Ascertaining pathogenicity of genetic variants: caution required

We thank the authors of the letter ‘High prevalence of pathogenic variants in Japanese children with steroid-resistant nephrotic syndrome without edema detected by urine screening program” for their comment on our own paper on a low diagnostic yield of genetic testing in children with SRNS [1]. The authors confirm our finding with results from their own cohort: of 29 children with SRNS, none of them had an identified underlying causative genetic variant and all responded to extended immunosuppression. Yet, as the title indicates, the most interesting aspect of their comment concerns the genetic findings in 6 patients with asymptomatic proteinuria (ASP), which they define as proteinuria (without oedema) identified by population urine screening. Interestingly, despite the absence of nephrotic syndrome, all 6 patients appear to have been treated with immunosuppression (cyclosporine), but with no apparent response. Moreover, 5 of these 6 patients are described as having causative variants in SRNS disease genes. The authors reach the fascinating conclusion that the yield of genetic testing of SRNS disease genes may increase by testing patients with ASP, rather than SRNS! How about that?! When it comes to testing of SRNS genes, forget about SRNS! Just focus on ASP!

Before we embark on such dramatic paradigm shift in SRNS genetic testing, we would like to express some caution. First, we must be careful when reaching conclusions from such a small number of patients. Next, the authors did not detail the exact definition of ASP. Was there a minimum level of proteinuria? Apparently, all 6 later developed plasma albumin levels <25 g/l, but it is unclear, if that was part of the definition of ASP. Most importantly, the authors do not provide details on the genetic variants they identified, nor the criteria used to ascertain pathogenicity. Yet, provision of these details is critical to verify the accuracy of their findings. Establishing a genetic diagnosis can have profound consequences, such as termination of subsequent pregnancies found to carry this variant, or on assessing family members for kidney donation [2]. Thus, causality of genetic variants for a given phenotype should be considered very carefully. We note that in the letter, all the identified variants are in dominant disease genes: *WT1, TRPC6, ACTN4* and *INF2.* This can present challenges in variant assessment, for instance when using the ACMG criterion “PP3” (Multiple lines of computational evidence support a deleterious effect). These computer algorithms are designed to detect variants that disrupt function of the gene product, which is useful for recessive disorders, where loss-of-function is the typical disease mechanism and similarly for dominant disorders where the established disease mechanism is haploinsufficiency. But the algorithms are not designed to predict gain-of-function variation, which, for instance, is the initially reported disease mechanism for *TRPC6*-associated kidney disease [3]. For instance, we identified a heterozygous *TRPC6* nonsense variant (c.7C>T; p.(Gln3*)) in a 7-month-old girl with nephrotic syndrome, unresponsive to a short course of steroids. Was this the explanation for her kidney disease? In such cases,

ascertaining the inheritance of the variant can be very helpful. In this girl, it was inherited from the asymptomatic mother, and we therefore decided that the variant was unlikely to be disease-causing. Of note, the nephrotic syndrome went into remission associated with tacrolimus treatment and she has remained in remission even after weaning of the drug.

Conversely, we identified a heterozygous missense variant in *ACTN4* (c.720G>A; p.(Met240Ile)) in a 13-year-old girl who presented with clinical features of nephrotic syndrome and advanced chronic kidney disease. She commenced peritoneal dialysis a month after presentation. There was no family history of nephrotic syndrome or kidney failure, but of parental consanguinity, so the identification of a variant in a dominant disease gene raised concerns about its pathogenicity, even though another variant of the same amino acid (c.719T>C; p.(Met240Thr)) has been reported as pathogenic with functional data to support this [4] In this case, parental testing identified that the variant was *de novo*, providing strong evidence for its classification as pathogenic for her condition.

We perform genetic testing to find a cause for a patient's phenotype and therefore we may be biased in assuming pathogenicity for interesting variants identified. Yet, the null hypothesis, that there is no association between phenotype and variant may still be true! These two cases highlight the importance of gathering sufficient evidence before deciding on the pathogenicity of a given genetic variant. All relevant evidence may have been assiduously ascertained in the Japanese cohort, but without provision of these details, we should wait before we focus genetic testing on ASP.

References:

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