Mendelian Steroid Resistant Nephrotic Syndrome in childhood: is it as common as reported?

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Abstract:

Background:

Primary steroid resistant nephrotic syndrome (SRNS) is thought to have either genetic or immune-mediated aetiology. Knowing which children to screen for genetic causes can be difficult. Several studies have described the prevalence of genetic causes of primary SRNS to be between 30-40%, but these may reflect a selection bias for genetic testing in children with congenital, infantile, syndromic or familial NS and thus may overestimate the true prevalence in a routine clinical setting.

Methods:

Retrospective electronic patient record analysis was undertaken of all children with non-syndromic SRNS and presentation beyond the first year of life, followed at our centre between 2005 and 2020.

Results:

Of the 49 children who met the inclusion criteria, 5 (10%) had causative variants identified, predominantly in *NPHS2*. None responded to immunosuppression. Of the 44 (90%) who had no genetic cause identified, 33 (75%) had complete or partial remission after commencing second-line immunosuppression and 67% of these had eGFR >90 ml/min/1.73m^2 at last clinical follow-up. Of the children who did not respond to immunosuppression, 64% progressed to kidney failure.

Conclusions:

In our cohort of children with non-syndromic primary SRNS and presentation beyond the first year of life, we report a prevalence of detectable causative genetic variants of 10%. Those with identified genetic cause were significantly (p= 0.003) less likely to respond to immunosuppression and more likely (p=0.026) to progress to chronic kidney disease. Understanding the genetics along with response to immunosuppression informs management in this cohort of patients and variant interpretation.

Introduction:

Nephrotic Syndrome (NS) is the commonest glomerular disease of childhood [1]. Response to immunosuppression in the form of steroids, typically 4 weeks of 60 mg/m^2 of oral prednisolone, defines its steroid sensitive (SSNS) or steroid resistant (SRNS) nature [2]. Primary SRNS is thought to be of either genetic or immune-mediated aetiology [3].

 Children with pathogenic variants in SRNS disease genes generally tend to not respond to immunosuppression and typically progress to kidney failure during childhood [4, 5]. However, some forms of genetically acquired SRNS allow for targeted therapies such as in the case of coenzyme Q$_{10}$-related genes, where supplementation has been reported to slow disease progression [6]. Those without identifiable causative variants and especially those with secondary steroid-resistance are thought to have a disease process secondary to circulating factors. The role of Calcineurin-Inhibitors (CNIs) and Renin-Angiotensin system (RAS) inhibition in establishing disease remission in some patients in this cohort of children has been well documented [7]. Early determination of either genetic or immune-mediated forms informs management early in the disease process and can prevent exposure to potentially harmful immunosuppressive agents.

Knowing which children to screen for genetic causes can also be difficult. Numerous studies describe how genetic causes can make up to 30-40% of the SRNS cohort in childhood [3, 4], but these cohorts usually combine all childhood SRNS, including congenital nephrotic syndrome (CNS) and infantile NS where children present with NS during the first 3 months and first year of life, respectively, as well as syndromic forms. Moreover, these studies typically originate from genetic laboratories and may be subject to referral bias in that patient with young age of onset, a positive family history, syndromic features or a background of consanguinity may be more likely to undergo genetic testing. The positive predictive value of genetic testing is dependent on the prevalence of detectable Mendelian disorders in the test population and this informs variant interpretation through criterion PP4 (patient's phenotype is

highly specific for the disease associated with the respective gene) [8]. There are little data available on the frequency of causative variants in an unbiased cohort of children with SRNS. We therefore aimed to look at the yield of pathogenic variants in our SRNS cohort of children over a 15-year period. We specifically excluded those with CNS/Infantile NS or syndromic NS. We also reviewed the response to immunosuppressive medications and how that might influence future management options.

Methods:

A retrospective electronic patient record (EPR) analysis of all children (ages between 1 to 16 years) who presented to our tertiary paediatric nephrology centre, between 2005 and 2020, with primary SRNS was undertaken. Children who had received 4 weeks of 60 mg/m^2 of prednisolone and had not entered remission, at the time of referral to our centre, were deemed primary steroid resistant as per the widely accepted International Study of Kidney Disease in Children (ISKDC) SRNS definition [2]. Our routine clinical practice includes performing genetic testing in children with SRNS and subjects were identified from the database in our clinical genetic laboratory that handles all genetic testing.

Children with CNS and infantile NS, and those with associated syndromic features (suggesting a specific inherited disorder, such as Schimke's disease) were excluded from the analysis.

Parents/guardian were consented for genetic analysis as part of the clinical care. All children were tested through the National Health Service genetic testing service, which is free at the point of care. The service uses a panel of genes that is regularly updated to provide comprehensive testing for pathogenic variants responsible for SRNS [9]. The panel uses massively parallel sequencing (MPS) to identify candidate variants which are then confirmed by Sanger sequencing [10].

Data collected from EPR included demographics, age of onset of NS, any atypical features associated with NS, any extra-renal features or dysmorphism, family history of NS, kidney histopathology results where available and validated genetic variant reports.

All statistical analysis were performed using SPSS Version 27.

Results:

49 children met the inclusion criteria. Mean age at the time of diagnosis was 5.5 years with a range of 1-16 years (pertinent details are summarised in Table 1).

Family History:

None of the children had a known family history of SRNS.

Genetic cause identified (Table 2):

5 (10%) of the 49 children had pathogenic variants identified which were deemed causative for their phenotype. Mean age at the time of diagnosis was 5.2 years (range 1-13 years). Mean follow-up period was 5.2 years with a range of 3 -10 years. Four (80%) children had pathogenic variants in *NPHS2* and one in *NPHS1*. None of the children in this cohort responded to a trial of immunosuppression.

No genetic cause identified:

44 (90%) of the 49 children who presented with primary SRNS had no genetic cause identified. Mean age at the time of diagnosis was 6.0 years with a range of 1 to 16 years. Mean follow-up period for this cohort was 7.2 years with a range of 1 to 15 years.

1) Complete/partial response to immunosuppression (33/44):

Of the 44 children with no identified genetic cause, 33 (75%) went into partial or complete remission following initiation of alternate immunosuppression and/or RAS inhibition. All those who went into complete remission (22/33, 67%) had estimated glomerular filtration rates (eGFR) greater than 90 ml/min/1.73m^2 (modified Schwartz formula) at the last clinical encounter (median time of follow-up 6.8 years). Only 2 (2/11, 18%) of the children who went into partial remission had eGFRs less than 90 ml/min/1.73m^2. Four children (4/33, 12%) in this cohort received Rituximab, after trial of alternate immunosuppression, to achieve remission, whereas the rest achieved remission after initiation of tacrolimus and RAS inhibition. The histology findings of the 28 children who underwent a kidney biopsy are detailed in Table 1.

2) No response to immunosuppression (11/44):

25% (11/44) children did not respond to any immunosuppression and RAS inhibition. 7 (64%) of these progressed to kidney failure during the follow-up period (median 20 months post-diagnosis). Of the remaining 4; 3 have an eGFR of >90 ml/min/1.73m^2 at an average follow-up period of 8.5 years and one child has CKD stage 3 at 10 years follow-up. All children underwent a kidney biopsy. 10 (91%) had FSGS whilst 1 (9%) had MCD as the associated histological feature.

Overall, there was no difference between the two cohorts (genetic cause identified and not identified) in terms of age (p=0.165), gender (p= 0.873), ethnicity (p= 0.976), and associated histology (p=0.873) but there was a statistically significant difference in response to immunosuppression (p= 0.003) and progression to chronic kidney failure (p=0.026) (Figure 1).

Discussion:

Our study in an unbiased clinical cohort identifies causative variants in 10% (5/49) of children with non-syndromic SRNS and presentation beyond the first year of life. This is substantially lower than those reported by genetic testing centres for SRNS in general and suggests that the reported diagnostic yield is inflated by a referral bias that selects those cases for genetic testing that have features suggestive of an inherited basis [6, 11, 12], Indeed, while the reported diagnostic yield for SRNS in a national UK cohort was 26.2%, this reduced to 14.5%, when excluding those with onset in the first year of life, family history or extra-renal manifestations consistent with a syndromic disorder [13]. This yield of 14.5% is much closer to the one observed in our cohort and some of the remaining difference may be due to uncertainty from the small sample size in our cohort. But it may also reflect that referring clinicians not always detail clinical features suggestive of inherited disease when sending a sample for genetic testing, whereas these were readily available in our own cohort.

Our observation informs the interpretation of variants identified in children with sporadic SRNS, especially of variants of uncertain significance (VUS). The criteria defined by ACMG for variant assessment have deliberately put a high threshold for assigning pathogenicity to avoid false positive genetic diagnoses, with the threshold for "likely pathogenic" being a likelihood of ≥90% [14]. If the prevalence of detectable Mendelian disease is only 10%, as in our cohort, the positive predictive value of a VUS is at best around 50% and therefore unlikely to be causative [8].

In our cohort, 80% of the children who had a pathogenic variant identified had the variant in *NPHS2* gene. Although the numbers are small, this is in keeping with previous reports for children older than 1 year of age and with non-syndromic SRNS in European and North American cohorts [6, 15]. *NPHS2* variants are less frequently reported as a cause of SRNS in cohorts outside these geographical areas [16–18]. Unsurprisingly, 80% of these

progressed to kidney failure during the follow-up period of this study. In those without identified genetic cause, most (75%) achieved partial or complete remission in response to CNIs and/or RAS inhibition and had excellent overall kidney function. 64% of children who did not respond to any immunosuppression and RAS inhibition progressed to kidney failure, at a median 20 month after disease onset, during the follow-up period of this study.

Response to immunosuppression in children with SRNS, regardless of genetic status, is a known predictor of progression to kidney failure [19, 20]. Children with immune-mediated SRNS have a better prognosis in terms of disease remission and overall progression to kidney failure. Almost three-quarter of children in our cohort achieved complete or partial remission in response to a CNI-based therapy with or without RAS inhibition. All children who achieved complete remission and 83% of those with partial remission had an eGFR of > 90 ml/min/1.73m^2 at the end of follow-up period in our study. Commencing CNIs early, after establishing SRNS as the diagnosis, can serve as an effective strategy in achieving disease remission whilst awaiting results of genetic testing which may take up to a few months. Our results suggest that given the low prevalence of identifiable genetic causes, the number of patients exposed to unnecessary immunosuppression would actually be very low. Immunosuppression should be discontinued in those without apparent response once a genetic cause is identified as it is unlikely to provide any benefit. Arguably, genetic analysis should be prioritised only to those patients who have not responded to second line immunosuppressants, such as CNI. Although this is in contrast to current guidance, this could be particularly relevant in countries with low resources for genetic testing [21, 22]. In our cohort, the prevalence of detectable genetic causes increased to 31%, when assessing only patients with no response to such immunosuppression.

Our study has limitations in that it was a retrospective, single centre review with small numbers. While requesting genetics is part of our protocol for managing SRNS, it is possible that this may have been missed in some. Yet, this would likely have biased our cohort towards an increased prevalence of identifiable genetic causes, as those patients

with persistent resistance to immunosuppression and progressive chronic kidney disease would have eventually been tested, whereas those entering remission (and thus unlikely to have a Mendelian cause) may never have had genetic testing performed and thus would not have been included in this study. To minimise the referral bias for genetic testing, we also included only those children who received genetic testing through our centre. In our clinic are further patients, who transferred from other centres and had genetic testing performed there (n=4). Of these, only one had a likely pathogenic variant (c.1228+5G>A, *de novo*) in *WT1* (a boy with a history of hypospadias, who presented age 3 years with NS) identified. The others reportedly had no suspicious variants identified. Thus, by excluding these, we did not substantially change our assessment of the prevalence of genetic causes.

During the analysis period from 2005 to 2020, several new SRNS disease genes were identified, and it is therefore possible that causative variants may have been missed, if the respective gene had not yet been recognised at the time of testing. However, the NHS genetic testing service is associated with an active research program and unexplained patients are routinely re-analysed with updated reports issued to clinicians if a cause had subsequently been identified. As we had not received any such updated report, we consider the likelihood of such incomplete testing as very low.

In summary, in our cohort of children with non-familial, non-syndromic primary SRNS, we report a prevalence of detectable causative genetic variants of 10%. Although our numbers are small, this is substantially lower than the prevalence reported by genetic centres and suggests that those cohorts are biased by referral, as patients with features suggestive of an underlying genetic cause, such as presentation in the first year of life, a positive family history or a specific syndrome may be more likely to be referred for genetic testing than sporadic cases without those features.

Understanding the genetics along with response to immunosuppression informs the management of these children. Our findings can help clinicians especially in centres where genetic testing is not readily available in guiding management of this challenging disorder.

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	Genetic Cause identified (n=5)	Genetic cause not identified (n= 44)	Chi-Square Tests
Age	5.2 years (range 1-13)	6.0 years (range 1- 16)	p=0.165
Sex	3 (60%) girls	28 (64%) girls	p= 0.873
Ethnicity	3 (60%) White 1 (20%) Asian 1 (20%) Black	23 (52%) White 11 (25%) Asian 9 (21%) Black 1 (2%) mixed	p= 0.976
Histology	4 (80%) FSGS 1 (20%) MCD	29 (74%) FSGS 5 (12%) MCD 2 (5%) C1q nephropathy 1 (2%) membranoproliferative nephropathy 1 (2%) thin-membrane disease 1(2%) focal mesangial hypercellularity	p= 0.873
Response to immunosuppression	0 (0%)	33 (75%)	p= 0.003
Progression to chronic kidney disease	4 (80%)	9 (21%)	p= 0.026

Table 1: Cohort characteristics of children with an identifiable genetic cause and those without.

Patient Number	Age at Diagnosis (years)	Sex	Ethnicity	Pathogenic Variant	Variant classification according to ACMG guideline		Histology	Response to Immunosu-ppression	CKD stage
					Allele1	Allele2			
1	5	F	White	*NPHS2* c.413G>A p.(Arg138Gln) & c.855_56del p.(arg286Thrfs*17)	PM2 PP3 PS4_Mod PS3_Mod PM3_St Class 5	PVS1 PM2 PS4_Mod PM3 Class 5	FSGS**	None	On peritoneal dialysis
2	13	F	Black	*NPHS1* hom c,1756A>G p.(arg586Gly) likely pathogenic	PS4_Mod PM2 PM3_mod PP1_st Class 5		FSGS	None	1
3	4.5	M	White	*NPHS2* hom c.413G>A p.(Arg138Gln)	PM2 PP3 PS4_Mod PS3_Mod PM3_St Class 5		FSGS	None	Post-transplant
4	3.5	F	Asian	*NPHS2* hom c.562G>T p.(Glu188*)	PVS1 PM2 Class 5		FSGS	None	Post-transplant
5	1.1	M	White	*NPHS2* app hom c.378+5G>A	PM2 PP3 PS4_supp PM3* Class 4		MCNS***	None	5

Table 2: Characteristics of children with pathogenic variants responsible for their phenotype.

*Bierzynska et al. (2017) Kidney Int 91(4): 937-47 **FSGS- Focal Segmental Glomerulosclerosis, ***MCNS- Minimal Change Nephrotic Syndrome
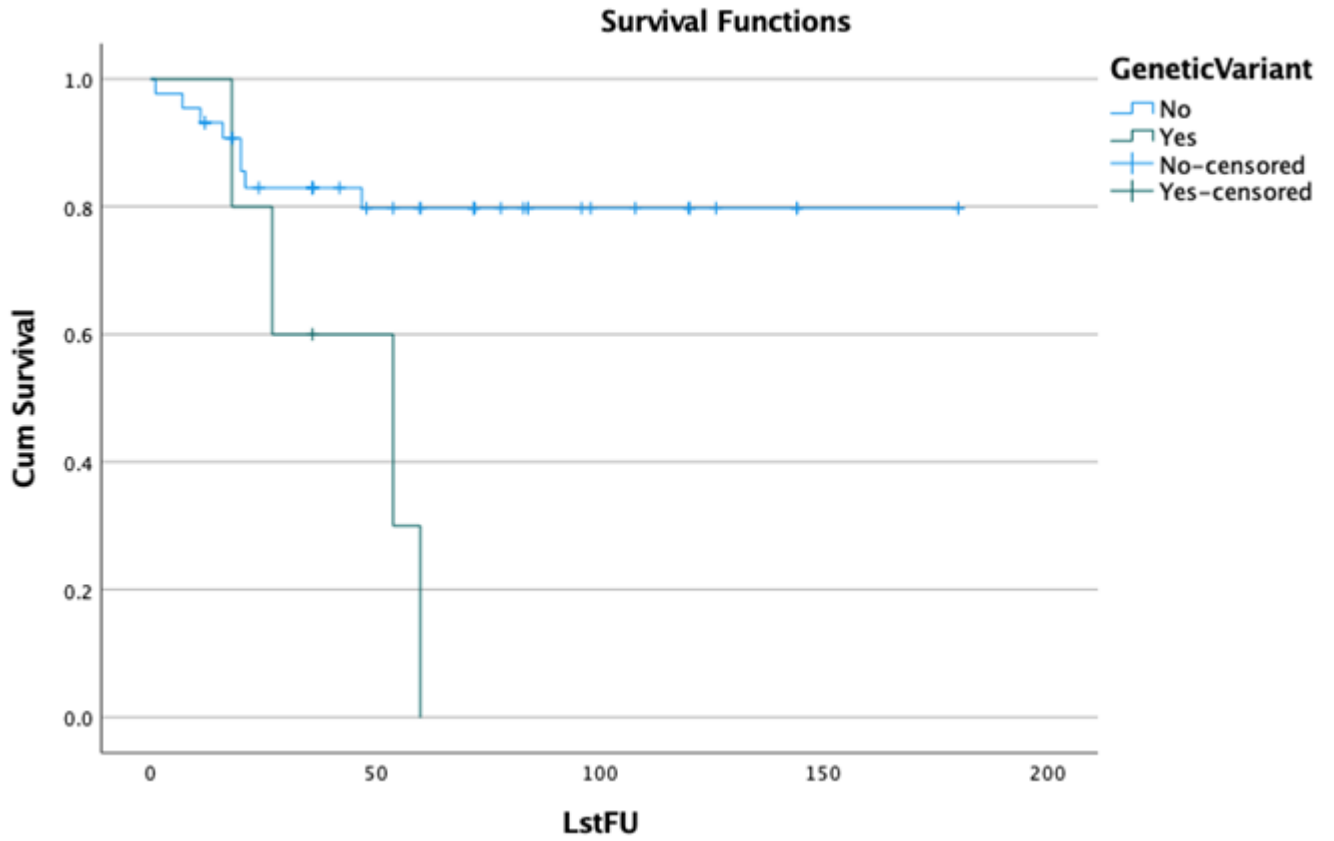
Figure 1- Kaplan-Meier curves illustrating progression to chronic kidney disease in children with an identified genetic variant with those with no genetic variant identified.