original article

Steroid-resistant nephrotic syndrome: impact of genetic testing

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BACKGROUND AND OBJECTIVES: Mutations in several genes are known to cause steroid-resistant nephrotic syndome (SRNS), most commonly in *NPHS1*, *NPHS2*, and *WT1*. Our aims were to determine the frequency of mutations in these genes in children with SRNS, the response of patients with SRNS to various immunosuppressants, and the disease outcome, and to review the predictive value of genetic testing and renal biopsy result.

DESIGN AND SETTINGS: A retrospective review was performed of the medical records for all children with SRNS who were treated and followed-up in the Pediatric Nephrology Unit of King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia from 2002–2012.

PATIENTS AND METHODS: We retrospectively reviewed the medical records of children above 1 year of age, who presented with SRNS to KAUH, Jeddah, Saudi Arabia, in the 10-year interval from 2002–2012 and for whom the results of genetic testing for *NPHS1*, *NPHS2*, and *WT1* were available. We compared the clinical phenotype, including response to treatment and renal outcome to genotype data.

RESULTS: We identified 44 children with a clinical diagnosis of SRNS in whom results of genetic testing were available. Presumably disease-causing mutations were detected in 5 children (11.4%) of which 3 (6.8%) had *NPHS2* mutation and 2 (4.5%) had *NPHS1* mutation. Renal biopsy revealed minimal change disease (MCD) or variants in 17 children, focal segmental glomerulosclerosis (FSGS) in 23 children, membranoproliferative changes (MPGN) in 2 children, and IgA nephropathy in another 2 children. Children with MCD on biopsy were more likely to respond to treatment than those with FSGS. None of those with an identified genetic cause showed any response to treatment.

CONCLUSION: The frequency of identified disease-causing mutations in children older than 1 year with SRNS presented to KAUH was 11.4%, and these patients showed no response to treatment. Initial testing for gene mutation in children with SRNS may obviate the need for biopsy, and the use of immunosuppressive treatment in children with disease due to *NPHS1* or *NPHS2* mutations. Renal biopsy was useful in predicting response in those without genetic mutations.

The diagnosis and management of steroid-resistant nephrotic syndrome (SRNS) remains a challenge to pediatric nephrologists. Several genes have been found to cause SRNS.

NPHS2 and *NPHS1* mutations are the most common genetic causes in children with SRNS and congenital nephrotic syndrome, respectively.[1] Many different mutations of the *NPHS2* gene have been reported in several Western, European, Middle Eastern, and Asian countries.[1-9] The prevalence of *NPHS2* mutations varies in these different populations, and underlies 26% of cases with SRNS in American[2] and 24.7% of Turkish patients,[3] compared to 9% in Greek,[4] 4 % in Indian[5] 3.4% in Pakistani,[6] 4.3 % in Chinese[7] and 0% in Japanese[8] and Korean[9] children with SRNS.

Mutations in *WT1*, *NPHS3*, *TRPC6*, *CD2AP*, *PLCE1*, *INF2*, *ACTN4*, and *ITGA3* have also been reported as causes of SRNS.[5,10-13]

Identification of an underlying genetic basis allows clinical observations in molecularly defined patient cohorts, which impacts prognosis and treatment.[14] Indeed, several reports so far indicate that children with SRNS

caused by mutations in *NPHS1* and *NPHS2* do not respond to immunosuppressive treatment,[2,15-17] whereas some with an underlying mutation in *WT1* appear to respond to treatment with cyclosporin.[18]

In this study, we reviewed clinical features of children older than 1 year with SRNS presenting to a King Abdulaziz University Hospital (KAUH) in Jeddah, Saudi Arabia, including response to treatment, biopsy findings, disease outcome, and the frequency of *NPHS1*, *NPHS2*, and *WT1* mutations in this cohort.

PATIENTS AND METHODS

A retrospective review was performed of the medical records for all children with SRNS who were treated and followed-up in the Pediatric Nephrology Unit of AUH, Jeddah, Saudi Arabia, from 2002–2012. The diagnosis of NS was based on clinical and laboratory findings of nephrotic range proteinuria, hypoalbuminemia, and hyperlipidemia. Only children with primary nephrotic syndrome (NS) who had undergone renal biopsy and had genetic testing results available for *NPHS2*, *NPHS1*, and *WT1* were included in the study. We excluded children with (a) an underlying, secondary cause for NS (such as lupus nephritis, infections, or neoplasm), (b) congenital and infantile NS, or (c) steroid-sensitive nephrotic syndrome (SSNS). Permission to conduct the study was granted by the Ethics Research Committee of King Abdulaziz University.

For all children included in the study, blood urea and serum creatinine measurements were performed regularly to monitor kidney function. Other investigations included hepatitis B and C serology, human immunodeficiency virus 1 and 2 serology, and complement C3 and C4 levels; antinuclear antibody assays were performed for selected cases with older age at presentation.

The diagnosis of SRNS was made if the child did not respond to the standard steroid therapy with oral prednisone 60 mg/m^2/d for 4 weeks. Secondary steroid resistance was defined as no response after 4 weeks of prednisone 60 mg/m^2/d in a child previously known to have a steroid-sensitive course. All children received intravenous pulse methyl prednisolone 600 mg/m^2 daily for 3 consecutive days after failure of the 4-week treatment with oral prednisolone.

Ultrasound-guided kidney biopsy was performed in all patients. The biopsy specimens were examined by light, electron, and immunofluorescence microscopy. An adequate biopsy was defined as the presence of at least 5 glomeruli in the specimen on light microscopy.

Based on the histopathologic diagnosis, children were treated with 1 or more of the following regimens:

For children with focal segmental glomerulosclerosis (FSGS), the following treatments were given consecutively: (1) calcineurin inhibitor (CNI), mainly cyclosporin, 4 to 6 mg/kg/d in 2 divided doses, (2) mycophenolate mofetil (MMF) 500 mg/m^2/dose twice daily, and (3) intravenous rituximab (375 mg/m2/dose) 2 doses given 2 weeks apart, in addition to alternate-day oral prednisolone.

For children with minimal change disease (MCD), the following treatments were given consecutively: (1) oral cyclophosphamide 2.5 mg/kg/d was given for 8 weeks, (2) children who did not respond to cyclophosphamide were treated with cyclosporin for 3 to 6 months, and (3) if no response to cyclosporine was observed, then the children were treated with MMF for 3 to 6 months. Children with membranoproliferative glomerulonephritis (MPGN) were treated with low-dose aspirin, 8-week course of cyclophosphamide (2.5 mg/kg/d), and prolonged alternate day course of prednisolone at 40 mg/m^2 for 6 months, followed by 30 mg/m^2 for another 6 months, then 20 mg/m^2 for 6 months, and 10 mg/m^2 for 6 months.

Genetic testing for mutations in the *NPHS1*, *NPHS2*, and *WT1* genes was performed as detailed previously.[19]

The treatment response was evaluated by measuring the degree of proteinuria and serum albumin levels. The reports can be listed as follows: (1) complete remission was defined as negative dipstick test result (normal protein excretion) in a spot first-morning urine sample or quantitative urine protein test result of ≤300 mg/L and serum albumin level ≥35 g/L. (2) Partial remission was defined as a reduction in urine protein excretion in a spot morning urine sample below the nephrotic range in the pediatric age group (i.e., <1000 mg/m^2/d) and serum albumin between 25 and 34 g/L. (3) Failure to respond was defined as persistent proteinuria (≥1000 mg/m^2/d) and failure of serum albumin to increase to 25 g/L or more.

The follow-up was done twice monthly initially and subsequently every 1 to 3 months. On each visit, the child was evaluated clinically for the evidence of disease activity and complications (infections and drug adverse effects). When the child did not respond to a given drug for 3 to 6 months it was discontinued.

Statistical analysis

Statistical analysis was performed using the SPSS, version 16.0 (SPSS Inc., Chicago, IL USA). For categorical variables, descriptive statistics were performed and the results were expressed as frequencies and percentages. Descriptive statistics were performed using median and inter-quartile range.

Table 1. Summary of the mutations detected in children with SRNS.

	Age at presentation	Follow-up duration	Histopathologic examination	Primary or secondary	Mutation found	Consequence for protein	Homozygous (H) or heterozygous (h)	Response to treatment
1	1.5	10	FSGS	Primary	*NPHS1: c.2215G>A NPHS2: G688A*	*A739T R229Q*	H h	Did not respond to cyclosporine or MMF
2	2	5	FSGS	Primary	*NPHS1: c.2215G>A NPHS2: G688A*	*A739T R229Q*	H h	Did not respond to cyclosporine
3	3.5	1.5	FSGS	Primary	*NPHS2: Ex6:779 T>A*	*V260E* (Weber et al[37])	H	Did not respond to cyclosporin
4	1	3	FSGS	Primary	*NPHS2:Exon 6*	*779T>A=V260E* (Weber et al[37])	H	Did not respond to cyclosporin
5	4	11	FSGS	Primary	*NPHS2: Ex5:538 G>A*	*V180 M* (Boute et al[22])	H	Did not respond to cyclosporin

SRNS: Steroid-resistant nephrotic syndrome, FSGS: focal segmental sclerosis.

Table 2. Summary of the therapeutic Interventions performed and the response according to the histopathologic examination.

Histopathologic examination	IV MP	Cyclophosphamide	Cyclosporine	MMF	Rituximab
MCD (n=17) Number received the drug Response (complete + partial)	17 2 (2+0)	15 6 (5+1)	9 7 (5+2)	2 1 (1+0)	1 0
FSGS (n=23- with negative genetic testing= 18) Number received the drug Response (complete + partial)	23 1(0+1)	0	23 8 (5+3)	9 8 (7+1)	4 1 (0+1)
MPGN n=(2) Number received the drug Response (complete + partial)	2 0	2 2 (2+0)a	0	0	0
IgA nephropathy (n=2) Number received the drug Response (complete + partial)	2 0	0	2 1 (0+1)	1 1 (1+0)	0

IV: Intravenous, MMF: mycophenolate mofetil, MCD: minimal change disease, FSGS: focal segmental sclerosis, MPGN: membranoproliferative glomerulonephritis, IgA: immunoglobulin A.

[a]Patients also received alternate days prednisolone at 40 mg/m^2 for 6 months, followed by 30 mg/m^2 for another 6 months, then 20 mg/m^2 for 6 months and 10 mg/m^2 for 6 months.

RESULTS

Patient characteristics

A total of 242 children with NS were followed up at the Pediatric Nephrology Unit of KAUH between 2002 and 2012. Idiopathic NS was diagnosed in 214 patients older than 1 year of age (88.4%), SSNS was diagnosed in150 patients (62.0%), and SRNS in 64 patients (26.4%).

Patients with SRNS were classified to have either primary or secondary SRNS; 53 patients (82.8%) had primary SRNS while 11(17.2%) had secondary SRNS.

Genetic testing results were available for 44 children of which 36 had primary SRNS and 8 had secondary SRNS. Five children (11.4%) had presumably disease-causing mutations identified (mut+). The 2 patients with *NPHS1* mutations were sisters homozygous for the same missense variant c.2215G>A (p.A739T). The pathogenicity of this variant is unclear, but is not a recognized single nucleotide polymorphism (SNP), and the amino acid is reasonably conserved. They also carried the known SNP in *NPHS2 G688A* (p.R229Q). Another 3 children had homozygous mutations in *NPHS2* (**Table 1**). There was no underlying *WT1* mu-

tation identified in any patient. **Table 1** gives a summary of the mutations detected.

All mut+ children presented with primary SRNS and did not respond to treatment with immunsuppressives (**Table 1**). There were 2 children with a history of affected siblings who tested negative for mutations in *NPHS1*, *NPHS2*, and *WT1* and thus their disease was likely caused by another gene not tested for.

Biopsy findings were as follows: MCD[25] or MCD variants (immunoglobulin M [IgM] nephropathy and C1q nephropathy) in 17 children, FSGS in 23 children, MPGN in 2 children, and IgA nephropathy in another 2 children. All mut+ children presented a histological picture of FSGS on biopsy.

Treatment

Initially all patients received oral prednisone 60 mg/m^2/d for 4 to 8 weeks. All patients received intravenous methyl prednisolone pulse therapy (600 mg/m^2 body surface area/d) for 3 consecutive days, followed by 3 doses on alternate days if the child did not respond after the 3 initial doses. Seventeen children received oral cyclophosphamide 2.5 mg/kg/d for 8 weeks. Thirty-four patients received cyclosporin for a mean duration of 2.91 years. Twelve patients received MMF for a mean duration of 2.57 years, and 5 patients received rituximab 375 mg/m^2 body surface area, of which only 1 had a partial remission while the other 4 patients had no clinical or laboratory response. **Table 2** summarizes the treatment modalities and the response according to the histopathologic examination." Children who relapsed after remission were managed by pulse methyprednisolone, ranging from 3 to18 pulses (median, 6 pulses) during follow-up, in addition to changing the steroid-sparing immunosuppressive therapy. A total of 26 children received 2 immunosuppressants, in addition to methyl prednisolone, 5 children received 3, and 1 child received 4 different immunosuppressive drugs (apart from steroids) during the course of their illness.

Complications

The following complications, due to SRNS or as a result of treatment, were observed: cushingoid appearance in 20 patients (45%), growth retardation in 17 patients (38.6%), hypertension in 9 patients (27.3%), recurrent infections in 9 patients (20.5%), acute renal failure in 8 patients (18.2%), 2 resistant edema in 2 patients, and simultaneous onset of type 1 diabetes mellitus in 2 patients (4.5%); however, 4 patients (9.1%) did not develop overt complications.

Disease outcome

A total of 29 children without identified genetic cause responded to immunosuppressive treatment and achieved remission (19 complete, 10 partial). Sixteen children who achieved remission had MCD or MCD variants (94%), 10 had FSGS (55%), 2 with MPGN, and 1 with IgA nephropathy. None of the 5 mut+ children responded to a 3- to 6-month course of cyclosporin and one of them had an extra course of MMF for another 3 months without apparent response.

Two patients (4.5%) progressed to chronic kidney disease (stage 3 and 4), 3 (6.8%)progressed to end-stage kidney disease and 2 (4.5%) died. None of the mut+ children achieved remission.

DISCUSSION

The primary goals of this study were to determine the frequency of *NPHS2* and *WT1* mutations in children with SRNS, the response to various immunosuppressants, and the disease outcome. Our results showed that 4.5% and 6.8% of children with primary SRNS had mutations in the *NPHS1* and *NPHS2* genes, respectively; none of the patients with secondary SRNS had a mutation in the *NPHS1* or *NPHS2* genes. This is lower than the podocin mutation frequency of 18.1% reported by Hinkes et al in 430 children with SRNS.[16] This could be explained by the small number of our cohort or more likely reflects the lower frequency of these mutations in Saudi Arabia. Indeed, our results are comparable to those of Abid et al from Pakistan who found a frequency of 5.5% *NPHS1* and 3.4% *NPHS2* mutations in children with SRNS.[6] This low mutation frequency indicates that the availability of rapid genetic testing for *NPHS1*, *NPHS2*, and *WT1* would influence the management of only a minority of patients. However, we observed non-response in these children and therefore recognizing these children would spare them a renal biopsy and prolonged exposure to immunosuppressive drugs. We did not test for other potential recessive genes such as *PLCE1 (NPHS3)* that could be the underlying genetic cause in some of our patients. Other unidentified autosomal recessive genes could also be the underlying cause in other nonresponding children.

We had 2 children (40%) with biallelic mutations explaining their disease. Biallelic and triallelic mutations were reported to be implicated in genotype/phenotype correlations.[20,21] Schultheiss et al[20] reported 5 patients with mutations in both the *NPHS1* and the *NPHS2* genes out of 62 children with SRNS and CNS. Koziell et al[22] showed that an overlap in the *NPHS1*/*NPHS2* mutation spectrum with the characterization

of a unique digenic inheritance of *NPHS1* and *NPHS2* mutations, which results in a multiple allelic hits that modify an autosomal recessive disease phenotype in humans from CNS to FSGS. This may result from an epistatic gene interaction and functional inter-relationship between *NPHS1* and *NPHS2* in human nephrotic disease.)

In those children without identified mutations, a biopsy can still direct treatment. Our results here are biased, as not all patients received the same treatment from the outset. Nevertheless, the high subsequent response rate to cyclosporin and cyclophosphamide in patients with MCD supports the initiation of treatment with these agents.

More than half of the patients in our cohort (23 of 44; 52.3%) had a histologic diagnosis of FSGS. Similar to our findings, other data show that FSGS is the most common histopatholgical subtype in children with SRNS.[23,24] Approximately 75% of patients with SRNS exhibit renal histologic features of FSGS while 20% show MCD.[25] Although the role of kidney biopsy has increasingly become restricted,[26] the underlying histologic features in NS are of significance in determining the outcome[27] as children with SRNS and the histological picture of MCD are more likely to achieve remission. In one report on 136 patients with SRNS, it was shown that following extended immunosuppressive therapy, children with MCD had significantly greater remission rates compared to those with other histopathologic subtypes.[23] The treatment options in our study were also based on the histopathologic findings, and the response was better in children with MCD.

About 80% of children with idiopathic NS responded to corticosteroids;[28] however, some authors suggest that testing for *NPHS2* mutations should be performed in children with a first episode of NS to avoid an unnecessary steroid course in those testing positive.[4,16] In our current practice, it is not possible to base initial treatment decisions on genetic testing, as results become available only after several months. In our case, we had to weigh the benefit of initiating treatment with corticosteroids against the potential complications that could arise as a result of non-treatment.

At present, no optimal treatment has been reported to meet the goals of therapy for SRNS, which are to achieve complete resolution of proteinuria and to preserve kidney function. Immunosupressive therapy has been used to induce complete remission in patients with SRNS; however, there is only partial remission in some cases, especially in genetic forms of SRNS, which are typically refractory to immunosuppressive therapy. In one report on patients with SRNS, none of the 29 cases with *NPHS2* mutations who were treated with cyclosporin or cyclophosphamide demonstrated complete remission.[4] Similar observations were made by other authors who found that none of the 43 patients with an identified genetic cause of SRNS demonstrated complete response to cyclosporin therapy, yet 2 achieved a partial response.[29]

A considerable number of our cohort achieved either complete or partial remission on cyclosporin therapy. This is in line with the Cochrane review of 14 randomized controlled trials of 449 children with SRNS, which found that cyclosporin when compared with placebo or no treatment significantly increased the number of children who achieved complete remission.[29] Other investigators also reported a high response rate to cyclosporin in SRNS without identified disease-causing mutations. Recently, cyclosporin was reported as having an antiproteinuric effect independent of its immunosuppression action.[31]

A total of 10 of the 12 patients (83%) who received MMF in this study demonstrated complete remission of NS, and one additional patient (8.5%) demonstrated partial remission. Moreover, all of those patients received MMF after relapsing or not achieving remission with either cyclophosphamide and/or cyclosporin. In the published reports, there are inconsistent results regarding the efficacy of MMF in patients with SRNS. In one clinical trial of 138 patients with SRNS, only 33% of the patients achieved a partial or complete response. However, the patients in their study were randomly assigned to a combination therapy comprising oral pulse dexamethasone and MMF.[32] Other observational studies demonstrate that MMF has variable benefits in children with SRNS.[33,34] The role of rituximab in SRNS is still uncertain: It has been demonstrated in several case series that rituximab, in combination with a corticosteroid and/or CNI, improves remission rates in patients with SRNS.[35,36] We cannot, however, compare our findings with those of other authors because the proportion of patients who received rituximab in our study was very small.

In conclusion, the frequency of identified disease-causing mutations (*NPHS1* and *NPHS2*) in children with SRNS presented to KAUH is 11.4%, and they show no response to treatment. Initial testing for gene mutation in children with SRNS would save them unnecessary treatment. Renal biopsy was useful in deciding immunosuppression regimen and predicting prognosis for those with negative mutations. MMF was a highly effective treatment in those children with no response to cyclophosphamide and or cyclosporin.

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