

A

Exon 16
 GACAAACTGTGTGAATATTTTAGTAACAACACTACTCAGCTCATCCAGCATTG
 GCGAAAGTCCTGCTGTATGGGTTAGGAATTGTATTTCCAATAGAAAATATTT
 ACAGTGCAACTAAAAATAG

Exon 17
 TAAAGAAAGCTGTTTGGAGAAATAATTAAGGCTTGGAGAAAGCTGTA
 TTAATGTTTGTATAGAGATAAGTGTACAAAGAAAGAAAGAGGAGLAAAGAAAG

c.1698
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Exon 18
 CACGGGATGCCCTTCTGGAGGATCTCCAGCCACTCGGACCTCATGGCCCTGC
 ACCAAGCCTTGGAACTGGAGTACCTGTAAAGCGCTCGGCACCTTGACAGCG
 CACAG...

B

ERVFIWDLDETIIVFHSLTGSYANRYGRDPPTS SVSLGLRMEEMIFNLADTH
 LFFNDLEECDQVHIDDVSSDDNGQDLSTYNFGTDGFPAAATSANLCLATGVR
 GGVDWMRKLAFRYRRVKEIYNTYKNNVGGLLGPAKREAWLQLRAEIEALTDS
 WLTALKALSLSIHSRTNCVNILVTTTQLIPALAKVLLYGLGIVFPIENIYSA
 TKIGKESCFERIIQRFGRKVVYVVI GDGVVEEQGAKKHAMPFWRISSHSDLM
 ALHHALELEYL

ERVFIWDLDETIIVFHSLTGSYANRYGRDPPTS SVSLGLRMEEMIFNLADTH
 LFFNDLEECDQVHIDDVSSDDNGQDLSTYNFGTDGFPAAATSANLCLATGVR
 GGVDWMRKLAFRYRRVKEIYNTYKNNVGGLLGPAKREAWLQLRAEIEALTDS
 WLTALKALSLSIHSRTNCVNILVTTTQLIPALAKVLLYGLGIVFPIENIYSA
 TKIARDALLEDLQPLGPHGPAPRLGTGVPVTALGTLTAHSCSVTRDRSSRPQ
 SRISAGLQNLAISAW

C

Human	ERVFIWDLDETIIVFHSLTGSYANRYGRDPPTS SVSLGLRMEE
Chimpanzee	ERVFIWDLDETIIVFHSLTGSYANRYGRDPPTS SVSLGLRMEE
Mouse	ERVFIWDLDETIIVFHSLTGSYANRYGRDPPTS SVSLGLRMEE
Zebrafish	ERVFIWDLDETIIVFHSLTGSYANRYGRDPPTS SVSLGLRMEE
Human	MIFNLADTHLFFNDLEECDQVHIDDVSSDDNGQDLSTYNFGTD
Chimpanzee	MIFNLADTHLFFNDLEECDQVHIDDVSSDDNGQDLSTYNFGTD
Mouse	MIFNLADTHLFFNDLEECDQVHIDDVSSDDNGQDLSTYNFGTD
Zebrafish	MIFNLADTHLFFNDLEECDQVHIDDVSSDDNGQDLSTYNFGTD
Human	GFPAAATSANLCLATGVRGGVDWMRKLAFRYRRVKEIYNTYKN
Chimpanzee	GFPAAATSANLCLATGVRGGVDWMRKLAFRYRRVKEIYNTYKN
Mouse	GFPAAATSANLCLATGVRGGVDWMRKLAFRYRRVKEIYNTYKN
Zebrafish	GFPAAATSANLCLATGVRGGVDWMRKLAFRYRRVKEIYNTYKN
Human	NVGGLLGPAKREAWLQLRAEIEALTDSWLTALKALSLSIHSRT
Chimpanzee	NVGGLLGPAKREAWLQLRAEIEALTDSWLTALKALSLSIHSRT
Mouse	NVGGLLGPAKREAWLQLRAEIEALTDSWLTALKALSLSIHSRT
Zebrafish	NVGGLLGPAKREAWLQLRAEIEALTDSWLTALKALSLSIHSRT
Human	NCVNILVTTTQLIPALAKVLLYGLGIVFPIENIYSA TKIGKES
Chimpanzee	NCVNILVTTTQLIPALAKVLLYGLGIVFPIENIYSA TKIGKES
Mouse	NCVNILVTTTQLIPALAKVLLYGLGIVFPIENIYSA TKIGKES
Zebrafish	NCVNILVTTTQLIPALAKVLLYGLGIVFPIENIYSA TKIGKES
Human	CFERIIQRFGRKVVYVVI GDGVVEEQGAKKHAMPFWRISSHSD
Chimpanzee	CFERIIQRFGRKVVYVVI GDGVVEEQGAKKHAMPFWRISSHSD
Mouse	CFERIIQRFGRKVVYVVI GDGVVEEQGAKKHAMPFWRISSHSD
Zebrafish	CFERIIQRFGRKVVYVVI GDGVVEEQGSKKHAMPFWRISSHSD
Human	LMALHHALELEYL
Chimpanzee	LMALHHALELEYL
Mouse	LMALHHALELEYL
Zebrafish	LMALHHALELEYL

Figure S1. (A) cDNA sequence of the mutant *EYA1* transcript corresponding to the lower band in the middle lane of the gel image depicted in Figure 2C. The sequence shows the skip of exon 17 (highlighted in red) and the ensuing frameshift as a consequence of c.1698+1G>A. Sanger sequencing also revealed c.1755T>C which is highlighted in yellow. Grey, not sequenced; blue, protein-coding bases covered by Sanger sequencing; orange, part of 3' untranslated region covered by Sanger sequencing. For reverse transcription, RNA was extracted from dermal fibroblasts derived from patient III-1. (B) Wild-type (top) and predicted mutant (bottom) EYA domain protein sequences based on NP_000494.2, showing frameshift-induced aberrant C-terminal residues (red vs. green letters). As a consequence of c.1755T>C, the position highlighted in yellow harbors an arginine instead of a cysteine residue. (C) Alignment of EYA domain protein sequences (EYA1 amino acid positions 322-592) from different species, demonstrating high conservation of the protein domain. Red boxes highlight the residues affected by c.1698+1G>A. Blue and black letters mark consecutive exons, respectively, and red letters indicate residues which overlap splice sites.

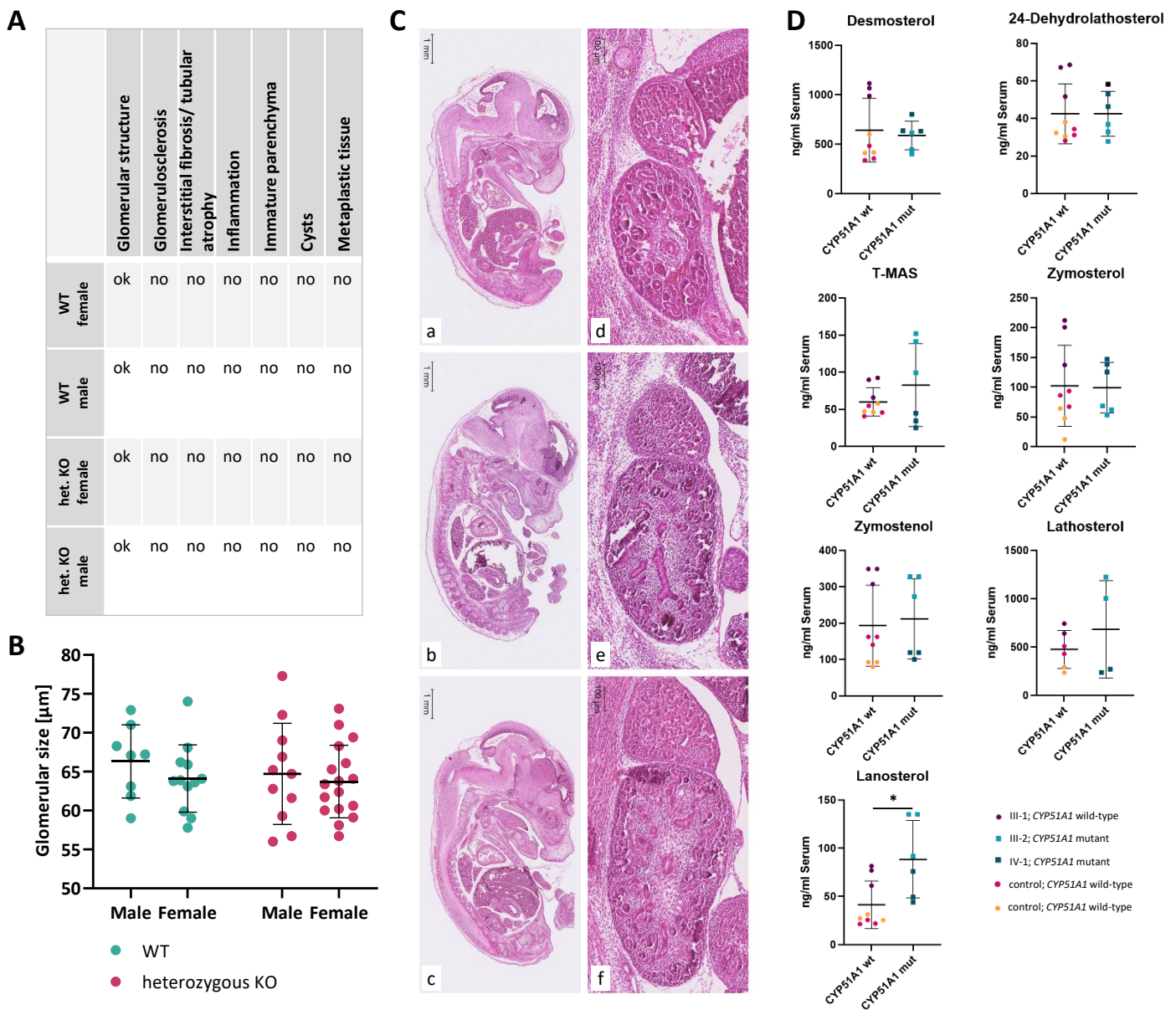


Figure S2. Histopathological analysis of kidneys from *Cyp51^{+/-}* and *Cyp51^{-/-}* mice and measurement of sterol intermediates in patient sera. (A) Histopathological analysis of kidney sections from *Cyp51^{+/-}* (heterozygous knock-out) and wild-type mice did not show any significant differences with respect to glomerular structure, glomerulosclerosis, interstitial fibrosis/ tubular atrophy, inflammation, immature parenchyma, cysts, or metaplastic tissue. (B) Glomerular sizes did not significantly differ between kidneys from *Cyp51^{+/-}* mice and those from wild-type animals. Two-way ANOVA, mean \pm SD. (C) For *Cyp51^{-/-}* (a,d), *Cyp51^{+/-}* (b,e), and wild-type (c,f) mouse embryos at E14.5, representative sagittal sections (left) are shown alongside higher magnifications of the kidneys (right). Normal renal development with primitive glomeruli in the cortex as well as collecting ducts and stromal cells in the medulla are present in all three genotypes. (D) Quantitative analysis of seven main cholesterol intermediates in three family members (III-1, *CYP51A1* wild-type, violet; III-2, *CYP51A1* mutant, light turquoise; IV-1, *CYP51A1* mutant, dark turquoise) and two control subjects (*CYP51A1* wild-type, pink and orange, respectively) with three technical replicates each. The measurements were not significantly different ($p > 0.7$) between samples harboring wild-type and mutant *CYP51A1* except for the lanosterol analysis ($p = 0.0256$). Mann Whitney tests, mean \pm SD. het., heterozygous; KO, knock-out; WT/ wt, wild-type; mut, mutant, i.e., harboring *CYP51A1* c.770+1G>A in a heterozygous fashion; T-MAS, testis meiosis-activating sterol.

Gene	Variant	Poly-Phen-2	PROVEAN	MaxEnt, NNSPLICE	Allele frequency*	IV-1	IV-2	III-1	III-2
<i>CYP51A1</i>	c.770+1G>A p.?	n/a	n/a	-100.0% -100.0%	not known	het	het		het
<i>LRRD1</i>	c.1419dup p.(Asn474*)	n/a	deleterious	no predicted impact	not known		het	het	
<i>SPAG9</i>	c.2081A>G p.(Asn694Ser)	possibly damaging	deleterious	no predicted impact	0.0002402	het	het		het

Table S1. Candidate genes from exome sequencing identified in family members IV-1, IV-2, III-1, or III-2. *LRRD1* and *SPAG9* were not considered further due to their implausible segregation or high allele frequency, respectively. n/a, not available; het, heterozygous; * gnomAD database (European, non-Finnish population).