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Supplemental Data

Heterozygous Loss-of-Function *SEC61A1* Mutations Cause Autosomal-Dominant Tubulo-Interstitial and Glomerulocystic Kidney Disease with Anemia

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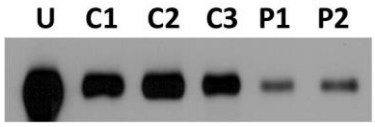
Supplementary Figures and Table

Figure S1: Western blot of urinary uromodulin



U-umod isolated from urine (0,26 µg)
C1,2,3-controls (urinary creatinine 1.9, 9.4 and 18.3 respectively)
P1-patient (father = 2-II:2)
P2-patient (index case = 2-III:1)

Urines loaded relatively to urinary creatinine.

Figure S2: Results of linkage analysis

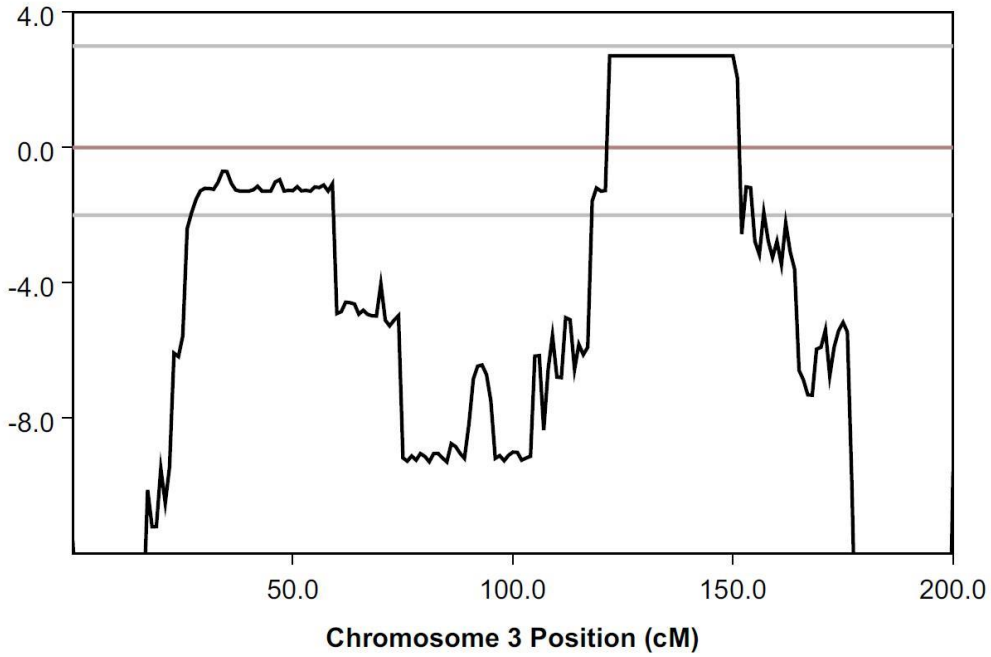
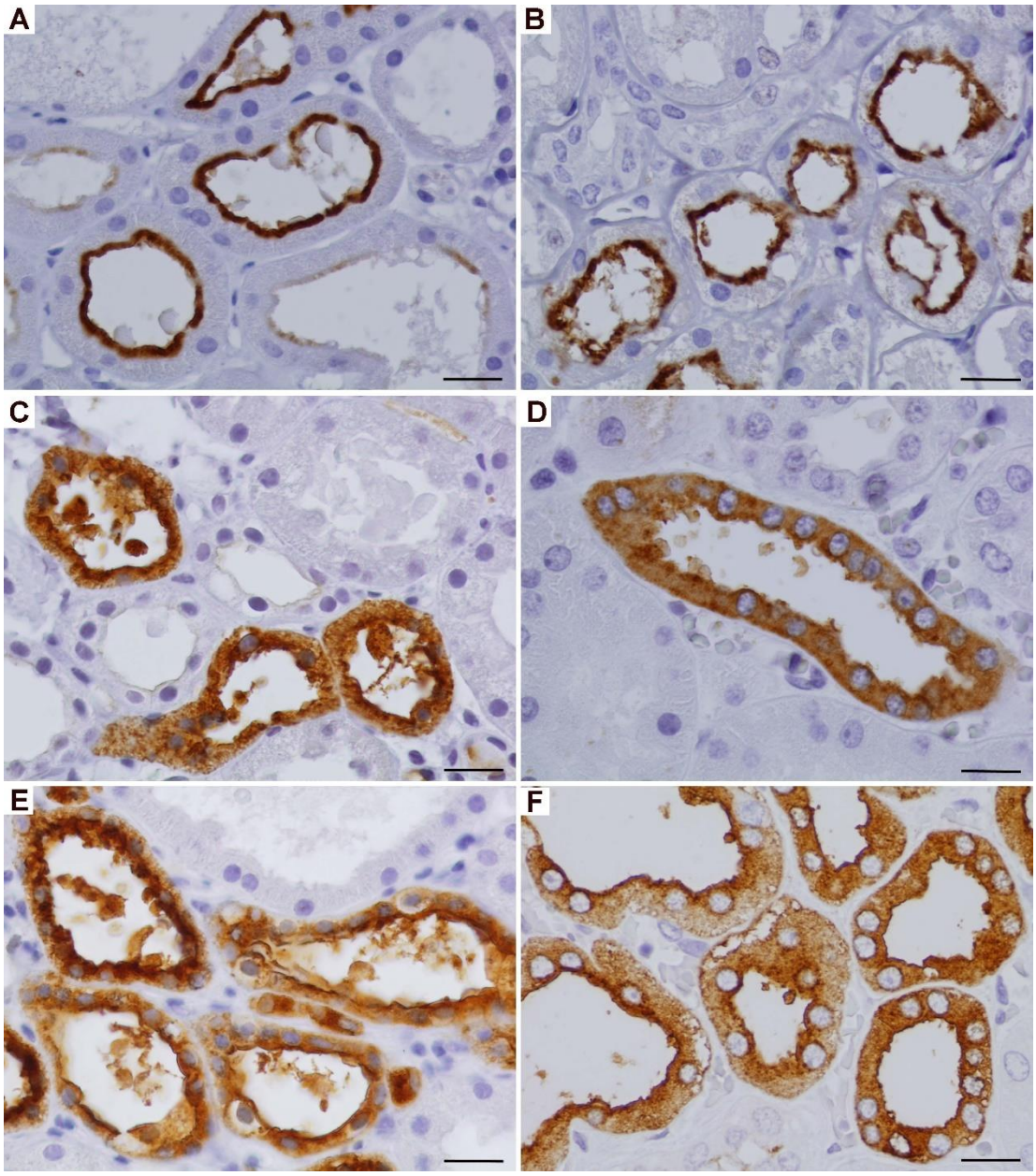


Figure indicates candidate region with a maximum LOD-score of 2.7 on chromosome 3q14-25.1. The candidate region spans genomic positions 120M – 152M bp.

Figure S3: Immunohistochemical detection of several glycosylated proteins at apical membranes of renal tubular cells in family 1 member II:3 with the p.Thr185Ala mutation



Urat transporter SLC22A12 at apical membranes of proximal tubules in a patient (A) and a control (B). Uromodulin intracytoplasmic positive staining accented at apical membranes of distal tubules in a patient (C) and a control (D). Strong positivity for MUC1 protein at apical membranes of tubular cells in a patient (E) and a control (F). Scale bars represent 30µm.

Figure S4: High magnification images of mislocalisation of SEC61A1 after transient expression

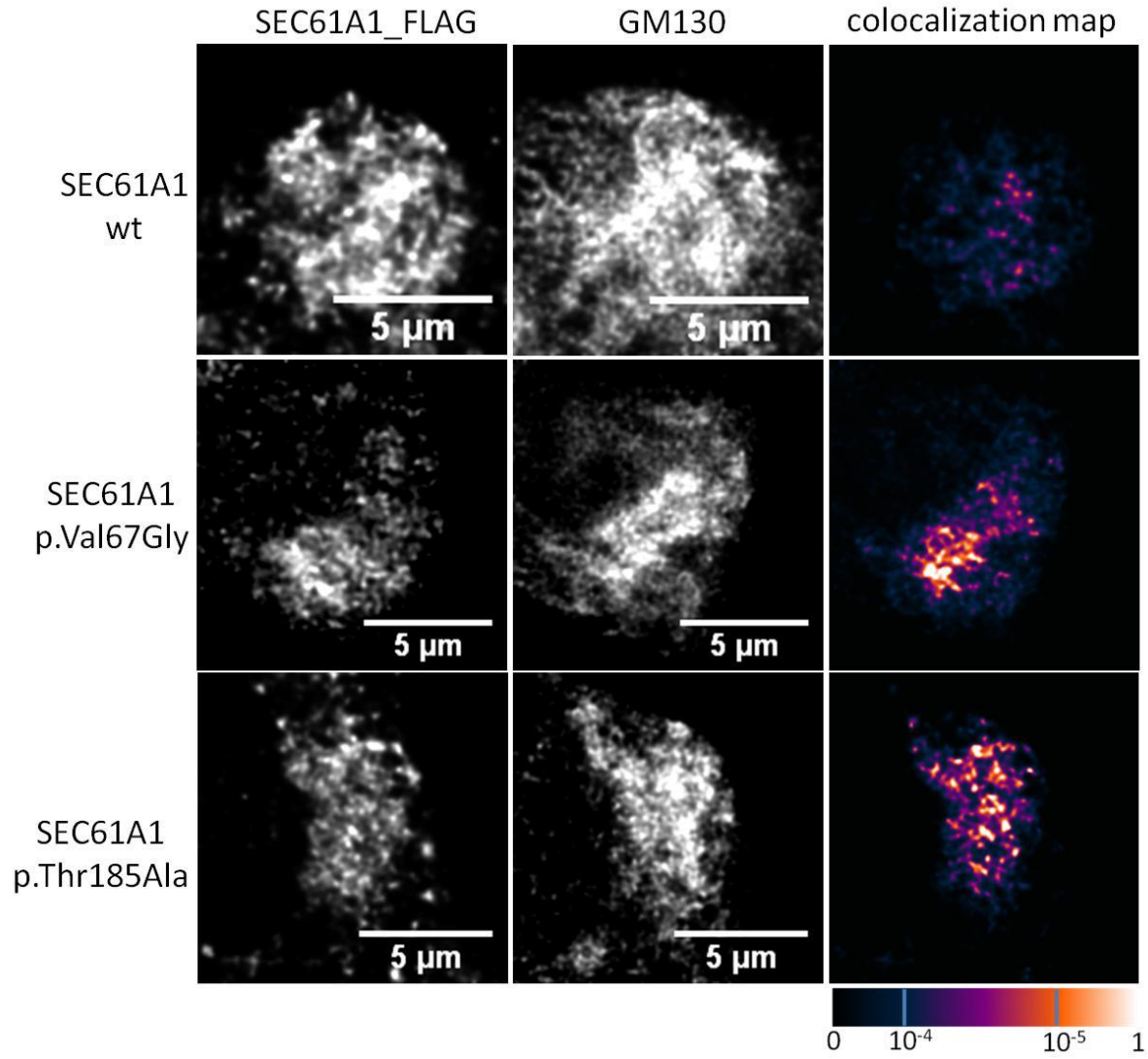


Figure S5: High magnification images of mislocalisation of SEC61A1 in kidney biopsy

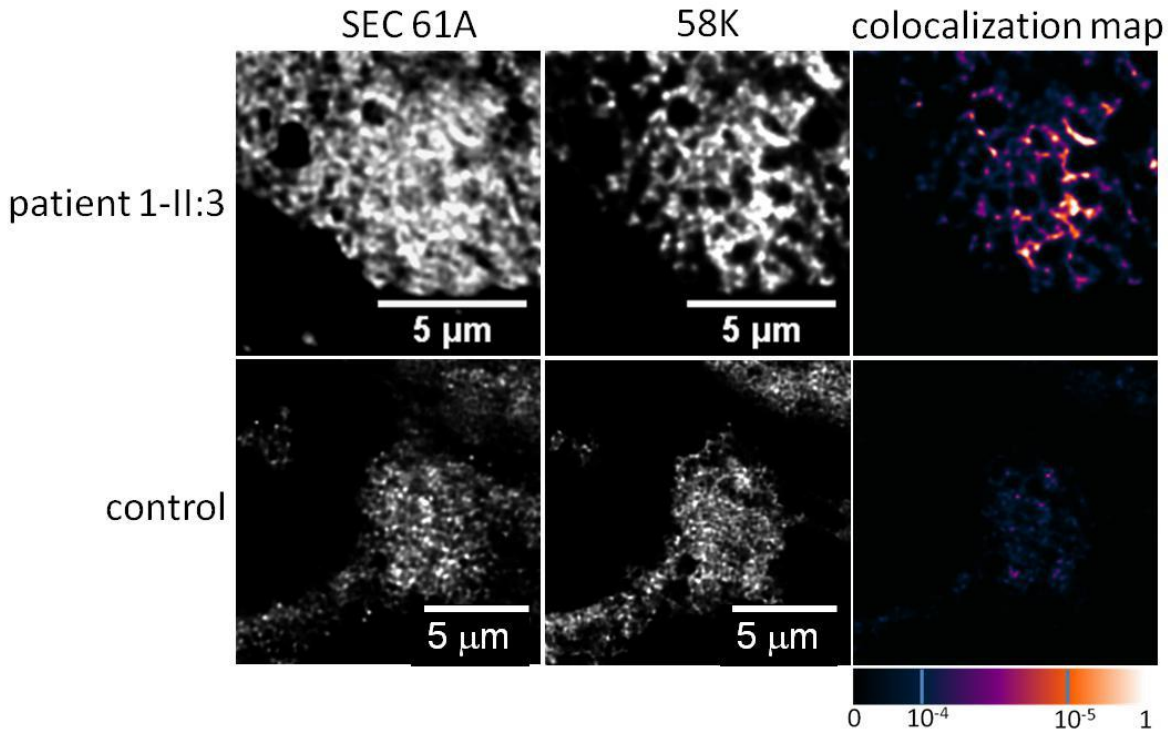
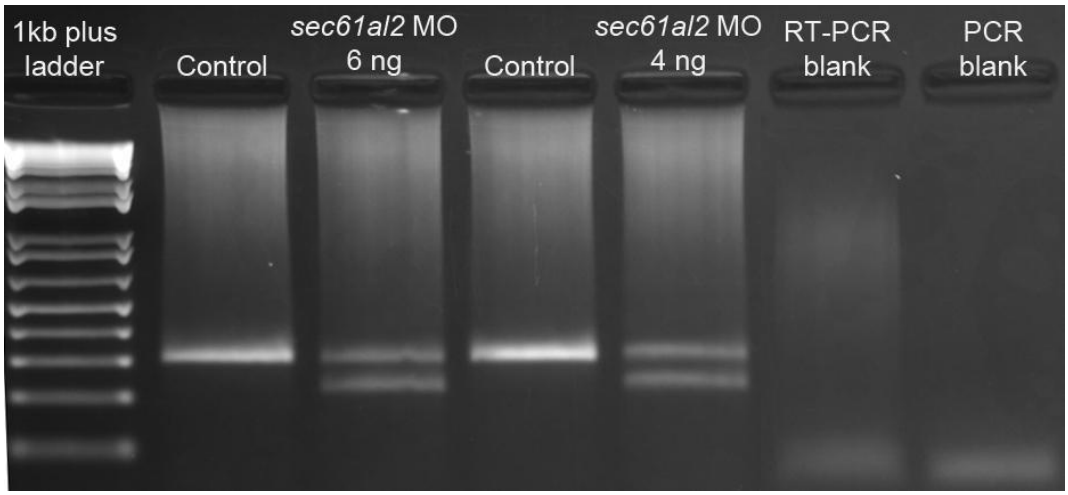
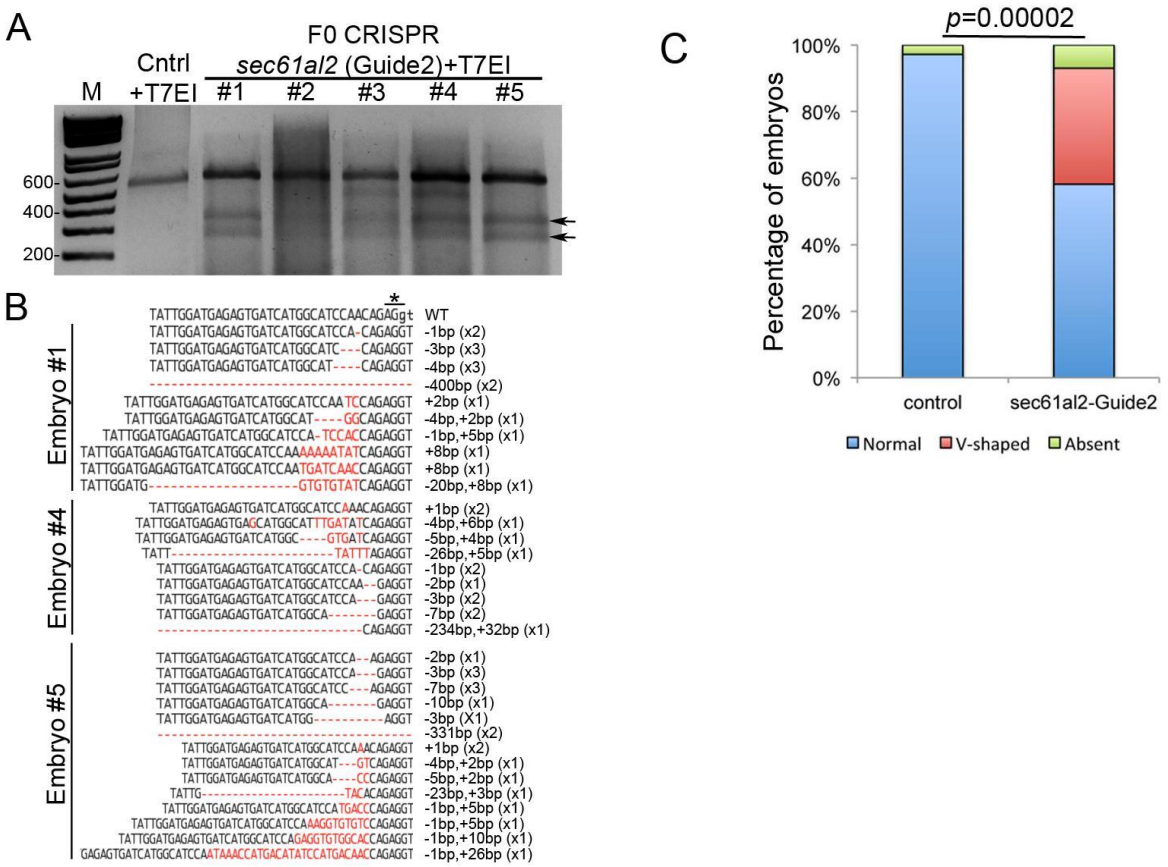


Figure S6: *sec61al2* MO efficiently disrupts the splicing of its zebrafish endogenous message.



Injection (at 4 dpf) of sec61al2 splice-blocking morpholino (4 ng and 6 ng) results in abnormal splicing as shown by PCR amplification of cDNA reverse transcribed from extracted total mRNA. β-actin was used as a control. A minimal dose of 4 ng of MO shows knockdown of the endogenous message and was utilized in the zebrafish experiments performed in this study.

Figure S7. CRISPR mediated deletion of Sec61al2-CRISPR in Zebrafish Embryos



Sec61al2-CRISPR Zebrafish Embryos Phenocopy sec61al2 Morphant Phenotypes, Related to Figure 6. (A) At 2 dpf, a total of 16 founders and 10 controls were randomly selected and were subjected to T7 endonuclease I (T7EI) assay. Representative gel picture shows one control and five founders subjected to T7EI assay. T7EI fragments are noted with black arrows for the positive founders #1, #3, #4 and #5. Lanes from left to right: M: 100 bp DNA ladder; Cntrl+T7EI: PCR of exon4 of *sec61al2* after denaturation/reannealing, and after T7EI digest for control 1; F0 #1, #2, #3, #4, #5: random selection of five embryos injected with *sec61al2*-gRNA2. We noted the presence of a T7EI fragment for a total of 9 out 16 founders subjected to T7EI assay, indicating that 56% of the founders have indels of exon 4 of *sec61al2*. No T7EI fragment was detected in the 10 controls tested. (B) Sequence alignment of reference sequence (top) to three sec61al2-CRISPR F0 mutants generated by Sanger sequencing. The underlined black star marks the putative CRISPR cut site based on the location of the PAM recognition motif (i.e., AGG). For each embryo tested, we observed 50% of disruptive events (deletions and insertions/deletions) in the T7EI positive embryos compared to control. (C) Four days after sec61al2-gRNA2 and Cas9 protein co-injection, the pronephric tubules were stained for both control and gRNA/Cas9 injected embryos and the convolution of the pronephric tubules was quantified. Bar graph represents qualitative scoring of the convolution of the pronephric tubules into three categories: normal, V-shaped, and absent (see representative pictures on the Figure 6). A total of 55% of the injected embryos showed a convolution defect compared to controls. A Fisher's exact test was performed and the corresponding p value is denoted on the bar graph.

Table S1: PCR and sequencing primers

Name	Forward	Reverse	Chrom	length (bp)	PCR Additive
SEC61A1					
SEC61A1EX1-2	CTGCATGCCGGGGCTTGAGGTC	GTCACCTTTCGACAGCTCTCCGG	3	785	DMSO
SEC61A1EX3-4	AGCTGGGGTGATGCCTGTAGGT	TGATGGTATGCCTTGGTGCTGAACT	3	642	x
SEC61A1EX5	CTGTTTAGACACCATGTGACTTCC	CCAACTATAGACATCAGATTATTCAC	3	412	x
SEC61A1EX6	CCCCGTGCCTGGCGTTGAATT	ATCACATCCTCAGCCCCAGAGAGC	3	393	x
SEC61A1EX7	AAGCAGAAGCCTAAGACTCTGGGTT	TGCTGAAAGCTGTCGCCACCAG	3	387	x
SEC61A1EX8	TCCCTGTGGCTATGGGCACCATG	CCACAGAGTGGGGGACTGCACA	3	379	x
SEC61A1EX9	GTGGCCCACTGGACAGTCCC	AACCAAACGCATGTGAGTGCTCCT	3	548	x
SEC61A1EX10	ACTGTGGGCACCGAGTAAAATTGCA	ACTGCCCCCTAAAGGACGACGA	3	434	x
SEC61A1EX11	CGTCGTCCTTTAGGGGGCAGTTCAG	TGTCCACAGACGGGAGGTTGGG	3	380	x
SEC61A1EX12	GTCACAGTCCCCTGGCCACGTGT	TCCCACTTCTCTGGCAGTCGGC	3	643	x
SEC61A1altEX1	CACACCGCCATGCTTTGCCC	GGTGTGTCACGCCTCCCGAG	3	537	DMSO
NPHP3					
NPHP3EX7	GCTGCCACAATGCAGTATAGCACAC	TGGCCTCCGAGGTTCTTCACAA	3	388	x

The above primers for SEC61A1 were designed based on ENSEMBL transcript ID ENST00000243253. All above exons were amplified using the touchdown PCR method : Step 1: 94^0C→ 3 mins, Step 2: 94^0C→ 30 seconds, Step 3: 65^0C-55^0 (-0.5^0/ cycle) → 30 seconds**,** Step 4: 72^0C→ 45 seconds, Step 5: Repeat Step 2- 4 x 20 cycles, Step 6: 94^0C→ 30 seconds, Step 7: 55^0C→ 30 seconds**,** Step 8: 72^0C→ 45 seconds, Step 9 : Repeat Step 6-8 x 15 cycles, Step 10: 72^0C→ 10 mins, Step 11: 10^0C→ 1 min. The above primers for NPHP3 exon 7 were designed based on the ENSEMBL Transcript ID ENST00000337331. Exon 7 was amplified under Standard PCR conditions: 94^0C→ 3 mins, Step 2: 94^0C→ 30 seconds, Step 3: 55^0C→ 30 seconds**,** Step 4: 72^0C→45 seconds, Step 5: Repeat Step 2- 4 x 34 cycles, Step 6: 72^0C→ 10 mins, Step 7: 10^0C→1 min