Guaragna MS, Lutaif ACGB, Belangero VMS, Maciel-Guerra AT, Guerra-Junior G, De Mello MP.
J Rare Dis Res Treat. (2017) 2(3): 49-51

www.rarediseasesjournal.com

Journal of Rare Diseases Research & Treatment

Commentary Open Access

Commentary: NPHS2 mutations account for only 15% of nephrotic syndrome cases

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Article Info

Article Notes
Received: April 30, 2017
Accepted: May 24, 2017

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Background

Nephrotic syndrome (NS) represents one of the most common kidney conditions that affect children. It manifests as proteinuria, edema, hypoalbuminemia and hyperlipidemia. According to the response to standardized corticosteroid therapy, 80 to 90% of children that respond well to the treatment are classified as steroid sensitive (SSNS), whereas the remaining 10 to 20% that are unresponsive to the treatment are classified as steroid resistant (SRNS)[1]. NS is a highly heterogeneous condition and many SRNS cases remain idiopathic, although a fraction of them have a clear genetic etiology. The most frequent renal histologic feature of SRNS is focal segmental glomerulosclerosis (FSGS). Almost 40% of SRNS/FSGS children develop end-stage renal disease (ESRD) before adulthood and may receive kidney transplantation with 10 to 50% risk of FSGS recurrence in the allograft kidney[2–4]. Conversely, the post-transplant risk of proteinuria recurrence in patients with inherited forms of SRNS is only 1-2%[5].

In the last 15 years, more than 50 genes have been associated to hereditary forms of SRNS/FSGS, whose proteins are mainly expressed in the visceral epithelial podocytes, specialized cells of the outer surface of the glomerular filtration barrier[6]. Two recent studies in large SRNS cohorts report a monogenic cause of the disease in approximately 30% of cases in whom mutations were found in one of those 50 genes[7,8]. Despite the increasing number of genes associated with SRNS, there are three that are frequently mutated in patients with early onset of the disease: *NPHS1*, *NPHS2* and *WT1*[7–9]. *NPHS1* (OMIM *602716) mutations are typically associated with congenital nephrotic syndrome, with proteinuria onset before 3 months of age, although there are reports on mutations identified in childhood onset cases of SRNS[10–12]. *NPHS1* encodes nephrin, an essential protein of the glomerular slit diaphragm formed between adjacent podocytes[13]. *NPHS2* (OMIM *604766) mutations are responsible for 6-17% and 40% of sporadic and familial forms of SRNS in childhood, respectively, and encodes podocin, a close interactor of nephrin[14–16]. The third main gene associated with early onset SRNS is *WT1* (OMIM *607102), which encodes a transcription factor that plays a key role during kidney and genital development. Dominant *de novo*

mutations in exons 8-9 have been associated to 2 to 7% of sporadic cases of SRNS in childhood[17].

Our results

Our publication "*NPHS2* mutations account for only 15 % of nephrotic syndrome cases" was the first cohort study focusing on genetics of SRNS in Brazilian children. In this study we included 27 SRNS patients that enrolled from 2008 to 2013 in the reference public hospital of clinics from the South East region of Brazil[18]. The patients comprised seven unrelated familial cases and 20 sporadic cases with onset of proteinuria between 3 months and 18 years of age. We performed sequence analysis of the entire *NPHS2* and *NPHS1* genes and of exons 8 and 9 of the *WT1* gene in all the patients. A genetic cause of the NS was identified in only four cases (4/27, 14.8%), two sporadic (2/20, 10%) and two familial (2/7, 28.5%) (Table 1). All variants considered as the cause of the NS in these patients were identified in *NPHS2* gene. Therefore, mutations in *NPHS1* and *WT1* genes have not been identified in those patients. The sporadic patients were compound heterozygous for [p.Arg229Gln];[p.Ala284Val] and [p.Arg229Gln];[p. Glu310Lys] *NPHS2* mutations, as indicated by the segregation of the variants in the family. Those results confirmed the autossomal recessive inheritance in both cases. The association of those mutations have been already described as the cause of SRNS by other groups[19–22]. In the case of the two familial cases, the heterozygosis for *NPHS2* mutations were, respectively, [p.Arg229Gln (;) p.Ala284Val] and [p.Lys239Argfs*13 (;) p.Val260Glu]. We also identified the same combinations in the affected siblings, but we could not evaluate the segregation in these two families although we considered the compound heterozygosis as the most probable genotype. We are not aware if other members of the family have renal disease. The age at onset of NS in the two patients with the p.Arg229Gln variant along with the p.Ala284Val mutation was 12 and 13 years old, confirming a later onset of the disease as the result of this association[21]. One of them progressed to CKD and received kidney transplant with no FSGS recurrence. On the other hand, the other two cases presented NS symptoms with 1.2 and 2.2 years old and with no progress to ESRD yet.

Our findings also included SRNS patients with only one heterozygous alteration in the *NPHS2* gene: three sporadic cases presented promoter variants c.-164C>T, c.-196C>G and c.-537_-531delCTTTTTT and a familial case presented the p.Arg229Gln variant. Two of these patients progressed to ESRD and both received kidney transplant, although one of them presented FSGS recurrence post-transplant. Regarding promoter variants, Di Duca et al.[23] studied regulatory elements in the *NPHS2* promoter and described the c.-537_-531delCTTTTTT variant (rs146791300, MAF = 0.069; http://www.ncbi.nlm.nih.gov/) as a "functional polymorphism" that downregulated the gene expression of podocin by 85% when transfected in podocytes. Since there are no records of association of c.-164C>T and c.-196C>G variants with SRNS patients and our *in-silico* predictions indicated a possible creation of a new WT1 transcription binding site after c.-164C>T transition, we are investigating their role on podocin expression using reporter vector transfection assays in podocytes. For the familial case presenting the p.Arg229Gln, he and his father presented this variant and both had already received kidney transplant with no FSGS recurrence, clearly indicating a genetic background of their NS. Therefore, we performed whole exome sequencing (WES) for this family and the results will be published further.

Limitations and strengths

Our paper's main limitation was the sample small size. Approximately 200 patients with NS are routinely followed up in our service, one of the reference centers in Brazil; our 27 SRNS patients represent a frequency of 13.5% (27/200), which is in line with the reported worldwide frequency of 10-20% for this form of the condition. However, the sample is considered small for mutation frequency and epidemiological estimations[16,24]. An example of this limitation is the *NPHS2* mutation rate – 28.5% - detected for our four out of seven familial cases. We cannot assure that the frequency described by us for the

Table 1: Clinical data from SRNS patients with *NPHS2* variants.

Patient Number	Age onset (years)	Sporadic/familial[a]	Renal biopsy[b]	Renal transplant (yes/no)	NPHS2
					Two heterozygous variants
P6	12	spo	FSGS	Yes	[p.Ala284Val] ; [p.Arg229Gln]
P67	2.2	spo	DMP	No	[p.Glu310Lys] ; [pArg229Gln]
P103	1.2	fam	FSGS	No	[p.Lys239Argfs*13 (;) p.Val260Glu]
P154	13	fam	FSGS	No	[p.Arg229Gln (;) p.Ala284Val]
One heterozygous variant					
P111	3	spo	FSGS	No	c.-164C>T
P68	4	spo	CO	No	c.-196C>G
P85	6.11	spo	CO	Yes	c.-537_531delCTTTTTT
P72	16	fam	FSGS	Yes	p.Arg229Gln

[a]Spo: sporadic; Fam: familial;[b]FSGS:focal segmental glomerular sclerosis; DMP: diffuse mesangial proliferation; CO: complex = minimal change disease or diffuse mesangial proliferation or focal segmental glomerular sclerosis.

Brazilian population is lower then those described by others in different populations (~ 40%), since our cohort is not robust enough for making such comparisons. The Brazilian population is highly miscegenated and presents diverse regional features therefore our cohort from South East did not represent the entire country. The novelty of the work was the genetic study in Brazilian SRNS patients and the identification of a new frameshift mutation associated with SRNS.

Conclusions

Since we were able to identify the genetic origin of the NS only in 14.8% of our SRNS cases, our conclusion was that target-oriented next generation sequencing of glomerulopathy related genes is recommended to search for mutations in other genes related to SRNS in the remaining 74.2% patients. It is worth to mention that such studies are being carried out. Finally, the unequivocal molecular genetic diagnose and the correct establishment of genotype-phenotype correlation are essential because they can influence physicians' decision on individual treatment, as patients carrying mutations can be spared the side effects of immunosuppressive therapy and ultimately can be considered for kidney transplantation from a living donor.

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 478444/08-7 to G.G-J and 141072/2010 to MSG) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 2012/51109-0 to MPdeM).

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