High Incidence of COL4A Genetic Variants Among a Cohort of Children With Steroid-Resistant Nephrotic Syndrome From Eastern India

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Childhood nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, hypercholesterolemia, and edema. Approximately 10% to 15% of the patients do not achieve complete remission even after 4 to 6 weeks of corticosteroid therapy and are termed as having steroid-resistant NS (SRNS).1 Up to 30% of children with SRNS may have underlying monogenic etiologies with high risk of progressing to end-stage kidney disease, yet often paradoxically protective against disease recurrence post-kidney transplant.1,2 With >50 genes linked to monogenic SRNS, massive parallel sequencing (MPS) has become an indispensable diagnostic tool as it enables simultaneous sequencing of multiple genes quickly. Genetic variability with ethnicity underscores the need for local data. Data based primarily on North American and Western European population suggest that approximately a third of SRNS can be secondary to an underlying genetic etiology with podocin (NPHS2) variant being most common.1,2 Similar data are lacking from the Indian subcontinent.3 We undertook a retrospective cohort review between May 2016 and December 2020 across 4 tertiary pediatric nephrology centers from Eastern India. The objective was to identify children aged <18 years with congenital NS (CNS) and SRNS who had undergone MPS. Our cohort was subgrouped into CNS (onset ≤3 months), infantile NS (onset 3–12 months), and childhood SRNS (cSRNS: 1–18 years of age). NS and SRNS were defined as per standard definitions, and all children except those with having CNS failed 6 weeks of steroids before being labeled SRNS.51 A custom kit was used to capture exome sequencing for the targeted gene. The libraries were sequenced to mean 80× to 100× coverage on Illumina sequencing platform. The sequences were aligned to human reference genome (GRCh37/hg19). All variants identified were classified as per the American College of Medical Genetics and Genomics criteria into pathogenic, likely pathogenic, nonpathogenic, and variant of uncertain significance.52 Further detail of genetic sequencing is provided in the Supplementary Material, which also includes details of the 61 kidney-related genes screened (Supplementary Table S1), descriptions of the significant variants identified and their phenotypic details (Supplementary Table S2), details of all variant of uncertain significance identified (Supplementary Table S3), and Supplementary References. Parents of index children positive for a novel genetic variant (variant of uncertain significance) that was correlating with phenotypic presentation were tested by Sanger sequencing. Post-test counseling was done for all families, and parental segregation study and targeted genetic testing for sibling were advised as necessary. Standard statistical analysis was done with continuous variables presented as median and range and categorical variables as number and percentage. For comparing the variables, Mann-Whitney U test was used for the continuous variables and \( \chi^2 \) test was used for the categorical variables. The study was approved by local institutional ethic boards.

Of 77 children who had undergone MPS, an underlying monogenic etiology was identified in 19 children resulting in an overall genetic yield of 25% (Table 1). There were 62 relevant genetic variants identified, of which 39% (n = 24) were deemed...
Table 1. Yield of next-generation sequencing among current cohort of children with congenital NS, infantile NS, and childhood steroid-resistant NS

| Classification            | Number of patients (n) | Age of onset: Median (IQR), mo | Female (n) | Number of relevant variants detected (n) | Number of pathogenic/likely pathogenic variants detected (n) | Number of children with pathogenic/likely pathogenic variants detected (n) | Genes with pathogenic/likely pathogenic variants detected (number of children) and histology if available |
|---------------------------|------------------------|--------------------------------|------------|-----------------------------------------|-------------------------------------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| Congenital NS (0 to ≤3 mo) | n = 6                  | 1.8 (1.3–2.3)                  | 3 (50%)    | 9                                       | 8                                                          | 4/6 (67%)                                                             | NPHS1: (4)                                                                                                           |
| Infantile NS (>3 to ≤12 mo) | n = 23                 | 8 (7–10)                       | 9 (39%)    | 16                                      | 5                                                          | 4/23 (17%)                                                            | PLCE1: (1)–MCNS, WT1: (1)                                                                                      |
| Childhood steroid-resistant NS (1 to ≤18 yr) | n = 48                 | 52.8 (24–102)                  | 26 (54%)   | 37                                      | 11                                                         | 11/48 (23%)                                                           | COL4A5: (5)–FSGS, COL4A4: (2)–FSGS, COL4A3: (1)–FSGS, NPHS2: (1)–MCNS, WT1: (1)–FSGS, INF2: (1)–FSGS |

FSGS, focal segmental glomerular sclerosis; IQR, interquartile range; MCNS, minimal change nephrotic syndrome; NS, nephrotic syndrome.

significant (NPHS1 n = 10, 42%; COL4 n = 8, 34%; PLCE1 n = 2, 8%; WT1 n = 2, 8%; NPHS2 n = 1, 4%; IFN2 n = 1, 4%) (Supplementary Table S2). The patients with CNS had the highest MPS yield (67%), whereas among those with cSRNS, an underlying genetic etiology was identified in 23% (Table 1). Infective etiology as per the STORCH (Syphilis, Toxoplasma, Rubella, Cytomegalovirus and Herpes) screen was found to be negative among all those having CNS. Among the 19 children identified with having significant variant, 42% (n = 8) were in COL4A4 genes, which was the most common identified gene among cSRNS beyond infancy (73%, 8 of 11). All children with significant COL4A4 variants had focal segmental glomerulosclerosis histopathology (Supplementary Table S2). Despite thorough clinical evaluation postavailability of the genetic reports, none had ocular or hearing abnormalities of classical Alport syndrome phenotype. Electron microscopy report was available for 4 of 8 children, and diffuse foot process effacement was reported in all with variable thinning of the glomerular basement membrane in 2 children (Supplementary Table S2).

In summary, in our contemporary cohort of children with SRNS from India, compared with previous mainly Western European/North American studies, we found similar MPS yield of 25% but much higher incidence of COL4A4 variants. Phenotypic spectrum for COL4A4 mutations has expanded beyond classical Alport syndrome phenotype and has recently been found to include SRNS/focal segmental glomerular sclerosis (FSGS). In 2015, Malone et al. reported COL4A4 mutations in families with nephrotic-range proteinuria and variable hematuria but without having all features consistent with classical Alport syndrome. Subsequently, Gast et al. found the COL4A4 variants to be the most common significant genetic variant among adult cohorts of FSGS (38% with familial FSGS and 3% with sporadic FSGS). Although initially the PodoNet Registry did not report on COL4A4 variants, it recently reported 2.5% COL4A4 mutations among 2041 children of predominantly Western European and North American heritage, with NPHS2 being the predominating variant. Among more recent publication, Bierzynska et al. identified an underlying genetic etiology among 26% of their predominantly Caucasian cohort of cSRNS (n = 187), with podocin (NPHS2) again being the most common gene involved. Although they identified only 2 children with significant variant in COL4A4, it needs to be highlighted that most of their variant of uncertain significance (13 of 17) were related to COL4A4 genes. Abnormal matrix–podocyte interactions and defective expression or trafficking of the glomerular basement membrane matrix components by the podocytes have been postulated as possible explanation for the link between COL4A4 mutation and SRNS/FSGS. Pattern of nephrotic gene involvement in cSRNS does vary with region as reports from major Asian countries, including China, South Korea, and Japan, have failed to confirm podocin predominance. Even the previous Indian study focusing on identifying podocin variants among cSRNS yielded very low positive results. An intriguing though yet to be proven hypothesis might be that the higher incidence of COL4A4 mutations in ours and other Asian populations may be secondary to yet to be identified modifier genes existing in these populations and changing the phenotype found with these variants. This is of particular interest in that some of the exact COL4A4 variants found in our cohort (among children with COL1, COL2, COL5, and COL6) had been previously described presenting with a more classical Alport syndrome phenotype. The main component of the glomerular basement membrane is the α3/α4/α5
network which is encoded by COL4A3 to COL4A5 genes, respectively, and nature of underlying mutation might also explain some of the observed genotype-phenotype variability. Stop codons, frameshift mutations, and donor splice site alterations often result in more severe phenotype in comparison to missense codon which accounted for 80% of our COL4A5 variants.

We do acknowledge various limitations of our study, which mostly attributed to our retrospective design. Detailed follow-up clinical information was often hard to extract from case notes, and hence, we have focused primarily on the genetic yield. Although post-test counseling was done for all families and recommendation for parental segregation study and targeted genetic testing for sibling were advised as necessary, funding constraint prevented tests to be done in all. In addition, owing to funding constraint, we were unable to undertake any in-depth studies among our cohort of negative genetic yield, which could have resulted in improving our yield and identifying any novel gene or hidden variant within a known gene.

In conclusion, among this largest group of children with SRNS undergoing MPS published to date from India, we found a genetic yield of 25%. We confirmed podocin gene involvement to be rare among our population and found COL4A variants to be the most common among children older than 1 year. Larger multicentric pediatric studies among a pan-Indian/Asian cohort of cSRNS are required to further corroborate our novel finding.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. List of 64 kidney genes analyzed.

Table S2. Clinical details of children detected with significant variants, including variant description.

Table S3. List of variant of unknown significance found in our cohort.

Supplementary References.

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