SUPPLEMENTAL INFORMATION

TABLE S-1. Clinical and demographic characteristics of the patients with mutations in the *ADCK4* gene.

ID	Mutation	Gender	Age at 1st manifestation (years)	Symptoms at onset	Initial creatinine [mg/dL]	Initial proteinuria	Initial GFR ml/min/1.73m^2	Hematuria	Histopathology	Age at ESRD (years)	Response to immunosuppresive treatment	Neurological signs and symptoms	Other extra-renal signs	Lactate (mmol/L)	CK (U/L)
I-1	c.1339dupG (p.Glu447Glyfs*10) hom.	M	14	mild edema	0.9	2.1 g/d	73	yes	FSGS	17.7	no response to steroids, CsA	—	—	1.2 (2011) 1.9 (2014)	153
I-2	c.1339dupG (p.Glu447Glyfs*10) hom.	F	7.3	mild edema	0.6	0.78 g/d	>75	no	FSGS	12.6	no response to steroids; no CsA/other treatment	epilepsy: generalised bioccipital spike and wave pattern; normal brain MRI	Large ASD, intermediate ASVD, surgically corrected	1.6	163
I-3	c.1339dupG (p.Glu447Glyfs*10) hom.	F	17	mild edema	NA	NA	NA	no	not performed	18	not treated with steroids/CsA/other	—	—	NA	CK-MB 16 U/L
I-4	c.1339dupG (p.Glu447Glyfs*10) hom.	F	27	mild edema, hypertension	NA	NA	NA	no	not performed	31	not treated with steroids/CsA/other	—	—	NA	CK-MB 23 U/L
I-5	c.1339dupG (p.Glu447Glyfs*10) hom.	F	7	incidental	NA	5-6mg/m^2/d	NA	macroscopic	not performed	— (5.3 yrs obs)	not treated with steroids/CsA/other	—	—	2.5	90
II-1	c.1339dupG (p.Glu447Glyfs*10) hom.	F	25.7	edema, vague loin pain, headaches, medullary nephrocalcinosis, hypertension	0.98	3.6 g/d	63	no	FSGS	35.4	no response to steroids; no CsA/other treatment	—	—	1.39	NA
II-2	c.1339dupG (p.Glu447Glyfs*10) hom.	M	16.7	incidental	14.2	300 mg/dL in spot urine	4.9	no	not performed	16.7	not treated with steroids/CsA/other	—	trace MI, TI	NA	101
II-3	c.1339dupG (p.Glu447Glyfs*10) hom.	M	13.5	incidental	0.99	6.76 g/d	120	no	FSGS/GGS	16.6	no response to steroids; no CsA/other treatment;	mild mental retardation	—	1.9	NA
II-4	not tested	M	22	headaches	NA	NA	<15	NA	not performed	22	not treated with steroids/CsA/other	NA	NA	NA	NA

III-1	c.293T>G (p.Leu98Arg) hom.	F	5.9	incidental	0.4	1.39 g/m^2/d	>75	no	FSGS	-- (8.4 yrs obs)	partial response to steroids; partial response to CsA	—	primary nocturnal enuresis	0.9	99
III-2	c.293T>G (p.Leu98Arg) hom.	M	13.3	mild edema	0.6	2 g/m^2/d	>75	no	FSGS	14	partial response to steroids	—	primary nocturnal enuresis	0.7	144
IV-1	c.532C>T (p.Arg178Trp) hom.	M	14.3	hypertension, polyuria, polidypsja	14.6	1.5 g/d	7.5	no	FSGS	14.3	not treated with steroids/CsA/other	—	hypermetropia, astigmatism Pre-term (born at 34wks)	NA	NA
IV-2	c.532C>T (p.Arg178Trp) hom.	M	9.8	mild edema, hypertension	13.4	2.5 g/d	2.5	no	FSGS	9.8	not treated with steroids/CsA/other	—	hypermetropia, astigmatism	NA	NA
V-1	c.293T>G (p.Leu98Arg) hom.	F	13.5	moderate edema	1.5	+++ (dipstick)	64	no	FSGS/GGS	16.1	not treated with steroids/CsA/other	—	lupus-like symptoms	0.5-3.3	NA
V-2	c.293T>G (p.Leu98Arg) hom.	F	27	incidental	0.51	0.38 g/d	136	no	not performed	— (0.4 yrs obs)	not treated with steroids/CsA/other CoQ10 supplementation	—	—	NA	normal
VI-1	c.1339dupG (p.Glu447Glyfs*10) hom.	M	14.9	chronic renal failure	15	1.3 g/m^2/d	3.5	yes	not performed	14.9	not treated with steroids/CsA/other	—	—	NA	2098 at acute renal decompensation 69 (2014)
VI-2	c.1339dupG (p.Glu447Glyfs*10) hom.	F	13.2	hypertension	5.8	0.36 g/mmol uAlbCr	12	yes (+++)	not performed	13.2	not treated with steroids/CsA/other	Epilepsy (grand mal) diagnosed at 10yrs	—	0.9	88
VI-3	c.1339dupG (p.Glu447Glyfs*10) hom.	M	18	chronic renal failure	NA	NA	NA	NA	not performed	18	not treated with steroids/CsA/other	—	—	NA	NA
VI-4	c.1339dupG (p.Glu447Glyfs*10) hom.	M	9	incidental, asymptomatic	0.53	0.044 g/mmol uAlbCr	142	no	not performed	— (0.3 yrs obs)	not treated with steroids/CsA/other CoQ10 supplementation	—	—	1.1	78

VII-1	c.748G>A (p.Asp250Asn) hom.	M	16.9	hypertension, headaches	2.5	2.64g/m^2/d	28	yes	FSGS	17.4	no response to steroids; no CsA/other treatment	—	—	NA	NA
VII-2	c.748G>A (p.Asp250Asn) hom.	F	13.4	mild edema, hypertension, headaches	4.0	0.13g/m^2/d	15	no	FSGS	13.7	no response to steroids; no CsA/other treatment	—	—	NA	NA
VIII-1	c.645delT (p.Phe215Leufs*14) hom.	M	15.1	mild edema, hypertension	7.4	0.18g/m^2/d	9.5	no	FSGS	15.8	no response to steroids; no CsA/other treatment	—	—	NA	NA
IX-1	c.1199_1200dupA (p.His400Asnfs*11) hom.	M	10.8	edema	NA	NA	NA	no	FSGS – tip lesion	15.9	no response to steroids; no CsA/other treatment	—	—	NA	NA
X-1	c.929C>T (p.Pro310Leu) htz; c.1493_1494CC>AA (p.Ala498Glu) htz.	F	5.1	incidental	0.83	0.3 g/d	57	no	FSGS NOS	13.6	not treated with steroids/CsA/other	Generalized seizures 2° to hypertension while on PD; brain MRI: PRES; inconstant defects of visual fields; agoraphobia	—	0.81	38
XI-1	c.645delT (p.Phe215Leufs*14) hom.	M	14.2	edema, fatigue, chronic renal failure and 2° heart failure	18.4	0.56 g/mmol uPCr	4	no	FSGS NOS	15.2	not treated with steroids/CsA/other	—	retinitis pigmentosa; hypospadias	NA	1179 at acute renal decompensation and 2° heart failure
XII-1	c.1339dupG (p.Glu447Glyfs*10) hom.	M	17.6	chronic renal failure	NA	'nephrotic-range'	NA	yes	cFSGS	18.0	no response to steroids, MMF	—	—	NA	NA

Legend:

NA – not available; hom – homozygous; htz – heterozygous; M – male; F – female; uPCr – urinary protein/creatinine ratio; uAlbCr – urinary albumin/creatinine ratio; FSGS – focal segmental glomerulosclerosis; cFSGS – collapsing FSGS; NOS – non otherwise specified; GGS – global glomerulosclerosis; ESRD – end-stage renal disease; CsA – *ciclosporin A*; MMF – *Mycophenolate mofetil*; PD – peritoneal dialysis; US – ultrasound; MRI – magnetic resonance imaging; EEG – electroencephalography; PRES – *posterior reversible encephalopathy syndrome;* MI – mitral insufficiency; TI – triscuspid insufficiency; ASD – atrial septum defect; ASVD – atrioventricular septal defect;   CK – creatine kinase; CK-MB – creatine kinase MB isoenzyme;

S-2 Detailed clinical information on the subjects placed on CoQ10 supplementation at early stage of *ADCK4* glomerulopathy.

Patient VI-4 was diagnosed with mild albuminuria at still normal GFR at 9 years of age. After mutation confirmation he received CoQ10 supplementation at 30mg/kg per day. Six weeks later albuminuria had dropped by 80%. The patient complained of mild side effects of treatment including loss of appetite and abdominal pain.

Patient V-2 was diagnosed with mild proteinuria at age 27 years. After confirmation of the mutation she received CoQ10 at a starting dose of 10mg/kg per day. Albuminuria decreased by approximately 50% within 6 weeks. Moreover, she subjectively reported better physical fitness and reduced fatigue and weakness. The patient refused to increase the COD10 dose to the recommended 30 mg/kg/d due to financial constraints.

Figure S-3. Family Pedigrees

Family pedigrees showing affected members with confirmed *ADCK4*-related glomerulopathy.

Due to limited space and/or scarce family information, some family members are shown jointly (i.e. the number of siblings is indicated by the corresponding digit within the ideogram).
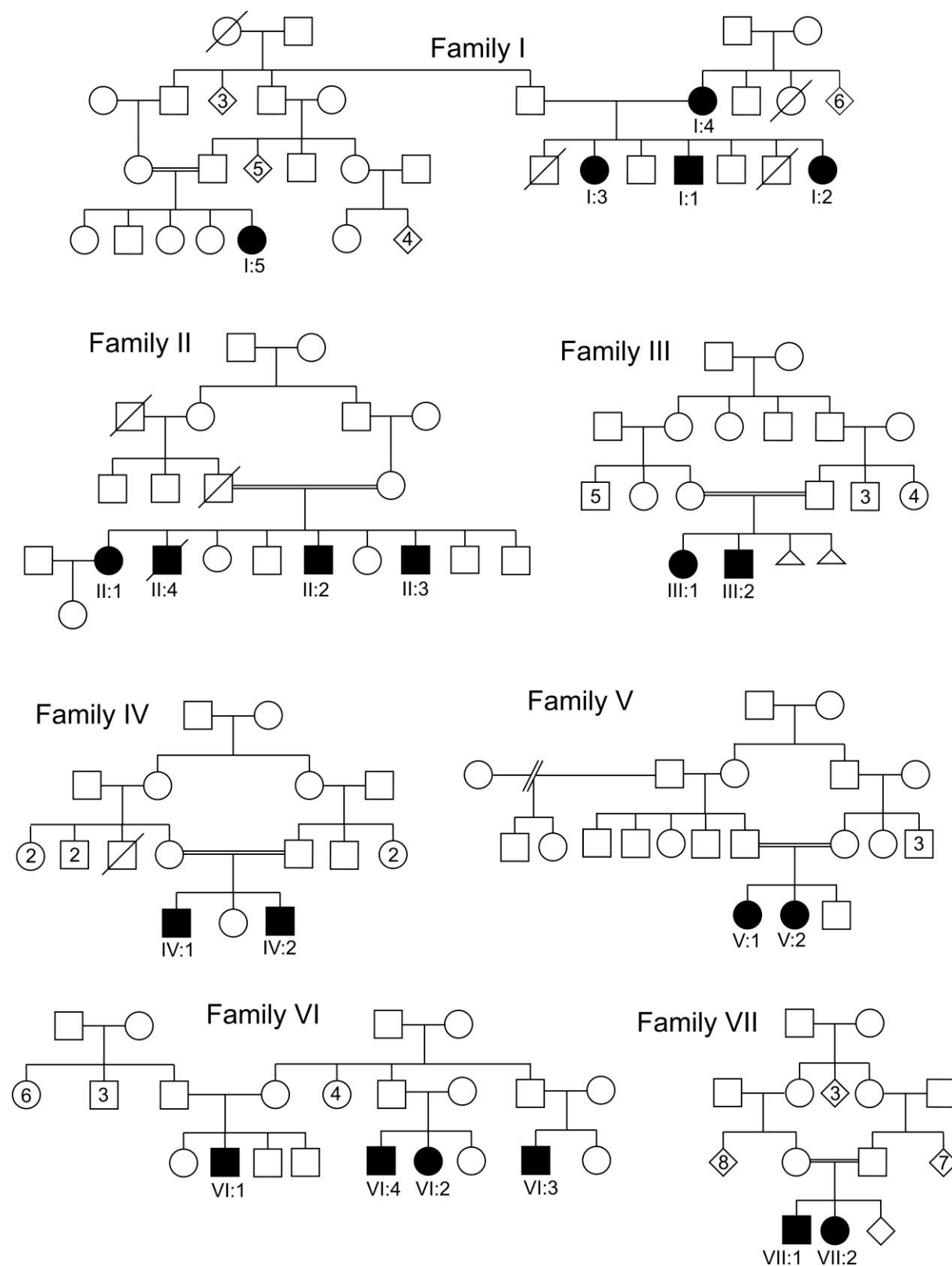
S-4   EVALUATION OF THE PATHOGENIC CHARACTER OF THE DETECTED NOVEL SEQUENCE VARIANTS

A. In-silico analyses of the effect on protein structure and function

The absence of structural information for ADCK4 precludes detailed assessment of mutation effects on protein structure and function (Protein Data Bank; PDB last accessed 12 Oct, 2014). Nevertheless, the variants are highly likely to be pathogenic in view of the results obtained from a number of *in-silico* prediction tools (as summarized in Table 1):

1. Three databases, namely ExAC, NHLBI ESP and 1000Genomes, comprising jointly sequencing information of ~ 70,000 individuals were searched to estimate minor allele frequency of the detected variants. The three missense variants were found to be **novel**, while c.1339dupG allele has been reported at a frequency of 3:100,000. The latter is in line with our observation that this variant is present in general Turkish population with an estimated frequency of ~ 3:1,000 (Table 1). It most likely points towards a putative **founder effect** in the local population. Indeed, this *ADCK4* mutation was found in homozygous state in four apparently unrelated Kurdish families originating from a region in South Eastern Turkey.

2. Multiple-alignment analysis of orthologs of ADCK4 from different species was performed using ClustalW algorithm in order to identify conserved amino acid residues. c.293T>C is predicted to produce a residue change from hydrophobic leucine to hydrophilic arginine in a highly conserved lipophilic helical transmembrane domain. The predicted change most likely disturbs the location of helical structure as it changes its hydrophobicity profile significantly, probably causing its misplacement in the membrane. c.929C>T is predicted to affect another highly conserved residue, Pro310 lying in the critical ABC1 sub-domain of the protein kinase. The exceptional conformational rigidity of proline affects the secondary structure of protein near a proline residue. Its replacement by a hydrophobic branched-chain leucine possibly would result in disruption of the domain structure and hence its proper functioning. The third missense variant c.1493_1494CC>AA is predicted to affect an evolutionarily not conserved alanine residue at the C-terminal part of the protein. Nevertheless, the residue lies adjacent to a region of high homology and the steric hindrance caused by significantly bigger side chain of glutamine may influence protein folding in that region. The forth variant c.1339duG results in a frameshift and leads to premature truncation of the predicted protein.

3. The three most commonly used bioinformatical predictors of variants' pathogenecity, namely PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/) and Mutation Taster (http://www.mutationtaster.org/) predicted all novel variants to be highly deleterious (Table 1). The only exception regarded p.Ala498Glu substitution, that was classified as pathogenic by SIFT and Mutation Taster but tolerable by PolyPhen-2.

B. *Verification of splicing signals*

The effect of the detected mutations on pre-mRNA splicing was performed using web *in silico* predictor Human Splicing Finder 3.0 (http://www.umd.be/HSF3/) to estimate possible alterations in either exonic splicing enhancer or the splice site acceptor/donor motifs. All variants were identified as having potential effect on splicing. The results of the analyses are summarized in the Table 1.

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S-5 DETAILED METHODS DESCRIPTION

Sanger sequencing

DNA was extracted from peripheral blood following the standard phenol-chloroform protocol or using commercially available kits (Qiagen; Hilden, Germany). The exons of *ADCK4* gene with mutations identified by high-throughput sequencing together with their adjacent intronic junctions were analyzed by direct sequencing using ABI3130 Genetic Analyser (Applied Biosystems, Foster City, CA). The NCBI 544aa isoform A of *ADCK4* (NM_024876.3), corresponding to ENSEMBL transcript ENST00000324464 (ENSG00000123815) was used as reference sequence.

Homozygosity mapping

In Family I, the index patient (I-3) and her relatives (cases: I-1, I-2 and five non-affected members of the family) were genotyped using 250K SNP array (Affymetrix, Santa Clara, CA) according to the manufacturer's protocol. Genotype files (CHP files) were generated with Affymetrix GTYPE software and were transferred to the VIGENOS (Visual Genome Studio) program (Hemosoft, Ankara, Turkey), which facilitates visualization of a large quantity of genomic data in comprehensible visual screens. We identified only one uninterrupted homozygous region across the genome, which was located on chromosome 19.

Exome Capture and Sequencing

Illumina system (Illumina, San Diego, CA) was used for whole-exome sequencing experiments. Genomic DNA samples were prepared for sequencing by using the Illumina TruSeq Sample Preparation kit. Exonic regions of the samples were captured by Illumina TruSeq Exome Enrichment Kit. Illumina TruSeq PE Cluster Kit v3-cBot-HS was used for paired-end cluster generation and TruSeq SBS Kit v3-HS reagent kit used for sequencing the post-capture libraries. Initial clustering was performed on an Illumina cBot machine. Paired-end sequencing was done on an Illumina HiSeq 2000 system with read length of 104. All procedures were carried out according to the manufacturer's instructions. Base calling and image analysis was done using Illumina's Real Time Analysis software version 1.13 with default parameters.

High-throughput Sequencing

Different approaches were performed. In the first one, all exons and adjacent intronic boundaries of *ADCK4* were targeted by a custom SeqCap EZ choice sequence capture library (NimbleGen, Madison, Wisconsin, USA) as part of a large NGS multi-gene panel for FSGS and related glomerulopathies and subsequently sequenced on an Illumina MiSeq or HiSeq platform according to the manufacturer´s protocol and as described previously. Patients described in this manuscript were analyzed with an

average coverage of more than 200-fold. Bioinformatic analysis was performed using SeqPilot SeqNext module[TM] (v3.5.2, JSI medical systems, Kippenheim, Germany) and in-house bioinformatic pipelines established at the Center for Human Genetics at Bioscientia. All mutations were confirmed by Sanger sequencing.

The second approach comprised a custom-designed multiplex PCR kit (MASTR FSGS, Multiplicom, Niel, Belgium) was used to amplify 546 coding exons of 31 glomerulopathy-related genes, *ADCK4* included. PCR performed on 8 to 16 different DNA samples were therefore barcoded, pooled and sequenced paired end (2x300 bp) with MiSeq® Reagent Kit v3 on the MiSeq System (Illumina, San Diego, CA) generating a maximum of 25 million reads and 15 Gb of sequence. The average coverage was more than 500-fold. The pilot study used the beta (evaluation) version of the kit, hence further technical description of the methodology remains confidential at this point.

Alignment, post-processing, calling and annotation

Aligment was made by BWA. Downstream processing was carried out with the Genome Analysis Toolkit (GATK), SAMtools, and Picard, following documented best practices *(www.broadinstitute.org/ gatk/guide/topic?name=best-practices)*. Variant calls were made both by the GATK Unified Genotyper and SAM-tools. The two calling sets were merged using GATK combined variant.

The variants were evaluated using a software system developed by the Paris Descartes University Bioinformatics platform. All the annotation processes were based on the Ensembl database (ver. 74), dbSNPs (ver. 135), EVS (ESP6500SI-V2), 1000 Genomes Project (release date: 21.05.2011), and local SNPs databases (>3000 full exomes).

Statistics

STATISTICA 9.1 (StatSoft; Tulsa, OK, USA) was the data analysis software system used for statistical analyses. Frequencies were compared using chi-squared tests with continuity corrections or Fisher exact test with Freeman-Halton extension for 2x3 and 2x4 tables when applicable. For continuous variables, differences between groups were evaluated using Kruskal-Wallis test for global and Mann-Whitney U tests for pairwise comparisons. Kaplan-Meier survival probability estimates and log-rank tests were performed to estimate the effect of mutations on long-time kidney survival rates. Due to the exploratory character of the analysis, no adjustment for multiple comparisons was done.

S -6 PODONET COLLABORATORS

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