Supplemental Methods – Sequencing and variant selection

The genomic data presented in this paper is derived from SOLiD 5500XL system (Life Technologies) sequencing data. All sequencing and variant selection was performed at the ISO15189 accredited Genome Diagnostics section of the Department of Genetics, UMC Utrecht (The Netherlands). This laboratory has now moved from SOLiD sequencing to whole exome sequencing on an Illumina HiSeq platform, allowing for all genepanels to be derived from whole exome data (see Supplemental Table 1).

SOLiD sequencing

For each patient genomic DNA was isolated from a peripheral blood sample. Subsequently, a sequencing library was prepared from the sheared genomic DNA. Each sequencing library, corresponding with a single patient, received a unique 10 nucleotide barcode allowing a cost-effective approach of ~50 samples per single enrichment procedure. Libraries were pooled and target DNA capture was performed with a custom-designed Agilent SureSelectXT assay containing the ~225 gene panel ('RENome', see Supplemental Table 1). All sequencing acquired an average depth of ~100X horizontal coverage to allow for optimal variant calling.

Variant calling and filtering

Variant calling and filtering was performed using the Cartagenia BENCHlab NGS module (V.3.1.2), with a validated filtering tree. To exclude common variants, variants were compared with our in-house database, the Exome Variant (6500 exomes), dbSNP, and GoNL (Genome of the Netherlands) databases. Non-synonymous variants, nonsense variants, essential splice site variants or coding frame-shift insertions or deletions were selected. Variants were subsequently analyzed with in silico prediction programs (a.o. Polyphen2, SIFT, GERP and Grantham scores, and multiple splice-site prediction programs) in the Alamut mutation interpretation software program (V.2.6.0) to determine the possible clinical relevance. All probable pathogenic mutations were independently validated by Sanger sequencing.

Mosaicism analysis

To determine whether a patient was mosaic for a specific variant, genomic DNA was isolated from a peripheral blood sample and a semi-quantitative Sanger analysis was applied. In this analysis the height and intensity of the Sanger sequencing peak at the genomic location of the variant of interest is compared with those in healthy controls and family members who are proven not to be mosaic for that variant. The relative height and intensity provides an estimation of the mosaicism percentage in white blood cells. In this paper, semi-quantitative Sanger analysis was only performed in peripheral blood, not in any other tissues.

Supplemental Table 1 - Genes included in the genepanels mentioned in this paper*, in alphabetical order, per targeted genepanel

FSGS	Nephrotic syndrome and FSGS	Broad renal diseases ('RENome')							
ACTN4	*ACTN4*	*ACE*	*BBS2*	*CHRM3*	*FAM58A*	*IFT140*	*NPHP1*	*SARS2*	*THBD*
APOL1	*ADCK4*	*ACTN4*	*BBS4*	*CLCN5*	*FAN1*	*IFT172*	*NPHP3*	*SCARB2*	*TMEM138*
ARHGAP24	*ARHGAP24*	*ADAMTS13*	*BBS5*	*CLCNKA*	*FAT1*	*IFT43*	*NPHP4*	*SCNN1A*	*TMEM216*
CD2AP	*ARHGDIA*	*ADCK4*	*BBS7*	*CLCNKB*	*FGF23*	*IFT80*	*NPHS1*	*SCNN1B*	*TMEM231*
CFH	*CD151*	*AGT*	*BBS9*	*CLDN16*	*FGFR1*	*INF2*	*NPHS2*	*SCNN1G*	*TMEM237*
CLCN5	*CD2AP*	*AGTR1*	*BICC1*	*CLDN19*	*FH*	*INPP5E*	*NR3C2*	*SDCCAG8*	*TMEM67*
COL4A3	*COQ2*	*AGXT*	*BSND*	*CNNM2*	*FLCN*	*INVS*	*OCRL*	*SEC63*	*TRIM32*
COL4A4	*COQ6*	*AHI1*	*C3*	*COL4A3*	*FN1*	*IQCB1*	*OFD1*	*SIX1*	*TRPC6*
COL4A5	*CUBN*	*ALDOB*	*C5orf42*	*COL4A4*	*FRAS1*	*ITGA3*	*PAX2*	*SLC12A1*	*TRPM6*
GLA	*DGKE*	*ALMS1*	*CA2*	*COL4A5*	*FREM1*	*ITGA8*	*PAX8*	*SLC12A3*	*TSC1*
INF2	*FAT1*	*ANKS6*	*CACNA1S*	*COQ2*	*FREM2*	*JAG1*	*PDSS1*	*SLC22A12*	*TSC2*
LMX1B	*INF2*	*APOL1*	*CASR*	*CPT2*	*FXYD2*	*KAL1*	*PDSS2*	*SLC2A2*	*TTC21B*
MYH9	*ITGA3*	*APRT*	*CC2D2A*	*CSPP1*	*G6PC*	*KCNJ1*	*PHEX*	*SLC2A9*	*TTC8*
MYO1E	*ITGB4*	*AQP2*	*CD151*	*CTNS*	*GALNT3*	*KCNJ10*	*PKD1*	*SLC34A1*	*UMOD*
NPHS1	*LAMB2*	*ARHGDIA*	*CD2AP*	*CUL3*	*GALT*	*KIF7*	*PKD2*	*SLC34A3*	*UPK3A*
NPHS2	*LMX1B*	*ARL13B*	*CD46*	*CYP24A1*	*GATA3*	*KLHL3*	*PKHD1*	*SLC37A4*	*VHL*

PAX2	*MYO1E*	*ARL6*	*CDKN1C*	*DGKE*	*GDNF*	*LAMB2*	*PLCE1*	*SLC3A1*	*WDPCP*
TRPC6	*NPHS1*	*ARSA*	*CEP164*	*DMP1*	*GLA*	*LMX1B*	*PRKCSH*	*SLC41A1*	*WDR19*
TTC21B	*NPHS2*	*ATP6V0A4*	*CEP290*	*DSTYK*	*GLIS2*	*LRIG2*	*PSAP*	*SLC4A1*	*WDR35*
WT1	*PDSS1*	*ATP6V1B1*	*CEP41*	*DYNC2H1*	*GPC3*	*LZTFL1*	*PTPRO*	*SLC4A4*	*WDR60*
	PDSS2	*ATP7B*	*CFB*	*EGF*	*GRHPR*	*MET*	*PYGM*	*SLC7A9*	*WNK1*
	PLCE1	*AVP*	*CFH*	*EHHADH*	*GRIP1*	*MKKS*	*REN*	*SLC9A3R1*	*WNK4*
	PTPRO	*AVPR2*	*CFHR1*	*EVC*	*HNF1B*	*MKS1*	*RET*	*SMARCAL1*	*WNT4*
	SCARB2	*B9D1*	*CFHR3*	*EVC2*	*HOGA1*	*MYH9*	*ROBO2*	*SOX17*	*WT1*
	SMARCAL1	*B9D2*	*CFHR4*	*EYA1*	*HPRT1*	*MYO1E*	*RPGRIP1*	*STX16*	*XDH*
	TRPC6	*BBS1*	*CFHR5*	*FAH*	*HPSE2*	*NEK1*	*RPGRIP1L*	*TCTN1*	*XPNPEP3*
	TTC21B	*BBS10*	*CFI*	*FAHD2A*	*HSD11B2*	*NEK8*	*SALL1*	*TCTN2*	*ZNF423*
	WT1	*BBS12*	*CHD7*	*FAM20A*	*IFT122*	*NOTCH2*	*SALL4*	*TCTN3*	

FSGS=focal segmental glomerulosclerosis,

* The panels in this table are the genepanels our facility offered up to December 2017. Currently all genepanels are derived from whole exome sequencing data, although one can still have only a specific genepanel analyzed to reduce the risk of incidental findings. For the full list of up-to-date whole exome sequencing-derived panels see https://www.umcutrecht.nl/nl/Ziekenhuis/Professionals/Diagnostiek-aanvragen/Genoomdiagnostiek/Aanvraagformulieren