Supplementary Materials and methods

Recruitment of patients and clinical investigation A total of 69 patients affected by sporadic (*i.e.* not familial), non-syndromic and non-congenital nephrotic syndrome (NS) referred to Meyer Children's Hospital of Florence (Italy) between 2000 and 2013 and who provided informed consent were included in the study (Supplementary Table 1). Only patients affected by NS that did not exhibit extra-renal symptoms, that did not have a familial history of renal disease or of consanguinity, and that did not have a congenital NS were included in this study. Steroid-resistance was defined as failure to achieve remission after 8 weeks of prednisone 60 mg/m^2/day or 2 mg/kg/day for 4 weeks followed by 40 mg/m^2 or 1.5 mg/kg on alternate days for 4 weeks, according to Kidney Disease: Improving Global Outcomes (KDIGO) guidelines.[1] None of these patients exhibited a phenotype of late-responder to steroid treatment. Complete remission was defined as urine protein:creatinine ratio (uPCR) <200 mg/g or <1+ of protein on urine dipstick for 3 consecutive days; partial remission was defined as proteinuria reduction of 50% or greater from the presenting value and absolute uPCR between 200 and 2,000 mg/g; finally, no remission was defined as failure to reduce urine protein excretion by 50% from baseline or persistent excretion uPCR >2,000 mg/g, according to KDIGO guidelines.[1] On the basis of these criteria, we selected 31 cases of steroid-resistant NS (SRNS), as well as 38 patients with the same clinical phenotype but that were sensitive to steroid treatment. All information about clinical features, disease onset, response to treatment, histologic findings and final outcome were collected retrospectively. Relatives were either included prior to this study or requested to participate after the identification of potentially causative variants in the proband. The study protocol was approved by the local Ethics Committee of the Meyer Children's Hospital and informed written consent was obtained from the parents or legal guardians of each study participant.

Exon sequencing array design The probe design was made by Roche NimbleGen Inc. (Roche, Madison, Wisconsin, USA) in a solution based method to capture all coding exons and flanking regions of 46 genes. These genes comprised the 19 known genes responsible for SRNS and 27 candidate genes associated with onset of proteinuria in animal models and expressed in the glomerular filtration barrier (Supplementary Table 2A-B). The probe design comprised a total of 110,884 bases targeting 46 genes consisting of 610 coding exons.

DNA Sequencing DNA was extracted from peripheral blood using a QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany). The DNA library were prepared and were hybridized to the exon sequencing array according to the manufacturer's protocol (NimbleGen Arrays User's Guide: 454 Optimized Sequence Capture). To characterize simultaneously different patients, Roche FLX Titanium MID-Adapter oligonucleotides were ligated to the ends of the libraries fragments. The enriched libraries were sequenced using the Roche 454 sequencing FLX platform according to the Roche manufacturer's protocols (emPCR Method Manual-Lib-L LV-Sequencing Method Manual). We obtained an average of 125,742 mapped reads *per* sample, resulting in an evenly distributed average depth of 71X *per* sample. On average, 84% of all bases were covered at least 10X. Among the 610 targeted exons, only 23 exons (4%) were poorly covered, due either to high GC content or the abundant presence of repetitive sequences. The poorly covered regions were successively resequenced by Sanger method. According to the literature,[2] the accuracy of our probe design was 95%.

Assembly and Variant Calling Reads were mapped to the reference human genome (UCSC build *hg19*) with the GS Mapper software version 2.6 (Roche Diagnostic, Basel, Switzerland). Variants were annotated with chromosome location, genomic coordinate, reference and variant nucleotide, absolute number and percentage of reads containing variant nucleotide, reference and variant amino acid, coding frame, gene name and overlap with a known single nucleotide polymorphism from dbSNP 135. Coverage statistics and mean depth were extracted from the mapping output files using custom scripts.

Identification of pathogenic variants In order to identify genetic variants with a possible pathogenic significance, the following criteria were applied: variants were either non-synonymous, short insertion/deletion or splice-site variants (60 bp splice acceptor, 60 bp splice donor); variants were not included in dbSNP135 unless they were reported in HGMD (www.hgmd.cf.ac.uk), were previously demonstrated to alter the function of the mutated protein or represented the second mutated allele in presence of a surely pathogenic variant;[3] variants did not occur in healthy controls within the "1000 Genomes Project" database (www.1000genomes.org) at a frequency greater than 5%; variants did not occur within the Kaviar-hg19 database (http://db.systemsbiology.net/kaviar/) at a frequency greater than 5%; variants were not found in the "Exome Variant Server" of the "NHLBI Exome Sequencing Project (ESP)"

(http://evs.gs.washington.edu/EVS/, release version: v.0.0.10) at a frequency greater than 5%. Subsequently, a systematic classification scheme was applied using a combination of prediction programs. The missense variants were analyzed with the dedicated softwares, PolyPhen (http://genetics.bwh.harvard.edu/pph2/), and PMut (http://mmb.pcb.ub.es/PMut/PMut.jsp) in order to distinguish potentially damaging variants from those predicted to have neutral effect. Intronic variants localized ≤60 bp were analyzed with Netgene2 (http://genes.mit.edu/GENSCAN.html) and BDGP splice site prediction (http://www.fruitfly.org/seq_tools/splice.html web server). The *in silico* analysis was performed to assess the effect of sequence variants identified on structure and function of respectively encoded proteins. For the missense variants identified we used dedicated prediction tools such as PolyPhen-2 and PMut. PolyPhen-2 (Polymorphism Phenotyping v2) is a software which predicts possible impact of amino acid substitutions on the structure and function of human proteins using straightforward physical and evolutionary comparative considerations. Detailed results of the PolyPhen-2 analysis for a single variant query included:- the strength of the putative damaging effect of the variant classified as probably damaging, possibly damaging, or benign (assessed using the HumDiv-trained predictor, preferred for evaluating rare alleles, dense mapping of regions identified by genome-wide association studies, and analysis of natural selection), -the multiple sequence alignment that displays a fixed 75-residue wide window surrounding the variant position, -3D-protein structure loaded and zoomed into the mutation residue. PMut is a prediction tool based on the use of evolutionary and structure information with residue properties. All these informations are input to a neural network that provides the final prediction on whether the target mutation is pathological, or neutral. The comparison of the aminoacidic sequence of all newly identified variants with those of phylogenetically distant species was performed using CLUSTALW algoritm. On the basis of the results, the variants were classified as potentially pathogenic variants, variants of unknown clinical significance, or benign variants, in accordance with the interpretation guidelines of the American College of Medical Genetics and Genomics (ACMG).[4-6]

Sanger sequencing A total of 17 potentially pathogenic variants detected by 454 FLX analyzer were selected and confirmed by Sanger sequencing (Supplementary Table 4). Parental samples, if available, were also analyzed to determine whether the mutated allele had been transmitted.

Immunofluorescence and Confocal Microscopy Confocal microscopy was performed on 5-μm sections of frozen renal biopsies,[7] using a Leica TCS SP5-II laser confocal microscope (Leica, Milan, Italy). The anti-podocin pAb (Abcam, Cambridge, UK) was used as primary antibody and Alexa Fluor goat anti-rabbit 488 was used as secondary antibody (Invitrogen). For the detection of nuclei, staining of cells with To-pro-3 (Molecular Probes from Invitrogen) was performed.

Statistical analysis Statistical analysis was performed using SPSS software (SPSS, Inc., Evanston, IL). Frequencies between groups were compared by $\chi 2$ test, applying Yate's correction when appropriate. Between-group comparisons were made by means of Student t test for unpaired data or Mann–Whitney U-test owing to normal or nonparametric distribution. A p-value <0.05 was considered as statistically significant.

Patient	Type	Age at onset	Gender	Ethnicity	Lenght of Follow-up	Time to ESRD	Age at Tx
Case 1	SRNS	3 yr	M	Caucasian	3 yr, 5 m	no	no
Case 2	SRNS	4 m	F	Caucasian	2 yr, 2 m	no	no
Case 3	SRNS	1 yr, 2 m	M	Caucasian	3 yr, 11 m	4 yr, 8 m	no
Case 4	SRNS	6 yr, 4 m	M	Caucasian	2 yr, 3 m	no	no
Case 5	SRNS	5 yr	F	Caucasian	10 yr	no	no
Case 6	SRNS	18 m	F	Caucasian	20 yr, 6 m	18 m	I:2 yr, 6 m II:8 yr
Case 7	SRNS	3 m	F	Caucasian	11 yr	3 yr	4 yr
Case 8	SRNS	7 yr, 7 m	F	Caucasian	6 yr, 5 m	8 yr, 9 m	11 yr, 11 m
Case 9	SRNS	3 yr, 8 m	F	Caucasian	9 m	yes	no
Case 10	SRNS	7 yr, 5 m	M	Tunisian	1 yr, 5 m	no	no
Case 11	SRNS	6 yr	M	Caucasian	5 yr, 8 m	no	no
Case 12	SRNS	9 yr	M	Brazilian	7 yr, 1 m	no	no
Case 13	SRNS	4 yr	F	Caucasian	4 yr, 5 m	no	no
Case 14	SRNS	5 yr, 5 m	F	Caucasian	15 yr, 3 m	16 yr, 6 m	19 yr, 7 m
Case 15	SRNS	2 yr, 3 m	M	Caucasian	8 yr, 11 m	11 yr	no
Case 16	SRNS	5 yr	M	Caucasian	6 yr	no	no
Case 17	SRNS	9 yr	F	Caucasian	7 yr	no	no
Case 18	SRNS	12 yr	M	Caucasian	9 yr	15 yr	17 yr
Case 19	SRNS	2 yr	F	Caucasian	16 yr	no	no
Case 20	SRNS	8 m	F	Moroccan	4 yr, 4 m	1 yr, 9 m	2 yr, 6 m
Case 21	SRNS	2 yr, 6 m	F	Caucasian	4 yr, 6 m	no	no
Case 22	SRNS	2 yr, 6 m	F	Caucasian	6 yr, 7 m	6 yr, 4 m	no
Case 23	SRNS	3 yr, 5 m	F	Caucasian	13 yr	5 yr, 6 m	5 yr, 9 m
Case 24	SRNS	2 yr, 8 m	M	Caucasian	4 yr	no	no
Case 25	SRNS	13 yr	F	Caucasian	7 yr	15 yr	15 yr
Case 26	SRNS	1 yr, 8 m	M	Caucasian	3 yr, 1 m	no	no
Case 27	SRNS	4 yr, 2 m	M	Caucasian	11 yr, 4 m	no	no
Case 28	SRNS	13 yr, 11 m	M	Caucasian	6 yr, 6 m	no	no
Case 29	SRNS	13 yr, 8 m	F	Caucasian	7 yr	no	no
Case 30	SRNS	15 yr	M	Caucasian	4 yr, 6 m	no	no
Case 31	SRNS	9 yr, 6 m	F	Caucasian	1 yr	no	no
Case 32	SSNS	4 yr, 5 m	F	Caucasian	7 yr, 7 m	no	no
Case 33	SSNS	7 yr, 8 m	M	Caucasian	7 yr, 3 m	no	no
Case 34	SSNS	16 m	F	Caucasian	7 yr, 8 m	no	no
Case 35	SSNS	3 yr, 3 m	F	Caucasian	7 yr, 4 m	no	no
Case 36	SSNS	3 yr, 2 m	F	Moroccan	11 yr, 8 m	no	no
Case 37	SSNS	5 yr, 2 m	M	Caucasian	4 yr, 7 m	no	no
Case 38	SSNS	2 yr, 9 m	F	Caucasian	2 yr, 6 m	no	no
Case 39	SSNS	4 yr	M	Moroccan	2 yr, 3 m	no	no
Case 40	SSNS	3 yr, 5 m	F	Caucasian	7 yr, 4 m	no	no
Case 41	SSNS	3 yr, 5 m	M	Caucasian	3 yr, 7 m	no	no
Case 42	SSNS	6 yr, 5 m	M	Caucasian	11 m	no	no
Case 43	SSNS	11 yr, 6 m	M	Caucasian	4 yr, 10 m	no	no
Case 44	SSNS	3 yr	F	Chinese	9 yr, 11 m	no	no
Case 45	SSNS	10 yr, 3 m	M	Chinese	5 yr, 7 m	no	no
Case 46	SSNS	2 yr, 1 m	M	Caucasian	4 yr, 5 m	no	no
Case 47	SSNS	8 yr, 8 m	M	Albanian	7 yr, 5 m	no	no
Case 48	SSNS	5 yr, 7 m	M	Caucasian	9 m	no	no
Case 49	SSNS	1 yr, 9 m	M	Caucasian	8 m	no	no
Case 50	SSNS	2 yr	F	Caucasian	5 yr, 9 m	no	no
Case 51	SSNS	4 yr, 5 m	M	Indian	8 yr, 1 m	no	no
Case 52	SSNS	2 yr, 9 m	F	Caucasian	3 yr, 4 m	no	no
Case 53	SSNS	7 ys, 5 m	F	Senegalese	1 yr,	no	no

Case 54	SSNS	10 yr, 11 m	F	CapeVerdean	6 m	no	no
Case 55	SSNS	3 yr, 4 m	M	Caucasian	2 yr, 8 m	no	no
Case 56	SSNS	3 yr, 6 m	F	Caucasian	2 yr, 9 m	no	no
Case 57	SSNS	2 yr, 11 m	M	Caucasian	1 yr, 4 m	no	no
Case 58	SSNS	10 yr	M	Caucasian	10 m	no	no
Case 59	SSNS	2 yr, 6 m	M	Macedonian	13 yr, 2 m	no	no
Case 60	SSNS	3 yr, 2 m	F	Caucasian	8 yr, 9 m	no	no
Case 61	SSNS	9 yr, 2 m	M	Caucasian	7 yr	no	no
Case 62	SSNS	2 yr, 1 m	F	Caucasian	1 yr, 5 m	no	no
Case 63	SSNS	2 yr, 9 m	M	Caucasian	2 yr	no	no
Case 64	SSNS	5 yr, 11 m	M	Caucasian	2 yr, 3 m	no	no
Case 65	SSNS	4 yr, 5 m	M	Chinese	10 m	no	no
Case 66	SSNS	3 yr, 2 m	M	Caucasian	2 yr, 8 m	no	no
Case 67	SSNS	4 yr, 11 m	M	Caucasian	3 m	no	no
Case 68	SSNS	2 yr, 7 m	M	Caucasian	2 yr, 7 m	no	no
Case 69	SSNS	4 yr, 1 m	M	Caucasian	4 yr	no	no

Supplementary Table 1. Clinical profile of patients. SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; ESRD, end-stage renal disease; Tx, transplant; yr, years; m, months; M, male; F, female.

Gene	Protein	Locus	Exons	RefSeq	Reference
WT1	Wilms tumor	chr11:32409325-32457087	10	NM_024426	8
LMX1B	LIM homeobox transcription factor 1, beta	chr9:129376748-129463311	8	NM_002316	9-10
NPHS1	nephrin	chr19:36316274-36342739	29	NM_004646	11
NPHS2	podocin	chr1:179519677-179545084	8	NM_014625	12
CD2AP	CD2-associated protein	chr6:47445525-47594994	18	NM_012120	13
ACTN4	actinin, alpha 4	chr19:39138327-39221170	21	NM_004924	14-15
INF2	inverted formin 2	chr14:105155943-105185947	23	NM_022489	16
ITGA3	integrin alpha 3	chr17:48133340-48167848	26	NM_002204	17
TRPC6	transient receptor potential cation channel,6	chr11:101322296-101454659	13	NM_004621	18
LAMB2	laminin, beta 2	chr3:49158548-49170599	33	NM_002292	19
PLCE1	phospholipase C, epsilon 1	chr10:95753746-96088146	32	NM_016341	20
SCARB2	scavenger receptor class B, member 2	chr4:77079894-77135035	12	NM_005506	21
CoQ2	para-hydroxybenzoate-polyprenyltransferase,	chr4:84184979-84205964	7	NM_015697	22
PDSS2	prenyl diphosphate synthase, subunit 2	chr6:107473761-107780779	8	NM_020381	23
SMARCAL1	SWI/SNF-related matrix-associated	chr2:217277473-217347772	18	NM_001127207	24
ZMPSTE24	zinc metallo-peptidase STE24	chr1:40723733-40759855	10	NM_005857	25
MYH9	myosin, heavy polypeptide 9, non-muscle	chr22:36677324-36784063	42	NM_002473	26
PTPRO	receptor-type protein tyrosine phosphatase O	chr12:15475487-15750335	27	NM_030667	27
MYO1E	myosin IE	chr15:59428564-59665071	28	NM_004998	28

Supplementary Table 2A. List of known genes responsible for nephrotic syndrome included in the sequencing array.

Gene	Protein	Locus	Exons	RefSeq	Reference
WTIP	Wilms tumor 1 interacting protein	chr19:34972880-34992084	10	NM_001080436	29
BASP1	brain abundant, membrane attached signal protein	chr5:17217750-17276935	2	NM_006317	30
PAX2	paired box protein 2	chr10:102505468-102589697	10	NM_003990	31
LDB1	LIM domain binding 1	chr10:103867327-103880210	11	NM_001113407	32
TCF21	transcription factor 21	chr6:134210259-134213391	2	NM_003206	33-34
MAFB	transcription factor MAFB	chr20:39314519-39317876	1	NM_005461	35
FOXC2	forkhead box C2	chr16:86600857-86602535	1	NM_005251	36
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	chr6:139693397-139695785	2	NM_006079	37
NF-Kb1	nuclear factor kappa-B, subunit 1	chr4:103422486-103538458	24	NM_003998	38
SMAD7	SMAD family member 7	chr18:46446224-46477081	4	NM_005904	38
TGFB	transforming growth factor, beta 1	chr19:41836651-41859816	7	NM_000660	38-39
ZHX1	zinc fingers and homeoboxes 1	chr8:124260697-124286547	4	NM_007222	40
ZHX2	zinc fingers and homeoboxes 2	chr8:123793901-123986755	4	NM_014943	40
ZHX3	zinc fingers and homeoboxes 3	chr20:39807089-39928739	4	NM_015035	40
RBPJ-K	recombining binding protein suppressor of hairless	chr4:26322448-26433278	11	NM_203284	41
SYNPO	synaptopodin	chr5:150020220-150038792	3	NM_007286	42
PALLD	palladin	chr4:169418217-169849607	21	NM_016081	43
ITGB1BP1	integrin cytoplasmic domain-associated protein 1	chr2:9545823-9563643	7	NM_004763	44-45
CTSL1	cathepsin L1	chr9:90340974-90346382	8	NM_145918	46

KIRREL	kin of IRRE like protein1	chr1:157963063-158065842	15	NM_018240	47
KIRREL3	kin of IRRE like protein 3	chr11:126293397-126870766	16	NM_032531	48
PODXL	podocalyxin	chr7:131185023-131241376	8	NM_005397	49
CoQ10A	coenzyme Q10 homolog A	chr12:56660642-56664748	5	NM_144576	50
CoQ10B	coenzyme Q10 homolog B	chr2:198318231-198339851	5	NM_025147	50
CD151	CD151 antigen	chr11:832952-838834	9	NM_004357	51
LMNA	lamin A	chr1:156084461-156109878	12	NM_170707	52
NOTCH1	notch1	chr9:139388897-139440238	34	NM_017617	53

Supplementary Table 2B. List of candidate genes associated with onset of proteinuria in animal models and expressed in the glomerular filtration barrier, included in the sequencing array.

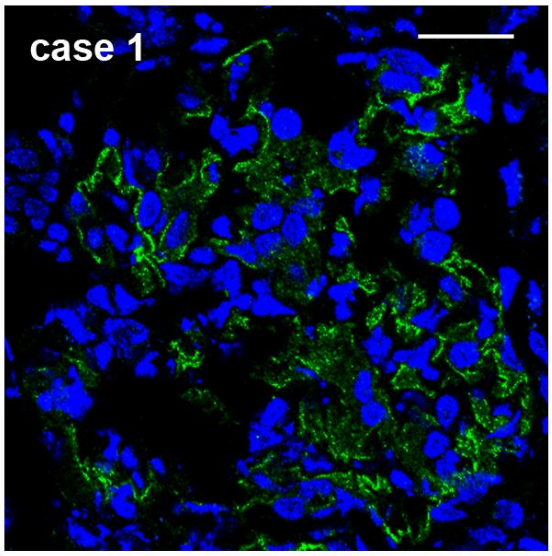
Patient	Gene	Pathol	Type	Immunosuppressive drugs								RAS-Is	R	Remission
				CnIs	R	CF	R	MMF	R	Rtx	R			
Case 1	*NPHS2*	MCD	SRNS	0		1	0	0		0		1	1	C
Case 2	*NPHS2*	no	SRNS	0		0		0		0		1	0	N
Case 3	*NPHS2*	FSGS	SRNS	0		0		0		0		1	0	N
Case 4	*NPHS2*	FSGS	SRNS	0		0		0		0		1	1	P
Case 5	*NPHS2*	FSGS	SRNS	1	0	1	0	0		0		1	0	N
Case 6	*PLCE1*	FSGS	SRNS	0		1	0	0		0		1	0	N
Case 7	*PLCE1*	DMS	SRNS	0		0		0		0		1	0	N
Case 8	*ACTN4*	FSGS	SRNS	1	0	1	0	0		0		1	0	N
Case 9	*ACTN4*	FSGS	SRNS	1	0	0		0		0		0		N
Case 10	*LMX1B*	MCD	SRNS	1	0	0		0		0		1	1	P
Case 11	none	FSGS	SRNS	1	1	1	0	1	0	1	1	1	1	P
Case 12	none	MCD	SRNS	1	1	1	0	0		0		0		C
Case 13	none	FSGS	SRNS	0		1	1	0		0		1	1	C
Case 14	none	FSGS	SRNS	1	0	1	0	0		1	0	1	0	N
Case 15	none	FSGS	SRNS	1	0	1	0	1	0	0	0	1	0	N
Case 16	none	MCD	SRNS	1	1	0		0		0		0		C
Case 17	none	FSGS	SRNS	1	0	1	0	0		1	0	1	0	N
Case 18	none	FSGS	SRNS	1	0	1	0	0		0		1	0	N
Case 19	none	FSGS	SRNS	0		0		0		0		1	0	N
Case 20	none	DMS	SRNS	0		0		0		0		1	0	N
Case 21	none	FSGS	SRNS	1	0	1	Ø	0		0		1	1	C
Case 22	none	MCD	SRNS	1	1	0		1	0	1	0	1	0	N
Case 23	none	FSGS	SRNS	1	0	1	0	0		0		1	0	N
Case 24	none	FSGS	SRNS	0		1	0	1	0	0		1	1	P
Case 25	none	FSGS	SRNS	0		0		0		1	0	1	0	N
Case 26	none	no	SRNS	1	0	0		0		1	1	0		C
Case 27	none	FSGS	SRNS	1	1	1	0	1	1	0		1	1	C
Case 28	none	DMS	SRNS	1	1	1	0	0		0		1	1	P
Case 29	none	MCD	SRNS	1	1	0		0		0		0	0	C
Case 30	none	no	SRNS	0		1	1	0		0		1	1	C
Case 31	none	DMS	SRNS	1	1	0		0		0		1	1	P
Case 32	none	no	SSNS	1	1	0		0		0		0		C
Case 33	none	no	SSNS	1	1	0		1	1	0		0		C
Case 34	none	no	SSNS	0		0		0		0		0		C
Case 35	none	no	SSNS	1	1	0		1	Ø	0		0		C
Case 36	none	no	SSNS	1	1	1	Ø	1	1	1	—	0		C
Case 37	none	no	SSNS	0		0		0		0		0		C
Case 38	none	no	SSNS	0		0		0		0		0		C
Case 39	none	no	SSNS	1	—	0		0		0		0		C
Case 40	none	no	SSNS	0		0		0		0		0		C
Case 41	none	no	SSNS	1	1	1	1	0		0		0		C
Case 42	none	no	SSNS	1	—	0		0		0		0		C
Case 43	none	no	SSNS	1	0	1	1	1	0	1	—	0		C
Case 44	none	no	SSNS	1	1	0		0		0		0		C
Case 45	none	no	SSNS	1	0	1	1	1	1	0		0		C
Case 46	none	no	SSNS	0		0		0		1	—	0		C
Case 47	none	no	SSNS	1	1	0		0		1	—	0		C
Case 48	none	no	SSNS	0		0		0		0		0		C
Case 49	none	no	SSNS	0		0		0		0		0		C
Case 50	none	no	SSNS	1	1	0		0		0		0		C
Case 51	none	no	SSNS	0		0		0		0		0		C

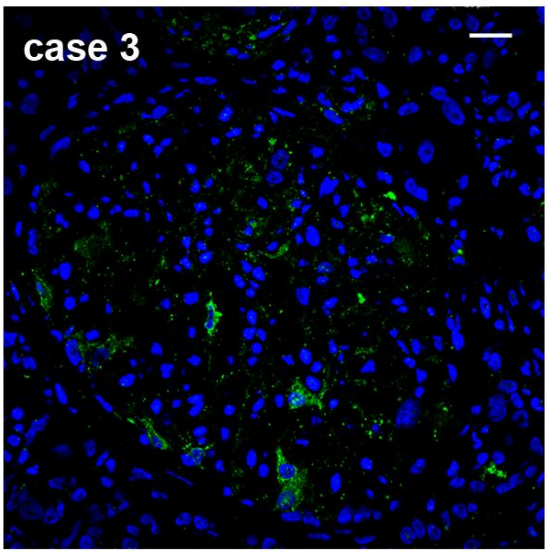
Case 52	none	no	SSNS	1	1	0		0		0		0		C
Case 53	none	no	SSNS	0		0		0		0		0		C
Case 54	none	no	SSNS	0		0		0		0		0		C
Case 55	none	no	SSNS	0		0		0		0		0		C
Case 56	none	no	SSNS	1	1	0		0		0		0		C
Case 57	none	no	SSNS	0		1	—	0		0		0		C
Case 58	none	no	SSNS	0		0		0		0		0		C
Case 59	none	no	SSNS	1	1	1	1	0		0		0		C
Case 60	none	no	SSNS	1	1	1	1	0		0		0		C
Case 61	none	no	SSNS	1	1	0		0		0		0		C
Case 62	none	no	SSNS	1	1	0		0		0		0		C
Case 63	none	no	SSNS	0		1	1	0		0		0		C
Case 64	none	no	SSNS	0		1	1	0		0		0		C
Case 65	none	no	SSNS	1	1	0		0		0		0		C
Case 66	none	no	SSNS	0		1	1	0		0		0		C
Case 67	none	no	SSNS	0		0		0		0		0		C
Case 68	none	no	SSNS	0		0		0		0		0		C
Case 69	none	no	SSNS	0		0		0		0		0		C

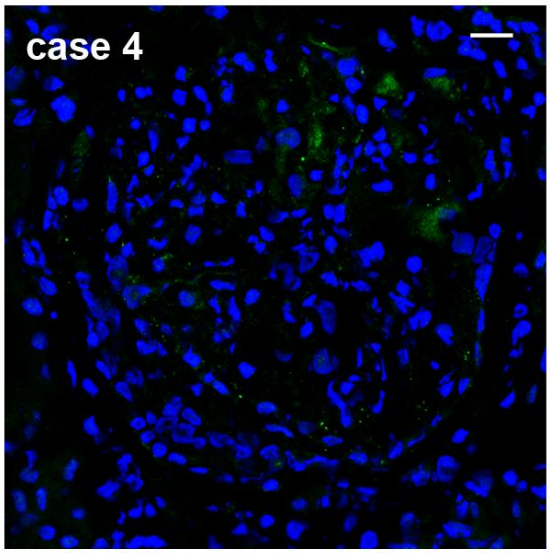
Supplementary Table 3. Genotype in relation to pathology, response to treatment and outcome in patients affected by SRNS and SSNS. Pathol, pathology; FSGS, focal segmental glomerulosclerosis; MCD, minimal-change disease; DMS, diffuse mesangial sclerosis; SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; CnIs, calcineurin inhibitors; CF, cyclophosphamide; MMF, mycophenolate mofetil; Rtx, rituximab; RAS-Is, renin-angiotensin system inhibitors; R, type of response; 1, response; 0, no response; —, response not evaluable because the drug has been started too recently; Ø, suspension of the drug because of the onset of major side effects; C, complete remission; P, partial remission; N, no remission.

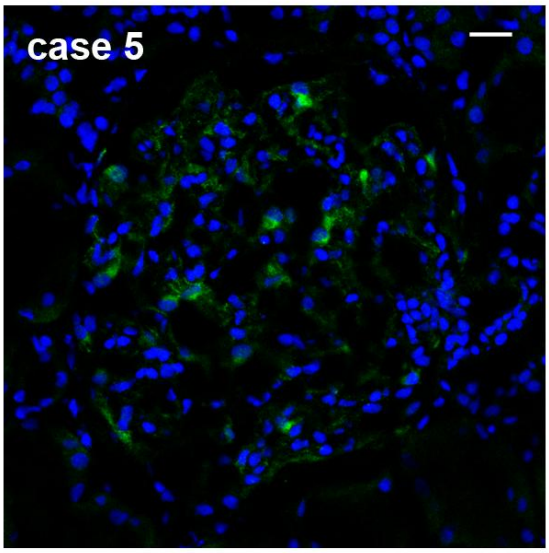
Gene	Forward	Reverse
ACTN4 ex4	5'TAACGACCTTCTGCCCTCTG-3'	5'-CAGGAAGAGGCTTGAAGCAT-3'
ACTN4 ex8	5’-TCACTCTGCAGGTCCCTCTC-3’	5’-CGTCTGCAAGAGAAATGAGG-3’
PLCE1 ex6	5’GAATTTAGGCTCCTTGCTGTAAAC-3’	5’-GACAGGGCCTGATGAGACAG -3’
PLCE1ex7	5'-TCCAGGAAAAGCTCCTTGAA-3'	5'-GTGGCAGGTTTTGATGAGGT-3'
PLCE1ex17	5’-CGA GGC TTT ATC TCC AGG TG -3’	5’-AACAGAGCGAGAACCCG -3’
PLCE1ex19	5'-GGCATTGATTGTATGTTTTCCTT-3'	5'-TAGGCACACTGCCTTTTCAT-3'
LMX1B ex6	5’-GCCAGAAGACTACGGTCCAG-3’	5’-CTTGGTGGAAGGCTTTTGAG-3’
NPHS2 ex1	5’-GCAGCGACTCCACAGGGACT-3’	5’-GAACCTGAGCATCCAGCAAT-3’
NPHS2 ex3	5’-TCTTATGCCAAGGCCTTTTG-3’	5’-CCAATTCTCTCTCTTGGCTACC-3’
NPHS2 ex4	5’-CCCAGTTTGCTGGGATAATC-3’	5’-CCCTAGATTGCCTTTGCACT-3’
NPHS2 ex5	5'-GACCTGGTTTGGAATCAACTG-3'	5'-GCTATGAGCTCCCAAAGGGA-3'
NPHS2 ex8A	5’-GTCTCCCCAGCTCAAGACC-3’	5’-GGATGGTGCATTGTGACTTC-3'

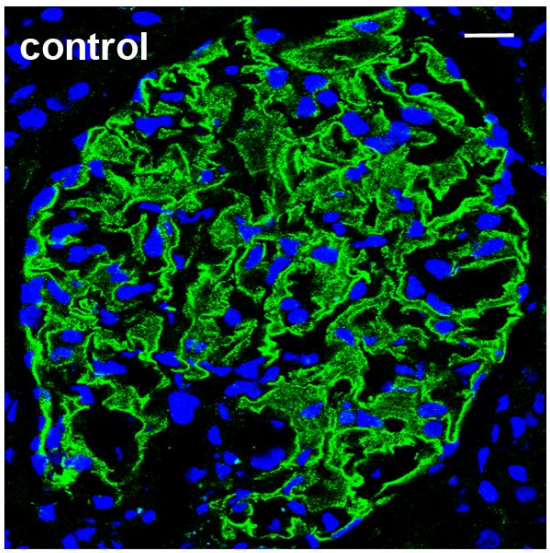
Supplementary Table 4. Primer Sequences. Primer sequences used to confirm potentially pathogenic variants identified with next generation sequencing.


case 1


case 3

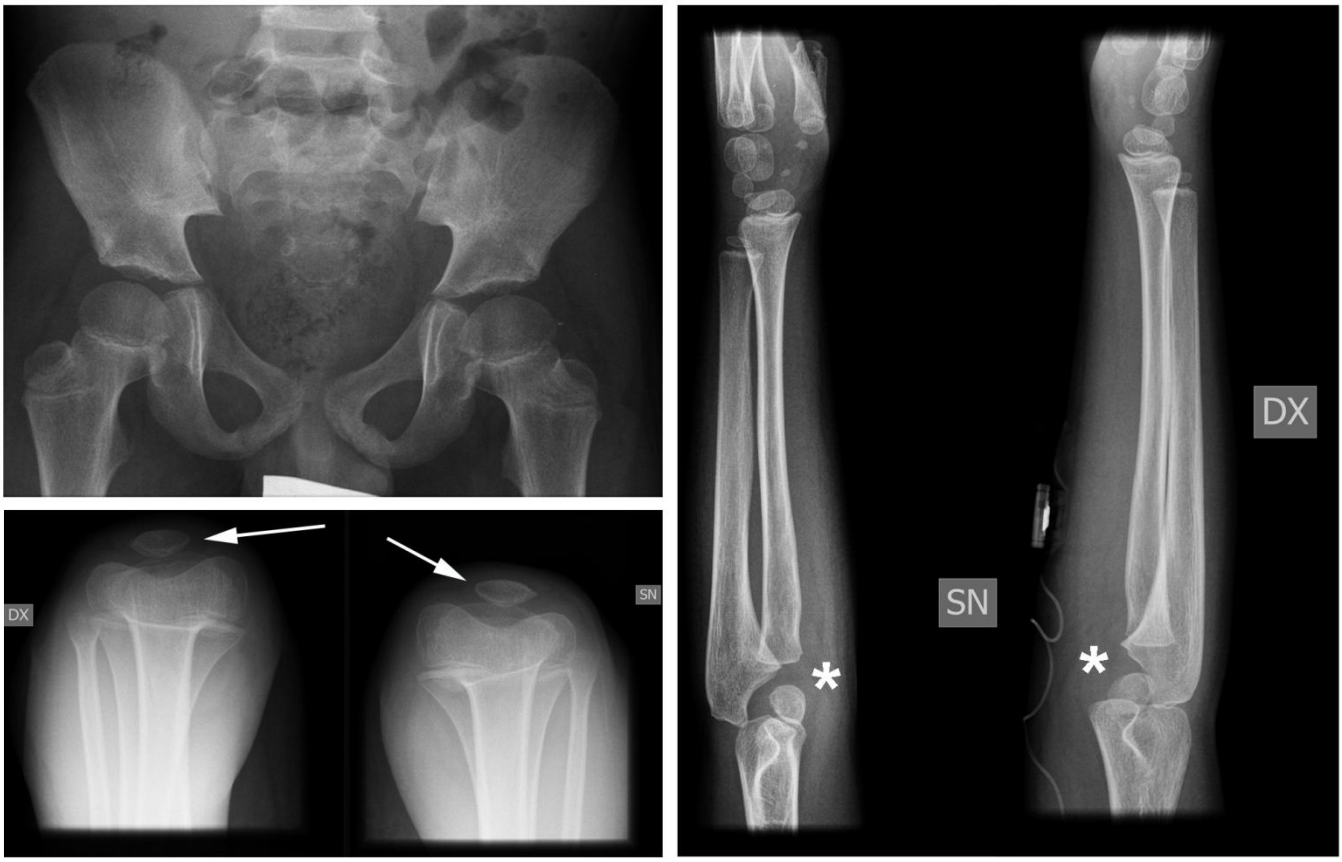

case 4


case 5


control

Supplementary Figure 1. Immunofluorescence staining for podocin on human biopsies of SRNS patients with *NPHS2* mutations. Representative micrograph of kidney sections stained with podocin (green) on human biopsies of children affected by SRNS with potentially pathogenic variants in *NPHS2* gene, as assessed by confocal microscopy. A healthy glomerulus is shown as a comparison. To-pro-3 counterstains nuclei (blue). Bars 20µm.

Supplementary Figure 2. Comparison of the aminoacidic sequences of all newly identified variants (*NPHS2, PLCE1, ACTN4* and *LMX1B*) with those of phylogenetically distant species, illustrating the degree of conservation of the altered amino acid residue.



Supplementary Figure 3. X-ray exams of patients with *LMX1B* mutation (case 10). Radiologic examinations showing lack of iliac horns (upper left), bilateral normal kneecaps (down left, arrows) and bilateral lack of ossification of the proximal radial epiphysis (*).

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