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ORIGINAL ARTICLE

New insights from the genetic work-up in early onset nephrotic syndrome: report from a registry in western India

Jyoti Sharma[1] · Anshuman Saha[2] · Alpana Ohri[3] · Vaishali More[4] · Fagun Shah[5] · Jalpa Dave[6] · Brinda Panchal Jain[7] · Manoj Matnani[8] · K. Sathe[9] · Pankaj Bhansali[10] · Puneet Chhajed[11] · Pawan Deore[12] · Nivedita Pande[13] · Chintan Shah[14] · Vala Kinnari[2] · Jyoti Singhal[1] · Nisha Krishnamurthy[15] · Meenal Agarwal[1] · Uma Ali[15]

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Abstract

Background Eighty-five percent of infants with congenital nephrotic syndrome (CNS) and 66% with infantile NS (INS) are likely to have a monogenic etiology. There exists a significant genetic variability between different regions and ethnic groups. This study aimed to determine the genetic defects in children with CNS and INS by establishing a registry in western India.

Methods In this cross-sectional study, pediatric nephrologists from 13 private and government institutions shared relevant clinical data and details of the genetic evaluation of children presenting with NS within the first year of life.

Results The median age at presentation was 9 months (range 1–23, IQR 3–13 months), history of consanguinity between parents existed in 14 patients (34%), family history of similar illness in 6 (15%), and extra-renal manifestations in 17 (41%). Twenty-five (61%) were confirmed to have a monogenic etiology. *NPHS1* gene was the most implicated (9/25) followed by *PLCE1* (5/25). There were 12 variants of uncertain significance (VUS) involving 10 genes (10/25, 40%), and no definite genetic abnormality was found in 4 (25%). A re-analysis of these VUS attempted 2–3 years later facilitated reclassification of 7/12 (58%); increasing the diagnostic yield from 61 to 68.2%.

Conclusions Consistent with worldwide data, variants in *NPHS1* gene were the most common cause of NS in infancy; however, *PLCE1* was implicated more frequently in our cohort. *NUP93* and *COL4A3* were reported in early onset NS for the first time. Reclassification of VUS should be attempted, if feasible, since it may lead to a useful revision of diagnosis.

Keywords Congenital nephrotic syndrome · Infantile nephrotic syndrome · Molecular testing in NS · Variants of uncertain

Introduction

Children with congenital nephrotic syndrome (CNS) develop heavy proteinuria, hypoalbuminemia, and edema, in utero or within the first 3 months of life. Those with onset of nephrotic syndrome (NS) from 4–12 months are said to have infantile NS (INS) [1]. Guidelines recommend genetic testing for all patients with CNS. Genetic testing is also recommended for two other groups of children with NS, i.e., those who demonstrate primary steroid resistance and those

✉ Jyoti Sharma
jyotivsharma@gmail.com

1 KEM Hospital, Pune, India

2 IKDRC-ITS-GUTS, Ahmedabad, India

3 B J Wadia Hospital for Children, Mumbai, India

4 Tara Children's Kidney Care, Wockhardt Hospital and SRCC Children's Hospital, Mumbai, India

5 Child's Kidney Care Centre, Surat, India

6 Tender Kidney Care, Vadodara, India

7 Namaha Hospital, Kandivali and Thunga Hospital, Malad, Mumbai, India

8 Jehangir Hospital, Pune, India

9 Sir HN Reliance Foundation Hospital and Research Centre, Mumbai, India

10 Orchid Pediatric Superspeciality Clinic, Aurangabad, India

11 Malad, Mumbai, India

12 Nashik, India

13 BYL Nair Hospital, Mumbai, India

14 Division of Pediatric Nephrology, B J Wadia Hospital for Children, Mumbai, India

15 SRCC Children's Hospital, Mumbai, India

who have extra-renal manifestations [2]. Eighty-five percent of infants with CNS and 66% of those with INS are likely to have a monogenic etiology [3–6]. The most common gene implicated is *NPHS1* followed by *NPHS2, WT1, PLCE1*, and *LAMB2* [2, 7, 8]. There exists significant genetic variability in people from different regions and ethnicities. The availability of variant frequency data within a population forms the major basis for the interpretation of genomic variants. Hence, a registry was established in western India to enhance the information regarding genomic variants underlying kidney diseases in children. In the present work, we report the variants associated with CNS and INS.

Methods

Pediatric nephrologists from 13 private and government institutions from the states of Maharashtra and Gujarat contributed data to this cross-sectional study. Permission from respective ethics committees was obtained. Relevant clinical details including age of onset, age of presentation at the pediatric nephrology center, family history of similar illness, presence of dysmorphism, details of anthropometry, histopathology, and results of molecular testing were recorded. All patients but one had undergone a large next-generation sequencing (NGS) panel (clinical exome or whole exome sequencing). One subject who was phenotypically a female but XY on karyotyping underwent targeted sequencing for the *WT1* gene. For the present work, a single clinical geneticist went through all the genetic variants and raw data (wherever available) obtained from 5 laboratories across the country. Variant interpretation was done by the criteria laid down by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP). Definite monogenic etiology was considered "confirmed" when a heterozygous pathogenic/likely pathogenic (P/LP) variant in one of the genes associated with autosomal dominant mode of inheritance (e.g., *WT1*) or biallelic (in a homozygous or compound heterozygous state) P/LP variants in genes associated with autosomal recessive mode of inheritance were identified [9]. Samples with VUS or negative findings were considered unsolved. The complete NGS raw data set was re-analyzed whenever possible. Reclassification of VUS was done 2–3 years later by an in silico analysis using available databases, published literature, and laboratories' in-house databases. Parents' segregation studies and functional analysis were not performed.

Statistical analysis

Data was summarised as median (interquartile range, IQR) for continuous variables and as proportions for categorical variables.

Results

Of the 41 subjects from 13 centers, 18 (44%) were girls. A monogenic etiology was confirmed in 25/41 (60.9%) subjects. The median age at presentation to the pediatric nephrology center was 9 months (range 1–23, IQR 3–13 months). There was a history of consanguinity between parents in 14/25 (56%) and a family history of similar illness in 7/25 (28%) subjects. In each of the latter, a sibling was affected. Extra-renal manifestations were seen in 16 (64%). Some of the characteristic extra-renal manifestations were as follows: the 2 siblings with a variant in *SGPL1* had hypothyroidism, adrenal insufficiency and ichthyosis; short trunk dwarfism, T cell immunodeficiency, and hematological abnormalities were associated with the *SMARCAL1* variant; the child with a variant in *WDR73* had typical features of global developmental delay, facial dysmorphism, ocular defects and MRI brain findings of cerebral, cerebellar atrophy with gliosis, vermian hypoplasia and hyperintense signal in bilateral posterior putamina; the presence of low set ears, nystagmus, generalized hypotonia and MRI findings of a leukodystrophy were present in the child with the *COQ2* variant. Four children had seizures, one of whom had West syndrome with microcephaly; two had infection with cytomegalovirus and associated chorioretinitis and additionally cerebral calcification in one.

The *NPHS1* gene was most often implicated (9/25, 21.9%) (Fig.1) followed by *PLCE1* (5/25, 20%). In twelve (12/41, 29.2%), one or more variants of uncertain significance (VUS) were identified involving 10 genes (Supplementary Table 1).

Re-analysis of 14 VUS facilitated re-classification of 7/14 (50.8%); 3 VUS, possibly pathogenic (PP), 3 likely benign and one (in *DSTYK* gene) was discarded due to lack of phenotype correlation; increasing the diagnostic yield of monogenic etiology from 61 to 68.3% (28/41). Of the 4 (4/41, 9.6%) cases where no clinically relevant variant was obtained, NGS data was reanalyzed, and potentially relevant variants were detected in 3 of them. One case presented at 3 months of age with NS and neurological manifestations, cerebral MRI showing polymicrogyria, and delayed myelination. His raw data revealed 3 potentially relevant variants in 3 genes. All 3 variants were classified as VUS based on the standard in silico analysis (a) c.2138C > T, p.Thr713Ile in the *ALMS1* gene in a homozygous state [OMIM # 203800]. This gene is associated with multisystem manifestations of the kidneys, nervous system, hearing loss, and rod-cone dystrophy; (b) c.211G > A, p.Gly71Arg in *TCF21* gene in a homozygous state [OMIM * 603306]. The gene currently does not have any OMIM phenotype. However,

the gene has been recently shown to be associated with proteinuria in a mouse model c.1865C > T, p.Pro622Leu heterozygous variant in the *ANLN* gene. Heterozygous P/LP variants in *ANLN* gene are associated with focal segmental glomerulosclerosis (FSGS) type 8 (OMIM # 616032). Segregation analysis in the parents' samples or functional analysis could not be done as a part of this study. Another child who presented with SRNS at 11 months, focal segmental glomerulosclerosis (FSGS) on kidney biopsy, showed a homozygous missense variant in *TRIM8* gene (c.1034G>A, p.Arg345Gln) which was classified as a VUS. The third case had a homozygous missense variant in the *MXRA5* (c.3313A > G) gene. A likely pathogenic variant in the *MXRA5* gene has been recently identified in association with steroid-resistant nephrotic syndrome (*SRNS)*. Variants in all 3 subjects were reclassified as VUS at the time of secondary analysis. In 12 cases, variants of unknown significance (VUS) were found and, hence, the genetic etiology was considered "not established".

Kidney biopsy data was available in half of the subjects; all were children who had INS and demonstrated primary steroid resistance. The most common histopathological finding was FSGS seen in 9/20 (45%) followed by diffuse mesangial sclerosis (DMS) in 7/20 (35%) and minimal change disease (MCD) in 4/20 (20%). A subset analysis showed that all patients with *PLCE1* variants who underwent kidney biopsy (4/7) showed DMS on histology. Other genes affected in association with DMS were *WT1*(2), *SGPL1* (1), and *ANLN* (1).

Discussion

In this multicentric registry of genetic evaluation of early onset NS, we identified a definite etiology in 68.2% of cases, the majority being due to P/LP variant/s in the *NPHS1* gene.

When we compared our study with others, most were reports from multicentric studies and two had included both infantile and congenital nephrotic populations [3, 7] (Table 1).

Variants in *NPHS1* remain the most common etiology of early onset NS across all studies, worldwide; its prevalence varying from 22–69% [4, 7, 10–12]. *PLCE1* was the next most common gene implicated in our cohort accounting for 5 (22%) infants. Variants in *PLCE1* gene have also been implicated as a cause of early onset NS by other centers that have included it in their panel for testing, though the frequency has been much lower (0.03–2.5%) compared to ours (12%) (Table 1). With rapidly advancing technology in molecular medicine, genetic analysis has moved from time-consuming algorithmic-based capillary sequencing to more rapid, high-throughput NGS. Cil et al. aimed to determine genotype–phenotype correlations and prognosis in Turkish children with CNS and INS [3]. Extrapolating from previous studies, they looked

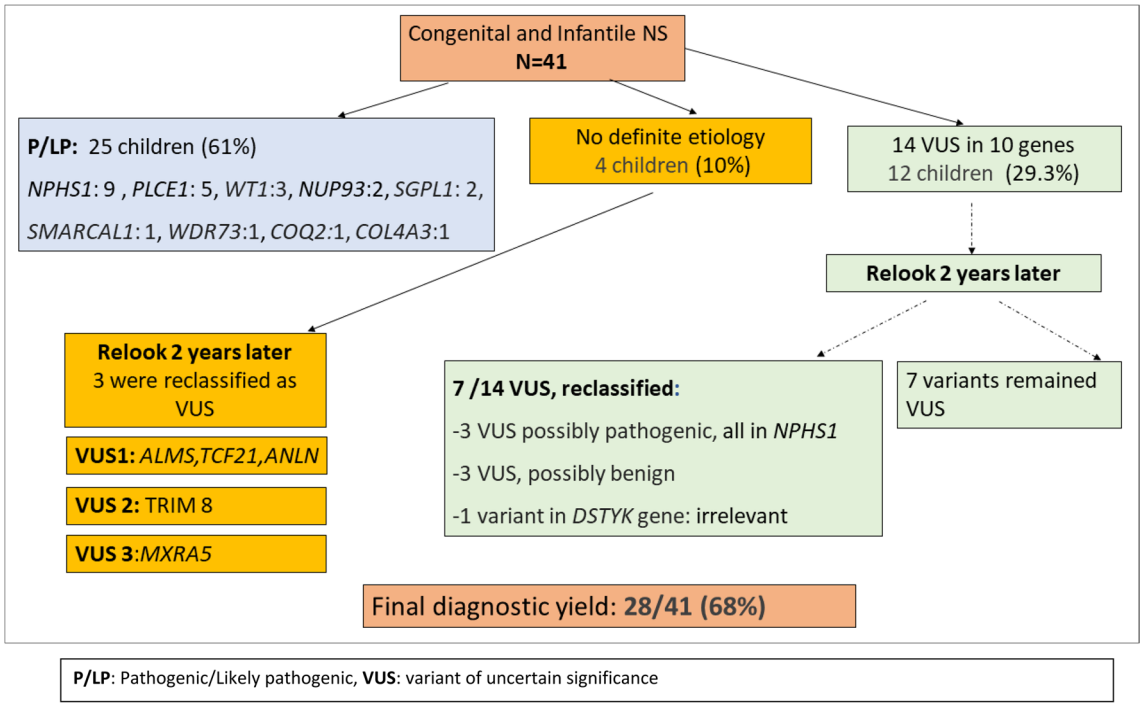

Fig. 1 Genetic variants identified on intial evaluation and VUS reclassification

Table 1 Genetic etiology of congenital and infantile nephrotic syndrome: a comparison with other studies

Authors [ref] (year of publication)	Cil et al. [3] (2015)	Dufek et al. [10] (2019)	Bérody et al. [4] (2019)	Sharief et al. [7] (2019)	Sinha et al. [11] (2022)	Joshi A et al. [12] (2021)	Our study (2022)
Center	Turkey	Europe 22 centers	France 18 centers	Jeddah, Saudi Arabia	India 12 centers	India 15 Centers	Western India 13 Centers
Population	80 CNS, 22 INS	CNS	CNS	20 CNS, 9 INS	CNS	CNS	9 CNS, 32 INS
Number	102	80	55	29, genetic testing: *20* (11CNS, 9 INS)	65, genetic testing: 15	34	41
Methodology	Sanger sequencing for *NPHS1, NPHS2, LAMB2,* exons 8 and 9 of *WT1*	Variable center-specific methods	Algorithmic analysis Method not mentioned	Method not mentioned	NGS or Sanger sequencing	Whole exome sequencing by NGS	Clinical exome/ whole exome sequencing by NGS
Genes affected, *n* (%)	Total 66/102 (64.7) *NPHS1*:38 (37.2) NPHS2 :16 (15.6) *WT1:* 8 (0.07) *LAMB2*: 4 (0.03)	Total 69/80 (86) *NPHS1*: 55(68) *WT1*: 9 (11) *PLCE1*: 1 (1.3) *NPHS2:* 1 (1.3) *LAMB2*: 2 (2.5) *SGPL1*: 1 (1.3)	Total 46/55 (83) *NPHS1*: 36 (65) *NPHS2*: 5 (9) *WT1*: 4 (7) *PLCE1*:1 (2)	Total 18/20 (90) *NPHS1*: 9 (45) *NPHS2:* 2 (10) *LAMB2:* 3 (15) *PLCE1*: 1 (0.5) *WT1*: 1 (0.5) *ITSN*: 2 (10)	Total 10/15 (66) *NPHS1*:8 (53) *PLCE1*:1 (0.06) *WT1:*1 (0.06)	Total 28/34 (82.5) *NPHS: 24 (70.6)* *NPHS2*: 1 (0.03) *OSGEP*:1 (0.03) *PLCE1:*1 (0.03) *LAMB2:*1 (0.03)	Total 25/41 (61) *NPHS1*: 9 (22) *PLCE1*: 5 (12) *WT1*: 3 (7) *NUP93*: 2 (5) *SGPL*: 2 (5) *SMARCAL1*:1 (0.02) *WDR73*:1 (0.02) *COQ2:*1 (0.02) *COL4A3:*1 (0.02)

CNS congenital nephrotic syndrome, *INS* infantile nephrotic syndrome, *NGS* next-generation sequencing

for variants in only 4 genes, i.e., *NPHS1, NPHS2, WT1*, and *LAMB2*, by Sanger sequencing and hoped to identify at least 66% of the causative variants. They acknowledged that they would be missing a few caused by variants in *COQ2*, *PLCE1*, and some yet unidentified genes. Berody et al. evaluated their subjects sequentially for variants in *NPHS1*, then in *NPHS2* if no *NPHS1* mutation was identified, and then for *WT1, LAMB2*, and *PLCE1* [4]. In addition, over the years, newer genes may have been implicated in the etiology of early onset NS. This is the likely explanation for the fewer genes identified as causative for CNS and INS in the studies performed almost a decade before the current study.

Variants in *NPHS2* were conspicuous by their absence in our study. This could reflect the pattern seen in the region and is consistent with a hypothesis that Asians may have a lower prevalence of *NPHS2* in children with early onset NS [13].

This is the first report from India of variants in *NUP93* causing INS. Braun et al. in a search for more genes causing SRNS, first demonstrated in 2015 the 2 different homozygous variants in *NUP93*. This gene (OMIM * 614351) codes for a nuclear pore protein 93 which is involved in the active transport of molecules between the nucleus and cytoplasm. The loss of function in P/LP variants in this gene has been shown to result in impaired podocyte migration, decreased proliferation of podocytes, and increased apoptosis in response to oxidative stress. Mutations in nuclear pore genes *NUP93*, *NUP205*, and *XPO5* cause SRNS. Histologically these patients have DMS or FSGS, and present with SRNS that rapidly progresses to kidney failure [14]. In addition, *NUP93* as a cause of SRNS has also been reported in case series from the UK, Czech Republic, and Slovakia and in a few case reports [15–17].

In our cohort, one 10-month-old male infant who presented with SRNS demonstrated FSGS on biopsy and had a pathogenic homozygous variant (p.Arg406T c.1216C > T er) in *COL4A3*. In the most recent classification, Alport syndrome (AS) is a descriptive term that includes a phenotype extending from non-progressive microscopic hematuria, proteinuria, and associated morbidity or a multisystem progressive disease involving the kidneys, eyes, and hearing. Genetically, it may be X-linked, autosomal dominant, or recessive. Patients with pathologic variants in *COL4A3, COL4A4,* or *COL4A5* have been found to have FSGS on biopsy in both sporadic and familial disease [18]. Genetic studies were performed in 95 Brazilian children with NS (excluding CNS) who eventually underwent kidney transplantation. Of the 149 variants identified, in 22 of 24 sequenced genes, one child presented at 2 years of age with histopathological evidence of FSGS and a heterozygous variant in *COL4A3* (pGly566Ala c.1697G > CI) [19]. Malone et al. described 7 out of 70 families with familial nephrotic range proteinuria, FSGS on histopathology, and variants in *COL4A3* or *COL4A4*. Some family members had proteinuria and hematuria [20]. Most of the genetic panels for a child with SRNS or isolated proteinuria and FSGS on biopsy included *COL4A3-5* genes [18]. However, we believe that this is the first report of infantile nephrotic syndrome with FSGS and a *COL4A3* variant.

With the availability of high throughput methods of genetic testing, sequencing of many genes or the entire genome is easily made available by molecular laboratories. This has a potential to increase the speed of definite genetic diagnosis and overall diagnostic yield. However, this also leads to the identification of many VUS. The ACMG guidelines recommend that as genetic databases are updated, VUS should be reviewed, and efforts should be made to reclassify them as benign or pathogenic [9, 21]. This sometimes requires a clinical evaluation, segregation testing in the parents and other affected/ unaffected family members, and non-genetic investigations. Reclassification of the VUS in our study, after 2 years, increased the genetic yield from 61 to 68%.

Guidelines recommend against performing kidney biopsies in children with CNS; however, a biopsy is indicated in infants 4–12 months of age with SRNS [6, 22]. Renal histology reports were available for 20 subjects. Our study reiterates the lack of association between histopathology and underlying genetic variant in early onset NS [4, 11, 23].

Extra-renal manifestations were noted in 16/25 (64%) patients. Co-infection with cytomegalovirus (CMV) was present. Two siblings had typical features of hyperpigmentation, ichthyosis, manifestations of hypothyroidism, aldosterone, and cortisol deficiencies due to variants in the *SGPL1* gene leading to sphingosine phosphate lyase insufficiency syndrome (SPLIS). Other clinical findings associated with specific syndromes were noted as expected: short-trunk dwarfism in a child with Schimke immuno-osseus dysplasia and variants in *SMARCAL1;* ataxia, hypotonia and characteristic findings on MRI scan of the brain in a child with variant in the *COQ2* gene; XY karyotype in a phenotypic female with a *WT1* variant; and a child with a mutation in *WDR3* had features of Galloway Mowat syndrome.

A limitation of our study is that we were able to capture data contributed by only 60% of pediatric nephrologists practicing in this part of the country.

Our study adds to the evidence that *NPHS2* variants are uncommon among Asians while *PLCE1* may be a common variant in western India. Furthermore, our study expands the genetic spectrum in two ways. First, we report variants in *NUP93* for the first time from India; also, a heterozygous variant in *COL4A3*, FSGS on histology and presentation as infantile SRNS. Second, we reiterate an important aspect of precision and personalized medicine, i.e., that even if genetic reports had failed to provide a definite etiology at the time of initial testing, follow-up of the patients for evolving clinical features and reanalysis of genomic data in the light of the ever-expanding genetic database might help to arrive at a final diagnosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00467-024-06295-8.

Data availability The data regarding this study will be made available on request.

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