Supplementary tables, methods and figures

SUPPLEMENTARY TABLE 1. Mutations in 48 different individuals with nephrotic syndrome used in the pilot study

No	Family number	Gene	Nucleotide change (Zygosity)	Exon (Zygosity)	Amino Acid Change	Consan guinity	Age of onset	Kidney disease, biopsy (at age [yr]), treatment	Ethnic origin	Reference	Results
1	F1082-21	*COQ6*	c.1058C>A	9 (H)	p.A353D	Y	2.5 yr	SRNS, DMS (3 yr), ND	Turkey	(1)	detected
2	A1072-21	*COQ6*	c.1058C>A	9 (H)	p.A353D	Y	6 yr	SRNS, FSGS (6 yr), CP-NR	Turkey	(1)	detected
3	A234-21	*COQ6*	c.763G>A	7 (H)	p.G255R	Y	3 mo	SRNS, DMS, ND	Turkey	(1)	detected
4	A3331-21	*COQ6*	c.1341G>A c.1383delG	11 (h) 12 (h)	p.W447X p.Q461fs*478	N	3 yr	SRNS, FSGS (3 yr), CP-R, ACE-I	Turkey	(1)	detected
5	F252-46	*COQ6*	c.763G>A	7 (H)	p.G255R	ND	3 mo	SRNS, FSGS, ND	Lebanon	(1)	detected
6	A 2410-21	*CUBN*	c.8355delA	53 (H)	p.S2785Sfs*19	Y	4 yr	ND	Egypt	(2)	detected
7	A3113-21	*ITGA3*	c.1883G>C	14 (H)	p.R628P	Y	4 mo	ND	Pakistan	(3)	faulty primer pair in exon14
8	A3503-21	*ITGA3*	c.1538-1G>A	12 (H)	Acceptor splice site	N	3 days	SRNS, FSGS	Israel	(3)	undetected splice-site mutation
9	A2327-21	*LAMB2*	c.3243delC c.3904C>T	22 (h) 25 (h)	p.N1082Tfs*69 p.R1302X	N	birth	ND	USA (Caucasian)	unpublished unpublished	detected
10	A3812-21	*LAMB2*	c. 2740G>A c.2773C>T	20 (h) 20 (h)	p.G914R p.R925W	N	4.5 yr	SRNS, FSGS (4.5 yr), ND	India	unpublished unpublished	detected
11	F1012-21	*LAMB2*	c.737G>A	7 (H)	p.R246Q	Y	2 mo	CNS, FSGS (7 mo), ND	Turkey	(4)	detected
12	A1193-21	*NPHS1*	c.1555C>T c.2596C>T	12 (h) 19 (h)	p.P519S p.R866X	N	4 mo	ND	Germany	(5)	low-coverage amplicons
13	A1801-21	*NPHS1*	c.1716C>G c.3478C>T	13 (h) 27 (h)	p.S572R p.R1160X	N	14 days	CNS, DMS (1yr), ND	Malta	(5) (6)	detected
14	A2030-21	*NPHS1*	c.1096A>C c.3478C>T	9 (h) 27 (h)	p.S366R p.R1160X	N	1 mo	CNS, Finnish type (1 mo), ND	Montenegro	(7)	detected

15	A2031-21	NPHS1	c.1096A>C	9 (H)	p.S366R	N	2 mo	CNS, Finnish type (2 mo), ND	Serbia	(7)	detected
16	A2249-21	*NPHS1*	c.2227C>T c.3442C>T	17 (h) 27 (h)	p.R743C p.Q1148X	N	5 mo	Finnish type (5 mo), ND	Great Britain	(7) (6)	detected
17	A2341-21	*NPHS1*	c.320C>T c.2600G>A	3 (h) 19 (h)	p.A107V p.G867D	N	2 mo	CNS, Finnish type (2 mo), ND	India	(8)	detected
18	A2535-21	*NPHS1*	c.574C>T c.2728T>C	5 (h) 20 (h)	p.Q192X p.S910P	N	9 mo	SRNS, IgM nephropathy (9 mo), CsA-NR	USA (African-American)	(8)	detected
19	A3326-21	*NPHS1*	c.139delG c.1701C>A	2 (h) 13 (h)	p.E46fsX127 p.C567X	N	1 mo	CNS, Finnish type (5 mo), ND	Argentina	(5) (6)	detected
20	A3792-21	*NPHS2*	c.686G>A c. 826_833dup8	5 (h) 7 (h)	p.R229Q p.T279fsX295	N	8 yr	SRNS, FSGS +MPG (8 yr), ND	Switzerland	(9) unpublished	detected
21	A4326-22	*NPHS2*	c.686G>A c.983A>G	5 (h) 8 (h)	p.R229Q p.Q328R	N	6 yr	SRNS, FSGS (6), ACE-I	USA (Caucasian)	(9) (10)	detected
22	A4342-21	*NPHS2*	c.372C>G c.855_856delAA	2 (h) 7 (h)	p.C124W p.R286Tfs*17	N	10 yr	SRNS, Mesangial proliferation, IgM deposit (11 yr), ND	Spain	(11) (12)	detected
23	A4382-22	*NPHS2*	c.103-126dup23 c.596_7 insA	1 (h) 5 (h)	p.A43fsX103 p.K199fsX212	Y	1.9 yr	SRNS, MCNS (2 yr), ND	Egypt	unpublished unpublished	large duplication (23 bp) in NPHS2
24	A4426-21	*NPHS2*	c.413G>A c.868G>A	3 (h/h) 7 (h/h)	p.R138Q p.V290M	N	27 yr	SRNS, MCNS (27 yr), FSGS (30 yr)	Germany	(12) (13)	detected
25	A674-21	*NPHS2*	c.413G>A c.535-1G>T	3 (h) 5 (h)	p.R138Q Acceptor splice site	N	2 mo	SRNS, MCNS (9 mo), FSGS (5 yr), CSA-CR	Germany	(12) (13)	detected
26	F1028-21	*NPHS2*	c.353C>T c.413G>A	2 (h) 3 (h)	p.P118L p.R138Q	N	1 mo	CNS, MPGN, ND	Germany	(14) (12)	detected
27	F1221-21	*NPHS2*	c.378G>T c.948delT	2 (h) 8 (h)	p.K126N p.L347X	N	birth	CNS, FSGS, dialysis, KT	Germany	(10) (10)	detected
28	F355-21	*NPHS2*	c.460_467insT c.413G>A	4 (h) 3 (h)	p.V165X p.R138Q	N	birth	CNS, FSGS with MPGN (1 yr), CSA-NR	Germany	(15) (12)	homopolymer regions
29	F515-21	*NPHS2*	c.413G>A c.419delG	3 (h), 3 (h)	p.R138Q p.G140Dfs*41	N	birth	CNS, not classifiable glomerulopathy, SR	Czech Republic	(12)	detected
30	F935-21	*NPHS2*	c.460-467insT c.506T>C	4 (h), 4 (h)	p.V165X p.L169P	N	birth	CNS, MCNS, SR	Germany	(15) (16)	detected
31	F942-21	*NPHS2*	c.413G>A c.503G>A	3 (h), 4 (h)	p.R138Q p.R168H	N	1 mo	CNS, FSGS, ACE-I, Diuretics	Germany	(12) (14)	detected
32	A1537-21	*NPHS1*	c.139delG c.840+26A>T	2 (h), 7 (h)	p.A47Pfs*94 Intronic	N	birth	CNS, MCNS (1mo), SR	USA (African American)	(5) NA	low-coverage amplicons

33	A1630-21	*PLCE1*	c.6178delT	29 (H)	p.F2060Ffs*2	Y	1.5 yr	SRNS, DMS, ND	Pakistan	(17)	detected
34	A3217-21	*PLCE1*	c.4977_4982delCAGA	22 (H)	p.Q1660Lfs*9	Y	4 yr	SRNS, ND	Israel	Unpublished	detected
35	A38-21	*PLCE1*	c.1477C>T	3 (H)	p.R493X	Y	3 mo	CNS, ND, CSA-NR	Israel	(18)	detected
36	A601-21	*PLCE1*	c.4451C>T	21 (H)	p.S1484L	Y	8.8 yr	SRNS, FSGS (9 yr), CP-NR	Turkey	(18)	detected
37	F331-21	*PLCE1*	c.3843delG	14 (H)	p.L1281fsX1308	Y	3 yr	SRNS, FSGS (3 yr), ND	Turkey	(18)	detected
38	F389-21	*PLCE1*	c.3346C>T	10 (H)	p.R1116X	Y	4.5 yr	SRNS, FSGS (15 yr), ND	Turkey	(18)	detected
39	F310-21	*SMARCAL1*	p.1756C>T	12 (H)	p.R586W	Y	8 yr	SRNS, MPGN, ND	Italy	(19)	detected
40	505-32	*TRPC6*	c.395T>C	2 (h)	p.M132T	Y	20 yr	SRNS, FSGS, dialysis (20 yr), KT (22 yr)	Germany	(20)	detected
41	F1280-21	*WT1*	c.1432+5G>A	9 (h)	Donor splice site	N	8 yr	SRNS, FSGS (8 yr), CSA-NR	Serbia	(21)	detected
42	A3808-21	*WT1*	c.1300C>T	8 (h)	p.R434C	N	1 mo	CNS, DMS (1 mo), SR	USA (Caucasian)	(22)	detected
43	A4602-21	*WT1*	c.1283G>A	8 (h)	p.C428Y	N	2 mo	ND	USA (Caucasian)	(23)	detected
44	A580-21	*WT1*	c.C1384T	9 (h)	p.R462W	N	3.5 yr	SRNS, FSGS (3.5 yr), ND	USA (Hispanic)	(24)	detected
45	A644-21	*WT1*	c.C1323A	8 (h)	p.H441Q	N	6 yr	SRNS, MCNS (6 yr), FSGS (9yr), Dialysis (11 yr)	USA (Hispanic)	(25)	detected
46	F1351-21	*WT1*	c.C1348T	8 (h)	p.P450S	N	10 yr	SRNS, FSGS (12 yr), CSA-PR	Serbia	(26)	detected
47	F734-21	*WT1*	c.A1394C	9 (h)	p.H465P	N	1.2 yr	SRNS, FSGS, CSA-PR	Germany	(10)	detected
48	F953-21	*WT1*	c.1432+4C>T	9 (h)	Donor splice site	N	14 yr	SRNS, FSGS (14 yr), ND	Germany	(27)	detected

H, homozygous; h, heterozygous; ACE-I, angiotensin-converting enzyme inhibitor; CSA-S, cyclosporine A sensitive; CSA-PR, cyclosporine A partial response; CSA-NR, cyclosporine A no response; ESRF, end-stage renal failure; KT kidney transplantation; SRNS, steroid resistant nephrotic syndrome; DMS, diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis; MCNS Minimal change nephrotic syndrome; mo, months; yr, years; ND, no data or DNA not available; Y, yes; N, no.

SUPPLEMENTARY TABLE 2. Diagnosis, median age of onset, gender and consanguinity in the pilot and diagnostic study

	Median age of onset in months (range)	Diagnosis						Gender		Consanguinity		
		DMS	FSGS	MCNS	MPGN	IgM Nephropathy	ND	F	M	Y	N	ND
Pilot Study (n=48)	6.5 (1-354)	5	23	3	2	2	13	28	20	15	32	1
Diagnostic Study (n=48)	12 (0-444)	2	10	3	1	3	28	27	21	10	35	3

DMS, diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis; MCNS Minimal change nephrotic syndrome; MPGN Membranoproliferative glomerulonephritis; ND, no data available; F, female; M, male; Y, consanguinity; N, non-consanguinity.

SUPPLEMENTARY METHODS

Cost of the Method

For each of our experiments, the total cost amounted to $65 per patient compared to sequencing 23,040 amplicons on an ABI capillary sequencer the cost would be reduced 1/29th, assuming $4 total cost per amplicon sequenced.

The combination of barcoded multiplex PCR combined with next generation sequencing allows rapid mutation analysis in < 3 weeks for 48 patients in 21 genes.

Web-based programs for variant analysis

PolyPhen 2

Predictions on the possible impact of an amino acid substitution on the chemical change, evolutionary conservation, and protein function were obtained by using the web-based PolyPhen2 software program (http://genetics.bwh.harvard.edu/pph2/).

Exome variant server database

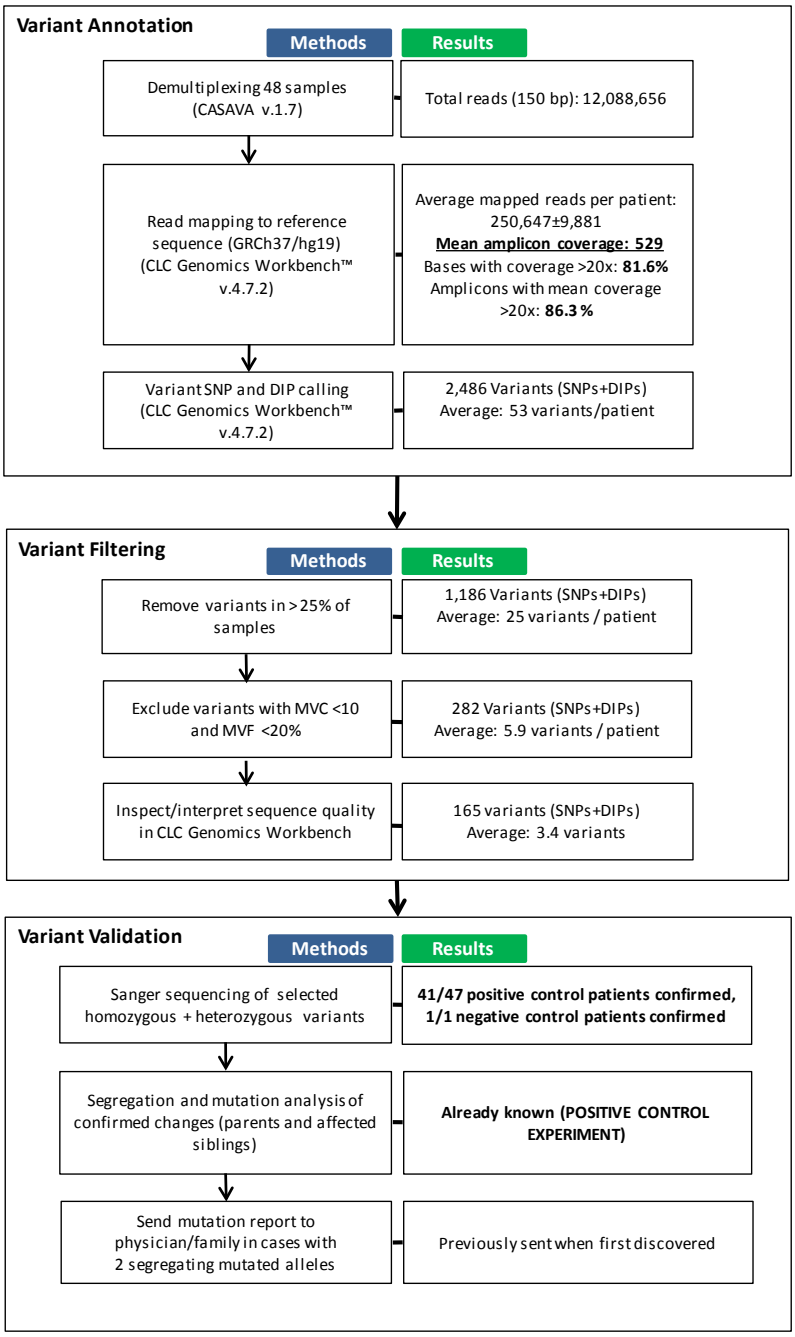
Using the exome capture and NGS data derived from about 6,500 individuals deposited in the Exome Variant Server database (EVS, http://evs.gs.washington.edu/EVS/).

HGMD® Human Gene Mutation Database – BioBase

Biobase provides data on published human inherited disease (http://www.biobase-international.com/product/hgmd)

Mutation Taster

Is online available to predict in-silico if a mutation changes the protein function (http://www.mutationtaster.org).



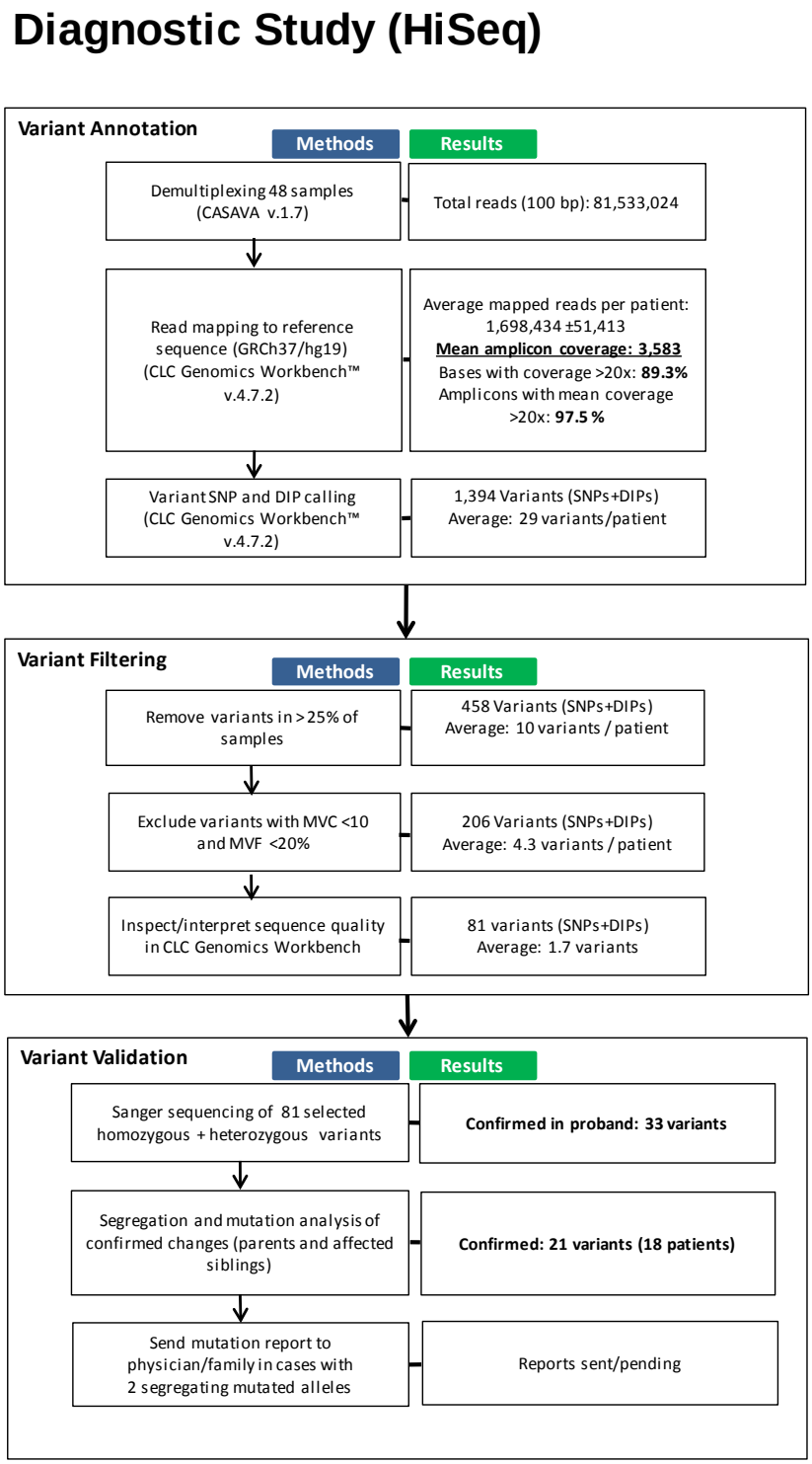
Supplementary Fig. 1 Variant annotation, filtering and validation in the pilot study.

(A) The methods (in blue) applied to raw sequence data are shown on the left of the chart for each subsequent step. The results (in green) are shown on the right of the chart for each subsequent step.

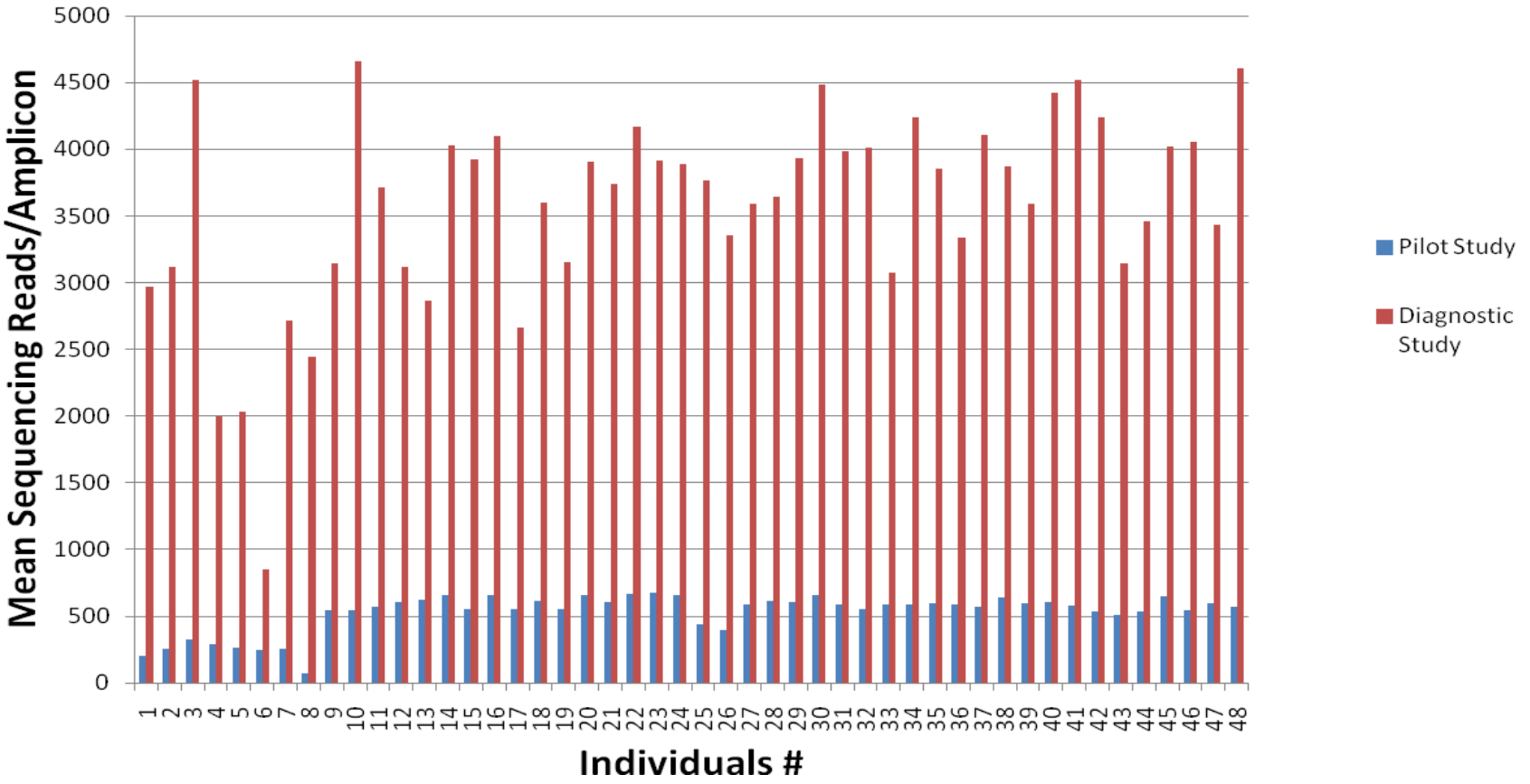
Sequencing reads were first demultiplexed by barcode using CASAVA v.1.7 software, and the reads from each barcode were mapped to the reference sequence in CLC Genomics Workbench software. Following read mapping, variant detection (SNPs and DIPs) was performed.

(B) Variants were then further filtered: Variants appearing in more than 25% of barcodes were discarded, as well as variants with minor variant count (MVC) of < 10 and minor variant frequency (MVF) of < 20%. Finally, sequences corresponding to the remaining variants were inspected for sequence quality in CLC Genomics Workbench software.

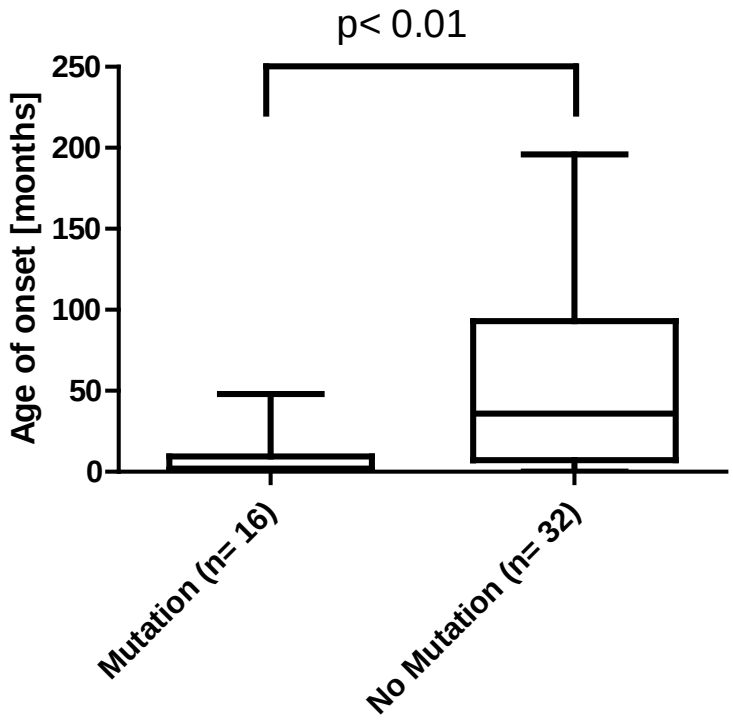
(C) Remaining "surviving" variants were Sanger sequenced to confirm their validity.



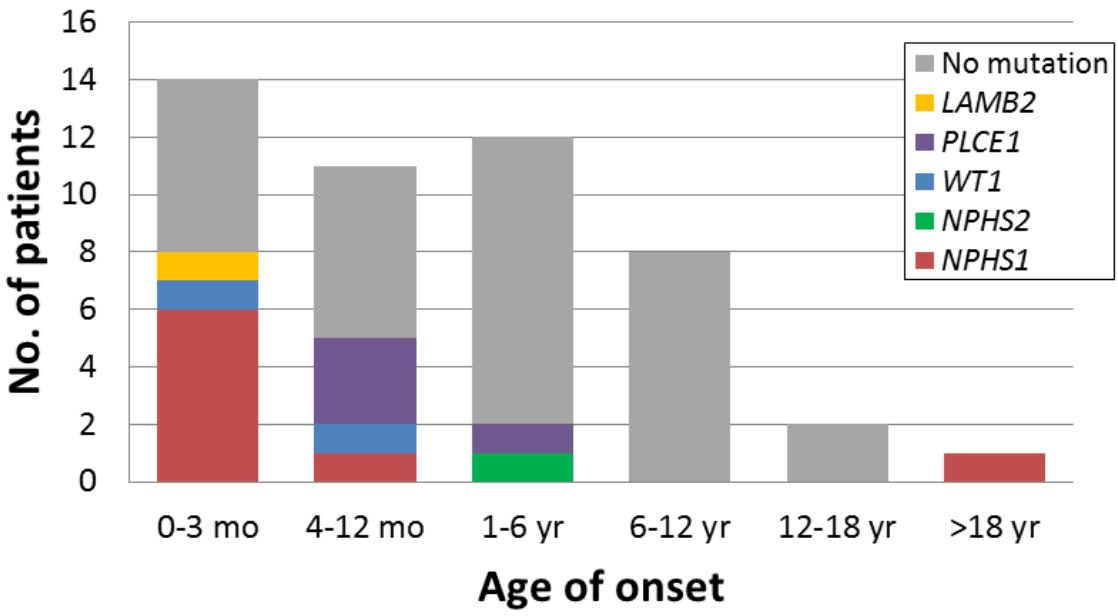
Supplementary Fig. 2. Variant annotation, filtering and validation in the diagnostic study. (A) The methods (in blue) applied to raw sequence data are shown on the left of the chart for each subsequent step. The results (in green) are shown on the right of the chart for each subsequent step. Sequencing reads were first demultiplexed by barcode using CASAVA v.1.7 software, and the reads from each barcode were mapped to the reference sequence in CLC Genomics Workbench software. Following read mapping, variant detection (SNPs and DIPs) was performed. (B) Variants were then further filtered: variants appearing in more than 25% of barcodes were discarded, as well as variants with minor variant count (MVC) of < 10 and minor variant frequency (MVF) of < 20%. Finally, sequences corresponding to the remaining variants were inspected for sequence quality in CLC Genomics Workbench software. (C) Remaining "surviving" variants were Sanger sequenced to confirm their validity. Segregation analysis was also performed to show segregation of the variants in family members.



Supplementary Fig. 3. Mean mapped reads per amplicon per individual. The y-axis plots the mean sequence reads per amplicon against each individual from 1-48 on the x-axis. In the pilot study (blue bars), a total of 12,088,656 reads aligned to the targeted genomic regions across all 48 individuals (mean coverage per amplicon/individual of 502 ± 20 SEM). In the diagnostic study (red bars), a total of 81,533,024 reads aligned to the targeted genomic regions across all 48 individuals (mean coverage per amplicon/individual of 3,583 ± 108 SEM.



Supplementary Fig. 4. Distribution for age of onset between individuals with mutations and without mutations.
Box plots show median, quartiles and range, p<0.01.



Supplementary Fig. 5. Distribution of detected mutations and age of onset in the diagnostic study.
The y-axis indicates the number of patients. The x-axis indicates the age of onset in ascending order. A total of 16 individuals had disease-causing mutations in the genes: *NPHS1, NPHS2*, *WT1*, *LAMB2* and *PLCE1*. In 32 patients no genetic cause of NS was detected (mo, months; yr, years).

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