

## Steroid-resistant nephrotic syndrome with mutations in *NPHS2* (podocin): report from a three-generation family

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### Abstract

Genetic causes of steroid-resistant nephrotic syndrome are being increasingly recognized. Mutations in *NPHS2*, which encodes the glomerular protein podocin, account for up to 17% of sporadic and 40% of familial cases, where they display an autosomal-recessive pattern of inheritance. This report describes a non-consanguineous family with three generations of individuals who are either compound heterozygotes for mutations in *NPHS2* or who have inherited a mutation and a non-neutral polymorphism (R229Q). As well as providing an aetiological explanation, identifying pathogenic mutations and considering genotype-phenotype correlations can provide prognostic information and lead to changes in genetic counselling and management.

**Keywords:** focal segmental glomerulosclerosis; *NPHS2*; podocin; steroid-resistant nephrotic syndrome

### Background

Nephrotic syndrome (NS) is defined by the presence of proteinuria, hypoalbuminaemia and oedema. The peak age of presentation is in pre-school children with the majority of cases responding to steroids [1]. However, around 10% of children and 40% of adults with NS fall into the 'steroid-resistant' category (steroid-resistant nephrotic syndrome: SRNS) [2]. The progression to end-stage renal disease (ESRD) occurs in 50–70% of patients with SRNS [3]. Inherited structural defects of the glomerular filtration barrier have been identified in isolated as well as familial cases of SRNS [2, 4, 5]. The genes involved in non-syndromic SRNS include *NPHS1*, *NPHS2*, *CD2AP*, *PLCE1*, *ACTN4*, *TRPC6*, *INF2*, *MYO1E*, *PTPRO* and *ARHGDI1A*. Syndromic SRNS is less common but the known genes are *WT1*, *LMX1B*, *LAMB2*, *ITGB4*, *SCARB2*, *COQ2*, *PDSS2*, *MTTL1*, *SMARCAL1*, *MYH9* and *NXF5* [6, 7].

The protein podocin, encoded by the *NPHS2* gene, is expressed exclusively in glomerular podocytes at the insertion site of the slit diaphragm [8, 9]. Mutations in *NPHS2* have been found in 40% of familial SRNS cases [where it follows an autosomal-recessive (AR) pattern of inheritance] as well as in 6–17% of sporadic SRNS cases [10]. *NPHS2* mutations lead to dysfunction of the glomerular filtration barrier with the prevailing renal pathology being focal segmental glomerulosclerosis (FSGS) [3, 9, 11]. The age of onset is variable but typically before 6 years of age [3, 12]. Late-onset (>18 years) cases of SRNS associated with *NPHS2* are also well recognized. These patients are frequently found to be compound heterozygotes for a

mutation and the non-neutral polymorphism, R229Q [3, 12–14].

This report describes a non-consanguineous Caucasian family in which two generations are affected with SRNS due to compound heterozygous mutations in *NPHS2* and with an older unaffected family member in an earlier generation who has inherited a mutation and a non-neutral polymorphism (R229Q) in the same gene. We illustrate how genetic testing in SRNS can lead to modified management for patients and define the risks for the wider family.

### Case report

The proband (III.1 in Figure 1) was 13 months old when she presented with 'puffy eyes', proteinuria and generally feeling unwell. She was diagnosed with NS and a renal biopsy showed minimal-change disease. Her family history was significant in that her father (II.1) had a renal transplant at the age of 10. He had been admitted at 11 months of age with respiratory symptoms, constant wet nappies and excessive thirst. Urinalysis revealed significant proteinuria and he was diagnosed with NS. The initial renal biopsy was suggestive of minimal-change disease but a repeat biopsy demonstrated FSGS. Both father and daughter had trials of various immunosuppressants that failed to improve their condition. The proband is now 9 years old and on furosemide and enalapril. Recent blood results showed sodium 140 mmol/L, potassium 5.0 mmol/L, urea 8.5 mmol/L, creatinine 27 µmol/L, calcium

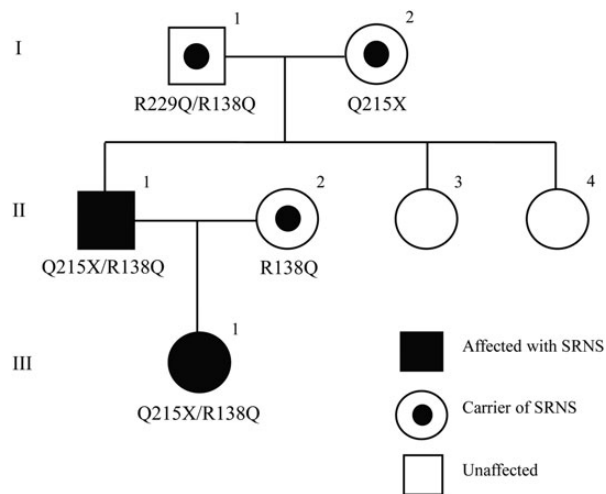


Fig. 1. Family pedigree showing the genotypes of those individuals who had *NPHS2* testing (written below the symbol) and their phenotype.

2.32 mmol/L, phosphate 1.61 mmol/L and albumin 21 g/L. Her father remains well since his renal transplant.

After referral to a regional clinical genetics centre, the family was counselled about SRNS following what appeared to be an autosomal-dominant (AD) pattern of inheritance but was also told that AR inheritance was a possibility. Genetic testing was undertaken and the father and daughter were both found to have two pathogenic *NPHS2* mutations (R138Q and Q215X). This particular gene and the presence of two mutations suggested an AR form of SRNS. In order to confirm this and to provide accurate recurrence risks, *NPHS2* genetic testing was offered to other family members. The mother (II.2) of the proband was a carrier for mutation R138Q therefore giving II.1 and II.2 a 50% risk of having another affected child. The paternal grandmother (I.2) was a carrier for mutation Q215X and the paternal grandfather (I.1) carried mutation R138Q, along with a well-known *NPHS2* variant (R229Q). At the present time, he has no evidence of renal dysfunction.

## Discussion

This family illustrates an interesting example of 'pseudo-dominant' inheritance. With no reported consanguinity, a father and daughter with SRNS would be suggestive of an AD mode of inheritance and a subsequent recurrence risk of 50% for any future children of an affected person. That both affected individuals carried well-known mutations in *NPHS2*, a gene that is only known to be associated with AR SRNS, was a surprising result in this family. The carrier frequency for any mutation in *NPHS2* is unknown but considered to be rare, making the genetic situation in this non-consanguineous family somewhat unique.

As well as confirming the aetiology, finding a pathogenic mutation in cases of SRNS has other positive outcomes for affected patients. Immunosuppressants, which have significant side effects and are unlikely to lead to an improvement in the condition, can be avoided [3, 10, 11]. Those with inherited defects as a cause of FSGS also have a lower post-transplant recurrence risk (3–8%) compared with those with FSGS due to non-inherited causes (30–45%) [3–5, 15]. In congenital cases, where the mutation pick-up rate is high, genetic testing offered early enough could

confirm the diagnosis before undertaking invasive renal biopsy [10]. Observed correlations with age of onset, renal biopsy findings and observed pattern of inheritance have led to proposed genetic testing strategies to assist clinicians with navigating the increasing number of genes associated with SRNS [2, 10]. Genetic testing in sporadic cases is also advocated. It has been suggested that mutations in *NPHS2* are frequent enough (9 out of 44 cases in a study by Caridi et al. [16]) to warrant *NPHS2* testing in all sporadic cases of SRNS with an onset beyond 3 months of age [2].

Genetic testing in affected individuals frequently leads to cascade testing in unaffected relatives. In this particular case, cascade testing had two aims. First of all, it confirmed that *NPHS2* was indeed the causative gene in this family. Both father and daughter carried the same two *NPHS2* mutations (R138Q/Q215X). Each mutation needed to be on a different copy of the *NPHS2* gene (*in trans*) to be deemed causative. If the father had had both mutations on one copy of *NPHS2* (*in cis*) while the other copy was 'mutation free', then that may also have explained why his daughter had inherited both mutations from him. However, the latter would not give a genetic explanation for the family's SRNS and other genetic causes would need to be looked for. This important point was clarified by testing the spouse and parents of II.1. All were found to be carriers of pathogenic *NPHS2* mutations and confirmed that II.1 and III.1 had inherited each of their mutations from a different parent. Therefore, the mutations were *in trans* and *NPHS2* was the causative gene for SRNS.

Knowing this, cascade testing could then answer questions about recurrence risk. II.1 and II.2 were told that they have a 50% chance of having another child affected with SRNS and that they may want to consider pre-natal testing in a future pregnancy if they feel that they would terminate an affected fetus. The genotype of an affected fetus may be either R138Q/Q215X or R138Q/R138Q. The latter is one of the genotypes associated with the earliest onset of *NPHS2*-associated SRNS, although there is no significant difference in progression to ESRD [12]. SRNS is not currently on the Human Fertilization and Embryology Authorities (HFEA) list of conditions licensed for pre-implantation genetic diagnosis (PGD) [17] but in theory would be possible, as causative mutations have been found. Although a recurrence risk of 50% is the same as the risk that this couple would have been given if the condition had been confirmed as following an AD pattern of inheritance, the risk to any future children of III.1 is no longer 50%. She now has a 25% risk of having a child with SRNS, but only if her partner also happens to be a carrier of a mutation in *NPHS2*.

Cascade testing in the proband's paternal grandfather (I.1) revealed an additional complexity in this case. He carried a pathogenic mutation (R138Q) along with a variant (R229Q). Variants (also called 'polymorphisms') are found in all genes. Many are not pathogenic, but some may have an as yet undefined disease-modifying role (a 'variant of unknown significance'). R229Q is the commonest *NPHS2* variant in people of European descent, with a frequency of between 2 and 3% in this population [3, 4]. However, it has a higher frequency in SRNS cases (5.3% in a study by Santin et al.) [3, 13]. When R229Q is inherited with a pathogenic *NPHS2* mutation (*in trans*), some individuals have developed SRNS with a significantly later onset compared to those with two *NPHS2* mutations [12–14]. Studies by Santin et al. and Machuca et al. reported that adult-onset SRNS cases with one *NPHS2* mutation all had the R229Q variant *in trans*, rather than a second *NPHS2* mutation [3, 14].

Therefore, the evidence suggests that R229Q had some role to play in the development of SRNS (i.e. a non-neutral polymorphism). Because of this, some advocated that the spouses of individuals with NPHS2-associated SRNS be offered genetic testing for the R229Q variant as there is up to a 50% risk of their children developing later onset SRNS [10]. However, R229Q inherited with a pathogenic mutation is not disease causing in all cases and at the age of 68, individual I.1 remains well. A recently published study by Tory *et al.* [18] shows that R229Q is only pathogenic when in *trans* association with specific NPHS2 mutations. Those mutations located in exons 1–6 (which includes R138Q) are unlikely to be pathogenic in association with R229Q, which is supported by our case. A greater understanding of the contribution of R229Q to the development of SRNS will enable more accurate counselling for families in the future.

This case, no doubt, illustrates that rare recessive conditions can manifest in more than one individual in a non-consanguineous family. The two carriers with R138Q (I.1 and II.2) are to the best of their knowledge, unrelated, as are II.1 and II.2. The R138Q mutation is the most frequently found pathogenic NPHS2 mutation and is believed to be a founder mutation in Europe [13, 19], therefore possibly explaining its presence in multiple individuals in the family.

We have shown that genetic testing in SRNS can be useful for aetiological confirmation and for providing accurate risk assessment and counselling in families. Significant progress has been made in understanding the genetics of SRNS. Further research is needed to understand the role of genetic variants and identify modifiers of disease that may explain why, for example, siblings with the same NPHS2 mutations have variability in the onset and presentation of SRNS [12, 20]. The ‘mainstreaming’ of genetic testing, and increasing use of next-generation sequencing means that clinicians will be faced more frequently with questions of how to interpret and use genetic information in the management and counselling of patients and their families.

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**Conflict of interest statement.** The results presented in this paper have not been published previously in whole or part, except in abstract format.

## References

1. McBryde KD, Kershaw DB, Smoyer WE. Pediatric steroid-resistant nephrotic syndrome. *Curr Probl Pediatr* 2001; 31: 275–307
2. Santin S, Bullich G, Tazon-Vega B *et al.* Clinical utility of genetic testing in children and adults with steroid-resistant nephrotic syndrome. *Clin J Am Soc Neph* 2011; 6: 1139–1148
3. Santin S, Tazon-Vega B, Silva I *et al.* Clinical value of NPHS2 analysis in early- and adult-onset steroid-resistant nephrotic syndrome. *Clin J Am Soc Neph* 2011; 6: 344–354

4. Karle SM, Uetz B, Ronner V *et al.* Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 2002; 13: 388–393
5. Weber S, Gribouval O, Esquivel EL *et al.* NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. *Kidney Int* 2004; 66: 571–579
6. Bouchireb K, Boyer O, Gribouval O *et al.* NPHS2 mutations in steroid-resistant nephrotic syndrome: a mutation update and the associated phenotypic spectrum. *Hum Mutat* 2013; 00: 1–9
7. Caridi G, Trivelli A, Sanna-Cherchi S *et al.* Familial forms of nephrotic syndrome. *Paed Nephrol* 2010; 25: 241–252
8. Boute N, Gribouval O, Roselli S *et al.* NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nature Genet* 2000; 24: 349–354
9. Schwarz K, Simons M, Reiser J *et al.* Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest* 2001; 108: 1621–1629
10. Benoit G, Machuca E, Antignac C. Hereditary nephrotic syndrome: a systematic approach for genetic testing and a review of associated podocyte gene mutations. *Paed Nephrol* 2010; 25: 1621–1632
11. Caridi G, Perfumo F, Ghiggeri GM. NPHS2 (Podocin) mutations in nephrotic syndrome. Clinical spectrum and fine mechanisms. *Pediatr Res* 2005; 57: 54R–61R
12. Hinkes B, Viangos C, Heeringa S *et al.* Specific Podocin mutations correlate with age of onset in steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 2008; 19: 365–371
13. Tsukaguchi H, Sudhakar A, Le TC *et al.* NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease-associated allele. *J Clin Invest* 2002; 110: 1659–1666
14. Machuca E, Hummel A, Nevo F *et al.* Clinical and epidemiological assessment of steroid-resistant nephrotic syndrome associated with the NPHS2 R229Q variant. *Kidney Int* 2009; 75: 727–735
15. Bertelli R, Ginevri F, Caridi G *et al.* Recurrence of focal segmental glomerulosclerosis after renal transplantation in patients with mutations of podocin. *Am J Kidney Dis* 2003; 41: 1314–1321
16. Caridi G, Bertelli R, Carrea A *et al.* Prevalence, genetics, and clinical features of patients carrying Podocin mutations in steroid-resistant nonfamilial focal segmental glomerulosclerosis. *J Am Soc Nephrol* 2001; 12: 2742–2746
17. Human Fertilisation and Embryology Authority. List of PGD conditions licensed by the HFEA. Updated 18th November 2013. <http://www.hfea.gov.uk/cps/hfea/gen/pgd-screening.htm> (18 November 2013, date last accessed)
18. Tory K, Menyhard DK, Woerner S *et al.* Mutation-dependent recessive inheritance of NPHS2-associated steroid-resistant nephrotic syndrome. *Nat Genet* 2014; <http://dx.doi.org/10.1038/ng.2898> (11 February 2014, date last accessed)
19. Hildebrandt F, Heeringa SF, Ruschendorf F *et al.* A systematic approach to mapping recessive disease genes in individuals from outbred populations. *PLOS Genet* 2009; 5: e1000353
20. Ekim M, Ozcakar B, Acar B *et al.* Three siblings with steroid-resistant nephrotic syndrome: new NPHS2 mutations in a Turkish family. *Am J Kidney Dis* 2004; 44: E22–E24

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