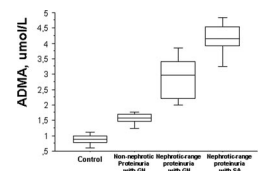
Specific Podocin Mutations Predict Phenotype

Steroid-resistant nephrotic syndrome can be caused by mutations in the gene encoding podocin (*NPHS2*). In a worldwide cohort study involving 430 affected children, Hinkes *et al.* identified a genotype–phenotype correlation between *NPHS2* mutations and age of onset of disease. The mean age of onset among children who had at least one truncation mutation or who were homozygous for the R138Q mutation was <2 yr. All other types of podocin mutations correlated with a mean age of onset of >4 yr. Even among those with identical mutations, however, age of onset varied by several years, suggesting the presence of additional disease modifiers. See Hinkes *et al.*, pages 365–371.



ADMA, Proteinuria, and Endothelial Dysfunction

Asymmetric dimethyl-arginine (ADMA), a breakdown product of arginine-methylated proteins, is a potent inhibitor of nitric oxide synthase. Yilmaz *et al.* report that serum levels of ADMA are higher in non-diabetic patients with proteinuria than in age-, sex-, and body mass index-matched healthy control subjects. This finding is particularly prominent among those with secondary amyloidosis, who are known to have increased protein turnover. Moreover, ADMA level is an independent predictor of endothelial dysfunction, providing a potential mechanistic link between proteinuria and cardiovascular morbidity and mortality. See Yilmaz *et al.*, pages 388–395.



Goodpasture T Cells May Exist in All of Us

Autoreactive T cells play a role in the pathogenesis of Goodpasture disease, but it is unknown how these cells escape tolerance mechanisms. Zou *et al.* hypothesized that proteases normally destroy the Goodpasture autoantigen before the immune system ever has a chance to recognize it as “self.” To test their hypothesis, the authors searched for quiescent $\alpha 3(\text{IV})\text{NC1}$ -specific T cells in healthy volunteers and found them in all. Furthermore, they show that the endosomal enzymes cathepsin D and E are likely responsible for the destructive processing of the Goodpasture autoantigen. These results suggest that peptide susceptibility to proteolytic cleavage may be a major determinant of T cell specificity in autoimmune disease. See Zou *et al.*, pages 396–404.

