## Supplementary Methods

### Patient Cohort

302 patients were referred with informed consent for diagnostic gene panel analysis via Consultant Nephrologists and Consultant Clinical Geneticists over a 26-month period with clinical data supplied by clinical proforma. DNA was prepared from venous blood samples using an Autopure Gentra system (Qiagen) or referred as DNA from external laboratories.

### Assay Design, Target Enrichment and Sequencing

A custom HaloPlex Target Enrichment System (Agilent) was designed to target 37 genes (exons and 25bp of flanking intron) associated with SRNS. Genomic DNA (225ng) was processed for each sample according to the manufacturer’s protocol. Library QC was performed using an Agilent TapeStation 2200. Samples were pooled in typical batches of 12-16 and sequenced using 2x150bp paired end sequencing on a MiSeq (Illumina) analyser following manufacturer’s protocol. Twenty-two patients were sequenced on an earlier version of the panel comprising 16 genes as indicated in Table 1. After initial analysis, gap filling by Sanger sequencing was undertaken on the rare occasions where a gap in coverage was found in a clinically relevant gene or where a single LP variant was detected in a recessive gene.

### Bioinformatic Analysis

Analysis was performed using a bespoke pipeline based on the Broad Institutes’ Best Practice guidelines [1, 2]. FASTQ’s were hard trimmed to remove HaloPlex adapter sequences and read through, the trimmed reads were then mapped to UCSC GRCh37/hg19 FASTA reference using BWA-MEM. GATK (version 1.6) unified genotyper was used for indel realignment and variant calling, with quality, capture and alignment metrics generated using Picard. Pindel was used for additional long insertion/deletion and structural variant detection. Variants were annotated and stratified for analysis using Geneticist Assistant (SoftGenetics Version 1.1.5 Release Build 189 Revision 6848).

### Variant Classification

Variants were classified according to the Association for Clinical Genetic Science best practice guidelines for the evaluation of pathogenicity and reporting of sequence variants: Class 1 - clearly not pathogenic, Class 2 - unlikely to be pathogenic, Class 3 - unknown significance, Class 4 - likely to be pathogenic and Class 5 - clearly pathogenic [3]. Variants were assessed using Alamut software v2.3.1 (Interactive Biosoftware, Rouen, France). Classification considered literature evidence, disease mechanism and phenotype, evolutionary conservation including relevant functional domains and population frequency (NHLBI Exome Variant Server, dbSNP and ExAC [total allele frequency]) (Supplementary Table 1). Variants with a frequency >1% in any population were excluded from further investigation (with the exception of the *NPHS2* p.(Arg229Gln) variant). In addition, web-based prediction tools PolyPhen-2, Align GVGD and SIFT were used for the assessment of missense variants and splice site variants were investigated with prediction programs SpliceSiteFinder, MaxEntScan, Human Splice Finder, NNSPLICE and GeneSplicer. Class 3, 4 and 5 variants were confirmed by Sanger sequencing (BigDye Terminator v3.1 Cycle Sequencing Kit and ABI 3730 Applied Biosystems).

### Variant Segregation Analysis

Analysis of parental samples and other available affected/unaffected relatives was undertaken where possible using Sanger sequencing to determine phase (*cis* or *trans*)and to gather evidence supporting pathogenicity by genotype/phenotype concordance in the family.

### Copy Number Analysis

Copy number variants (CNVs) were identified by CONTRA using log-ratios of GC corrected, library balanced, binned and interpolated read depth data [4]. CNVs were confirmed using Multiplex Ligation-dependent Probe Amplification (MLPA) with custom designed probes and the MRC-Holland P200-A1 Human DNA Reference-1 probe mix following the manufacturer’s protocol.

**Supplementary Table 1: Variant classification criteria**

|  |  |
| --- | --- |
| **Class 5**:Pathogenic | Reported in the literature as pathogenic supported by functional evidence **OR** segregation studies **OR** multiple independent case reports **AND**Consistent with phenotype of patient, inheritance and disease mechanism  |
| **Class 4**: Likely pathogenic | Minor Allele Frequency (MAF) <1%\* **AND**Not reported or literature evidence sparse**,** with no segregation studies or functional analysis available **AND** Consistent with phenotype of patient, inheritance and disease mechanism ***AND**** Missense variant in functional domain with high conservation and supporting *in silico* results (see supplementary methods) **OR**
* Nonsense or frame shift variant **OR**
* Invariant splice site (+/-2) variant or highly conserved synonymous variant with >3/5 *in silico* splice prediction tools returning a >10% difference in splice site prediction value between reference sequence and variant.
 |
| **Class 3**: Unknown Significance (VUS) | MAF <1% **AND*** Inconclusive or conflicting *in silico* results, not reported in the literature, but consistent with phenotype, inheritance and disease mechanism **OR**
* *In silico* predictions class the variant as Class 4, but not consistent with phenotype of patient
 |
| **Class 2:** Likely Benign | MAF <1% **AND*** *In silico* results indicate weak amino acid conservation and a benign impact on protein **OR**
* Synonymous or intronic change in a weakly conserved nucleotide with no *in silico* effect on splicing **OR**
* Inconclusive or conflicting *in silico* results, not reported in the literature, not consistent with phenotype of patient, inheritance, or disease mechanism **OR**
* Sparse literature evidence indicating benign status **OR**
* Limited segregation studies not supporting pathogenicity
 |
| **Class 1:**  Benign | MAF >1% **OR**MAF<1% and proven as non-pathogenic in published literature |
| \* <1% MAF in any population in EVS, dbSNP, 1000 genomes and ExAC databases; exception of non-neutral polymorphism *NPHS2*, c.686G>A p.(Arg229Gln). |

**Supplementary Table 2: Genotypes and phenotypes of patients with likely-pathogenic variants**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient number** | **Sex** | **Age at onset (years)** | **Presentation** | **Ethnicity; FH; Consanguinity** | **Biopsy** | **Clinical impact** | **Pathogenicity** | **Gene** | **Nucleotide; segregation** | **AA** | **Reference** † | **Mutation prediction: SIFT; PolyPhen** | **Allele frequency: dbSNP; EVS; ExAC** |
| 1 | M | 6\* | SRNS | ND; ND; ND | FSGS | ND | LP | ***ACTN4*** | c.776C>A | p.(Thr259Asn) | PS | Del; 1.00 | NL; NL; NL |
| 2 | F | 9 | Alport | W; Y; N | Alport | ND | LP | ***COL4A3*** | c.663\_664delAG | p.(Arg221Serfs\*5)  | [5]  |  |  |
|  |  |  |  |  |  |  | LP | ***COL4A3*** | c.3472G>C | p.(Gly1158Arg) | [5] |  |  |
| 3 | F | 16\* | Haematuria; proteinuria, ESRF; hearing loss | ND; Y; ND | ND | ND | LP | ***COL4A3*** | c.663\_664delAG | p.(Arg221Serfs\*5) | [5]  |  |  |
|  |  |  |  |  |  |  | LP | ***COL4A3*** | c.1985G>A | p.(Gly662Glu) | PS | Del; 1.00 | NL; NL; NL |
| 4 | F | 25\* | SRNS | ND; Y; ND | FSGS | ND | LP | ***COL4A3*** | c.698G>A; tracks with disease: affected brother | p.(Gly233Glu) | PS | Del; 0.999 | NL; NL; NL |
| 5 | M | 5 | Haematuria | ND; ND; ND | Alport | ND | LP | ***COL4A3*** | c.898G>A | p.(Gly300Arg) | [6] |  |  |
|  |  |  |  |  |  |  | LP | ***COL4A3*** | c.898G>A | p.(Gly300Arg) | [6] |  |  |
| 6 | F | 20 | Haematuria, proteinuria, TBMN | W; N; N | TBMN | ND | LP | ***COL4A3*** | c.2083G>A; pat | p.(Gly695Arg) | [7, 8]  |  |  |
|  |  |  |  |  |  |  | VUS | ***COL4A3*** | c.4981C>T; carried by unaffected son | p.(Arg1661Cys) | [9]  |  |  |
| 7 | M | 44\* | Alport | ND; ND; ND | ND | ND | LP | ***COL4A3*** | c.2452G>A; mat; tracks with disease: 4 affected, 2 unaffected | p.(Gly818Arg) | [5]  |  |  |
|  |  |  |  |  |  |  | VUS | *COL4A4* | c.4760C>G; mat; tracks with disease: 4 affected, 1 unaffected | p.(Pro1587Arg) | [8]  |  |  |
| 8 | M | 17\* | SRNS | S; Y; ND | FSGS | ND | LP | ***COL4A4*** | c.1598G>A | p.(Gly533Asp) | [5] |  |  |
|  |  |  |  |  |  |  | LP | ***COL4A4*** | c.1598G>A | p.(Gly533Asp) | [5]  |  |  |
| 9 | F | 78\* | Hypertensive nephrosclerosis, ESRF | ND; Y; ND | ND | ND | LP | ***COL4A4*** | c.2906C>G | p.(Ser969\*) | [5]  |  |  |
| 10 | F | 36\* | Alport | ND; ND; ND | ND | ND | LP | ***COL4A4*** | c.3052G>C; mat; tracks with disease: affected mother and mat aunt | p.(Gly1018Arg) | PS | Del; 1.00 | NL; NL; 0.00083% |
|  |  |  |  |  |  |  | VUS | *WT1* | c.541C>T; pat unaffected | p.(Pro181Ser)pat | [10]  |  |  |
|  |  |  |  |  |  |  | VUS | *WT1* | c.328G>T; pat unaffected | p.(Gly110Trp) | PS | Del, 0.00 | NL; NL; NL |
| 11 | F | 56\* | Haematuria, deafness, Alport | W; N; N | ND | ND | LP | ***COL4A4*** | c.4538G>A | p.(Cys1513Tyr)  | [5]  |  |  |
| 12 | F | 25\* | Haematuria | W; Y; ND | ND | ND | LP | ***COL4A5*** | c.367G>A | p.(Gly123Arg) | [11] |  |  |
| 13 | M | 18 | SRNS, bilateral undescended testes, FH severe adult-onset deafness | W; N; N | Not done | ND | LP | ***COL4A5*** | c.546+1G>T | p.(?) | PS | n/a; n/a | NL; NL; NL |
|  |  |  |  |  |  |  | VUS | *MYH9*  | c.2507C>T | p.(Pro836Leu) | [12] |  |  |
| 14 | M | 42\* | Haematuria, proteinuria, hearing loss | ND; ND; ND | ND | ND | LP | ***COL4A5*** | c.556G>A | p.(Gly186Ser) | PS | Del; 0.999 | NL; NL; NL |
| 15 | F | 34\* | Alport | ND; Y; ND | ND | ND | LP | ***COL4A5*** | c.1190G>T | p.(Gly397Val) | PS | Del; 1.00 | NL; NL; NL |
| 16 | F | 38\* | SRNS | ND; Y; ND | FSGS | ND | LP | ***COL4A5*** | c.1423G>A; c.4567C>A | p.(Gly475Ser); p.(Pro1523Thr) | [13, 14]  |  |  |
| 17 | M | 9 | Alport | BA; Y; N | Alport | ND | LP | ***COL4A5*** | c.1807G>T | p.(Gly603Cys) | PS | Del; 1.00 | NL; NL; NL |
| 18 | M | 46\* | Alport | ND; ND; ND | Alport | ND | LP | ***COL4A5*** | c.1826G>C; absent in mother (Haematuria detected opportunistically) and unaffected sister | p.(Gly609Ala) | PS | Del; 0.999 | NL; NL; NL |
| 19 | F | 3 | Alport | W; N; N | Alport | Imm not started | LP | ***COL4A5*** | c.1835G>T;neither parent | p.(Gly612Val) | PS. p.(Gly612Asp) [15]  | Del; 1.00 | NL; NL; NL |
| 20 | F | 22\* | Haematuria, proteinuria, hearing loss | ND; N; ND | Alport | ND | LP | ***COL4A5*** | c.3270C>G | p.(Tyr1090\*) | PS | n/a; n/a | NL; NL; NL |
| 21 | M | 28 | Alport | W; Y; N | FSGS | ND | LP | ***COL4A5*** | c.3319G>A; mat | p.(Gly1107Arg) | [16]  |  |  |
| 22 | F | 11 | SRNS | W; ND; N | FSGS | ND | LP | ***COL4A5*** | c.4015+2T>C  | p.(?) | PS | n/a; n/a | NL; NL; NL |
| 23 | F | 15 | Haematuria, proteinuria | Brazilian; Y; N | Not done | Avoid biopsy | LP | ***COL4A5*** | c.4415\_4416delinsCT | p.(Arg1472Pro) | PS | Del; 0.999 | NL; NL; NL |
| 24 | M | 0.5 | Haematuria, hearing loss | ND; Y; ND | Alport | ND | LP | ***COL4A5*** | c.4480delT; mat | p.(Ser1494Leufs\*60) | PS | n/a; n/a | NL; NL; NL |
| 25 | M | 38\* | CMT + NS | ND; Y; ND | FSGS | ND | LP | ***INF2*** | c.148T>G; tracks with disease: 5 affected, 1 unaffected | p.(Tyr50Asp) | PS | Del; 0.999 | NL; NL; NL |
|  |  |  |  |  |  |  | VUS | *COL4A4* | c.778G>A; does not track with disease: 5 affected | p.(Val260Ile) | PS | Del; 0.007 | NL; NL; 0.0042% |
| 26 | M | 24 | SRNS | W; N; N | FSGS | Imm not started | LP | ***INF2*** | c.494T>G; detected in affected son; absent in unaffected son and father | p.(Leu165Arg)  | PS. p.(Leu165Pro) [17] | Del; 0.999 | NL; NL; NL |
| 27 | F | 25 | SRNS, CKD | W; N; N | FSGS | No | LP | ***INF2*** | c.640C>T; pat; detected in affected father | p.(Arg214Cys)  | [17-19]  |  |  |
| 28 | M | 0.1 | CNS, Pierson syndrome | ND; ND; ND | Not done | Palliative care | LP | ***LAMB2*** | c.825T>A; mat | p.(Tyr275\*) | [20]  |  |  |
|  |  |  |  |  |  |  | LP | ***LAMB2*** | c.825T>A; pat | p.(Tyr275\*) | [20]  |  |  |
| 29 | M | 0 | CNS | W; N; N | ND | Antenatal testing in subsequent pregnancy | LP | ***LAMB2*** | c.1477delT; mat | p.(Cys493Alafs\*4) | [20, 21]  |  |  |
|  |  |  |  |  |  |  | LP | ***LAMB2*** | c.3523delC; pat | p.(Gln1175Serfs\*37) | PS | n/a; n/a | NL; NL; NL |
| 30 | M | 0 | CNS, died at 1 week | Ir; Y; Y | Not done | ND | LP | ***LAMB2*** | c.1814delG | p.(Gly605Valfs\*23) | PS | n/a; n/a | NL; NL; NL |
|  |  |  |  |  |  |  | LP | ***LAMB2*** | c.1814delG | p.(Gly605Valfs\*23) | PS | n/a; n/a | NL; NL; NL |
| 31 | F | 18 | SRNS | Pa; N; N | Tubulointerstitial disease | ND | LP | ***LMX1B*** | c.668G>A; neither parent | p.(Arg223Gln) | [22] |  |  |
| 32 | F | 17 | SRNS | W; N; N | FSGS | Imm not started | LP | ***LMX1B*** | c.737G>A; absent in unaffected father and brother | p.(Arg246Gln) | [23, 24]  |  |  |
| 33 | F | 14 | SRNS | W; Y; N | FSGS | ND | LP | ***LMX1B*** | c.737G>A | p.(Arg246Gln) | [23, 24]  |  |  |
| 34 | M | 2 | SRNS | ME; N; N | FSGS | Cessation of Imm | LP | ***LMX1B*** | c.737G>A | p.(Arg246Gln) | [23, 24]  |  |  |
| 35 | F | 13\* | SRNS, thrombocytopenia | ND; ND; ND | ND | ND | LP | ***MYH9*** | c.287C>T | p.(Ser96Leu) | [25, 26]  |  |  |
| 36 | F | 10\* | NS, thrombocytopenia, FH of same | ND; Y; ND | ND | ND | LP | ***MYH9*** | c.2152C>T | p.(Arg718Trp) | [27, 28]  |  |  |
| 37 | M | 0 | SRNS, hypomagnesaemia with secondary hypocalcaemia, focal seizures, hypothyroidism, Arnold-Chiari malformation | In; N; N | Finnish type | ND | LP | ***NPHS1*** | c.58+1G>T ; c.2600G>A; mat | p.(?) | [29]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.320C>T; pat | p.(Ala107Val) | [29]  |  |  |
| 38 | M | 0.1 | CNS, hypomagnesaemic seizures | Ban; N; Y | Not done | Imm not started | LP | ***NPHS1*** | c.320C>T; mat | p.(Ala107Val) | [29]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.320C>T; pat | p.(Ala107Val) | [29]  |  |  |
| 39 | F | 0 | CNS, IUGR | ND; N; ND | ND | ND | LP | ***NPHS1*** | c.325T>C; mat | p.(Tyr109His)  | PS | Del; 0.993 | NL; NL; 0.00087%  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.1868G>T; pat | p.(Cys623Phe) | [30]  |  |  |
| 40 | M | 0.1 | CNS, psychomotor delay | W; N; N | Not done | Imm not started | LP | ***NPHS1*** | c.325T>C; pat | p.(Tyr109His)  | PS | Del; 0.993 | NL; NL; 0.00087% |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.3442C>T; mat | p.(Gln1148\*)  | [31]  |  |  |
| 41 | M | 0 | CNS, deafness | W; N; N | FSGS | ND | LP | ***NPHS1*** | c.532C>T; mat | p.(Gln178\*) | [32] |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.1243dupC; pat | p.(Leu415Profs\*4) | PS | n/a; n/a | NL; NL; NL |
| 42 | M | 0 | CNS, placentomegaly and hepatosplenomegaly and single palmar creases | Filipino; N; ND | ND | ND | LP | ***NPHS1*** | c.565G>T | p.(Glu189\*) | [33]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.1379G>A | p.(Arg460Gln) | [32]  |  |  |
| 43 | F | 0 | CNS | W; N; N | Not done | ND | LP | ***NPHS1*** | c.736G>T | p.(Glu246\*) | [34]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.1868G>T | p.(Cys623Phe) | [30]  |  |  |
| 44 | F | 0 | CNS | W; N; N | Not done | ND | LP | ***NPHS1*** | c.866G>A; pat | p.(Trp289\*) | [29]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | Exon 23-29del; mat | p.(?) | PS | n/a; n/a | NL; NL; NL |
| 45 | F | 0.4\* | CNS | ND; ND; ND | DMS | ND | LP | ***NPHS1*** | c.1235delG | p.(Gly412Valfs\*2) | PS | n/a; n/a | NL; NL; NL |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.3481+1G>T | p.(?) | [29]  |  |  |
| 46 | M | 2\* | SRNS | ND; ND; ND | ND | ND | LP | ***NPHS1*** | c.1868G>T  | p.(Cys623Phe)  | [30]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.2335-1G>A | p.(?) | [35]  |  |  |
| 47 | M | 0 | CNS | W; N; N | Other | Assess risk of recurrent FSGS after transplant | LP | ***NPHS1*** | c.2227C>T; pat | p.(Arg743Cys) | [35-37]  |  |  |
|  |  |  |  |  |  |  | LP | *NPHS1* | c.2309C>T; c.2335-1G>A both mat | p.(Pro770Leu); p.(?) | PS; [35] | Del; 0.824 | rs115976159; NL; 0.0099% |
| 48 | F | 0.1 | CNS | W; N; N | Finnish type | Imm not started | LP | ***NPHS1*** | c.2335-1G>A; mat | p.(?) | [35]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS1*** | c.2335-1G>A; pat | p.(?) | [35]  |  |  |
| 49 | F | 11\* | SRNS | W; Y; N | ND | ND | LP | ***NPHS2*** | c.413G>A | p.(Arg138Gln) | [38]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS2*** | c.378+1\_378+2delinsTG  | p.(?) | PS | n/a; n/a | NL; NL; NL |
| 50 | M | 13 | SRNS | W; N; N | FSGS | ND | LP | ***NPHS2*** | c.413G>A | p.(Arg138Gln) | [38]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS2*** | c.868G>A | p.(Val290Met) | [39]  |  |  |
| 51 | M | 0.1 | CNS, ESRF, died at 2y | In; N; N | FSGS | Genetic testing done post-mortem | LP | ***NPHS2*** | c.419G>A; mat | p.(Gly140Glu) | PS | Del; 1.00 | NL; NL; 0.00082% |
|  |  |  |  |  |  |  | LP | ***NPHS2*** | c.419G>A; pat | p.(Gly140Glu) | PS | Del; 1.00 | NL; NL; 0.00082% |
| 52 | F | 3.5 | SRNS | Mix Afg/In; Y; Y | FSGS | Cessation of Imm | LP | ***NPHS2*** | c.562G>T; mat | p.(Glu188\*) | [40]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS2*** | c.562G>T; pat | p.(Glu188\*) | [40]  |  |  |
| 53 | M | 12\* | SRNS | ND; ND; ND | ND | ND | LP | ***NPHS2*** | c.871C>T | p.(Arg291Trp) | [41]  |  |  |
|  |  |  |  |  |  |  | LP | *NPHS2* | c.686G>A | p.(Arg229Gln) | [42, 43]  |  |  |
| 54 | F | 11 | SRNS | W; N; N | FSGS | Imm not started | LP | ***NPHS2*** | c.890C>T; pat | p.(Ala297Val)  | [44, 45]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS2*** | c.686G>A; mat | p.(Arg229Gln) | [42, 43]  |  |  |
|  |  |  |  |  |  |  | VUS | *COL4A4*  | c.232C>T  | p.(Pro78Ser)  | PS | Del; 0.008 | NL; NL; 0.00083% |
| 55 | M | 0.9\* | CNS, severe pulmonary valve stenosis | W; N; N | ND | ND | LP | ***NPHS2*** | c.1032delT; pat | p.(Phe344Leufs\*4) | [44]  |  |  |
|  |  |  |  |  |  |  | LP | ***NPHS2*** | Exon 2 del; mat | p.(?) | PS | n/a; n/a | NL; NL; NL |
| 56 | M | 2.5 | SRNS | K; Y; Y | FSGS | Imm not continued. Assess risk of recurrent FSGS after transplant | LP | ***PLCe1*** | c.1477C>T; mat | p.(Arg493\*) | [46, 47]  |  |  |
|  |  |  |  |  |  |  | LP | ***PLCe1*** | c.1477C>T; pat | p.(Arg493\*) | [46, 47]  |  |  |
|  |  |  |  |  |  |  | VUS | *APOL1* | c.558delA; pat | p.(Gly187Alafs\*19) | PS | n/a; n/a | NL; NL; 0.0041% |
| 57 | F | 2.8 | SRNS | Afg; N; N | FSGS | ND | LP | ***PLCe1*** | c.1477C>T | p.(Arg493\*) | [46, 47]  |  |  |
|  |  |  |  |  |  |  | LP | ***PLCe1*** | c.1477C>T | p.(Arg493\*) | [46, 47]  |  |  |
|  |  |  |  |  |  |  | VUS | *CD2AP*  | c.1511G>A | p.(Arg504His) | PS | Tol; 0.952 | NL; NL; 0.00249% |
| 58 | F | 22 | SRNS, tremor & ataxia, suspected AMRF | W; N; N | FSGS | Planned Imm not started until genetic test known | LP | ***SCARB2*** | c.434\_435dup | p.(Trp146Serfs\*16) | [48, 49]  |  |  |
|  |  |  |  |  |  |  | LP | ***SCARB2*** | c.704+5G>A  | p.(?) | [50]  |  |  |
| 59 | M | 7 | SRNS, short stature, mild central hypoventilation | W; N; N | FSGS | ND | LP | ***SMARCAL1*** | c.415\_416delTT | p.(Leu139Glufs\*3) | PS  | n/a; n/a | NL; 0.01%; 0.0016% |
|  |  |  |  |  |  |  | LP | ***SMARCAL1*** | c.2114C>T | p.(Thr705Ile)  | [51]  |  |  |
|  |  |  |  |  |  |  | VUS | *ALMS1* | c.11449C>T | p.(Gln3817\*) | [52]  |  |  |
|  |  |  |  |  |  |  | Carrier | *PMM2* | c.422G>A | p.(Arg141His) | [53]  |  |  |
| 60 | M | 39 | SRNS, unilateral blindness | W; ND; N | FSGS | ND | LP | ***TRPC6*** | c.2690A>C | p.(Glu897Ala) | PS. p.(Glu897Lys) [54]  | Del; 0.995 | NL; NL; NL |
| 61 | M | 37\* | SRNS | W; Y; ND | FSGS | ND | LP | ***WT1*** | c.1016A>G; tracks with disease: 6 affected (tested elsewhere, personal communication) | p.(His339Arg) | PS | Del; 0.991 | NL; NL; NL |
| 62 | M | 11 | SRNS, undescended testes | W; N; N | FSGS | Imm not started | LP | *WT1* | c.1091T>G | p.(Phe364Cys)  | PS | Del; 0.89 | NL; NL; NL |
| 63 | F | 2 | SRNS, seizures at 6m | W; N; ND | Not done | ND | LP | ***WT1*** | c.1097G>A | p.(Arg366His) | [55]  |  |  |
| 64 | M | 3\* | SRNS, undescended testes, penile anomaly | ND; ND; ND | ND | ND | LP | ***WT1*** | c.1133C>T; absent in both unaffected parents | p.(Thr378Ile) | [56]  |  |  |
|  |  |  |  |  |  |  | LP | ***COL4A3*** | c.2452G>A; pat unaffected | p.(Gly818Arg) | [5]  |  |  |
| 65 | F | 30 | Nephrotic in pregnancy | W; Y; N | FSGS | No planned Imm | LP | ***WT1*** | c.1169G>A; pat affected | p.(Arg390Gln) | PS. p.(Arg390\*) [57]  | Del; 0.999 | NL; NL; NL |
|  |  |  |  |  |  |  | VUS | *COL4A4* | c.4334-3C>T; pat | p.(?) | PS | n/a; n/a | NL; NL; NL |
| 66 | F | 0.6 | Proteinuria, ESRF | ND; ND; ND | Multisegmental sclerosing lesions | Already in ESRF at presentation | LP | ***WT1*** | c.1180C>T; absent in both unaffected parents | p.(Arg394Trp) | [55, 58] |  |  |
|  |  |  |  |  |  |  | VUS | *LAMB2* | c.4331G>A; mat | p.(Gly1444Glu) | PS | Del; 0.99 | NL; NL; NL |
| 67 | F | 1 | SRNS | ND; ND; ND | ND | ND | LP | ***WT1*** | c.1180C>T | p.(Arg394Trp) | [55, 58]  |  |  |
| 68 | M | 0.3\* | CNS | ND; ND; ND | ND | ND | LP | ***WT1*** | c.1181G>A; absent in both unaffected parents | p.(Arg394Gln) | [59, 60] |  |  |
| 69 | M | 3.3 | SRNS, ADHD | M; N; Y | DMS | Imm not started | LP | ***WT1*** | c.1228+5G>A; absent in both unaffected parents | p.(?) | [56, 61] |  |  |
| 70 | F | 3 | SRNS | W; N; N | FSGS | ND | LP | ***WT1*** | c.1228+5G>A; absent in both unaffected parents | p.(?) | [56, 61] |  |  |
| 71 | F | 2.5 | SRNS | W; N; N | FSGS | ND | LP | ***WT1*** | c.1228+5G>A; absent in both unaffected parents | p.(?) | [56, 61] |  |  |

\* Denotes age at genetic testing where age at disease onset was not available. † All references in this table are included as Supplementary Material.

Genes in bold type are the main causative gene in that patient.

**Legend:**

AA, amino acid; ADHD, attention-deficit hyperactivity disorder; Afg, Afghanistani; AMRF, action myoclonus renal failure syndrome; BA, Black African; Ban, Bangladeshi; CKD, chronic kidney disease; CMT, Charcot Marie Tooth disease; CNS, congenital nephrotic syndrome; Del, deleterious; DMS, diffuse mesangial sclerosis; ESRF, end stage renal failure; FH, family history; FSGS, focal segmental glomerulosclerosis; Imm, immunosuppression; In, Indian; Ir, Iranian; IUGR, intra-uterine growth restriction; K, Kurdish; m, months; LP, likely-pathogenic; M, Morrocan; mat, maternal; MCD, minimal change disease; ME, Middle Eastern; N, no; n/a, not available; ND, not done/no data; NL, not listed; NNP, non-neutral polymorphism; NS, nephrotic syndrome; Pa, Pakistani; pat, paternal; PS, present study; S, Slovakian; SRNS, steroid resistant nephrotic syndrome; TBMN, thin basement membrane nephropathy; Tol, tolerated; VUS, variant of unknown significance; W, White; y , years; Y, yes

**Supplementary Table 3: Patients with variants of unknown significance**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient number** | **Sex** | **Age at onset (years)** | **Presentation** | **Ethnicity; FH; Consanguinity** | **Biopsy** | **Clinical impact** | **Gene** | **Nucleotide; segregation** | **AA** |
| 72 | F | 53\* | SRNS | ND; ND; ND | ND | ND | *ACTN4* | c.2084G>A | p.(Arg695His) |
| 73 | M | 2 | SRNS | In; N; N | MCD | No change | *ACTN4* | c.2629G>A | p.(Glu877Lys) |
|  |  |  |  |  |  |  | *PTPRO* | c.2117G>A | p.(Cys706Tyr) |
| 74 | M | 13\* | Haematuria, familial microscopic haematuria | ND; Y; ND | ND | ND | *ALG1* | c.1127C>T | p.(Pro376Leu) |
|  |  |  |  |  |  |  | *ALG1* | c.1187+13C>A | p.(?) |
| 75 | M | 13 | NS, SCD | ND; ND; ND | ND | ND | *CD2AP* | c.1637C>T | p.(Ser546Phe) |
| 76 | F | 44\* | SRNS | ND; Y; ND | FSGS | ND | *COL4A3* | c.1855G>A | p.(Gly619Arg) |
| 77 | M | 30 | SRNS | W; N; N | FSGS | No change | *COL4A3* | c.2155T>C | p.(Ser719Pro) |
|  |  |  |  |  |  |  | *COL4A3* | c.4664C>T | p.(Ala1555Val) |
| 78 | M | 19 | Alport, FH haematuria and hearing loss | W; Y; N | Other | ND | *COL4A3* | c.2313\_2330del;mat; unaffected | p.(Leu775\_Gly780del) |
| 79 | M | 15\* | SRNS | ND; ND; ND | ND | ND | *COL4A4* | c.136C>A | p.(Pro46Thr) |
| 80 | M | 8\* | SRNS | ND; ND; ND | ND | ND | *COL4A4* | c.809G>A | p.(Gly270Glu) |
| 81 | M | 17\* | Haematuria and proteinuria | W; Y; ND | Alport | ND | *COL4A4* | c.4291C>T | p.(Arg1431Cys) |
| 82 | M | 49 | SRNS | Iraqi; Y; N | FSGS, laminopathy | Imm not started | *COL4A4* | c.4576A>G | p.(Asn1526Asp) |
|  |  |  |  |  |  |  | *COL4A4* | c.4810-15\_4810-14delTT | p.(?) |
| 83 | F | 53\* | Haematuria | W; Y; N | TBMN | Imm not started | *COL4A5* | c.466-3T>A | p.(?) |
|  |  |  |  |  |  |  | *COL4A5* | c.3285T>C | p.(=) |
| 84 | M | 32\* | SRNS | ND; Y; Y | ND | ND | *COL4A5* | c.2017A>G | p.(Arg673Gly) |
|  |  |  |  |  |  |  | *MYO1E* | c.3236A>G | p.(Asp1079Gly) |
| 85 | M | 24 | SRNS | W; Y; N | FSGS | Cessation | *COL4A5* | c.2326G>A; mat, tracks with disease in mother and brother | p.(Asp776Asn) |
| 86 | F | 2 | Steroid dependent NS | Pa; N; Y | MCD | ND | *COL4A5* | c.3691C>T | p.(Pro1231Ser) |
| 87 | M | 18\* | SRNS | ND; ND; ND | ND | ND | *COQ2* | c.286C>T | p.(Pro96Ser) |
| 88 | F | 50 | SRNS | W; Y; N | FSGS | ND | *INF2* | c.763G>T; also in son with proteinuria, absent in unaffected daughter | p.(Asp255Tyr)  |
|  |  |  |  |  |  |  | *LMX1B* | c.115C>A; absent in son with proteinuria, in unaffected mother | p.(Pro39Thr)  |
| 89 | M | 1 | SRNS | ND; ND; ND | ND | ND | *PTPRO* | c.1300G>A | p.(Glu434Lys) |
| 90 | F | 14\* | SRNS | W; Y; ND | Not done | Possibly change imm | *INF2* | c.2942G>C | p.(Arg981Thr) |
| 91 | F | 9\* | Haematuria | ND; Y; ND | ND | ND | *LAMB2* | c.1156T>C | p.(Cys386Arg) |
| 92 | M | 62\* | SRNS | ND; ND; ND | MPGN | ND | *LAMB2* | c.3533G>A | p.(Arg1178His) |
| 93 | M | 2 | SRNS | ND; Y; N | ND | ND | *MYH9* | c.1784A>G  | p.(Asn595Ser) |
| 94 | F | 9 | SRNS, single kidney | W; Y; N | Not done | Imm not started | *MYH9* | c.3215C>T; also in affected sister | p.(Ala1072Val) |
| 95 | F | 5.5 | SRNS | Ar; N; N | Not done | Variant found was not classified as likely-pathogenic therefore not influenced treatment strategy | *MYH9* | c.3838G>A | p.(Val1280Met) |
| 96 | F | 3\* | SRNS | ND; ND; ND | FSGS | ND | *MYO1E* | c.1547A>G | p.(Asp516Gly) |
| 97 | F | 2 | SRNS | W; N; N | FSGS | ND | *NPHS1* | c.2746G>T; pat | p.(Ala916Ser) |
| 98 | F | 17\* | NS, short stature | ND; ND; ND | ND | ND | *NPHS1* | c.2746G>T | p.(Ala916Ser) |
| 99 | M | 23 | SRNS | Pa; Y; N | FSGS | ND | *NPHS1* | c.3027C>G | p.(Tyr1009\*) |
| 100 | M | 42\* | Haematuria, hearing loss | W; Y; N | TBMN | ND | *NPHS2* | c.156delG | p.(Thr53Profs\*46)  |
| 101 | M | 22\* | SRNS | W; N; N | MPGN | ND | *NPHS1* | c.2591G>A | p.(Arg864His) |
| 102 | ND | 0 | SRNS | ND; ND; ND | ND | ND | *NPHS2* | c.860A>G | p.(Gln287Arg) |
| 103 | F | 12\* | SRNS | Samoan; N; N | ND | ND | *NPHS2* | c.1064A>G | p.(Asn355Ser) |
|  |  |  |  |  |  |  | *NPHS2* | c.138G>A | p.(=) |
|  |  |  |  |  |  |  | *PLCE1* | c.3580G>A | p.(Gly1194Arg) |
|  |  |  |  |  |  |  | *ARHGAP24* | c.1057\_1058delinsAA | p.(Ala353Asn) |
|  |  |  |  |  |  |  | *COL4A5* | c.4309C>G | p.(Gln437Glu) |
| 104 | F | 11\* | SRNS | ND; ND; ND | FSGS | ND | *PLCe1* | c.1478G>A | p.(Arg493Gln) |
| 105 | M | 18\* | SRNS | ND; ND; ND | FSGS | ND | *PLCe1* | c.2032A>G | p.(Met678Val) |
| 106 | M | 7 | SRNS | W; Y; N | ND | ND | *PMM2* | c.24\_delC | p.(Cys9Alafs\*27),  |
| 107 | M | 3.5 | SRNS | W; N; N | FSGS | Increase | *PMM2* | c.691G>A; pat | p.(Val231Met) |
| 108 | F | 19\* | SRNS | ND; ND; ND | FSGS | ND | *PTPRO* | c.1631C>T | p.(Thr544Met) |
| 109 | M | 14 | SRNS | ND; ND; ND | ND | ND | *TRPC6* | c.1A>G | p.(Met1?)  |
|  |  |  |  |  |  |  | *INF2* | c.395G>A | p.(Ser120Asn)  |
| 110 | F | 8\* | NS | ND; ND; ND | ND | ND | *TRPC6* | c.2392G>C | p.(Asp798His) |
| 111 | M | 4 | SRNS | W; N; N | Membranous | ND | *WT1* | c.844T>C | p.(Cys282Arg) |

\* Denotes age at genetic testing where age at disease onset was not available

Segregation analysis to clarify pathogenicity is ongoing in 15 of these patients with VUS.

**Legend:**

AA, amino acid; Ar, Arabic; CKD, chronic kidney disease; ESRF, end stage renal failure; FH, family history; FSGS, focal segmental glomerulosclerosis; Imm, immunosuppression; In, Indian; mat, maternal; MPGN, membranoproliferative glomerulonephritis; N, no; n/a, not available; ND, not done/no data; NS, nephrotic syndrome; Pa, Pakistani; pat, paternal; SCD, sickle cell disease; SRNS, steroid resistant nephrotic syndrome; TBMD, thin basement membrane disease; W, White; Y, yes

**Supplementary Table 4: Genotypes and phenotypes of patients with single heterozygous variants in *NPHS1* and *NPHS2***

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient number** | **Sex** | **Age at onset (years)** | **Presentation** | **Ethnicity; FH; Consanguinity** | **Biopsy** | **Pathogenicity** | **Gene** | **Nucleotide; segregation** | **AA** | **Reference** † | **Mutation prediction: SIFT; PolyPhen** | **Allele frequency: dbSNP; EVS; ExAC** |
| 112 | M | 4 | SRNS | W; N; N | MCD | LP | ***NPHS1*** | c.313G>A | p.(Asp105Asn) | [62]  |  |  |
| 113 | M | 3\* | SRNS | ND; ND; ND | ND | LP | ***NPHS1*** | c.895C>T | p.(Arg299Cys) | [29]  |  |  |
| 114 | F | 0 | CNS, clinodactyly 2nd toe | Jordanian; N; Y | FSGS | LP | ***NPHS1*** | c.1138C>T | p.(Gln380\*) | [63]  |  |  |
| 115 | M | 0.3 | CNS | W; N; N | Not done | LP | ***NPHS2*** | c.413G>A; pat | p.(Arg138Gln) | [38]  |  |  |
|  |  |  |  |  |  | NNP | *NPHS2* | c.686G>A; mat | p.(Arg229Gln) | [42, 43]  |  |  |
| 116 | F | 4\* | SRNS | ND; ND; ND | FSGS | LP | ***NPHS2*** | c.467dupT | p.(Leu156Phefs\*11) | [64] |  |  |
|  |  |  |  |  |  | NNP | *NPHS2* | c.686G>A | p.(Arg229Gln) | [42]  |  |  |
| 117 | F | 1.4 | SRNS | In; ND; ND | Not done | LP | ***NPHS2*** | c.872G>A | p.(Arg291Gln) | [45]  |  |  |
|  |  |  |  |  |  | LP | *NPHS1* | c.2512C>A | p.(Pro838Thr) | PS | Del; 1.00 | NL; NL; NL |

\* Denotes age at genetic testing where age at disease onset was not available. † All references in this table are included as Supplementary Material.

**Legend:**

AA, amino acid; CNS, congenital nephrotic syndrome; FH, family history; FSGS, focal segmental glomerulosclerosis; In, Indian; LP, likely-pathogenic; mat, maternal; N, no; ND, not done/no data; NL, not listed; NNP, non-neutral polymorphism; pat, paternal; PS, present study; SRNS, steroid resistant nephrotic syndrome; W, White; Y, yes

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