RESEARCH PAPER

Next-Generation Sequencing for Congenital Nephrotic Syndrome: A Multi-Center Cross-Sectional Study from India

ADITI JOSHI,[1*] ADITI SINHA,[2*] AAKANKSHA SHARMA,[2] UZMA SHAMIM,[1] BHARATHRAM UPPILLI,[1] POOJA SHARMA,[1] SANA ZAHRA,[1] SHAISTA PARVEEN,[1] ARADHANA MATHUR,[1] MONICA CHANDAN,[2] PRACHI TEWARI,[2] PRIYANKA KHANDELWAL,[2] PANKAJ HARI,[2] MITALI MUKERJI,[1] MOHAMMED FARUQ,[1] ARVIND BAGGA,[2] FOR THE NEPHQUEST CONSORTIUM

From [1]Genomics and Molecular Medicine, CSIR Institute of Genomics and Integrative Biology, Delhi; and [2]Division of Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi; India. [*]Joint first authors.

Correspondence to:
Professor Arvind Bagga,
Division of Nephrology,
Department of Pediatrics,
All India Institute of Medical Sciences,
New Delhi, India.
arvindbagga@hotmail.com
Dr Mohammed Faruq,
Genomics and Molecular Medicine,
CSIR Institute of Genomics and Integrative Biology,
Delhi, India.
faruq.mohd@igib.in

Objective: Information on etiology of congenital nephrotic syndrome in non-Caucasian populations is limited. This study aimed to determine the genetic basis of congenital nephrotic syndrome in Indian patients. **Methods:** In this observational, cross-sectional study, whole exome sequencing was performed on samples from all children diagnosed with congenital nephrotic syndrome, presenting at centers collaborating in a nationwide registry and biorepository. Analysis was targeted to focus on reported or novel, pathogenic or likely pathogenic variants in 89 genes implicated in etiology of nephrotic syndrome. Sanger sequencing was used to confirm disease-causing variants in patients and allelic segregation of compound heterozygous variants in samples from parents. Inheritance of a shared haplotype was analyzed among ten individuals carrying the most common variant. **Results:** During 2017-2019, 34 patients with congenital nephrotic syndrome were screened. Consanguinity and similar illness in siblings were reported in eleven patients each. Homozygous or compound heterozygous, pathogenic or likely pathogenic variants were found in *NPHS1* in 24 cases, including two novel variants. One patient each had homozygous pathogenic or likely pathogenic known or novel variant in *NPHS2*, *PLCE1, OSGEP* and *LAMB2* genes. Patients with *OSGEP* and *LAMB2* mutations had phenotype typical of Galloway Mowat and Pierson syndromes, respectively. Three variants in *NPHS1* were common to 16 individuals. One reported variant in exon 19 (c.2600G>A; p.Gly867Asp) appears to share a common founder. **Conclusion**: A genetic cause was determined for 82.4% patients with congenital nephrotic syndrome. Variants in *NPHS1* are most common in Indian patients and founder mutations might be present.

Keywords: *Nephrin, podocin, Galloway Mowat syndrome, Pierson syndrome, NPHS1*

Congenital nephrotic syndrome (NS) is a rare condition, characterized by nephrotic range proteinuria, hypoalbuminemia and edema before 3 months of age. Most patients show morbidities related to edema, infections and/or thrombosis, and progression to end stage renal disease (ESRD) in early childhood [1]. An inherited basis is reported in 60-80% patients; variants in *NPHS1*, which are most frequent and also cause the Finnish type of congenital NS [2], along with variants in *NPHS2*, *PLCE1*, *LAMB2* and *WT1*, result in defects affecting proteins in the podocyte slit diaphragm, actin cytoskeleton or transcription regulation [3-5]. Existing reports on variants in Asian patients are single-center and retrospective, screening for few genes [6-10]. We describe here the results of next-generation sequencing (NGS) in infants with congenital NS, enrolled prospectively from April, 2017 to June, 2019, in a multicenter collaboration on nephrotic syndrome.

METHODS

Following ethics approval and informed parental consent, clinical details and blood samples were collected from patients with congenital NS, diagnosed at seven tertiary care centres in the country. Diagnosis required the confirmation of nephrotic range proteinuria (spot urine protein to creatinine ratio >2.0 mg/mg or dipstick 3+/4+ on three occasions), hypoalbuminemia (serum albumin <3.0 g/dL) and edema beginning below 3-months of age. Intrauterine infections and structural renal anomalies were excluded by appropriate serology and ultrasonography, respectively. In consonance with current practice worldwide, kidney biopsy was not performed and echocardiography was performed if cardiac examination was abnormal. Management involved the use of furosemide (1-2 mg/kg daily, as

indicated), enalapril (0.3-0.4 mg/kg/day orally), intravenous infusions of albumin (1-2 g/kg once every 7-14 days), and supplements of thyroxine (5-10 µg/kg/day) and vitamins, while ensuring adequate nutrition. Parents were counselled regarding outcomes including risk of progression to end stage kidney disease, and families opted for a palliative care plan due to costs of kidney replacement therapy.

The methodology of NGS, performed at Institute of Genomic and Integrative Biology, Delhi, is detailed in **Supp. Methods**. Whole exome sequencing (WES) was performed using the Illumina HiSeq2000 or NovaSeq platforms, sequenced reads were mapped and aligned to the reference genome (GRCh37; hg19), and called and annotated variants in 89 genes associated with nephrotic syndrome (**Supp. Table SI**) [3,11-14] were prioritized based on rarity (minor allele frequency, MAF <0.1%), novelty in population databases [15-17], prediction of deleteriousness by *in silico* tools, and if previously reported with disease [18]. Only pathogenic and likely pathogenic variants, according to criteria of the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines [18,19] were considered causative, and were validated by Sanger sequencing. Sanger sequencing on parents' samples was used to confirm allele segregation for compound heterozygous variants. Haplotype studies were performed to determine if the *NPHS1* variant c.2600G>A (p.Gly867Asp) that segregated in 10 of 34 patients occurred on a common genetic background, suggesting inheritance from a common ancestor (founder mutation) (**Supp. Methods**) [20].

Statistical analyses: Data was summarized as median (interquartile range, IQR) for continuous variables and percentage with 95% confidence interval (CI) for dichotomous variables. Assuming 70% prevalence of pathogenic or likely pathogenic variations in genes encoding key podocyte proteins in patients with congenital nephrotic syndrome [1,3,12,13], 21 patients were required to be enrolled for a precision of 20%, at power of 80% and alpha error of 5%.

RESULTS

Samples were collected from 34 unrelated patients (53% boys) with congenital NS diagnosed at 7 centers across India. Onset of edema was at median age of 20 (IQR 15-45) days of life, and was associated with anasarca (91.2%), oliguria (41.2%), poor feeding (35.3%), seizures (32.3%), hypovolemia (23.5%), severe infections (20.6%) and/or lethargy (8.8%). Ten (29.4%) patients were born premature and 13 (38.2%) had low birth weight (**Supp. Table SII**). Consanguinity and similar illness in siblings were reported in 11 (32.4%) cases, each.

Median weight for age standard deviation score (SDS) was -3.1 (IQR -4.1, -1.9), length for age SDS was -3.9 (IQR -4.6, -2.2) and head circumference SDS was -3.2 (IQR -4.5, -2.2). Seven (20.6%) patients had hypertension. Isolated extrarenal features were observed in 9 patients (**Supp. Table SII**), while one patient each had features of Galloway-Mowat and Pierson syndrome. One patient had albinism and microcephaly and history of sibling death with similar symptoms.

The median blood level of albumin was 1.2 (IQR 0.9-1.4) g/dL, cholesterol 274 (234-349) mg/dL, creatinine 0.4 (0.3-0.7) mg/dL, and estimated glomerular filtration rate (eGFR) 60 (28.3-96) mL/minute per 1.73 m^2 [21]. Seven (20.6%) patients had eGFR <30 mL/minute per 1.73 m^2 at evaluation. Three (8.8%) patients had enlarged kidneys without hydronephrosis or venous thrombosis. There was no history of significant teratogenic drug intake during pregnancy or evidence of intrauterine infection.

WES with mean coverage of ≥30x (**Web Table SIII)** returned 16804 variants, of which 1370 variants were present in one or more of the targeted genes (**Fig. 1**). After filtering, 91 variants were shortlisted (**Supp. Table SIV**), of which 22 variants were prioritized in 28 patients (**Table I*;* Supp. Fig. S1)**. Pathogenic and likely pathogenic variants were inherited as homozygous and compound heterozygous variations in 20 and 8 patients, respectively. A monogenic cause was thus established in 82.4% (95% CI 66.9% to 92.5%) of 34 patients with congenital NS. Most variants were conserved across species (**Supp. Fig. S2**).

Variants in *NPHS1* were most common, including 16 reported [11,12,14,15,22-35] and two novel variants, segregated in 24 patients as homozygous (*n*=16) and compound heterozygous (*n*=8) variants (**Table I**). Reported variations included 7 pathogenic and 9 likely pathogenic variants. One novel homozygous variant in ID#181 was classified as likely pathogenic, while another novel *NPHS1* variant that segregated as compound heterozygous in ID#8, was assigned as pathogenic. **Fig. 2** indicates the distribution of defects in *NPHS1* across the structure of nephrin.

One previously reported [11,14] likely pathogenic *NPHS1* variant in exon 19 (c.G2600A; p.Gly867Asp) was inherited as homozygous in 7 and heterozygous in 3 patients from different ethnic and regional backgrounds, without any specific phenotype (**Tables I** and **SII**). Two other reported variations, p.Arg1160Ter [11,14,27] and p.Arg367Cys [14,25,27], were common to three patients each (**Table I**). In patients with *NPHS1* variants, atrial septal defect was seen in two patients, and developmental delay, facial dysmorphism, clubbing, café

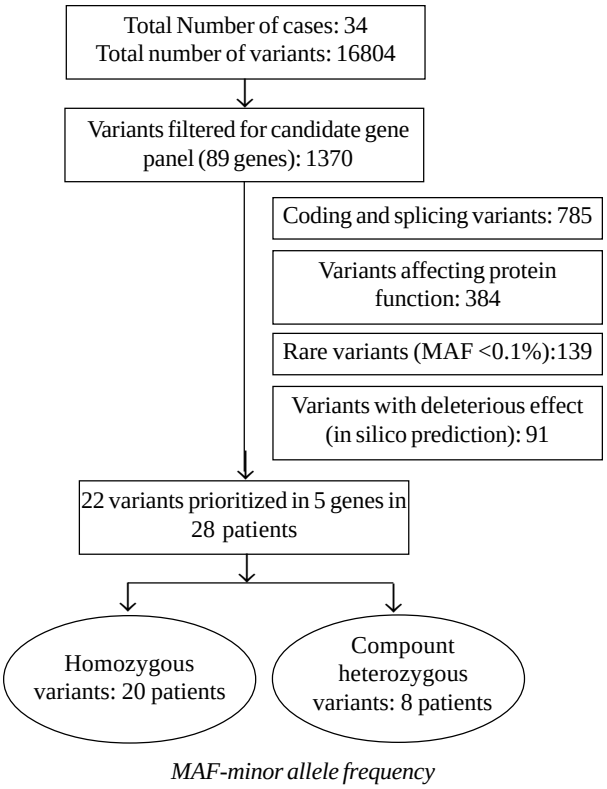


Fig. 1 Flowchart for variant filtering after whole exome sequencing.

au lait spots, hirsutism and aqueductal stenosis in one patient each (**Supp. Table SII**).

One patient each had homozygous likely pathogenic variants in *NPHS2* [34] and *OSGEP* [36], associated with an atrial septal defect and Galloway-Mowat syndrome, respectively. One patient each had novel pathogenic homozygous variations in *PLCE1* and *LAMB2* genes; the latter was associated with phenotype consistent with Pierson syndrome.

No variants were prioritized in two patients; four patients had heterozygous variations that were of unknown significance (**Supp. Table SIV**). Patients with causative variations also had additional heterozygous variations (**Supp. Table SIV**).

There were no differences in sex ratio, age at onset of symptoms, levels of serum albumin or estimated GFR between patients with *NPHS1* variations and those with other or no significant variations (P>0.05 each).

Forty-four of 900 single nucleotide polymorphisms (SNPs) (**Supp. Table SV**) in the region (±500 kbp) flanking the c.2600G>A (Gly867Asp) were selected for haplotype analysis in 33 patients. All 17 alleles carrying the c.2600G>A variant (homozygous in 7 and heterozygous in 3 patients) shared a core haplotype in the 500 kbp region between rs2230181 to rs466452 (**Supp. Table SVI)**. Thirteen of 17 alleles also shared a core haplotype extending to 800 kbp length. The 500 kbp core haplotype was observed in only one of 49 non-mutant chromosomes, suggesting a founder effect.

DISCUSSION

There is significant heterogeneity in prevalence of inherited defects across studies (**Supp. Table SVII**) [6-10,12,22-26,34]. Variants in *NPHS1* predominate even in non-Finnish cohorts, and contributions by *NPHS2*, *WT1* and *LAMB2* defects differ widely across populations. In the present study, the use of NGS enabled a diagnosis in 82% of 34 patients. These findings are unlike previous studies from non-Caucasian populations that report lower rates of inherited defects, perhaps due to focused testing including a few genes **(Supp. Table SVII**).

Two founder deletion mutations in *NPHS1*, accounting for the majority of cases of Finnish type of congenital nephrotic syndrome, were not observed in our patients, similar to reports from non-Finnish populations [2,3,6-10]. Over 200 *NPHS1* mutations are described worldwide in non-Finnish populations [29,32]. In our report, homozygous and compound heterozygous mutations in *NPHS1* accounted for 70.6% of cases of congenital NS, and 85.7% of cases with an identified genetic etiology. This proportion is higher than previous reports from Asia, in which *NPHS1* mutations accounted for 22-67% of cases, but similar to proportions reported in series including non-Finnish populations (**Supp. Table SVII**).

As shown in **Fig. 2**, variants in *NPHS1* were distributed all over the protein. Three patients shared the variant p.Arg1160Ter, responsible for premature truncation of protein in the intracellular domain that interacts with podocin. This variant, a founder mutation in Maltese patients, is associated with a different allele in Asian patients [25]. While Koziell, et al. reported a mild phenotype in affected girl infants [25], we and other authors [24,35] found a severe phenotype, irrespective of gender, indistinguishable from other *NPHS1* mutations. Three patients carried a variant (c.1099C>T; p.Arg367Cys), reported previously as a founder mutation from India [12]. One *NPHS1* variant, c.2600G>A (p.Gly867Asp), that translates into a change in the immunoglobulin-like domain 8, found in 10 unrelated patients from five states in north India (**Supp. Table SII**, **Table I** and **Fig. 1**), has been reported from India, Pakistan and Saudi Arabia [8,11,14,37], but not from east Asia [6,7,9] or Europe. Using statistical tools considered more efficient that conventional haplotyping [20], we show

Table I Pathogenic and Likely Pathogenic Variants in Patients With Congenital Nephrotic Syndrome[a]

Gene (chromosome); Chromosomal coordinate; change	*Exon*	*Variant change*		*ACMG# category*[b]	*Patient ID*[c]	*Reference*
		cDNA change	*Protein change*			
NPHS1 (19)						
36341349 T>C	5	c.527-2A>G		Pathogenic[b5]	165	33
36341342 G>A	5	c.532C>T	p.Gln178Ter	Pathogenic	8	26, 29, 34
36340176 G>A	7	c.802C>T	p.Arg268Ter	Pathogenic	80	26, 30
36337056 G>T	12	c.1481C>A	p.Ser494Ter	Pathogenic	180	29,31
36335078_36335079delGT	16	c.2138_2139delAC	p.Asp713Glyfs*12	Pathogenic[b6]	8	Novel
36330221 G>C	22	c.3027C>G	p.Tyr1009Ter	Pathogenic	**168**	23, 34
36321958 G>A	27	c.3478C>T	p.Arg1160Ter	Pathogenic	**150, 169,** 173	11,14,22,23,24,25,26,27,29,32, 35
36317522 TC>T	29	c.3619delG	p.Glu1207Lysfs*30	Pathogenic[b6]	4	ClinVar
36341889 G>A	4	c.500C>T	p.Pro167Leu	Likely pathogenic	267	11, 22
36340541-36340548 delCCGGGGTGinsAA	6	c.614_621delinsTT	p.Thr205_Arg207delinsIle	Likely pathogenic[b4]	180,228	22, 26,35
36339610 G>A	9	c.1099C>T	p.Arg367Cys	Likely pathogenic	**59**, 165, 267	14,22,25,26,29,30,33, 35
36339251 G>A	10	c.1219C>T	p.Arg407Trp	Likely pathogenic[b2]	**85**	22
36336350 T>C	14	c.1850A>G	p.His617Arg	Likely pathogenic[b1]	80	26,27,29,30
36335272 G>A	15	c.2020C>T	p.Pro674Ser	Likely pathogenic[b1]	**163**	24
36333370 G>T	18	c.2417C>A	p.Ala806Asp	Likely pathogenic[b2]	**157**	14, 26, 27,29
36333089 C>T	19	c.2600G>A	p.Gly867Asp	Likely pathogenic[b1]	4, **40, 47, 146, 162,** 173**, 196, 201**, 228, **235**	11,14, 34
36332715 A>G	20	c.2717T>C	p.Ile906Thr	Likely pathogenic[b1]	**181**	Novel
36322049 C>T	27	c.3388-1G>A		Likely pathogenic	**18**	26, 27
NPHS2 (1)						
179530456 C>T	3	c.419G>A	p.Gly140Glu	Likely pathogenic[b1]	**28**	34
PLCE1 (10)						
96058156 GC>AT	23	c.4264C>T	p.Gln1422Ter	Pathogenic	**46**	Novel
OSGEP (14)						
20920566 T>A	2	c.157A>T	p.Ile53Phe	Likely pathogenic[b7]	**154**	36
LAMB2 (3)						
49168499 G>A	8	c.799C>T	p.Arg267Ter	Pathogenic	**X1**	Novel

[a]Six patients did not have any pathogenic or likely pathogenic variants. ACMG-American College of Medical Genetics. [c]Patient IDs indicated in bold had homozygous variation; those not in bold were heterozygous variants. [b]Criteria were manually adjusted:[1]PM1, PM2, PP3& PP4; [2]PM1, PM2, PP4 & PP5; [3]PM1, PM2 & PP4; [4]PM1, PM2, PM3, PP4 & BP4; [5]PVS1, PM2, PP3, PP4; [6]PVS1, PM2, PP4; [7]PM1, PM2, PP2, PP4 & BP4

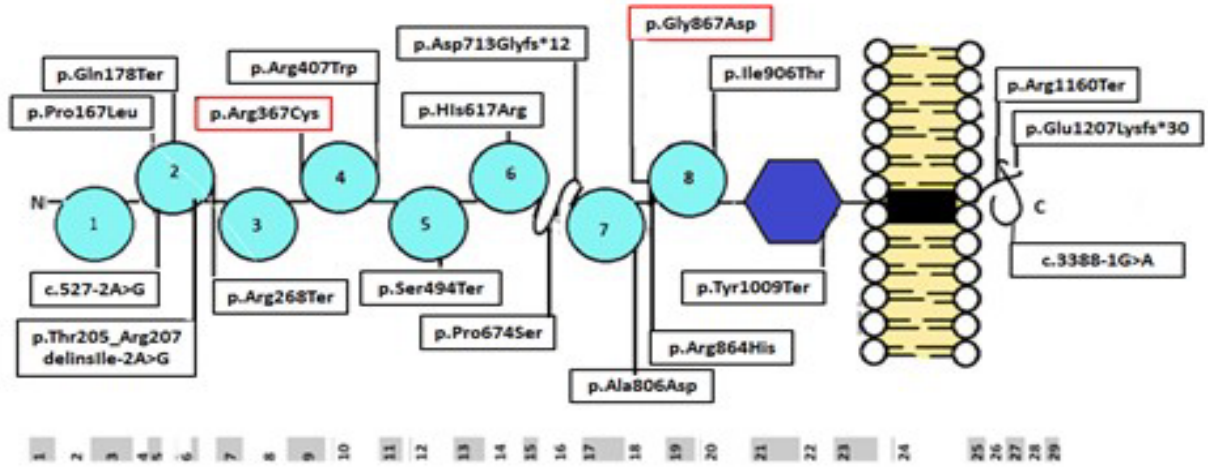


Fig. 2 Localization of novel variations and known mutations in the translated nephrin protein, comprised of eight extracellular immunoglobulin (Ig) -like domains (semi-circles), a fibronectin type III-like module (hexagon), a transmembrane domain (black rectangle) and a C-terminal (C) cytoplasmic domain (curled line). The bottom panel indicates the exons coding for the corresponding protein domains. Note that the 18 variations observed were spread throughout the protein. The variations with dotted lines are known or speculated to be founder mutations.

that c.2600G>A is possibly a founder mutation, as suggested by the lack of genetic variation in the 500-800 kbp length flanking regions [38]. The differences in frequency of the shared haplotype in various ethnicities in the 1000 genome database suggests a European origin for the mutation **(Supp. Table SVIII)** [15]. Our hypothesis requires confirmation by examining for the same shared haplotype in previously reported patients with the p.Gly867Asp mutation.

Mutations in *NPHS2* and *WT1* account for 0-51% and 0-40% cases, respectively, across populations, though *NPHS2* variants are uncommon in Asia (**Supp. Table SVII**). In this cross-sectional study, only one patient had homozygous mutations in *NPHS2*, and none had variants in *WT1*. Given the small study size, these findings have limited generalisability.

Confirming previous findings, we failed to find specific phenotypic associations in patients with *NPHS1*, *NPHS2* and *PLCE1* mutations [4,26,39]. The lone patient with homozygous *LAMB2* variant had findings of Pierson syndrome while another had Galloway-Mowat syndrome secondary to *OSGEP* mutation [36]. The latter patient had the same mutation and phenotype as an infant of Pakistani ethnicity described previously [36].

The present series underscores the utility of providing a genetic etiology in patients with congenital NS, thereby facilitating prenatal counseling and testing in subsequent pregnancies. One *NPHS1* mutation is hypothesized to have a founder effect in Indian population. Information on long term outcomes, including post-transplantation, is lacking since most children were lost to follow up after families chose a palliative care plan. Despite being a multicenter study, the findings of the relatively small sample size might not be generalizable.

Note: Supplementary material related to this study is available with the online version at *www.indianpediatrics.net*

Acknowledgments: We thank the following colleagues participating in the NephQuest network who contributed samples and details of their patients: SP Veeturi, Rainbow Children Hospital, Hyderabad; KL Tiewsoh, Postgraduate Institute of Medical Education and Research, Chandigarh; A Mittal, All India Institute of Medical Sciences, Jodhpur; S Krishnamurthy, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry; M Mantan, Maulana Azad Medical College, New Delhi; M Kumar and K Mishra, Chacha Nehru Bal Chikitsalaya, Delhi.

Ethics approval: Ethics committees at CSIR Institute of Genomics and Integrative Biology, Delhi and All India Institute of Medical Sciences, New Delhi; Sanction no. IECPG-616/ 21.12.2016. RT-33/22.03.2017 and 6/GAP127/CSIR-IGIB/2017

Contributors: All authors contributed to the study conception and design. AJ, AS, AS, MF, AB: material preparation, data collection and analysis were performed; AJ, AS: The first draft of the manuscript was written jointly. All authors commented on the manuscript, and approved the final manuscript.

Funding: Department of Biotechnology, Government of India (BT/11030/MED/30/1644/2016).

Competing interest: None stated.

WHAT IS ALREADY KNOWN?

- Genetic defects account for 60-80% of cases with congenital nephrotic syndrome
- Mutations in *NPHS1* are most common in Caucasians; *WT1* and *LAMB2* variants are probably more common in Asian patients

WHAT THIS STUDY ADDS?

- Genetic defects are present in more than 80% patients with congenital nephrotic syndrome in India
- Mutations in *NPHS1* account for more than 80% of patients with an inherited basis
- Common variants in *NPHS1* are those that are known (c.1099C>T; p.Arg367Cys) or speculated (c.2600G>A; p.Gly867Asp) to be founder mutations.

REFERENCES

1. Jalanko H. Congenital nephrotic syndrome. Pediatr Nephrol 2009; 24: 2121-28
2. Norio R. Heredity in the congenital nephrotic syndrome. A genetic study of 57 Finnish families with a review of reported cases. Ann Paediatr Fenn 1996;12:S27:1-94
3. Ha TS. Genetics of hereditary nephrotic syndrome: a clinical review. Korean J Pediatr 2017;60:55-63
4. Hinkes BG, Mucha B, Vlangos CN, et al; Arbeitsgemeinschaftfür Paediatrische Nephrologie Study Group. Nephrotic syndrome in the first year of life: Two thirds of cases are caused by mutations in 4 genes (NPHS1, NPHS2, WT1, and LAMB2). Pediatrics 2007;119: e907-19
5. Wang JJ, Mao JH. The etiology of congenital nephrotic syndrome: Current status and challenges. World J Pediatr 2016;12:149-58
6. Li GM, Cao Q, Shen Q, et al. Gene mutation analysis in 12 Chinese children with congenital nephrotic syndrome. BMC Nephrol 2018;19:382.
7. Sako M, Nakanishi K, Obana M, et al. Analysis of NPHS1, NPHS2, ACTN4, and WT1 in Japanese patients with congenital nephrotic syndrome. Kidney Int 2005;67: 1248-55
8. Sharief SN, Hefni NA, Alzahrani WA, et al. Genetics of congenital and infantile nephrotic syndrome. World J Pediatr 2019;15:198-203
9. Nishi K, Inoguchi T, Kamei K, et al. Detailed clinical manifestations at onset and prognosis of neonatal-onset Denys-Drash syndrome and congenital nephrotic syndrome of the Finnish type. Clin Exp Nephrol 2019;23:1058-65
10. Sinha R, Vasudevan A, Agarwal I, et al. Congenital nephrotic syndrome in India in the current era: A multicenter case series. Nephron 2019;144:1-9
11. Bierzynska A, McCarthy HJ, Soderquest K, et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. Kidney Int 2017;91:937-47
12. Sadowski CE, Lovric S, Ashraf S, et al; SRNS Study Group, Hildebrandt F. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol 2015;26:1279-89
13. Trautmann A, Lipska-Ziêtkiewicz BS, Schaefer F. Exploring the clinical and genetic spectrum of steroid resistant nephrotic syndrome: The PodoNet registry. Front Pediatr 2018;6:200
14. Lovric S, Fang H, Vega-Warner V, et al; Nephrotic Syndrome Study Group. Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2014;9:1109-16
15. 1000 Genomes Project Consortium; Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. Nature 2015; 526: 68-74
16. Karczewski KJ, Weisburd B, Thomas B, et al; The Exome Aggregation Consortium, Daly MJ, MacArthur DG. The ExAC browser: Displaying reference data information from over 60000 exomes. Nucleic Acids Res 2017;45 (D1): D840-5
17. Karczewski KJ, Francioli L, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2022;581:434-43.
18. Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2005;33:D514-7
19. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24
20. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978-89
21. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629-37
22. Schoeb DS, Chernin G, Heeringa SF, et al; Gesselschaftfür Paediatrische Nephrologie Study Group. Nineteen novel NPHS1 mutations in a worldwide cohort of patients with congenital nephrotic syndrome (CNS). Nephrol Dial Transplant 2010;25:2970-6
23. Lee JH, Han KH, Lee H, *et al*. Genetic basis of congenital

and infantile nephrotic syndromes. Am J Kidney Dis 2011;58:1042-3
24. Warejko JK, Tan W, Daga A, et al. Whole exome sequencing of patients with steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2018;13:53-62
25. Koziell A, Grech V, Hussain S, et al. Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional inter-relationship in glomerular filtration. Hum Mol Genet 2002;11:379-88
26. Machuca E, Benoit G, Nevo F, et al. Genotype-phenotype correlations in non-Finnish congenital nephrotic syndrome. J Am Soc Nephrol 2010;21:1209-17
27. Lenkkeri U, Männikkö M, McCready P, et al. Structure of the gene for congenital nephrotic syndrome of the Finnish type (NPHS1) and characterization of mutations. Am J Hum Genet 1999;64:51–61
28. Kestilä M, Lenkkeri U, Männikkö M, et al. Positionally cloned gene for a novel glomerular protein-nephrin-is mutated in congenital nephrotic syndrome. Mol Cell 1998;1:575-82
29. Beltcheva O, Martin P, Lenkkeri U, Tryggvason K. Mutation spectrum in the nephrin gene (NPHS1) in congenital nephrotic syndrome. Hum Mutat 2001;17: 368-73
30. Ulinski T, Aoun B, Toubiana J, Vitkevic R, Bensman A, Donadieu J. Neutropenia in congenital nephrotic syndrome of the Finnish type: Role of urinary ceruloplasmin loss. Blood 2009;113:4820-1
31. Bolk S, Puffenberger EG, Hudson J, Morton DH, Chakravarti A. Elevated frequency and allelic heterogeneity of congenital nephrotic syndrome, Finnish type, in the old order Mennonites. Am J Hum Genet 1999; 65: 1785-90
32. Heeringa SF, Vlangos CN, Chernin G, et al; Members of the APN Study Group. Thirteen novel NPHS1 mutations in a large cohort of children with congenital nephrotic syndrome. Nephrol Dial Transplant 2008;23:3527-33
33. Ashton E, Bockenhauer D, Boustred C, Jenkins L, Lench N. ASHG 2013 Poster: Development of a rapid genetic testing service for nephrotic syndrome using next generation sequencing. Conference: American Society of Human Genetics, At Boston, USA 2013; available at *https://www.researchgate.net/publication/258860074_ASHG_2013_Poster_Development_of_a_rapid_genetic_ testing_service_for_nephrotic_syndrom e_using_next_generation_sequencing*; last accessed 21 February 2020
34. Sen ES, Dean P, Yarram-Smith L, et al. Clinical genetic testing using a custom-designed steroid-resistant nephrotic syndrome gene panel: Analysis and recommendations. J Med Genet 2017;54:795-804.
35. Ovunc B, Ashraf S, Vega-Warner V, et al; Gesellschaft für Pädiatrische Nephrologie (GPN) Study Group. Mutation analysis of NPHS1 in a worldwide cohort of congenital nephrotic syndrome patients. Nephron Clin Pract 2012;120:c139-46
36. Domingo-Gallego A, Furlano M, Pybus M, et al. Novel homozygous OSGEP gene pathogenic variants in two unrelated patients with Galloway-Mowat syndrome: Case report and review of the literature. BMC Nephrol 2019;20:126
37. Abid A, Khaliq S, Shahid S, et al. A spectrum of novel NPHS1 and NPHS2 gene mutations in pediatric nephrotic syndrome patients from Pakistan. Gene 2012;502:133-7
38. Zlotogora J. High frequencies of human genetic diseases: founder effect with genetic drift or selection? Am J Med Genet 1994;49:10-3
39. Schultheiss M, Ruf RG, Mucha BE, et al. No evidence for genotype/phenotype correlation in NPHS1 and NPHS2 mutations. Pediatr Nephrol 2004;19:1340-8.

SUPPLEMENTARY METHODS

DNA extraction: DNA was extracted from EDTA blood using QIAamp® DNA Blood Mini Kit (Qiagen, Germantown, MD), as per manufacturer instructions. The quality and the quantity of DNA extracted was assessed using NanoDrop™ spectrophotometer (Thermo Scientific, Wilmington, DE) and 1% gel electrophoresis before next generation sequencing.

Whole exome sequencing (WES): DNA libraries were prepared using 100 ng of DNA, physically sheared on an ultrasonicator (Covaris), followed by ligation of adapter sequences on to fragmented DNA to generate indexed libraries, and exome enrichment using TruSeq Exome kit (Illumina, San Diego, CA), as per manufacturer protocol. Enriched libraries were quantified by Qubit fluorometer (ThermoFisher) and their size distribution measured using Bioanalyzer (Agilent). Three of 34 samples underwent cluster generation using Illumina Cbot followed by paired end sequencing (2x100 bp) using flowcell v3 on Illumina HiSeq2000 platform. The remainder were sequenced (2x150 bp) on Illumina NovaSeq platform using S2/S4 flowcell.

Processing of sequenced reads: Paired-end sequenced reads were processed using the Dynamic Read Analysis for GENomics Bio-IT (DRAGEN, Illumina) platform. The reads were demultiplexed and then mapped and aligned to the reference genome (GRCh37; hg19) using the seed generation algorithm followed by Smith-Waterman algorithm. This was followed by variant calling using Haplotype Caller (Dragen), merging of individual variant call files (VCF) using VCFtools (*vcftools.sourceforge.net*) and annotation of merged VCFs using ANNOVAR (*annovar.openbioinformatics.org*).

Variant prioritization: Based on literature search, 89 genes were considered relevant for genetic testing in nephrotic syndrome [i,ii,iii,iv,v]. The list of genes, along with the mean coverage of exonic regions, is provided in **Suppl. Table S1**. Variants in these genes were considered potentially disease causing if they fulfilled one of the following criteria: *(i) rare and deleterious,* with rarity defined as minor allele frequency (MAF) of less than 0.1% in the population databases of 1000 Genomes Project [vi], Exome Aggregator Consortium (Exac) [vii] and Genome Aggregation Database (gnomAD) [viii]; and deleteriousness predicted by assertion of pathogenicity on at least two computational tools, including Polymorphism Phenotyping v2 (PolyPhen2; *http://genetics.bwh.harvard.edu/pph2/*), Sorting Intolerant from Tolerant (SIFT; *https://sift.bii.a-star.edu.sg/*), Mutation taster v2 (*http://www.mutationtaster.org/ChrPos.html*), Combined Annotation Dependent Depletion (CADD; *https://cadd.gs.washington.edu/*) and Genomic Evolutionary Rate Profiling score

(GERP_RS;*http://varianttools.sourceforge.net/Annotation/dbNSFP*), Eigen (*https://omictools. com/eigen-tool*), and where relevant, Human Splicing Finder v3.1 (*http://umd.be/HSF3/*); *(ii) novel and deleterious*, with novelty defined by absence in the two population databases as well as in the Database of Single Nucleotide Polymorphism (DbsNP; *https://www.ncbi.nlm.nih.gov/snp/*) and ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar/*); or *(iii) reported,* in causative association with disease (congenital or steroid resistant nephrotic syndrome) in ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar*) or Human Genome Mutation Database (HGMD; *http://www.hgmd.cf.ac.uk*), particularly if reported as 'pathogenic' or 'likely pathogenic' in ClinVar.

Variants shortlisted based on above criteria were excluded if any of the following conditions were fulfilled: *(i) high (≥0.1%) MAF in south Asian population* of ExAC; *(ii) failing to have causative phenotype*, such as a variant called in heterozygous state in a gene following autosomal recessive pattern of inheritance [ix]; or *(iii) low depth:* variant with read depth of <10x. Prioritised variants were classified from benign to pathogenic using the web-based clinical INTERpretation of VARiants (wINTERVAR; *http://wintervar.wglab.org/*), with or without modifications to follow the criteria outlined by the 2015 guidelines of the American College of Medical Genetics and Genomics (ACMG) [x].

Variant validation: Variants considered causative of disease were validated by Sanger sequencing using the ABI3730 genetic analyzer (Applied Biosystems). Sanger sequencing on parents' samples was used to confirm allele segregation for compound heterozygous variations.

Haplotype analysis of p.Gly867Asp mutant allele: The NGS data was used to obtain all single nucleotide polymorphisms flanking the mutation (±500 kbp). Variants with MAF ≥0.05% and significant difference in frequency (P<0.05) in patients with and without the mutation (p.Gly867Asp) were selected for haplotype analysis by Phase v2.1 (*http://stephenslab.uchicago.edu/phase/download.html*) to obtain haplotypes segregating with Gly867 and Asp867 associated alleles.

Supplementary Table SI Panel of 89 genes examined for association with congenital nephrotic syndrome along with coverage

Gene	*Disease*	*Inheritance*	*Transcript*	*Mean Coverage*
ACTN4	Glomerulosclerosis, focal segmental, 1	AD	NM_004924.5	109.40
ALG1	Congenital disorder of glycosylation, type Ik	AR	NM_019109.4	126.06
ALMS1	Alstrom syndrome	AR	NM_015120.4	104.55
ANKS6	Nephronophthisis 16	AR	NM_173551.4	46.96
ANLN	Focal segmental glomerulosclerosis 8	AD	NM_001284301.2	61.67
APOL1	End-stage renal disease, nondiabetic, susceptibility to Glomerulosclerosis, focal segmental, 4, susceptibility to	-	NM_145343.2	51.61
ARHGAP24	-	-	NM_001025616.2	111.73
ARHGDIA	Nephrotic syndrome, type 8	AR	NM_001301242.1	44.86
AVIL	-	-	NM_006576.3	65.40
CD151	Nephropathy with pretibial epidermolysis bullosa and deafness	-	NM_001039490.1	49.87
CD2AP	Glomerulosclerosis, focal segmental, 3	-	NM_012120.2	124.88
CFH	Complement factor H deficiency (hemolytic uremic syndrome, atypical, susceptibility to, 1)	AR|AD	NM_000186.3	134.46
CLCN5	Nephrolithiasis, type I: Proteinuria, low molecular weight, with hypercalciuric nephrocalcinosis	XLR|XLR	NM_001127898.3	77.52
COL4A1	Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps	AD	NM_001845.5	126.02
COL4A3	Alport syndrome 2, autosomal recessive; Alport syndrome 3, autosomal dominant; hematuria, benign familial	AR|AD|AD	NM_000091.4	131.02
COL4A4	Alport syndrome 2, autosomal recessive| hematuria, familial benign	AR|AD	NM_000092.4	118.25
COL4A5	Alport syndrome 1, X-linked	XLD	NM_000495.4	90.51
COQ2	Coenzyme Q10 deficiency, primary, 1	AR	NM_015697.7	81.81
COQ6	Coenzyme Q10 deficiency, primary, 6	AR	NM_182476.2	149.02
COQ7	Coenzyme Q10 deficiency, primary, 8	AR	NM_016138.4	53.76
COQ8B	Nephrotic syndrome, type 9	AR	NM_001142555.2	52.54
COQ9	Coenzyme Q10 deficiency, primary, 5	AR	NM_020312.3	130.77
CRB2	Focal segmental glomerulosclerosis 9; ventriculomegaly with cystic kidney disease	AR|AR	NM_173689.6	41.61
CUBN	Finnish type	AR	NM_001081.3	136.24
CYP11B2	Hypoaldosteronism, congenital, due to CMO I deficiency; hypoaldosteronism, congenital, due to CMO II deficiency	AR|AR	NM_000498.3	121.62
DGKE	{Hemolytic uremic syndrome, atypical, susceptibility to, 7}| Nephrotic syndrome, type 7	AR|AR	NM_003647.2	58.76

E2F3	-	-	NM_001949.4	61.62
EMP2	Nephrotic syndrome, type 10	AR	NM_001424.5	45.32
EXT1	Chondrosarcoma| Exostoses, multiple, type 1	AR|AD	NM_000127.2	114.96
FAT1	-	-	NM_005245.3	75.17
FN1	Glomerulopathy with fibronectin deposits 2	AD	NM_001306129.1	127.17
G6PC	Glycogen storage disease	AR	NM_000151.3	126.16
GATA3	Hypoparathyroidism, sensorineural deafness, and renal dysplasia	AD	NM_001002295.1	94.05
GFND1	Glomerulopathy with fibronectin deposits 1	AD	MIM:137950	-
GLA	Fabry disease| Fabry disease, cardiac variant	X-linked	NM_000169.2	92.44
IGAN1	{IgA nephropathy, susceptibility to, 1}	?AD	MIM:161950	-
IGAN2	{IgA nephropathy, susceptibility to, 2}	?AD	MIM:613944	-
INF2	Glomerulosclerosis, focal segmental, 5	AD	NM_001031714.3	63.42
ITGA3	Interstitial lung disease, nephrotic syndrome, and epidermolysis bullosa, congenital	AR	NM_002204.3	52.16
ITGB4	Epidermolysis bullosa of hands and feet| Epidermolysis bullosa, junctional, non-Herlitz type| Epidermolysis bullosa, junctional, with pyloric atresia	AD|AR|AR	NM_001256876.1	101.91
KANK1	Cerebral palsy, spastic quadriplegic, 2	-	NM_001136191.2	70.34
KANK2	Nephrotic syndrome, type 16	AR	NM_001320269.1	45.10
KANK4	-	-	NM_006014.4	72.41
LAGE3	Galloway-Mowat syndrome 2, X-linked	XLR	NM_002292.3	24.05
LAMB2	-	-	NM_170708.3	126.50
LMNA	Cardiomyopathy, dilated, 1A| Charcot-Marie-Tooth disease, type 2B1| Emery-Dreifuss muscular dystrophy 2, autosomal dominant| Emery-Dreifuss muscular dystrophy 3, autosomal recessive| Heart-hand syndrome, Slovenian type| Hutchinson-Gilford progeria| Lipodystrophy, familial partial, type 2| Malouf syndrome| Mandibuloacral dysplasia| Muscular dystrophy, congenital| Restrictive dermopathy, lethal	AD|AR|AD|AR|AD| AR,AD|AD| AD|AR|AD|AR	NM_001282626.1	108.23
LMX1B	Nail-patella syndrome	AD	NM_001174146.1	56.96
LRP2	Donnai-Barrow syndrome	AR	NM_004525.2	130.34
MAFB	Multicentric carpotarsal osteolysis syndrome	AD	NM_005461.4	49.48
MAGI2	Nephrotic syndrome, type 15	AR	NM_001301128.1	66.23
MED28	-	-	NM_025205.4	79.14
MEFV	Familial Mediterranean fever | Familial Mediterranean fever	AD|AR	NM_000243.2	93.60
MT-TL1	-	-		-
MUC1	Medullary cystic kidney disease 1	AD	NM_002456.5	62.84

MYH9	Deafness, autosomal dominant 17| Macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss	AD|AD	NM_002473.5	112.51
MYO1E	Glomerulosclerosis, focal segmental, 6	AR	NM_004998.3	125.65
NEIL1	-	-	NM_001256552.1	62.27
NEU1	Sialidosis, type I| Sialidosis, type II	AR|AR	NM_000434.3	119.58
NPHP4	Nephronophthisis 4| Senior-Loken syndrome 4	AR|AR	NM_015102.4	52.17
NPHS1	Nephrotic syndrome, type 1	AR	NM_004646.3	112.08
NPHS2	Nephrotic syndrome, type 2	AR	NM_001297575.1	86.29
NUP107	Galloway-Mowat syndrome 7; nephrotic syndrome, type 11; ?ovarian dysgenesis 6	AR	NM_020401.3	60.98
NUP205	?Nephrotic syndrome, type 13	-	NM_015135.2	71.10
NUP93	Nephrotic syndrome, type 12	AR	NM_014669.4	56.54
NXF5	-	-	NM_032946.2	83.26
OCRL	Dent disease 2| Lowe syndrome	XLR; |XLR	NM_001587.3	94.16
OSGEP	Galloway-Mowat syndrome 3	AR	NM_017807.3	62.15
PAX2	Glomerulosclerosis, focal segmental, 7; papillorenal syndrome	AD; AD	NM_001304569.1	106.34
PDSS2	Coenzyme Q10 deficiency, primary, 3	AR	NM_020381.3	119.97
PLCE1	Nephrotic syndrome, type 3	AR	NM_016341.3	124.63
PMM2	Congenital disorder of glycosylation, type Ia	AR	NM_000303.2	133.97
PODXL	-	-	NM_001018111.2	61.63
PTPRO	Nephrotic syndrome, type 6	AR	NM_030668.2	137.09
SCARB2	Epilepsy, progressive myoclonic 4, with or without renal failure	AR	NM_001204255.1	123.97
SGPL1	Nephrotic syndrome, type 14	AR	NM_003901.3	66.27
SMARCAL1	Schimkeimmunoosseous dysplasia	AR	NM_001127207.1	122.11
SPRY2	IgA nephropathy, susceptibility to, 3	AD	NM_001318536.1	99.38
SYNPO	-	-	NM_001166208.1	87.84
TP53RK	Galloway-Mowat syndrome 4	AR	NM_033550.3	62.52
TPRKB	Galloway-Mowat syndrome 5	AR	NM_001330386.1	64.71
TRPC6	Glomerulosclerosis, focal segmental, 2	AD	NM_004621.5	96.58
TTC21B	Nephronophthisis 12	AR,AD	NM_024753.4	142.42
TUBAL3	-	-	NM_001171864.1	61.15
VIPAS39	Arthrogryposis, renal dysfunction, and cholestasis 2	AR	NM_001193314.1	125.19

VPS33B	Arthrogryposis, renal dysfunction, and cholestasis 1	AR	NM_001289148.1	137.52
WDR73	Galloway-Mowat syndrome 1	AR	NM_032856.3	73.33
WT1	Denys-Drash syndrome, Frasier syndrome, nephrotic syndrome, type 4	AD,somaticmutation	NM_000378.4	103.30
XPO5	-	-	NM_020750.2	57.90
ZMPSTE24	Mandibuloacral dysplasia with type B lipodystrophy; restrictive dermopathy; lethal	AR	NM_005857.4	118.18

Supplementary Table SII Clinical and demographic characteristics of included patients

ID	*Sex*	*Religion*	*State of origin*	*Age at onset, days*	*Consanguinity*	*Family history*	*Extra-renal features*	*Low birth weight*	*Prematurity*	*Seizures*	*Weight SDS*	*Length SDS*	*eGFR, ml/min per 1.73 m²*	*Serum albumin, g/dl*	*Total cholesterol, mg/dl*
4^1	Boy	Hindu	Haryana	5	0	0	Developmental delay	0	1	0	-1.05	-3.68	86.67	2.2	492
8	Boy	Hindu	Uttar Pradesh	30	0	0	0	1	1	1	-1.56	-4.46	60.00	1.5	171
18	Boy	Muslim	Uttar Pradesh	15	1	0	Clubbing	1	1	1	0.2	NA	60.00	0.6	201
28	Boy	Hindu	Punjab	20	0	0	Atrial septal defect	0	0	0	NA	NA	65.00	3.1	NA
30	Girl	Hindu	Delhi	NA	0	0	0	0	0	0	NA	NA	NA	NA	NA
40^1	Boy	Hindu	Delhi	5	0	1	0	1	0	1	NA	NA	22.60	1	NA
46	Boy	Hindu	NK		0	0	0	0	0	0	NA	NA	NA	NA	NA
47^1	Boy	Hindu	Rajasthan	30	0	0	0	0	0	0	-2.48	-4.63	24.00	2.3	283
51	Boy	Hindu	Delhi	90	0	1	Oculocutaneous albinism, developmental delay, microcephaly, hepatomegaly	0	0	1	NA	NA	4.24	2.1	NA
52	Girl	Hindu	Delhi	NA	0	0	0	0	0	0	NA	NA	NA	NA	NA
59^3	Girl	Hindu	Nepal	45	0	1	Atrial septal defect	1	1	0	-1.88	-4.06	21.85	1.1	391
80	Boy	Hindu	Telangana	30	0	0	0	1	1	0	-3.94	NA	NA	1.2	188
85	Girl	Hindu	Madhya Pradesh	15	0	1	0	0	0	0	-3.29	-11.73	49.00	1.3	349
146^1	Girl	Hindu	Delhi	4	0	0	0	1	1	1	-4.02	-3.54	46.40	1.2	233
150^2	Boy	Hindu	Punjab	60	0	0	0	1	1	1	-3.14	-2.16	36.00	1.1	247
154	Girl	Muslim	Uttar Pradesh	4	1	1	Hiatus hernia, microcephaly, developmental delay, hypotonia	1	0	0	-2.2	-1.3	64.00	0.9	406
157	Boy	Hindu	Uttar Pradesh	15	0	1	Aqueductal stenosis,	0	0	1	-4.61	-5.12	58.00	1.3	295

							obstructive hydrocephalus								
162^[1]	Boy	Muslim	Delhi	30	1	0	0	1	1	0	0.6	NA	96.00	1.3	428
163	Girl	3	Punjab	30	0	0	0	0	0	0	-4.55	-3.92	77.33	1.4	234
165^[3]	Girl	Hindu	Bihar	45	0	0	0	0	0	0	-4.22	-2.85	32.00	0.9	276
168	Girl	Muslim	Uttar Pradesh	20	1	1	Café au lait spot; hirsutism	1	0	0	-2.73	-1.71	104.00	1.7	243
169^[2]	Girl	Muslim	Uttar Pradesh	26	1	0	0	1	0	0	-3.61	-4.07	94.00	0.6	402
173^[1,2]	Girl	Hindu	Uttar Pradesh	60	0	0	0	1	1	1	-4.14	-5.17	17.00	0.7	172
180	Girl	Hindu	Uttar Pradesh	85	0	0	0	0	1	0	-4.32	-6.39	200.00	0.8	250
181	Boy	Muslim	Uttar Pradesh	12	0	0	Dysmorphic facies	0	1	0	0.8	NA	36.00	1.2	342
196^[1]	Boy	Muslim	Uttar Pradesh	15	1	0	0	1	0	1	-4.14	-4.52	28.29	0.9	474
201^[1]	Girl	Muslim	Delhi	20	1	1	0	0	0	0	-2.23	-3.37	196.00	0.56	237
217	Boy	Muslim	Rajasthan	2	1	1	0	NA	NA		-2.69	-0.23	23.66	1.44	309
228^[1]	Boy	Hindu	Uttar Pradesh	15	0	0	0	NA	NA	1	-4.91	-0.5	201.00	1.1	239
235^[1]	Girl	Muslim	Uttar Pradesh	45	0	0	0	1	0	0	-5.83	-5.17	123.90	1.4	200
240	Boy	Hindu	Puducherry	70	1	1	Dysplastic ears	NA	NA	0	-0.12	-0.59	134.23	0.9	271
266	Boy	Muslim	Delhi	15	1	0	0	NA	NA	NA	NA	NA	NA	1.23	296
267^[3]	Girl	Hindu	Bihar	15	0	1	Atrial septal defect	0	0	0	NA	NA	NA	1.2	NA
X1	Girl	Muslim	Punjab	15	1	0	Microcoria,microcornea	NA	NA	NA	NA	NA	NA	NA	NA

eGFR estimated glomerular filtration rate; NA not available

^Indicates patients that shared the following variations: [1]c.2600G>A; [2]c.3478C>T and [3]c.C1099C>T

Supplementary Table SIII Quality metrics based on raw (FASTQ) and mapped (BAM) reads

Sample ID	*Total mapped reads*	*Percentage of total mapped reads over reference genome*	*Mean region coverage depth*
4	36903956	73.80%	48.2
8	36883757	74.50%	48.9
18	39101579	76.00%	52.8
28	39925438	97.86%	57.45
30	33396553	97.6%	43.96
40	36402136	97.77%	49.82
46	46559034	97.63%	57.5
47	48347870	97.67%	51.46
51	41882937	98.14%	58.09
52	54820473	97.81%	59.33
59	43196032	97.71%	58.23
80	46494263	97.72%	57
85	41809738	97.7%	42.99
146	35916462	97.55%	42.14
150	48251461	97.55%	49.67
154	37484353	98.19%	50.85
157	47974741	97.63%	51.34
162	50014242	97.72%	54.71
163	33651571	97.79%	44.01
165	48225036	97.75%	61.04
168	47661449	98.01%	51.14
169	35009940	97.71%	41.01
173	33637511	97.71%	34.08
180	28249162	97.89%	37.79
181	32296239	97.56%	34.09
196	30935558	97.36%	35.64
201	33626554	98.26%	59.42
217	74654485	98.17%	115.89
228	55633896	98.08%	90.73
235	66570469	98.09%	97.98
240	35311365	97.81%	53.55
266	40554026	97.94%	66.39
267	48404929	98.04%	82.57
X1	53300045	99.17%	90.9

Supplementary Table SIV Lists of prioritized variants for individual patients. Variants in bold were considered relevant

Patient ID	*Gene*	*Chromosome: Position*	*Exon*	*Consequence (Base-pair and amino acid)*	*Zygosity*	*Change*	*Frequency in 1KG; Exac; Exac_SAS; gnomAD*	*Polyphen*	*CADD-Phred*	*Eigen_raw*	*GERP++_RS*
4	***NPHS1***	**19:36317523**	**29**	**c.3619delG:p.E1207Kfs*29**	**Heterozygous**	**fs*del**	**.; .; .; $4.0x10^{-6}$**	**.**	**.**	**.**	**.**
	NPHS1	**19:36333089**	**19**	**c.G2600A:p.G867D**	**Heterozygous**	**NS**	**.; 0.00002173; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	ITGB4	17:73753376	39	c.G5314A:p.E1772K	Heterozygous	NS	0.0002; 0.000059; 0; 0.000072	B	23	-0.305	3.03
8	***NPHS1***	**19: 36335078_36335079delGT**	**16**	**c.2138_2139delAC: p.D713Gfs*12**	**Heterozygous**	**fs*del**	**.; .; .; .**	**.**	**.**	**.**	**.**
	NPHS1	**19:36341342**	**5**	**c.C532T: p.Q178X**	**Heterozygous**	**Tr***	**.; .; .; 0.00001193**	**.**	**35**	**0.545**	**3.26**
	COQ8B	19:41198902	14	c.T1250C: p.L417P	Heterozygous	NS	.; .; .; .	D	25.6	0.831	5.37
18	***NPHS1***	**19:36322049**	**27**	**c.3388-1G>A**	**Homozygous**	**Sp**	**.; 0.0000083; 0; $3.98x10^{-6}$**	**.**	**22.2**	**0.771**	**4.1**
	NPHS1	19:36333098	19	c.G2591A: p.R864H	Homozygous	NS	0.0002; 0.0001; $7.2x10^{-5}$; 0.0001	D	34	0.501	4.93
	FN1	2:216242961	35	c.G5647C: p.V1883L	Heterozygous	NS	.; .; .; .	B	22.9	-0.123	4.6
28	***NPHS2***	**1:179530456**	**3**	**c.G419A: p.G140E**	**Homozygous**	**NS**	**.; $8.24 x10^{-6}$; $6.1 x10^{-5}$; .**	**D**	**32**	**0.983**	**5.82**
	MUC1	1:155162020	2	c.C113G: p.S38W	Heterozygous	NS	0.0004; 0.0002; 0.0013; 0.0002	D	22	-0.814	-3.48
30	No variants prioritized										
40	***NPHS1***	**19:36333089**	**19**	**c.G2600A: p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.000016**	**D**	**28.7**	**0.74**	**3.88**
	ALMS1	2:73799812	16	c.A10805G: p.N3602S	Heterozygous	NS	.; 0.0001; 0.001; 0.0001	D	24.5	0.46	4.44
	ARHGDIA	17:79826497	7	c.G758A: p.R253H	Heterozygous	NS	.; .; .; $7.50 x10^{-6}$	.	.	.	.
46	***PLCE1***	**10:96058156**	**24**	**c.C5188T: p.Q1730X**	**Homozygous**	**Tr***	**.; .; .; .**	**.**	**41**	**0.964**	**5.6**
	ALMS1	2:73653592	6	c.G1249T: p.G417W	Heterozygous	NS	.; .; .; .	D	26	0.325	3.8

	INF2	14:105181131	21	c.G3632T: p.R1211L	Heterozygous	NS	.; 0.0002; 0.0011; 0.000098	P	22.8	-0.822	-2.53
47	***NPHS1***	**19:36333089**	**19**	**c.G2600A: p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	COL4A3	2:228173699	49	c.G4547A: p.R1516Q	Heterozygous	NS	.; 0.000075; 0.0003; 0.000072	D	28.4	1.083	5.97
	CRB2	9:126128285	3	c.508_509del: p.C170fs	Heterozygous	fs*del	.; .; .; .	.	.	.	.
51	*COL4A4*	2:227924195	28	c.C2309T: p.P770L	Heterozygous	NS	.; 0.0000663; 0; 0.00003606	D	25.1	0.717	5.99
	NUP93	16:56864492	10	c.G980A: p.R327H	Heterozygous	NS	.; 0.0000165; 0.0001; 0.00002388	D	34	0.949	5.11
52	*ARHGAP24*	4:86921681	10	c.G2053A: p.D685N	Heterozygous	NS	0.0008; 0.0009; 0.0064; 0.0009	P	29.3	0.295	5.56
	SCARB2	4:77116941	2	c.A194G: p.Y65C	Heterozygous	NS	0.0006; 0.0003; 0.0021; 0.0003	D	25.3	0.53	4.35
59	***NPHS1***	**19:36339610**	**9**	**c.C1099T: p.R367C**	**Homozygous**	**NS**	**.; 0.00003308; 0.0001; 0.00003978**	**D**	**28.7**	**0.528**	**4.37**
	ARHGAP24	4:86921681	10	c.G2053A: p.D685N	Heterozygous	NS	0.0008; 0.0009; 0.0064; 0.0009	P	29.3	0.295	5.56
80	***NPHS1***	**19:36336350**	**14**	**c.A1850G: p.H617R**	**Heterozygous**	**NS**	**.; 0.0000084; 0; $7.98x10^{-6}$**	**D**	**22.8**	**0.43**	**4.56**
	NPHS1	**19:36340176**	**7**	**c.C802T: p.R268X**	**Heterozygous**	**Tr***	**.; 0.000034; $6.1x10^{-5}$; 0.00002407**	**.**	**35**	**0.166**	**2.65**
	COQ2	4:84188827	6	c.G1013A: p.G338E	Heterozygous	NS	.; .; .; 0.00001133	P	23.6	0.125	4.77
	CUBN	10:16918949	57	c.A9053C: p.Y3018S	Heterozygous	NS	.; 0.0001; 6.1 $x10^{-5}$; 0.0001	D	23.2	0.24	3.39
	TTC21B	2:166805994	3	c.C172T: p.R58X	Heterozygous	Tr*	.; 0.000058; 0.0002; 0.000040	.	36	0.751	4.52
85	***NPHS1***	**19:36339251**	**10**	**c.C1219T: p.R407W**	**Homozygous**	**NS**	**.; $8.2x10^{-6}$; 0; 0.000020**	**D**	**27**	**0.207**	**3.29**
	ANKS6	9:101558508	1	c.G266A: p.G89D	Homozygous	NS	.; .; .; 0	B	27.5	0.069	3.36
	KANK2	19:11304445	4	c.G311C: p.G104A	Heterozygous	NS	0.0004; 0.0005; 0.0037; 0.0004	D	13.59	-0.083	4.38
146	***NPHS1***	**19:36333089**	**19**	**c.G2600A: p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.000016**	**D**	**28.7**	**0.74**	**3.88**
	KANK1	9:710861	7	c.T95C: p.F32S	Heterozygous	NS	0.0006; 0.0004; 0.0023; 0.0003	P	12.73	0.092	3.58

150	***NPHS1***	**19:36321958**	**27**	**c.C3478T: p.R1160X**	**Homozygous**	**Tr***	**.; 0.000066; 0.0002; 0.00009943**	**.**	**37**	**0.251**	**3.74**
154	***OSGEP***	**14:20920566**	**2**	**c.A157T: p.I53F**	**Homozygous**	**NS**	**.; $2.47x10^{-5}$; 0.000182; $1.22x10^{-5}$**	**B**	**15.82**	**-0.4**	**0.619**
	ANLN	7:36459872	11	c.G1964A: p.R655Q	Heterozygous	NS	.; 0.000016; 0.0001; $7.96 x10^{-6}$	P	29.3	0.455	4.95
	MAGI2	7:77649189	22	c.G3811A: p.A1271T	Heterozygous	NS	0.0004; 0.0002; 0.0012; 0.0002	P	28.3	-0.075	4.59
157	***NPHS1***	**19:36333370**	**18**	**c.C2417A: p.A806D**	**Homozygous**	**NS**	**.; $8.24 x10^{-6}$; 0; $7.95 x10^{-6}$**	**D**	**23.7**	**0.374**	**4.46**
	INF2	14:105181022	21	c.G3523A: p.D1175N	Heterozygous	NS	.; 0.000017; 0; $8.15 x10^{-5}$	D	19.12	0.097	4.73
	NPHP4	1:5937173	20	c.C2797T:p.R933W	Heterozygous	NS	.; 0.0000416; 0; 0.00002938	P	16.48	-1.152	-9.61
162	***NPHS1***	**19:36333089**	**19**	**c.G2600A:p.G867D**	**Homozygous**	**NS**	**.; 0.000022; 0.0001; 0.000016**	**D**	**28.7**	**0.74**	**3.88**
	ALMS1	2:73747129	11	c.C9764G:p.S3255C	Heterozygous	NS	0.001; 0.0006; 0.0044; 0.0005	D	23.4	-0.174	3.76
	COL4A1	13:110817289	46	c.G4070C:p.G1357A	Heterozygous	NS	.; .; .; .	D	26	0.667	4.3
	ITGB4	17:73739874	26	c.C3043T:p.R1015C	Heterozygous	NS	.; 0.0001; 0.0007; 0.0001	D	28.9	0.186	2.94
	KANK1	9:742265	14	c.C3757T:p.L1253F	Heterozygous	NS	.; .; .; $3.98 x10^{-6}$	D	26.3	0.472	4.28
163	***NPHS1***	**19:36335272**	**15**	**c.C2020T:p.P674S**	**Homozygous**	**NS**	**.; .; .; .**	**D**	**27**	**0.728**	**3.67**
	KANK1	9:710853	7	c.87delC: p.D29fs	Heterozygous	fs*del	.; $8.4x10^{-6}$; $6.8 x10^{-5}$; $3.99 x10^{-6}$	.	.	.	.
	VPS33B	15:91561079	2	c.C133G: p.L45V	Heterozygous	NS	0.0002; 0.000058; 0.0004; 0.000043	D	25.7	0.668	5.45
165	***NPHS1***	**19:36339610**	**9**	**c.C1099T: p.R367C**	**Heterozygous**	**NS**	**.; 0.00003308; 0.0001; 0.00003978**	**D**	**28.7**	**0.528**	**4.37**
	NPHS1	**19:36341349**	**5**	**c.527-2A>G**	**Heterozygous**	**Sp**	**.; .; .; .**	**.**	**22.6**	**0.788**	**4.09**
	ALMS1	2:73680160	8	c.C6503T: p.S2168L	Heterozygous	NS	0.0002; 0.000075; 0.0005; 0.000060	D	25.2	0.094	3.48
168	***NPHS1***	**19:36330221**	**22**	**c.C3027G: p.Y1009X**	**Homozygous**	**Tr***	**.; $8.24 x10^{-6}$; $6.1 x10^{-5}$; $7.95 x10^{-6}$**	**.**	**38**	**0.513**	**1.06**
	ALMS1	2:737997787	17	c.A10771C: p.T3591P	Heterozygous	NS	0.0008; 0.0003; 0.0025; 0.0003	D	18.17	-0.413	-1.18
	ARHGAP24	4:86921681	10	c.G2053A: p.D685N	Heterozygous	NS	0.0008; 0.0009; 0.0064; 0.0009	P	29.3	0.295	5.56

	CRB2	chr9:126135651 Exon-10	10	c.2841delG: p.P947fs	Heterozygous	fs*del	.; .; .; .	.	.	.	.
169	***NPHS1***	**19:36321958**	**27**	**c.C3478T: p.R1160X**	**Homozygous**	**Tr***	**.; 0.000066; 0.0002; 0.000099**	**.**	**37**	**0.251**	**3.74**
	CD151	11:837277	6	c.A379C: p.K127Q	Heterozygous	NS	0.0002; 0.0004; 0.0031; 0.0004	B	15.64	-0.087	4.26
	TTC21B	2:166747104	24	c.C3148T: p.R1050W	Heterozygous	NS	.; .; .; 3**.99** x10^{-6}	D	34	0.891	4.76
173	***NPHS1***	**19:36321958**	**27**	**c.C3478T: p.R1160X**	**Heterozygous**	**Tr***	**.; 0.000066; 0.0002; 0.000099**	**.**	**37**	**0.251**	**3.74**
	NPHS1	**19:36333089**	**19**	**c.G2600A: p.G867D**	Heterozygous	NS	.; 0.000022; 0.0001; 0.000016	D	28.7	0.74	3.88
	ITGA3	17:48153013	12	c.C1588T: p.R530C	Heterozygous	NS	.; 0.00002481; 6.1x10^{-5}; 0.00002389	D	27.8	0.244	5.54
	SYNPO	5:150036540	3	c.G2603A: p.G868E	Heterozygous	NS	0.0002; 0.0004; 0.0019; 0.0001	D	17.24	0.444	4.91
180	***NPHS1***	**19:36337056**	**12**	**c.C1481A: p.S494X**	**Heterozygous**	**Tr***	**.; .; .; .**	**.**	**39**	**0.581**	**4.15**
	NPHS1	**19:36340541-36340548**	**6**	**c.614_621delinsTT: p.T205_A207delinsI**	**Heterozygous**	**fs*del**	.; **0.00001653**; **0.0001; 0.0000199**	**.**	**.**	**.**	**.**
	NPHP4	1:5937221	20	c.2749delG:p.E917fs	Heterozygous	fs*del	.; .; .; .	.	.	.	.
181	***NPHS1***	**19:36332715**	**20**	**c.T2717C:p.I906T**	**Homozygous**	**NS**	**.; .; .; .**	**D**	**26.8**	**0.821**	**4.78**
	FN1	2:216271099	19	c.C2848T:p.H950Y	Heterozygous	NS	.; 8.24x10^{-6}; 6.1x10^{-5}; 0.00003183	P	24.6	0.271	5.14
196	***NPHS1***	**19:36333089**	**19**	**c.G2600A:p.G867D**	**Homozygous**	**NS**	**.; 0.00002173; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	KANK2	19:11304341	4	c.G415T:p.A139S	Heterozygous	NS	.; .; .; .	D	22.6	0.161	4.38
	NPHP4	1:5947516	18	c.G2315A:p.R772H	Heterozygous	NS	.; 0.00001685; 0; 8.06x10^{-6}	D	33	0.754	5.46
201	***NPHS1***	**19:36333089**	19	**c.G2600A:p.G867D**	**Homozygous**	NS	**.; 0.00002173; 0.0001; 0.00001634**	**D**	**28.7**	**0.74**	**3.88**
	EXT1	8:119122904	1	c.G382T:p.A128S	Heterozygous	NS	.; 0.00001648; 0.0001; 0.0000159	B	12.59	-0.582	5.47
	LRP2	2:170068592	37	c.C6166T:p.R2056W	Heterozygous	NS	0.0004; 0.0001; 0.001; 0.0001	D	34	0.815	5.88
217	*ARHGDIA*	17:79826519	7	c.T736G:p.C246G	Heterozygous	NS	.; .; .; .	.	10.02	1.931	0.929
	FAT1	4:187527277	17	c.G10297A:p.V3433I	Heterozygous	NS	0.0006; 0.0002; 0.0014; 0.0002	B	19.37	-0.266	5.56
	ITGB4	17:73727328	10	c.G1094A:p.R365Q	Heterozygous	NS	.; 0.0001; 0.0006; 0.0001	P	24.3	-0.129	4.12

228	***NPHS1***	**19:36333089**	19	**c.G2600A:p.G867D**	**Heterozygous**	**NS**	**.; 0.00002173; 0.00001634**	**0.0001;**	**D**	**28.7**	**0.74**	**3.88**
	NPHS1	**19:36340541-36340548**	6	**c.614_621delinsTT; p.T205_A207delinsI**	**Heterozygous**	**fs*del**	**.; 0.00001653; 0.0000199**	**0.0001;**	**.**	**.**	**.**	**.**
	COQ6	14:74427966	9	c.G907A:p.A303T	Heterozygous	NS	0.0002; 0.0003; 0.0003	0.0023;	B	24.1	0.071	5.33
	COQ9	16:57490845	5	c.A524G:p.K175R	Heterozygous	NS	0.0002; 0.0001; 0.0001	0.0009;	P	23.6	0.464	5.68
	ITGB4	17:73745039	27	c.C3229T:p.R1077C	Heterozygous	NS	.; 0.00002598; 0.00003209	0;	D	32	0.584	4.93
235	***NPHS1***	**19:36333089**	19	**c.G2600A:p.G867D**	**Homozygous**	**NS**	**.; 0.00002173; 0.00001634**	**0.0001;**	**D**	**28.7**	**0.74**	**3.88**
	LAMB2	3:49162269	21	c.A2974G:p.I992V	Heterozygous	NS	0.0004; 0.0005; 0.0005	0.0038;	D	23.9	0.562	4.36
240	*WT1*	11:32450063	2	c.T749A:p.M250K	Heterozygous	NS	0.0002; 0.0003; 0.0002	0.0019;	B	24.4	0.09	5.62
266	No prioritized variations											
267	***NPHS1***	**19:36339610**	9	**c.C1099T: p.R367C**	**Heterozygous**	**NS**	**.; 0.000033; 0.0000398**	**0.0001;**	**D**	**28.7**	**0.528**	**4.37**
	NPHS1	**19:36341889**	4	**c.C500T: p.P167L**	**Heterozygous**	**NS**	**.; $8.3x10^{-6}$; $6.1x10^{-5}$; $3.9x10^{-6}$**		**D**	**27.6**	**0.555**	**5.99**
	FAT1	4:187540958	10	c.C6782T: p.T2261M	Heterozygous	NS	0.001; 0.0009; 0.0008	0.0033;	D	24.6	0.624	5.05
X1	***LAMB2***	3:49168499		**c.C799T; p.R267Ter**	**Homozygous**	**Tr**	**-, 0, 0, -**		**D**	**36**	**0.85**	**4.76**

B benign; D deleterious; del deletion; fs frameshift; NS non synonymous; P pathogenic; Tr truncating

Supplementary Table SV List of 44 single nucleotide polymorphisms (SNP), flanking p.Gly867Asp, selected for haplotype analysis

Haplotype region	*Chromosomal Coordinates*	*SNP ID*	*Reference allele A*	*Alternate; allele B*	*P-value**
5'-H2	chr19:35850672	rs142125121	T	C	0.0268
	chr19:35863180	rs201159994	A	G	0.0005
	chr19:35863226	rs150552589	G	T	2.2498E-08
5'-H1	chr19:35898796	rs112270905	T	TC	7.0269E-11
	chr19:35898899	rs16970294	A	G	7.0269E-11
	chr19:35899037	rs113510419	T	C	7.0269E-11
	chr19:35899068	rs142160831	GTGA	G	7.0269E-11
	chr19:35991373	rs2293695	C	T	0.025
	chr19:35998362	rs4254439	T	G	0.0459
	chr19:36004106	rs4806163	A	G	1.5754E-06
	chr19:36004171	rs12460932	C	A	0.0459
	chr19:36017928	rs10775583	G	C	0.0005
	chr19:36018272	rs12461911	C	T	0.0001
	chr19:36033460	rs2239945	C	T	0.0001
5'-H	chr19:36048741	rs2230181	G	T	0.0152
	chr19:36157740	rs61741212	C	T	0.0332
	chr19:36168914	rs2285421	T	C	2.0518E-05
	chr19:36218478	rs11670414	C	T	0.0047
	chr19:36224705	rs231591	A	G	5.3275E-08
	chr19:36233470	rs3746278	G	A	0.0113
	chr19:36234489	rs28656784	T	C	0.0332
	chr19:36235431	rs3761087	A	G	0.0256
	chr19:36236909	rs10402601	G	C	0.0256
	chr19:36246418	rs11549030	C	G	0.0429
	chr19:36269915	rs231230	T	C	0.0081
	chr19:36270052	rs231231	A	G	0.0081
	chr19:36273534	rs2291067	G	A	0.0035
	chr19:36275074	rs62112163	G	A	0.0035
	chr19:36278470	rs231235	C	G	0.0081

	chr19:36321910	rs731934	G	A	0.001
	chr19:36322270	rs2071327	C	T	0.0007
	chr19:36322509	rs466452	G	A	1.0815E-05
Mutation	chr19:36333089	G867D	C	T	3.53E-16
3'-H1	chr19:36351935	rs35854130	G	T	0.00006686
	chr19:36549684	rs61742664	G	A	0.015
3'-H2	chr19:36574063	rs45567532	C	T	7.6486E-08
	chr19:36577579	rs4806263	C	T	7.6486E-08
	chr19:36577742	rs77938609	G	A	0.015
	chr19:36583651	rs61494900	G	A	7.6486E-08
	chr19:36590329	rs2285745	T	C	0.0015
	chr19:36594063	rs17851502	C	T	7.6486E-08
	chr19:36595436	rs1008328	A	C	0.0044
	chr19:36603703	rs2072605	T	A	0.0005
	chr19:36674305	rs4805162	A	G	0.0066
	chr19:36727365	rs2070132	G	A	0.0013

Supplementary Table SVI Haplotype analysis of single nucleotide polymorphism markers flanking the mutation, indicating segregation of a core haplotype along with the Gly867Asp variant

No.	*~12 kbp region*	*~135 kbp region*	*Core-Haplotype (~500 kbp region)*			*~153 kbp region*	*Allele Count*
	rs142125121-rs150552589	*rs112270905-rs2239945*	*rs2230181-rs466452*		*rs35854130-rs61742664*	*rs45567532-rs2070132*	
	5'-H2	*5'-H1*	*5'-H*	***G867D***	*3'-H1*	*3'-H2*	
1	ABB	BBBBAAAABBB	AAAAAAAAAAAAAAAAAB	**B**	BA	BBABBBBBAB	13
2	ABB	BBBBAAAABBB	AAAAAAAAAAAAAAAAAB	**B**	BA	AAAABABBAB	1
3	AAA	BBBBAAAABBB	AAAAAAAAAAAAAAAAAB	**B**	BA	BBABBBBBAB	1
4	AAA	AAAABABABAA	AAAAAAAAAAAAAAAAAB	**B**	BA	AAAAAABABA	2
5	BAA	AAAAABAABBB	AAAAAAAAAAAAAAAAAB	A	BA	BBABBBBBAB	1
6	AAA	AAAABABABBB	AAAAAAAAAAAAAAAAAB	A	AA	AAAAAABAAA	1
7	BAA	AAAAAAAABBB	AAAAAAAAAAAAAAAAAB	A	AB	AABAAABAAB	1
	Other haplotypes						46

**Allele A refers to the major allele and B refers to the minor allele. In cases of Gly867Asp, B is a mutant allele. The grey shaded area refers to the mutant allele associated core haplotype*

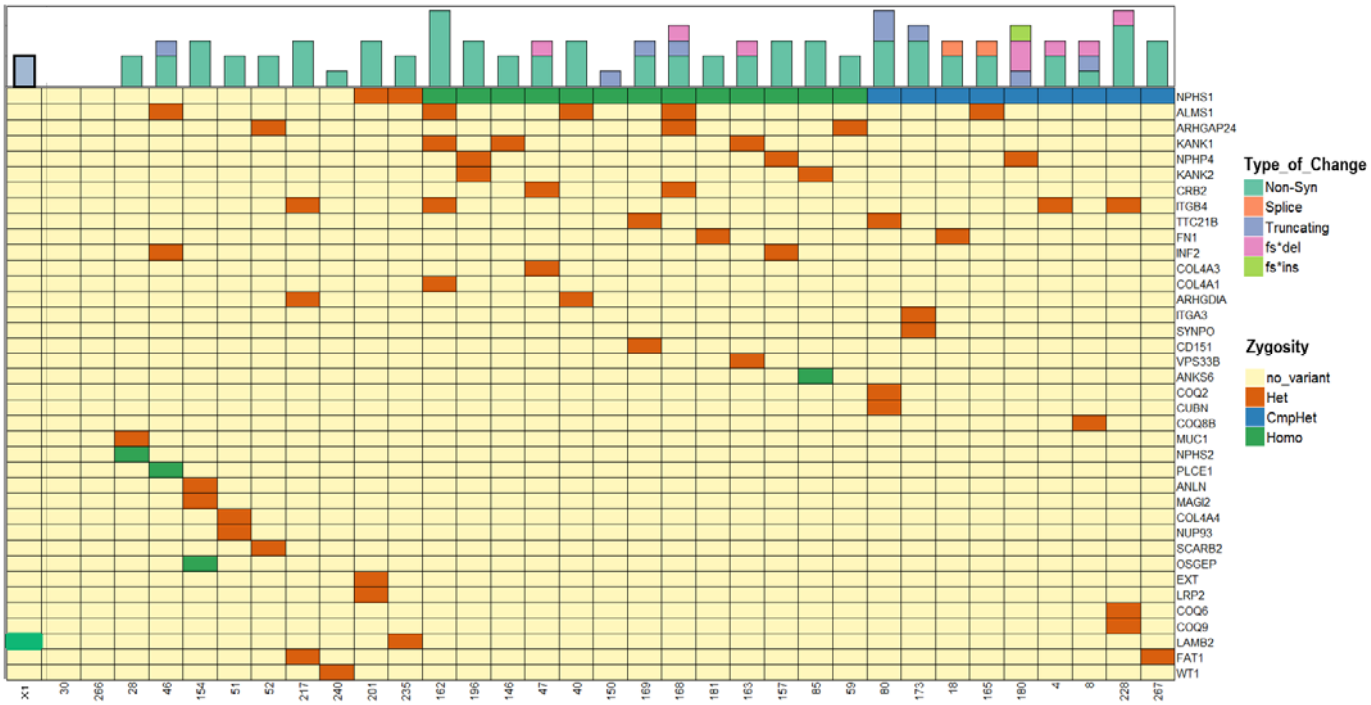
Supplementary SVII Studies examining the genetic basis of congenital nephrotic syndrome in 10 or more patients

Author, year	*N*	*Method of sequencing (number of genes)*	*Ethnicity*	*Etiology, %*						
				NPHS1	*NPHS2*	*WT1*	*PLCE1*	*LAMB2*	*Others*	*Unknown*
Koziell, 2002 [xi]	41	Sanger (2)	British, Maltese, Turkish, Asian	73	10	NT	NT	NT	NT	15
Sako, 2005 [xii]	13	Sanger (4)	Japanese	15	8	0	NT	NT	0 for *ACTN4*	77
Machuca, 2010 [xiii]	117	Sanger (8)	West Europe; Turkey; North Africa	61	15	2	2	2	0	19
Schoeb, 2010 [xiv]	67	Sanger (1)	Worldwide	58	Exc	Exc	NT	NT	NT	42
Buscher, 2010 [xv]	62	Sanger, panel (10)	German	53	13	23	2	5	2 (*ARGHDIA*)	3
Santin, 2011 [xvi]	15	Sanger (8)	Spanish	80	7	13	0	NT	NT	0
Lee, 2011 [xvii]	15	Not stated	Korean	40	7	40	0	7	0	7
Mbarek, 2011 [xviii]	12*	Linkage, Sanger (6)	Tunisian	60	0	0	0	40	0 for *CD2AP*	0
Cil, 2015 [xix]	80	Sanger (4)	Turkish; Middle East; East Europe	46	16	6	NT	4	NT	28
Sadowski, 2015 [xx]	235	Next-generation	Worldwide	40	11	9	2	6	3	31
Trautman, 2015 [xxi]	98	Sanger or next-generation	Europe, Middle East, Latin America	NA	NA	NA	NA	NA	NA	34
Sen, 2017 [xxii]	31	Next-generation	Worldwide	39	6	3	0	10	0	42
Wang, 2017 [xxiii]	12	Next-generation	Chinese	50	0	8	0	8	8 (*ADCK4*)	25
Li, 2018 [xxiv]	12	Sanger or next-generation	Chinese	67	0	8	0	0	8 (*COQ6*)	17
Sharief, 2019 [xxv]	11	Not stated	Arab, Asian, African	64	0	9	0	27	0	0
Nishi, 2019 [xxvi]	36	Not stated	Japanese	42	3	22	0	8	3 (*CRB2*)	22
Dufek, 2019 [xxvii]	69	Not stated	European	80	1	13	1	3	1 (*SGPL1*)	14
Berody, 2019 [xxviii]	55	Sanger (5)	European	65	9	7	2	0	0	16
Sinha, 2019 [xxix]	15	Next generation (27) or Sanger (<4)	Indian	53	0	7	7	0	0	33
Nagano, 2020 [xxx]	13	Targeted next generation	Japanese	31		15		31	8 (*LAMA5*)	15
Present study	34	Next-generation	Indian	74	4	0	4	4	4 (*OSGEP*)	11

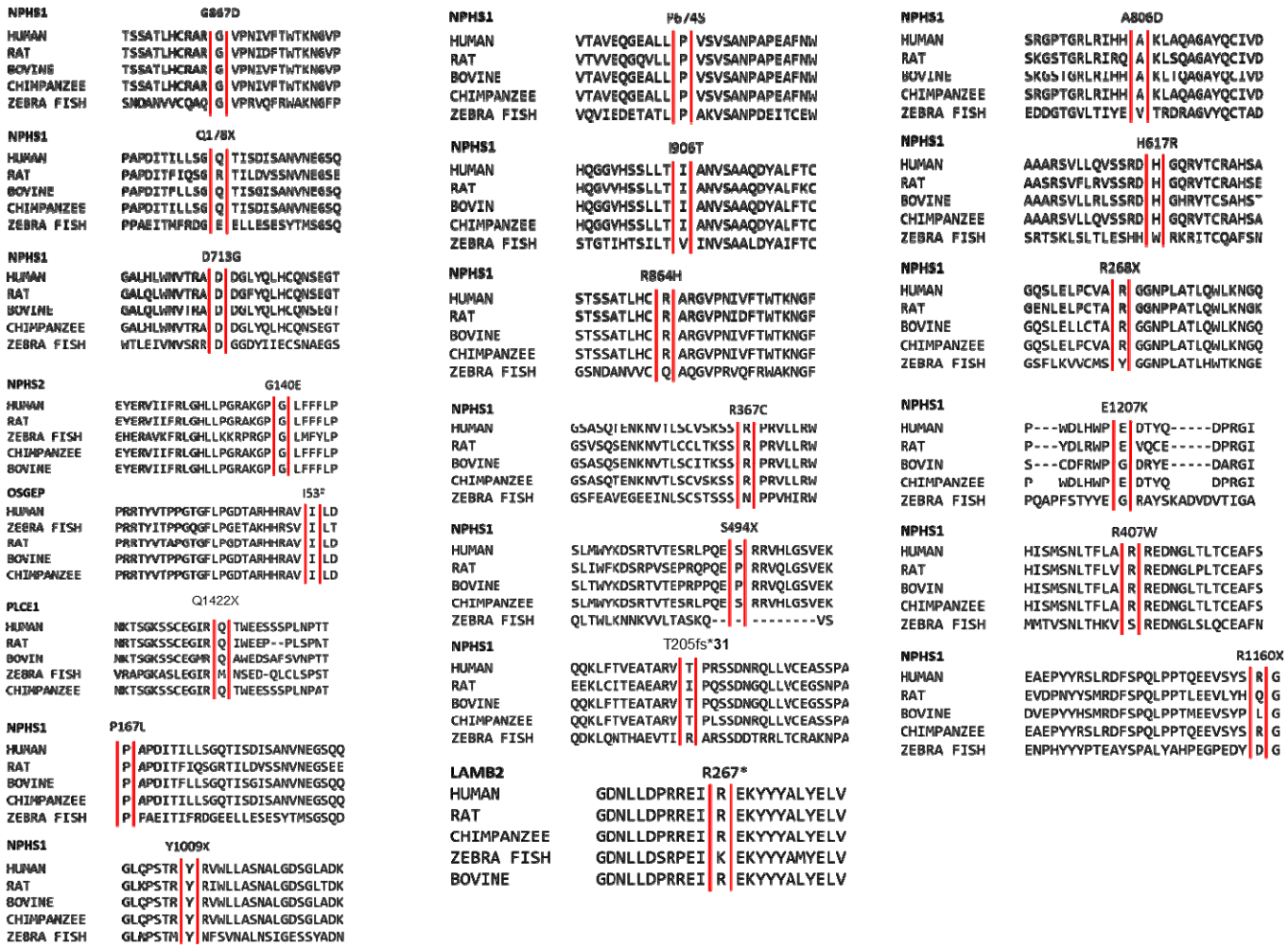
Exc excluded; NA not available; NT not tested
Only latest and largest paper for each group was included, unless overlap of patients between studies appeared unlikely
**Refers to 12 patients from 5 families*

Supplementary Table SVIII Frequency of haplotype markers of 5'-H1/5'H/3'-H1/3'-H2 region including Gly867Asp variant, (as indicated in Supplementary Table S6) from the1000 Genome population dataset

Sub-populations	*South Asian*	*European*	*East Asian*	*American*	*African*
Number of disease core haplotype carriers	2	20	1	7	1
Number of subjects	489	503	504	347	661
Frequency, %	0.20	1.98	0.09	1.008	0.07



Supplementary Figure S1 Heatmap representing prioritized variants per sample. Each column represents a patient while rows indicate genes. Individual cells are colored according to zygosity of variant while the type of change is indicated at the top of each column.



Supplementary Figure S2 Images indicating degree of conservation across species for variants to which pathogenicity was attributed

SUPPLEMENTARY REFERENCES

i Ha TS. Genetics of hereditary nephrotic syndrome: a clinical review. Korean J Pediatr 2017;60:55-63

ii Bierzynska A, McCarthy HJ, Soderquest K, et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. Kidney Int 2017;91:937-47

iii Sadowski CE, Lovric S, Ashraf S, et al; SRNS Study Group, Hildebrandt F. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol 2015;26:1279-89

iv Trautmann A, Lipska-Ziętkiewicz BS, Schaefer F. Exploring the clinical and genetic spectrum of steroid resistant nephrotic syndrome: The PodoNet registry. Front Pediatr 2018;6:200

v Lovric S, Fang H, Vega-Warner V, et al; Nephrotic Syndrome Study Group. Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2014;9:1109-16

vi 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. Nature 2015;526:68-74

vii Karczewski KJ, Weisburd B, Thomas B, et al; The Exome Aggregation Consortium, Daly MJ, MacArthur DG. The ExAC browser: Displaying reference data information from over 60 000 exomes. Nucleic Acids Res 2017;45 (D1):D840-45

viii Karczewski KJ, Francioli L, Tiao G, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. bioRxiv 2019;531210;doi: https://doi.org/10.1101/531210

ix Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2005;33:D514-7

x Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24

xi Koziell A, Grech V, Hussain S, et al. Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional inter-relationship in glomerular filtration. Hum Mol Genet 2002;11:379-88

xii Sako M, Nakanishi K, Obana M, et al. Analysis of NPHS1, NPHS2, ACTN4, and WT1 in Japanese patients with congenital nephrotic syndrome. Kidney Int 2005;67:1248-55

xiii Machuca E, Benoit G, Nevo F, et al. Genotype-phenotype correlations in non-Finnish congenital nephrotic syndrome. J Am Soc Nephrol 2010;21:1209-17

xiv Schoeb DS, Chernin G, Heeringa SF, et al; Gesselschaftfür Paediatrische Nephrologie Study Group. Nineteen novel NPHS1 mutations in a worldwide cohort of patients with congenital nephrotic syndrome (CNS). Nephrol Dial Transplant 2010;25:2970-6

xv Büscher AK, Beck BB, Melk A, et al. Rapid response to cyclosporin a and favorable renal outcome in nongenetic versus genetic steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2016;11:245-53

xvi Santín S, Bullich G, Tazón-Vega B, et al. Clinical utility of genetic testing in children and adults with steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2011;6:1139-48

xvii Lee JH, Han KH, Lee H, et al. Genetic basis of congenital and infantile nephrotic syndromes. Am J Kidney Dis 2011;58:1042-3

xviii Mbarek IB, Abroug S, Omezzine A, et al. Novel mutations in steroid-resistant nephrotic syndrome diagnosed in Tunisian children. Pediatr Nephrol 2011;26:241-9

xix Cil O, Besbas N, Duzova A, et al. Genetic abnormalities and prognosis in patients with congenital and infantile nephrotic syndrome. Pediatr Nephrol 2015;30:1279-87

xx Sadowski CE, Lovric S, Ashraf S, et al; SRNS Study Group, Hildebrandt F. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol 2015;26:1279-89

xxi Trautmann A, Bodria M, Ozaltin F, et al; PodoNet Consortium. Spectrum of steroid-resistant and congenital nephrotic syndrome in children: the PodoNet registry cohort. Clin J Am Soc Nephrol 2015;10:592-600

xxii Sen ES, Dean P, Yarram-Smith L, et al. Clinical genetic testing using a custom-designed steroid-resistant nephrotic syndrome gene panel: analysis and recommendations. J Med Genet 2017;54:795-804

xxiii Wang F, Zhang Y, Mao J, et al. Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. Pediatr Nephrol 2017;32:1181-92

xxiv Li GM, Cao Q, Shen Q, et al. Gene mutation analysis in 12 Chinese children with congenital nephrotic syndrome. BMC Nephrol 2018;19:382

xxv Sharief SN, Hefni NA, Alzahrani WA, et al. Genetics of congenital and infantile nephrotic syndrome. World J Pediatr 2019;15:198-203

xxvi Nishi K, Inoguchi T, Kamei K, et al. Detailed clinical manifestations at onset and prognosis of neonatal-onset Denys-Drash syndrome and congenital nephrotic syndrome of the Finnish type. Clin Exp Nephrol 2019;23:1058-65

xxvii Dufek S, Ylinen E, Trautmann A, et al; ESPN Dialysis Working Group. Infants with congenital nephrotic syndrome have comparable outcomes to infants with other renal diseases. Pediatr Nephrol 2019;34:649-55

xxviii Bérody S, Heidet L, Gribouval O, et al. Treatment and outcome of congenital nephrotic syndrome. Nephrol Dial Transplant. 2019;34:458-67

xxix Sinha R, Vasudevan A, Agarwal I, et al. Congenital nephrotic syndrome in India in the current era: A multicenter case series. Nephron 2019;144:1-9

xxx Nagano C, Yamamura T, Horinouchi T, et al. Comprehensive genetic diagnosis of Japanese patients with severe proteinuria. Sci Rep 2020;10;270