# THE MUTATION-DEPENDENT PATHOGENICITY OF THE *NPHS2* R229Q VARIANT

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## INTRODUCTION

*NPHS2*, encoding the membrane-anchored podocin, is the most frequently mutated gene in steroid-resistant nephrotic syndrome (SRNS) (1). Its mutations are inherited in an autosomal recessive fashion.

While mutations on both alleles ([mut];[mut]) lead to early-onset SRNS, the trans-association of the polymorphism c.686G>A, p.R229Q, and a mutation ([R229Q];[mut]) causes late-onset SRNS (2). In the homozygous state, R229Q does not cause SRNS (3). The allele frequency of R229Q in Europe (3-4%) is 15x higher than the cumulative allele frequency of all *NPHS2* mutations.

## AIM

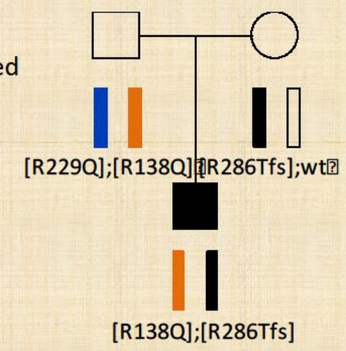
Despite the high frequency of R229Q, we noticed significantly less patients with [R229Q];[mut] than patients with [mut];[mut] in the medical literature. Therefore, we hypothesized that the [R229Q];[mut] is not pathogenic in all cases, and aimed to understand its pathogenicity.

## RESULTS

### The [R229Q];[mut] association is not pathogenic in all cases

We proved that the [R229Q];[mut] is not always pathogenic by its identification in 6/129 unaffected parents of children with *NPHS2* mutations. The unaffected parents carried the p.R138*, p.R138Q, p.R168H, c.534+1G>T, p.R238S mutations in trans with R229Q (Figure 1).

**Figure 1.** Identification of an unaffected father with [R229Q];[R138Q]. The index child developed SRNS due to [R138Q];[R286Tfs*17].




### The pathogenicity of R229Q depends on the trans-associated mutation

Next, we aimed to find the reason for the pathogenicity of [R229Q];[mut] in some cases. Interestingly, we found that the mutations associated to R229Q in the affected patients were missense mutations encoded by the last two exons (7-8). As these mutations are rare among *NPHS2* mutations, this resulted in a significant enrichment (Table 1). This striking enrichment pointed to the pivotal role of the associated mutation in determining the pathogenicity of R229Q.

**Table 1.** Three prime mutations are associated to R229Q in the affected patients

| | mutant alleles in 247 patients with [mutation];[mutation] | | mutant alleles in 71 patients with [p.R229Q];[mutation] | | |
|---|---|---|---|---|---|
| | *n* | ratio | *n* | ratio | |
| exons 1-6 | 419 | 85% | 8 | 11% | $P = 1.2 \times 10^{-35}$ |
| **exons 7-8** | **75** | **15%** | **63** | **89%** | |

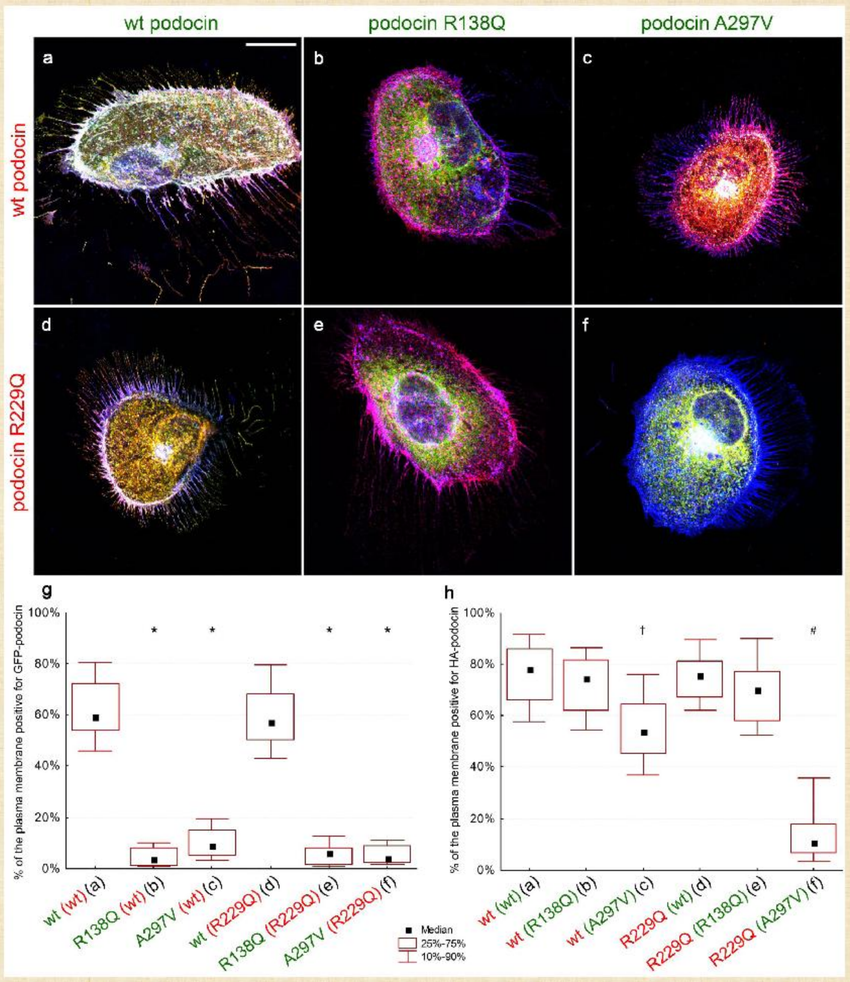
### Mutations that are pathogenic with R229Q alter the localization of podocin R229Q

In cultured podocytes we found the podocin R229Q to be mislocalized only when co-expressed with podocin mutants that are pathogenic with it, indicating that these mutants exert a dominant negative effect on podocin R229Q (Figure 2).

**Figure 2.** Membrane targeting of wt podocin and podocin R229Q as a function of the associated mutation in podocytes stably coexpressing podocin mutants. Podocin R229Q is not membrane-targeted only when coexpressed with podocin A297V.

Wt podocin (a-c) and podocin R229Q (d-f) are shown in red, the coexpressed GFP-tagged podocin in green. The plasma membrane is labeled with WGA and shown in blue. Both wt podocin and podocin R229Q are localized to the plasma membrane when coexpressed with wt podocin and with podocin R138Q, despite the retention of this latter in the endoplasmic reticulum. The membrane targeting of podocin R229Q in cells coexpressing podocin A297V is abolished (f). Scale bar = 20 µm.

g-h) Membrane targeting of podocin proteins quantified as the percentage of the plasma membrane (WGA) that is positive for GFP- (g) or HA-tagged podocin proteins (h), within 30 cells per group. * $P<3.3 \times 10^{-11}$ vs. wt (wt) [wt-GFP]; † $P=1.8 \times 10^{-6}$ vs. wt (wt) [wt-HA]; # $P=3.5 \times 10^{-11}$ vs. R229Q (wt)




We confirmed the mislocalization of podocin R229Q ex vivo, in urinary podocytes of a patient who is compound heterozygous for the pathogenic [R229Q];[A284V] association (Figure 3).

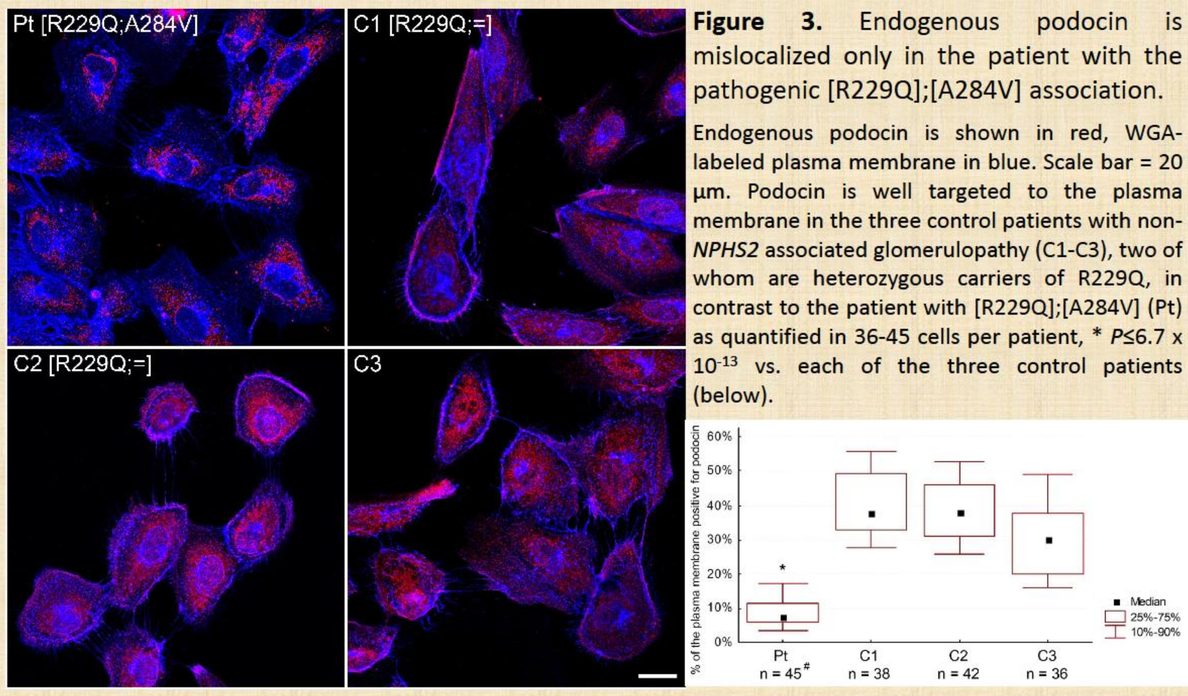



**Figure 3.** Endogenous podocin is mislocalized only in the patient with the pathogenic [R229Q];[A284V] association.

Endogenous podocin is shown in red, WGA-labeled plasma membrane in blue. Scale bar = 20 µm. Podocin is well targeted to the plasma membrane in the three control patients with non-*NPHS2* associated glomerulopathy (C1-C3), two of whom are heterozygous carriers of R229Q, in contrast to the patient with [R229Q];[A284V] (Pt) as quantified in 36-45 cells per patient, * $P \leq 6.7 \times 10^{-13}$ vs. each of the three control patients (below).

### The C-terminal missense mutations exert their dominant negative effect on podocin R229Q through an altered dimerization

According to the structural model of dimerization, the mutations that are pathogenic with podocin R229Q are all localized to the C-terminal helical tail of podocin (Figure 4a). This region is implicated in the dimerization by forming a coiled-coil type association. Significant structural reorganization appeared in pathogenic heterodimers of R229Q, but not in the non-pathogenic ones (Figure 4b-c).

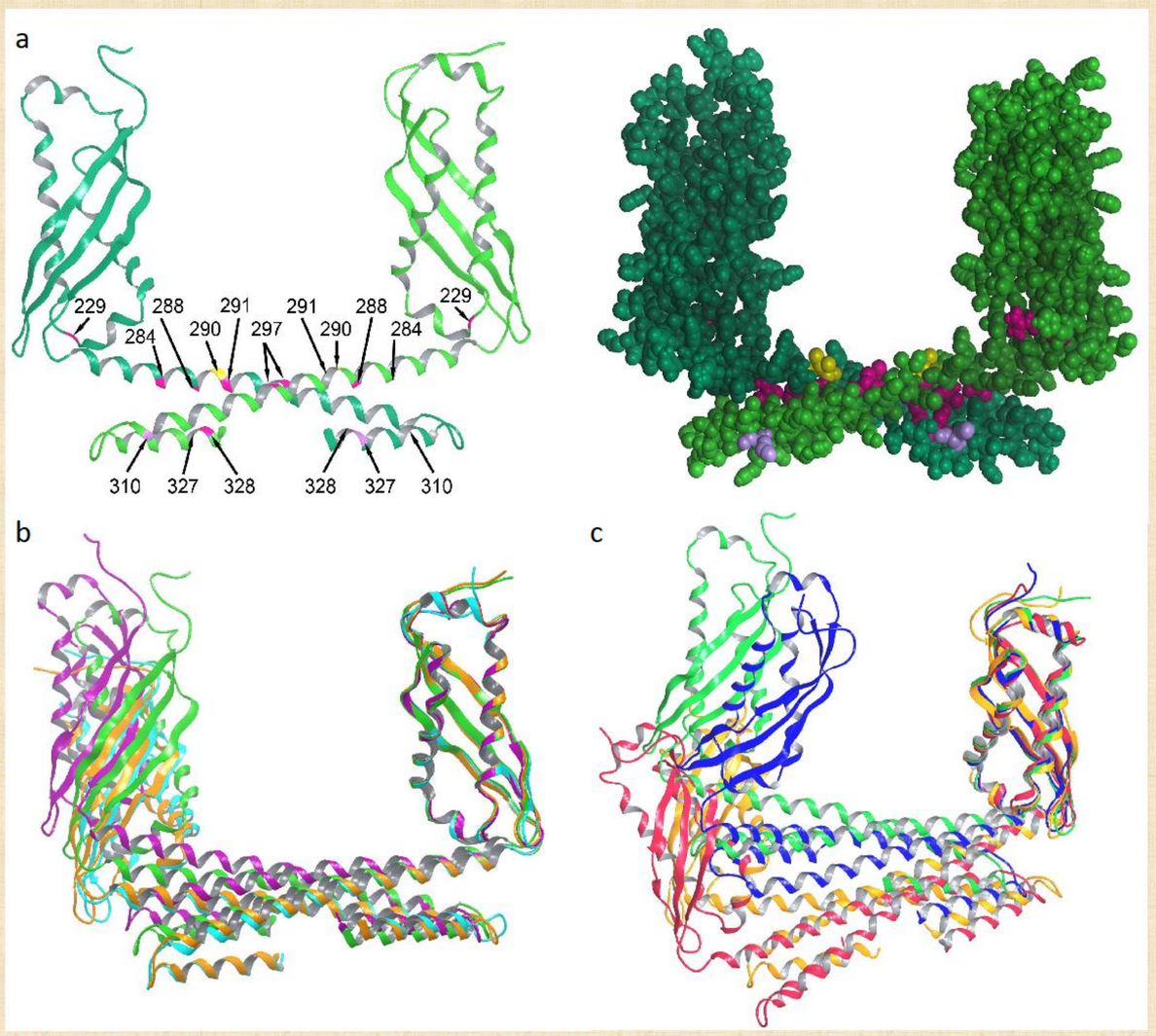



**Figure 4.** Structure of pathogenic and non-pathogenic podocin dimers

a) Calculated structure of the podocin wt/wt homodimer shown as ribbon diagram and an all-atom representation. Mutations that point inward to the head domain (229) and the coiled-coil region (284, 288, 291, 297, 328) are colored magenta. Mutations at these latter positions are expected to impair the mode of dimerization in contrast to V290 (in yellow) which points outward from the coiled-coiled domain. E310 and L327 (shown in lilac) seem like fenders to the coiled coil region.

b-c) Superimposed average structures of the non-pathogenic (b) and pathogenic dimers (c), overlaid based on their globular region of monomer A for comparison. b) Non-pathogenic dimers of podocin R229Q/R229Q (cyan), podocin V290M/R229Q (orange) and podocin A284V/wt (purple) are similar in structure to the podocin wt/wt homodimer (green). c) This is in contrast to the structures formed by the pathogenic dimers podocin A284V/R229Q (red), podocin A297V/R229Q (yellow) and the podocin A284V/A284V homodimer (blue) shown superimposed on the podocin wt/wt homodimer (green).

## CONCLUSIONS

- The R229Q variant is only pathogenic when trans-associated with specific mutations.
- Thus, *NPHS2*-associated nephrotic syndrome is the first autosomal recessive disorder, in which the pathogenicity of an allele is determined by the other. This phenomenon has direct clinical implications, and can open up a new aspect in the assessment of the pathogenicity of sequence variants (4).

## METHODS

A cohort of 129 healthy parents of 67 affected children with *NPHS2* mutations were screened for R229Q by ClaI digestion. Patients carrying *NPHS2* mutations were compiled from the literature. Human cultured podocytes were cotransfected with plasmids encoding HA-wt or HA-R229Q podocin and podocin-GFP mutants. The membrane-targeting of podocin proteins was characterized by the fraction of the perimembranous area that colocalized with podocin on confocal images. The structure of podocin dimers was calculated on the basis of a homology model based on the crystal structure of Pyrococcus horikoshii stomatin as detailed in Tory et al. Nat Genet, 2014 (4).

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