SUPPLEMENTARY APPENDIX

Mutations in *ANKS6* cause a Nephronophthisis-Like Phenotype with End Stage Renal Disease

Ekim Z. Taskiran, Emine Korkmaz, Safak Gucer, Can Kosukcu, Figen Kaymaz, Cansu Koyunlar, Elizabeth C. Bryda, Moumita Chaki, Dongmei Lu, Komal Vadnagara, Cengiz Candan, Rezan Topaloglu, Franz Schaefer, Massimo Attanasio, Carsten Bergman, Fatih Ozaltin

CLINICAL HISTORY OF THE REPORTED PATIENTS

Family PN25

The family was formed of 6 diseased individuals born from Turkish consanguineous parents. Of them 3 progressed to ESRD and 3 had CKD at the time of this report. (**Figure 1A and Table 1**).

The index patient (V-1) born to consanguineous parents presented with leg pain when she was 7-year-old (Figure 1A). A renal USG, which was performed due to history of CKD in relatives (III-3, III-5, III-6, III-7, and III-10) revealed increased echogenicity with normal sized kidneys. Renal functions were normal at that time. Specific gravity of the urine was 1.011. Microscopic examination of the urine showed 15 red blood cells per high-power field. A moderate proteinuria was detected (urine protein/creatinine ratio 0.47mg/mg.creatinine). A renal biopsy was performed due to decreased glomerular filtration rate (61 ml/min/1.73m^2) at the age of 10 years.

Light microscopy revealed 15 glomeruli in the corticomedullary junction. Of these, 10 were globally sclerotic. Periglomerular fibrosis was noted in non-sclerotic glomeruli.

Tubuli showed focal atrophy, basal membrane thickening and dilatation with occasional hyaline casts. Patchy infiltration with mononuclear inflammatory cells and moderate fibrosis were noted in the interstitium. There was also a mild thickening of arterioles (Supplemental Figure 1A). No immunoglobulin or complement protein deposits were detected by immunofluorescence microscopy. Electron microscopy showed no remarkable structural changes in the GBM. However, disruption of tubular basement membrane characterized by irregular thickening and splitting was prominent (Supplemental Figure 1B).

No mutation in *NPHS2* and *WT1* was detected. However, a heterozygous missense mutation was found in *NPHS1* (c.1339G>A; p.E447K) inherited from the father. The patient progressed to ESRD by the age of 13 years and underwent a living-related renal transplant from the mother. Her most recent eGFR is 114ml/min/1.73m^2.

Individual III-3 was diagnosed as CKD stage 2 while being searched for hypertension at the age of 31 years. His ultrasonography revealed increased echogenicity with normal sized kidneys. He is now 41 years old and his GFR is 59.5ml/min/1.73m^2.

Individual III-5 was diagnosed as ESRD accidentally when she was 31 years old. Polyuria and polydipsia had been present for a few years however it was not known certainly when they began. Hemodialysis had been commenced. The patient underwent a cardiac angiography at the age of 44 years, which disclosed insufficiency of the mitral valve. Thus she underwent a mitral valve replacement. She died at the age of 45 years.

Individual III-6 is sister of the individual III-5. She was diagnosed as ESRD accidentally during pregnancy when she was 25 years old. In the previous history, polyuria and polydipsia had been present for an unknown duration but not been realized. Hemodialysis had been started just after the delivery. Mitral and tricuspid

valve replacements were performed due to stenosis and regurgitation of mitral and tricuspid valves. She is 35 years old right now and is still under hemodialysis.

Individual III-7 presented with polyuria and polydipsia 4 years ago when she was 53 years old. Her initial biochemical evaluation was as follows: serum creatinine 1.3 mg/dL (normal<1 mg/dL), albumin 4.4 g/dL, pH 7.39, HCO3 24.3 mmol/L, intact parathyroid hormone 232 pg/mL (normal range 12-65), urine specific gravity 1.011, no proteinuria and normal urine sediment. Renal ultrasonography showed bilateral increased parenchymal echogenicity (i.e. grade 2) with normal sized kidneys. She was recently diagnosed as moderate mitral and aortic stenosis and underwent cardiac surgery. She is 57 years old now and her most recent eGFR is 41.5ml/min/1.73m^2.

Individual III-10 had been hospitalized due to chronic active hepatitis B at the age of 47 years and had been realized mild decrease in eGFR (95.8ml/min/1.73m^2). No proteinuria had been detected and urine specific gravity had been 1.010. He was operated for mitral insufficiency and aortic aneurism. He is 52 years old now and his most recent eGFR is 89ml/min/1.73m^2.

Individual V-2 is brother of the index case (i.e. individual V-1). He is 7 years old and apparently healthy now. Serum biochemistry was within normal limits with normal eGFR (110ml/min/1.73m^2). Urine specific gravity was 1.017 and no proteinuria was detected. No mutation in *NPHS1*, *NPHS2* and *WT1* was detected.

PN306 (DOB 20 November 1994)

A 16-year-old male presented with edema and chronic renal failure (GFR 32 ml/min/1.73m^2). His previous history was uneventful. There was no consanguinity

between parents. Severe proteinuria (2.8 grams/day) and microscopic hematuria were detected. Antinuclear and anti dsDNA were negative. Serum complement C3 and C4 levels were normal. Cardiac evaluation showed diastolic dysfunction associated with CKD. Renal ultrasound and 99^{m}Tecnetium-Dimercapto succinic acid scan disclosed only one atrophic kidney located ectopically just above the bladder. Voiding cystoureterogram showed a grade III vesico-ureteral reflux and a dilated ureter. A renal biopsy could not be performed since the patient had solitary atrophic kidney. He reached ESRD within 1 year and underwent hemodialysis. No mutation was detected in *NPHS2* and *WT1*.

PN516 (DOB 08 April 2010)

A 1.5-year-old male presented with edema and acute renal failure. Family history was remarkable with 2 renal transplantations of maternal uncle who died at the age of 30 years. Marked proteinuria (i.e. urinary protein/creatinine ratio was 49mg/mg.creatinine), hypoalbuminemia (1.7g/dL) and renal failure (eGFR 35ml/min) were noted. Serum complement C3 and C4 levels were normal. Tandem mass spectrometry and urinary organic acids were normal. Viral serology for TORCH, measles, mumps, rubella, HIV, hepatitis B, C, EBV, Parvo virus was negative. Renal ultrasonography showed bilateral enlarged kidneys and increased medullary echogenicities. Bilateral temporal hypoplasia and expansion of subarachnoid space secondary to cerebral atrophy with normal ventricular system was detected in cranial MRI, which was performed due to macrocephalie. Echocardiographic evaluation was normal. In renal biopsy, epithelial proliferation and sclerotic changes in 5/27 glomeruli, global sclerosis and adhesion in Bowman's capsule in 4/27 glomeruli, interstitial fibrosis, tubular atrophy were noted. Cyclophosphamide and prednisone

did not yield any benefit. The patient progressed to ESRD rapidly and hemodialysis was commenced. After 8 months under hemodialysis, a living related transplantation from the mother was performed at the age of 22 months. His most recent eGFR is 50ml/min. The patient had a 46, XY karyotyping and no mutation was detected in *NPHS1, NPHS2, WT1, COQ2, COQ6*.

PN521 (DOB: 19 December 2008)

He was born to non-consanguineous parents at 35.5th gestational week. When he was 40 days old, he was hospitalized due to cough and apnea. Small atrial septal defect was diagnosed. Renal USG showed increased echogenicity and loss of cortico-medullary differentiation. Laboratory values were as follows: Hemoglobin 11.3g/dL, white blood cell count 17.600/mm^3, platelet 605.000/mm^3, blood urea nitrogen 8.6mg/dL, serum creatinine 0.24mg/dL, Na^+ 118mEq/L, K^+ 5.1mEq/L, serum albumin 3.66g/dL, blood pH 7.24, $HCO3^-$ 19.4mmol/L, urine specific gravity 1004, urine protein 30mg/dL, sediment normal. Respiratroy distress gradually progressed and mechanical ventilation was required. The patient stayed in the hospital 10 months and renal functions impaired during this time window. Proteinuria (100mg/dL) and serum creatinine (3.7mg/dL) increased. Continuous veno-venous hemodiafiltration was commenced. The patient was discharged with tracheostomy and home ventilator. Marked developmental delay was observed. The patient was not able to sit, walk and speak at the age 14 months. The patient was hospitalized again due to cyanosis and hypoxia. At that time, urine protein was 300mg/dL while specific gravity was 1007. The patient progressed to end stage renal failure and continuous ambulatory peritoneal dialysis was started at the age of 15 months. He

has a completely healthy dizygotic twin who has a wild type sequence in both *ANKS6* and *NPHP2/INVS.*

4Cis3009 (DOB 18 January 1999)

This 9-year-old boy was evaluated due to elevated serum creatinine level (1.7mg/dL; eGFR 51.8ml/min) and proteinuria (500mg/d). In his previous history, polyuria, polydipsia and secondary nocturnal enuresis were present since 6 years of age. The parents were 1st degree cousin. Family history was unremarkable. No hematuria or edema was seen. Growth and development were comparable with his peers. His initial laboratory values were as follows: Hemoglobin 9.9 g/dL, white blood cell count 7.000/mm^3, platelet 494.000/mm^3, urine specific gravity 1.005, urine protein 1(+) with dipstick, urine sediment normal; serologic tests for hepatitis B and C negative; complement C3 and C4 normal, anti nuclear and anti double stranded DNA antibodies negative. A renal ultrasound showed increased echogenicity (i.e. grade 2) in normal sized kidneys. Biopsy proven nephronophtisis was diagnosed. Global sclerosis in 4 and periglomerular fibrosis in 2 out of 10 glomeruli as well as glomerular hypertrophy were noted. In electron microscopy, increased mesangial matrix and marked microvillus transformation in epithelial cells were seen. He progressed to ESRD within 3 years. He underwent hemodialysis for 1 month and was transplanted from living-related donor. Graft functions were normal for the first year however an acute rejection episode was observed due to non-compliance to the immunosuppressive drugs. He is 14 years old now and current eGFR is 30ml/min/1.73m^2.

Supplemental Table 1. The summary NGS data analysis output.

family No	target region (chr 9: 97400090-103653346)	matched Reads	total structural variants	exonic structural variants	Total SNVs	novel SNVs (dbSNP)	exonic SNVs	splice site mutation	exons not covered by NGS	SNVs in the non-coverd exons (sanger sequencing)
PN25	6 Mb	% 97,63	2	0	3739	147	0	1	19	0

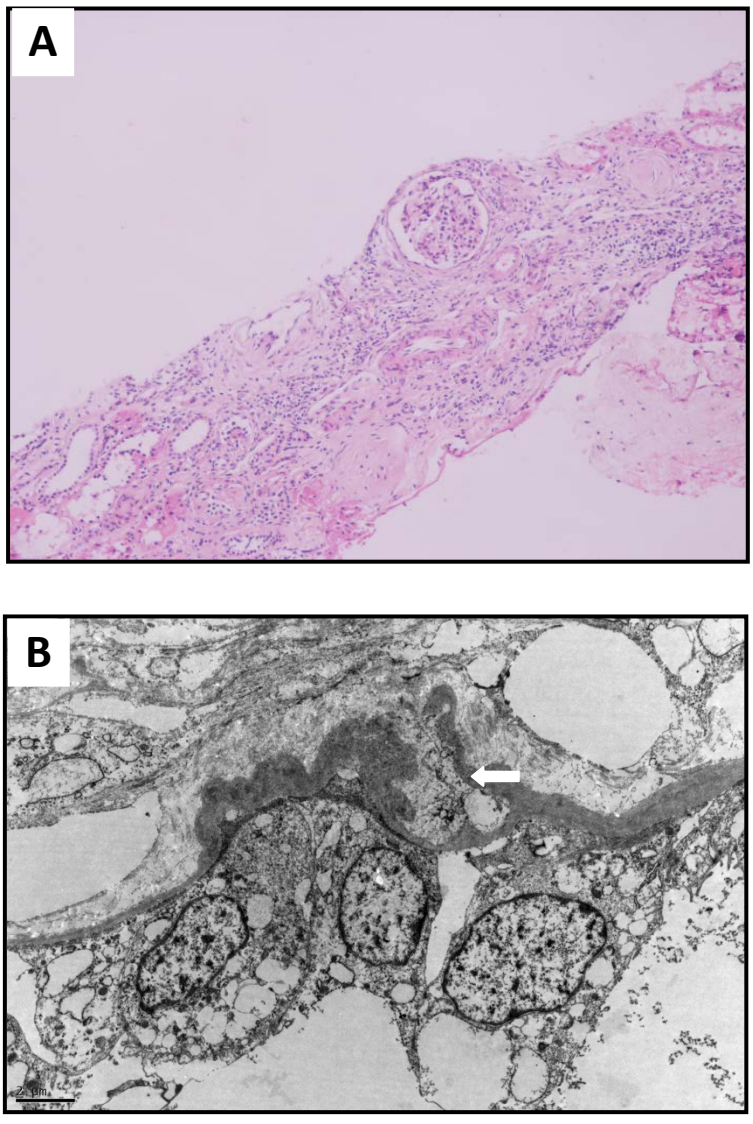
Mb: Megabase SNV: single nucleotide variant, NGS: next generation sequencing, Structural variants include insertions, deletions and inversions

Supplemental Table 2. Primer pairs used in PCR-based sequencing

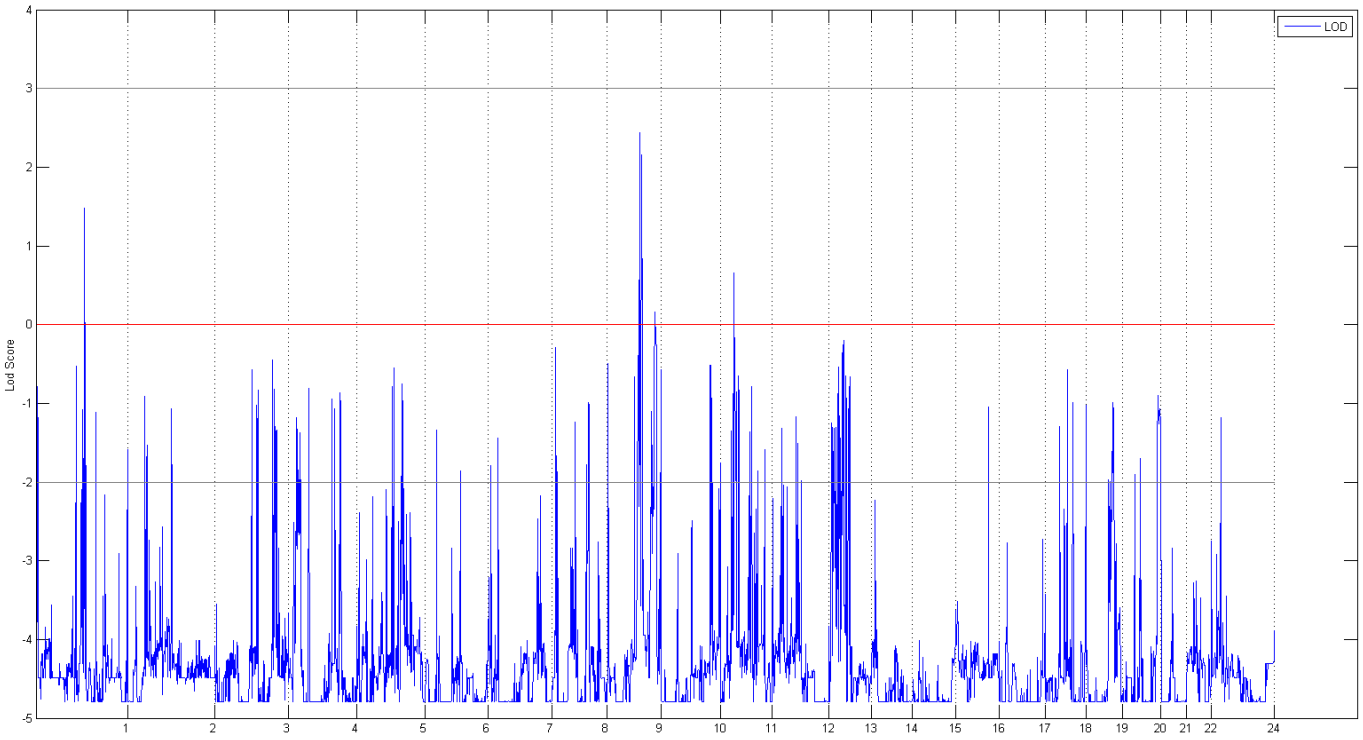
Primer name	Primer sequences (5'-3')
ANKS6-exon1	F: CGAAGAGGCAAAAGGGAACA
	R: AGGAGTCCTTAACTCTTCACCT
ANKS6-exon2	F: GCAGCTGTCAGGACTAATAA
	R: AGTAGAGCAATCCTCAGCTT
ANKS6-exon3	F: CTGTGTTGTTACACCCTTG
	R: GTCTGGAGTCTATGTGTAGAG
ANKS6-exon4	F: GTTACATGAACCACAGGAGA
	R: GCCTCAATGTCGTCATAGT
ANKS6-exon5	F: AGAGACTGAGCTTTGTAGTT
	R: AACACAAGTCACAGAGGA
ANKS6-exon6	F: GAATTACACAGACCCTGGA
	R: GACTCCCTAGCATAAACCA
ANKS6-exon7	F: TGAATGTAGTCACTGTCTCT
	R: CCCATTCCAACATCATCTTA
ANKS6-exon8	F: ACCCAGGAGAGTTGGTTT
	R: CCCTATCTGTGACCACTACAA
ANKS6-exon9	F: GAGAGCTGTAGATGCCAC
	R: AGTTGCTCTGTAGGGCTT
ANKS6-exon10	F: AGTCAGCACATAGAAGCAAG
	R: CTCTGAATTGGGAATTCACTGA
ANKS6-exon11	F: CCAATCATCACACGACAGT
	R: TGTCTTTAGTCCTGTCCCAT
ANKS6-exon12	F: GAATCAAATACCACCCATGT
	R: CCATACCTTTAGCATAGTGAA
ANKS6-exon13	F: GTTTGAAGACCACTTTACCA
	R: TCAGTGCAAGAGATCAATC
ANKS6-exon14	F: GCACTCATAGCTCATAATCAT
	R: GGTGAAGCTCTCAGATGT
ANKS6-exon15	F: ACTCGGTACTCTTTCAGTTAG
	R: AACAACATGACTAAGGCACA
INVS-exon13	F: GTGATGTAGTAGCTCCTCTT
	R: GAGGAAACAAGTAGAACACAA
FOXE1-exon1	F: CCTGAGCTCTCCGCAGAA
	R: GCAGTCGTTGAGTGTGAGGT
FOXE1-exon2	F: GCGGCATCTACAAGTTCATCA
	R: CCCGTAGAAGTCCACCGT
FOXE1-exon3	F: AGTGCGATCTTTGCCGCT
	R: CTCCTCCCGTTTACAGAGTACA
NANS-exon1	F: GTCCGAGACCACGCTCTG
	R: GATCCGAAGGATTCCGAAA
NANS-exon2	F: TGAAAGATGTCCTAATGTGTGTT
	R: GATCCGAAGGATTCCGAAA

GALNT12-exon1	F: CACTTGAAGACCCTGCCGA
	R: AGACTCAATAAGACGCGCCA
ALG2-exon1	F: GCTGATGTGGCCTAACTGAC
	R: ACTTCTCCATGTCAGTTCCGA
PTCH1-exon1	F: CGCAATGTGGCAATGGAA
	R: GCGATCCCAAAGAGTTAGAG
PTCH1-exon2	F: TGAATATTGTCTGTGTCGAGT
	R: GCGCCCAAACAATAAACAA
SLC35D2-exon1	F: CTGAGACTTACTGAAGCGTT
	R: AGTGTAGAAAGCTGAGCGA
SLC35D2-exon2	F: CTCAGATTGTTTAATAACTTGTCA
	R: GTGTCAATATGTCCTGTTGT
HABP4-exon1	F: ATAGCAAATACGGTCGCAG
	R: CTCAACTCTCCCTACTGTTC
XPA-exon1	F: GGATGACAAGAGAGCAGGTA
	R: ACAGGACGCTTTGACAAG
GABBR2-exon1	F: GGCGTGATTGATCCGTCA
	R: GGCAAATGTTACCAGCTGTG
CDC14B-exon1	F: GCCTCCATGAAGCGGAAA
	R: ACCGGTGATGTCCAGGTA
TGFBR1-exon1	F: CCGAGCAGTTACAAAGGG
	R: CCATGTTTGAGAAAGAGCAG
NR4A3-exon3	F: CAGCACCTCCATGTACTTCA
	R: TGCCGATTACAGCTCTCAA
TRIM14-exon1	F: CTCTCCTAGCTGGCTCATTA
	R: CGCTCTGAACTTTCGGTC
TRIM14-exon6	F: GCAGCAGAGTGATCTCTTC
	R: GCACTGTAGGCGTGATTG
ANKS6-cDNA-1	F: GCCTTTGCCTTTCTCTGAC
	R: TTTCTCCTCTTGTGCTCCC
ANKS6-cDNA-2	F: TGAACTGACTGGAATCCTTAA
	R: TACTACCAATGAATGCCGT
ACTB-cDNA	F: CGCAAAGACCTGTACGCCAAC
	R: GAGCCGCCGATCCACACG

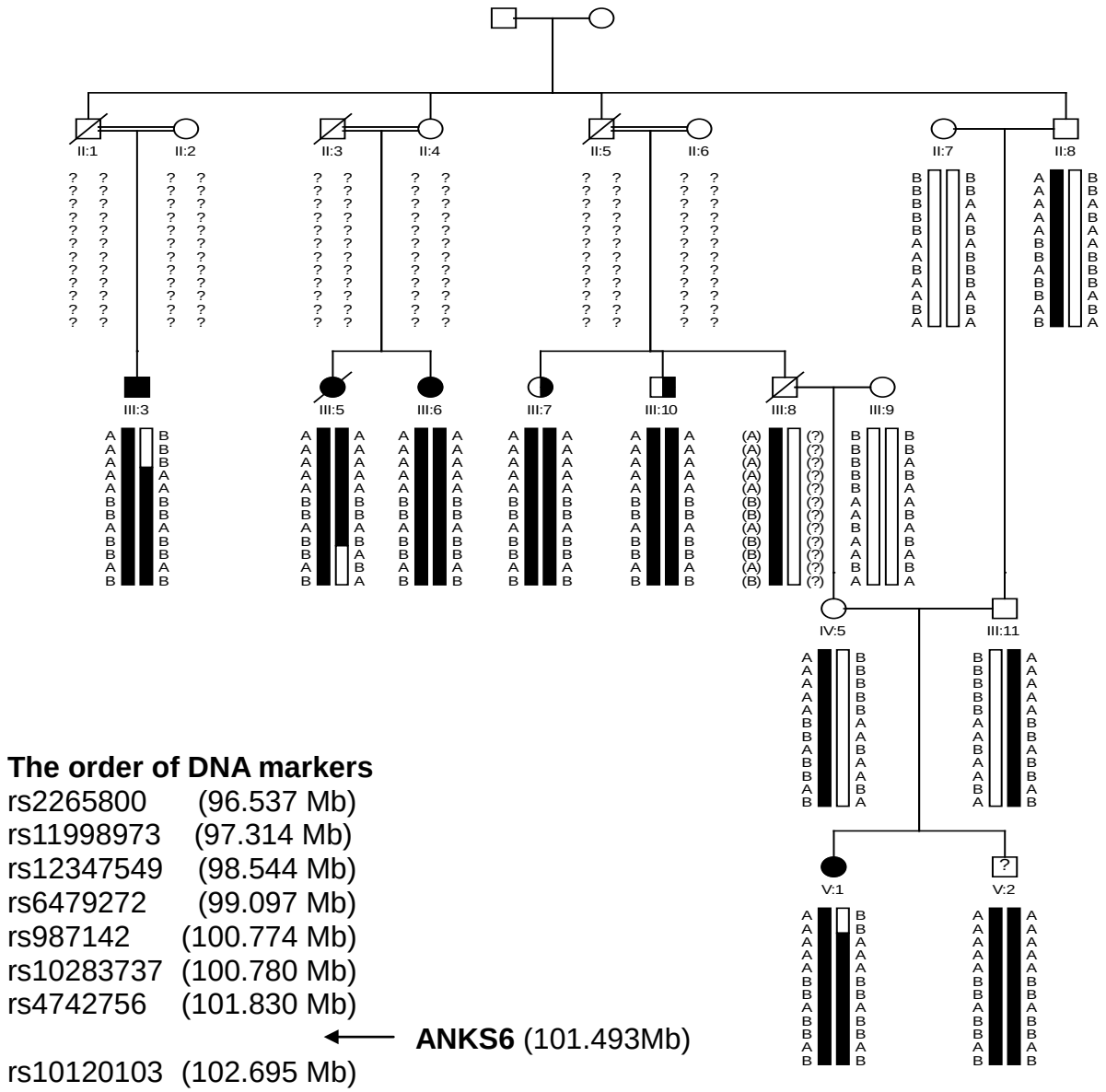
Supplemental Figure 1. (A) Light microscopy shows two sclerotic glomeruli, a glomerulus with periglomerular fibrosis, marked tubular atrophy and interstitial infiltration of mononuclear inflammatory cells (HEx200). (B) Electron microscopy shows irregular thickening and splitting (arrow) of tubular basement membrane (x8000).



Supplemental Figure 2 Graphical representation of multipoint LOD scores using the parametric linkage analysis program (MERLIN).[1] Maximum LOD scores for NPHP were observed on chromosome 9q22 residing between 97-104 cM interval.



Supplemental Figure 3: Haplotype structures with informative SNP markers selected from The critical region on chromosome 9q22. Solid bars below each individual represent affected haplotype. The order of the DNA markerswere shown on the left.



The order of DNA markers

rs2265800 (96.537 Mb)
rs11998973 (97.314 Mb)
rs12347549 (98.544 Mb)
rs6479272 (99.097 Mb)
rs987142 (100.774 Mb)
rs10283737 (100.780 Mb)
rs4742756 (101.830 Mb)

← **ANKS6** (101.493Mb)

rs10120103 (102.695 Mb)
rs2787365 (102.993 Mb)
rs1933796 (103.603 Mb)
rs10819898 (103.859 Mb)
rs1329071 (104.020 Mb)

Chromosome 9q22

Supplemental Figure 4. Wild type and mutant ANKS6 protein sequences. The sequence highlighted in blue represents aminoacids coded by exon 15 (34 aminoacids). The mutation (c.2512+2A>C) causes retention of intron 14. The aminoacid sequence highlighted in purple is predicted to be translated from intron 14 (31 aminoacids). Asterisk indicates termination codon of protein translation.

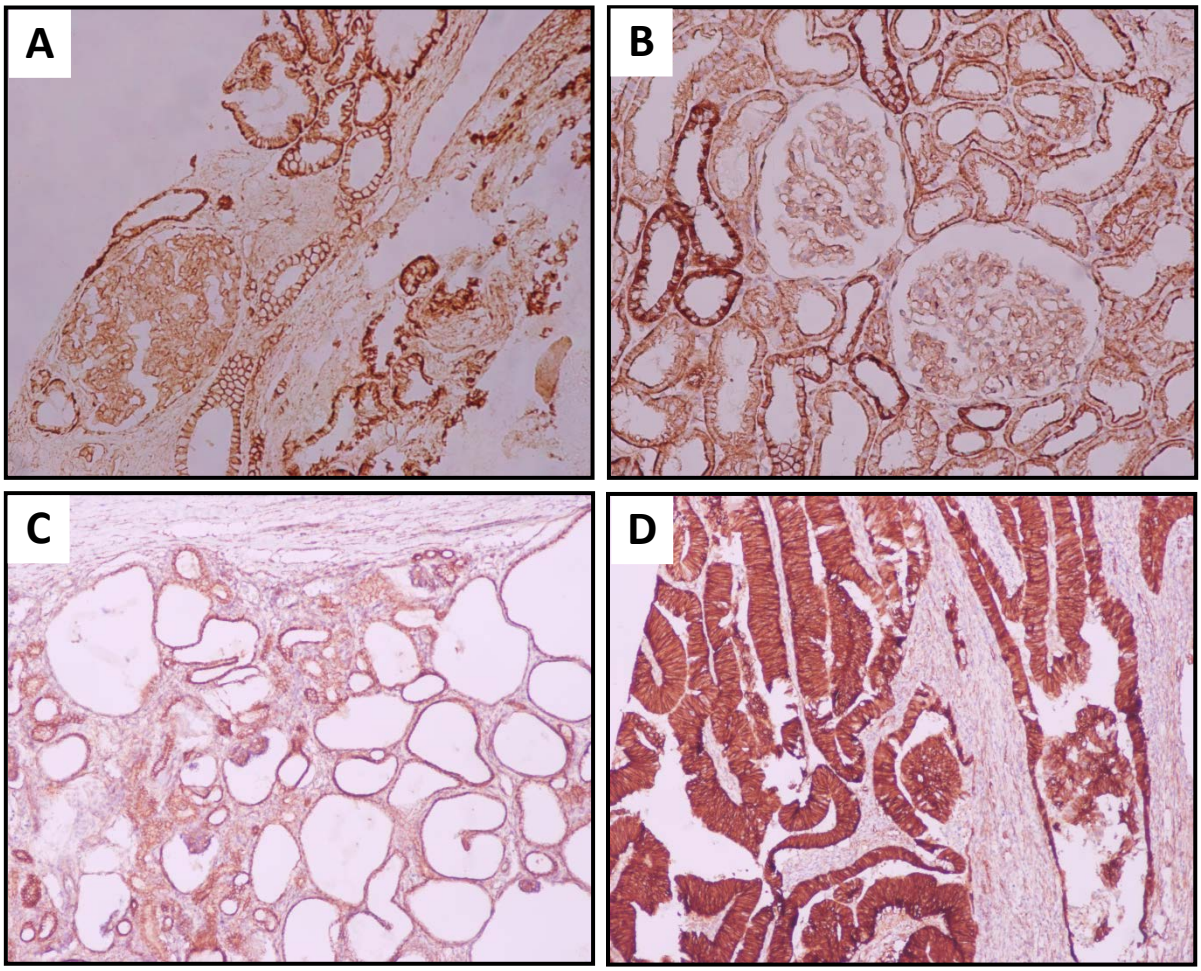
wild type protein sequence

MGEGGLPPAFQLLLRACDQGDTETARRLLEPGAAEPAERGAEPEAGAEPAGAEVAGPGAA
AAGAVGAPVPVDCSDEAGNTALQFAAAGGHEPLVRFLLRRGASVNSRNHYGWSALMQAAR
FGHVSVAHLLLDHGADVNAQNRLGASVLTVASRGGHLGVVKLLLEAGAFVDHHHPSGEQL
GLGGSRDEPLDITALMAAIQHGHEAVVRLLMEWGADPNHAARTVGWSPLMLAALTGRLGV
AQQLVEKGANPDHLSVLEKTAFEVALDCKHRDLVDYLDPLTTVRPKTDEEKRRPDIFHAL
KMGNFQLVKEIADEDPSHVNLVNGDGATPLMLAAVTGQLALVQLLVERHADVDKQDSVHG
WTALMQATYHGNKEIVKYLLNQGADVTLRAKNGYTAFDLVMLLNDPDTELVRLLASVCMQ
VNKDKGRPSHQPPLPHSKVRQPWSIPVLPDDKGGLKSWWNRMSNRFRKLKLMQTLPRGLS
SNQPLPFSDEPEPALDSTMRAAPQDKTSRSALPDAAPVTKDNGPGSTRGEKEDTLLTTML
RNGAPLTRLPSDKLKAVIPPFLPPSSFELWSSDRSRTRHNGKADPMKTALPQRASRGHPV
GGGGTDTTPVRPVKFPSLPRSPASSANSGNFNHSPHSSGGSSGVGVSRHGGELLNRSGGS
IDNVLSQIAAQRKKAAGLLEQKPSHRSSPVGPAPGSSPSELPASPAGGSAPVGKKLETSK
RPPSGTSTTSKSTSPTLTPSPSPKGHTAESSVSSSSSHRQSKSSGGSSSGTITDEDELTG
ILKKLSLEKYQPIFEEQEVDMEAFLTLTDGDLKELGIKTDGSRQQILAAISELNAGKGRE
RQILQETIHNFHSSFESSASNTRAPGNSPCA*

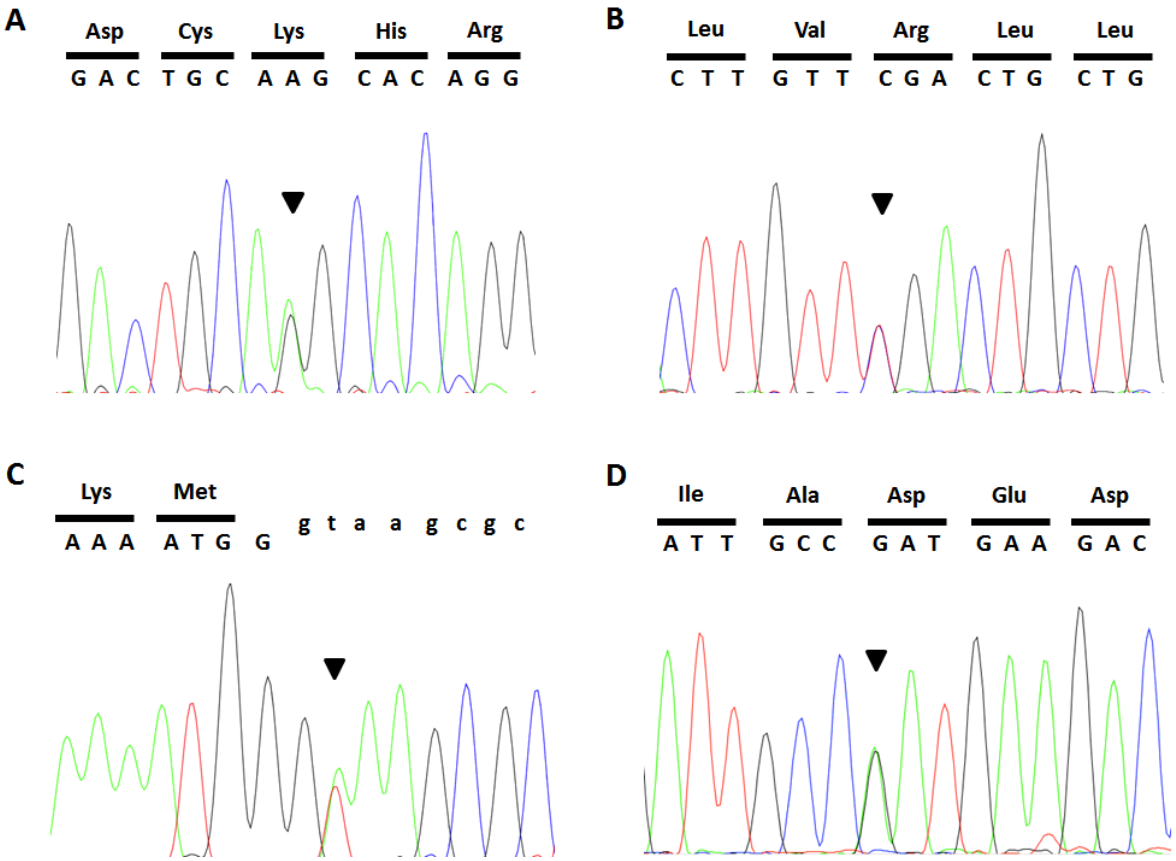
mutant protein sequence

MGEGGLPPAFQLLLRACDQGDTETARRLLEPGAAEPAERGAEPEAGAEPAGAEVAGPGAA
AAGAVGAPVPVDCSDEAGNTALQFAAAGGHEPLVRFLLRRGASVNSRNHYGWSALMQAAR
FGHVSVAHLLLDHGADVNAQNRLGASVLTVASRGGHLGVVKLLLEAGAFVDHHHPSGEQL
GLGGSRDEPLDITALMAAIQHGHEAVVRLLMEWGADPNHAARTVGWSPLMLAALTGRLGV
AQQLVEKGANPDHLSVLEKTAFEVALDCKHRDLVDYLDPLTTVRPKTDEEKRRPDIFHAL
KMGNFQLVKEIADEDPSHVNLVNGDGATPLMLAAVTGQLALVQLLVERHADVDKQDSVHG
WTALMQATYHGNKEIVKYLLNQGADVTLRAKNGYTAFDLVMLLNDPDTELVRLLASVCMQ
VNKDKGRPSHQPPLPHSKVRQPWSIPVLPDDKGGLKSWWNRMSNRFRKLKLMQTLPRGLS
SNQPLPFSDEPEPALDSTMRAAPQDKTSRSALPDAAPVTKDNGPGSTRGEKEDTLLTTML
RNGAPLTRLPSDKLKAVIPPFLPPSSFELWSSDRSRTRHNGKADPMKTALPQRASRGHPV
GGGGTDTTPVRPVKFPSLPRSPASSANSGNFNHSPHSSGGSSGVGVSRHGGELLNRSGGS
IDNVLSQIAAQRKKAAGLLEQKPSHRSSPVGPAPGSSPSELPASPAGGSAPVGKKLETSK
RPPSGTSTTSKSTSPTLTPSPSPKGHTAESSVSSSSSHRQSKSSGGSSSGTITDEDELTG
ILKKLSLEKYQPIFEEQEVDMEAFLTLTDGDLKELGIKTDGSRQQILAAISELNAGKVPF
SVSCISVFQTSESFTRVLGSWEGLTSSL*

Supplemental Figure 5. Beta-catening staining in the kidney biopsies. (A) Staining in the kidney section of the index case (V-1) (x200). Tubules are strongly stained. However, staining in the glomerulus is moderate. (B) The kidney tissue of a case with minimal change disease. A group of distal and collecting tubuli shows strong staining. (C) The kidney section of a neonate with autosomal dominant polycystic kidney disease. Both cystic glomeruli and tubuli are strongly stained. (D) Control staining of beta-catenin in a case with colon adenocarcinoma.



Supplemental Figure 6. The sequence electropherograms of the ANKS6 mutations with heterozygous condition. c.806A>G (p.Lys269Arg) (A), c.1234C>T (p.Arg412X) (B), c.907+2T>A (C) and c.937G>A (p.Asp313Asn) (D) mutations were represented (arrowheads). Codons and the corresponding amino acids are displayed above the chromatograms. Lowercase letters indicate the intronic sequence.



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