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Supplemental Data

Mutations in *KDSR* Cause Recessive Progressive Symmetric Erythrokeratoderma

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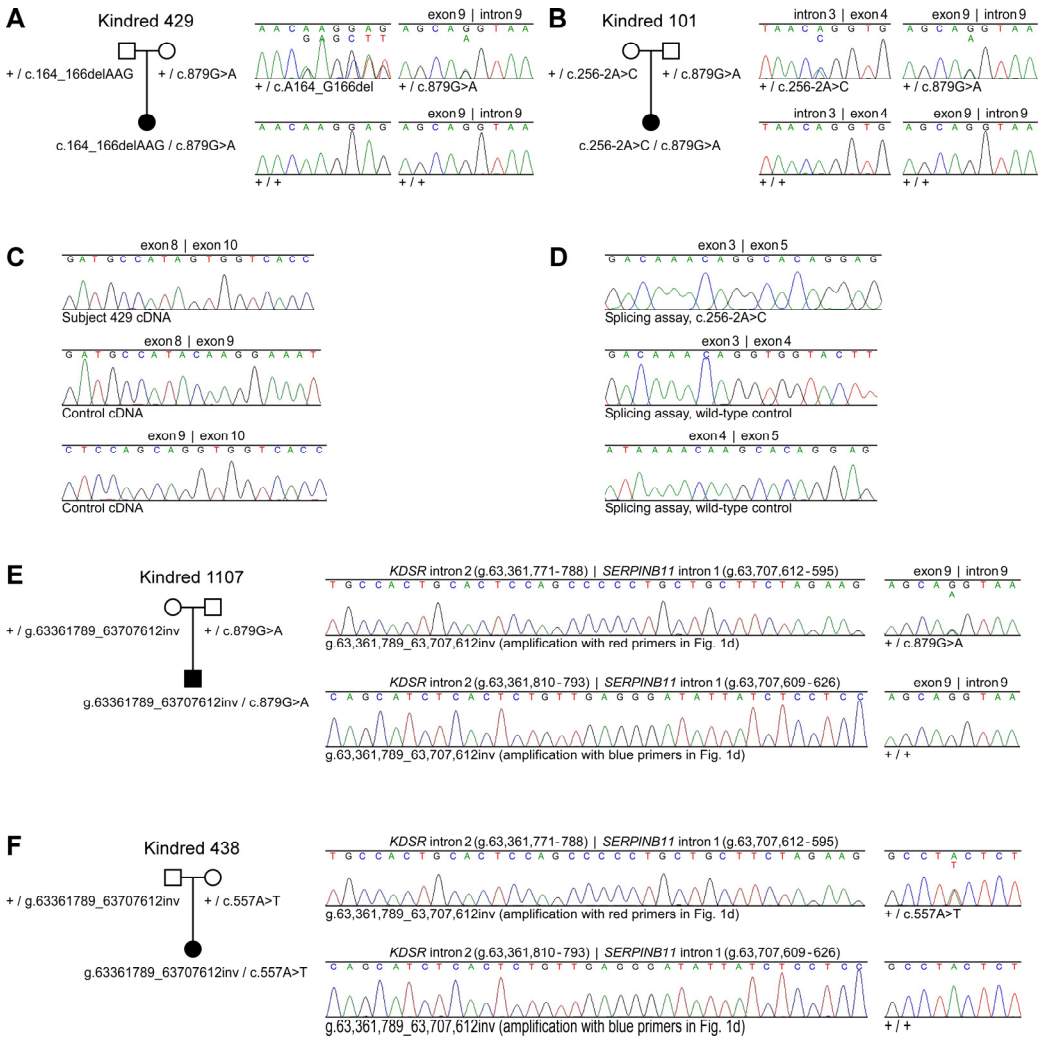
Figure S1. Sanger sequencing confirmation of *KDSR* mutations and aberrant splicing



In pedigree drawings, subjects are denoted with black (affected) and white (unaffected) symbols. *KDSR* alleles are denoted as '+' (wild-type) or by the sequence change. Genomic base position is the hg38 version of the human genome.

(**A**) Subject 429 is compound heterozygous for *KDSR* mutations c.164_166delAAG and c.879G>A.

(**B**) Subject 101 is compound heterozygous for *KDSR* mutations c.256-2A>C and c.879G>A.

(**C**) Reverse transcription and amplification of RNA from Subject 429, and gel isolation of the smaller band (**Figure 1A**), shows *KDSR* mutation c.879G>A results in skipping of exon 9.

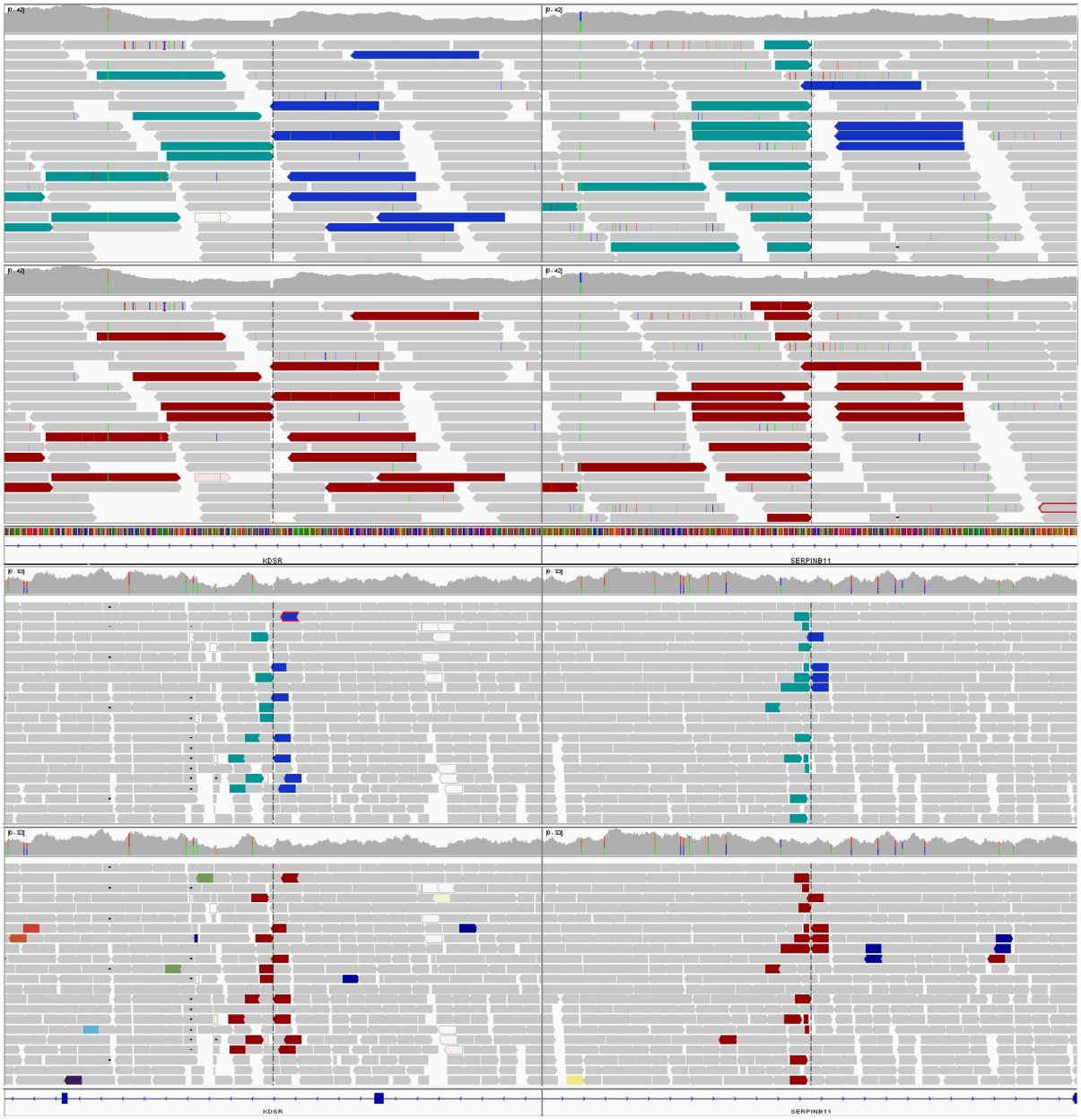
(**D**) Reverse transcription and amplification of RNA from cells transfected with a *KDSR* c.256-2A>C mutation construct shows skipping of exon 4.

(**E**) Subject 1107 is compound heterozygous for a 346 bp inversion on chromosome 18 (g.63,361,789_63,707,612inv), which disrupts the 5' end of *KDSR*, and mutation c.879G>A.

(**F**) Subject 438 is compound heterozygous for a 346 bp inversion on chromosome 18 (g.63,361,789_63,707,612inv), which disrupts the 5' end of *KDSR*, and mutation c.557A>T.

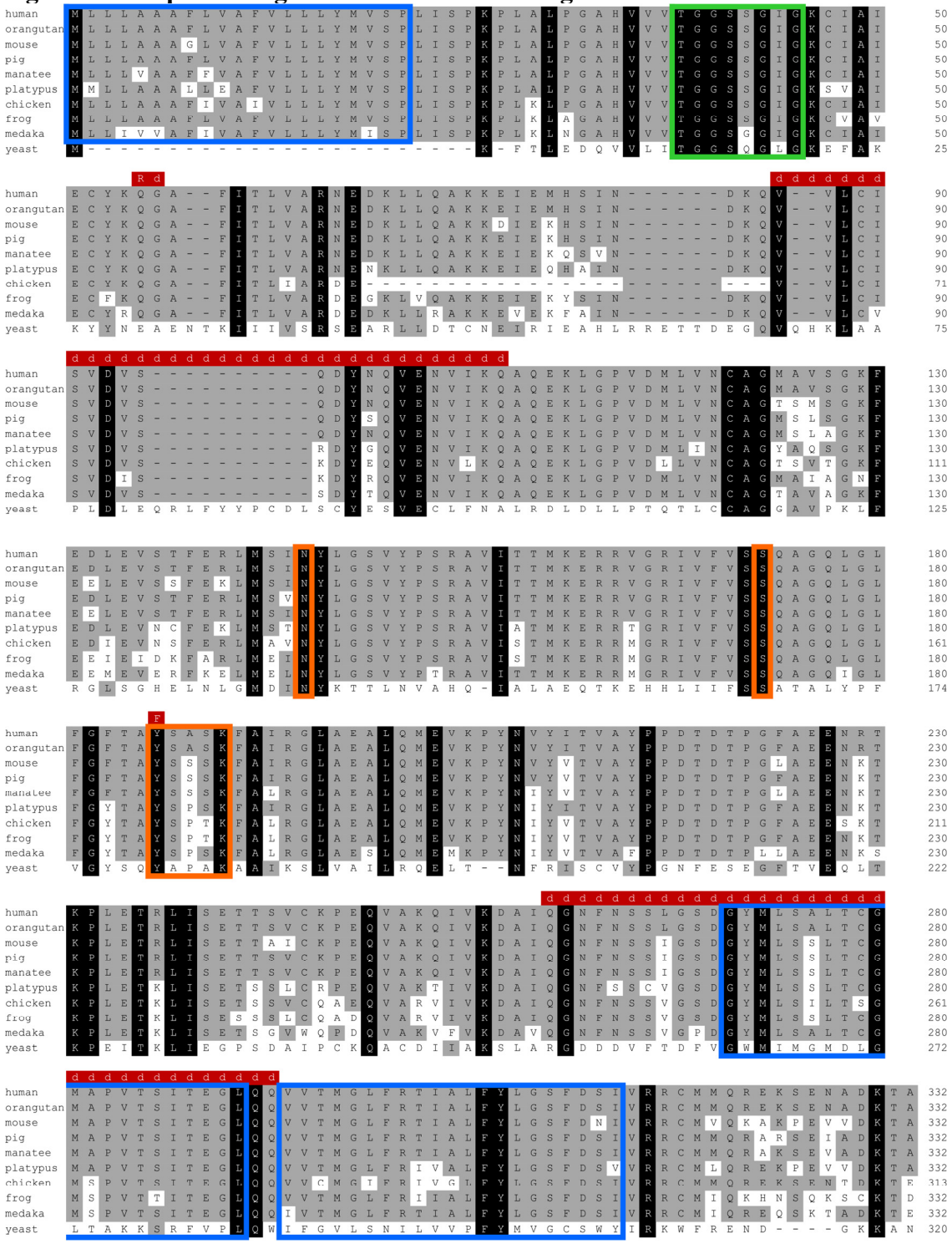
Note in (**E-F**) that at the distal junction of the inversion g.63,361,789-792 is lost and g.63,707,609-612 is retained.

Figure S2. Identification of a 346 kb inversion affecting the 5' end of *KDSR* by genome sequencing



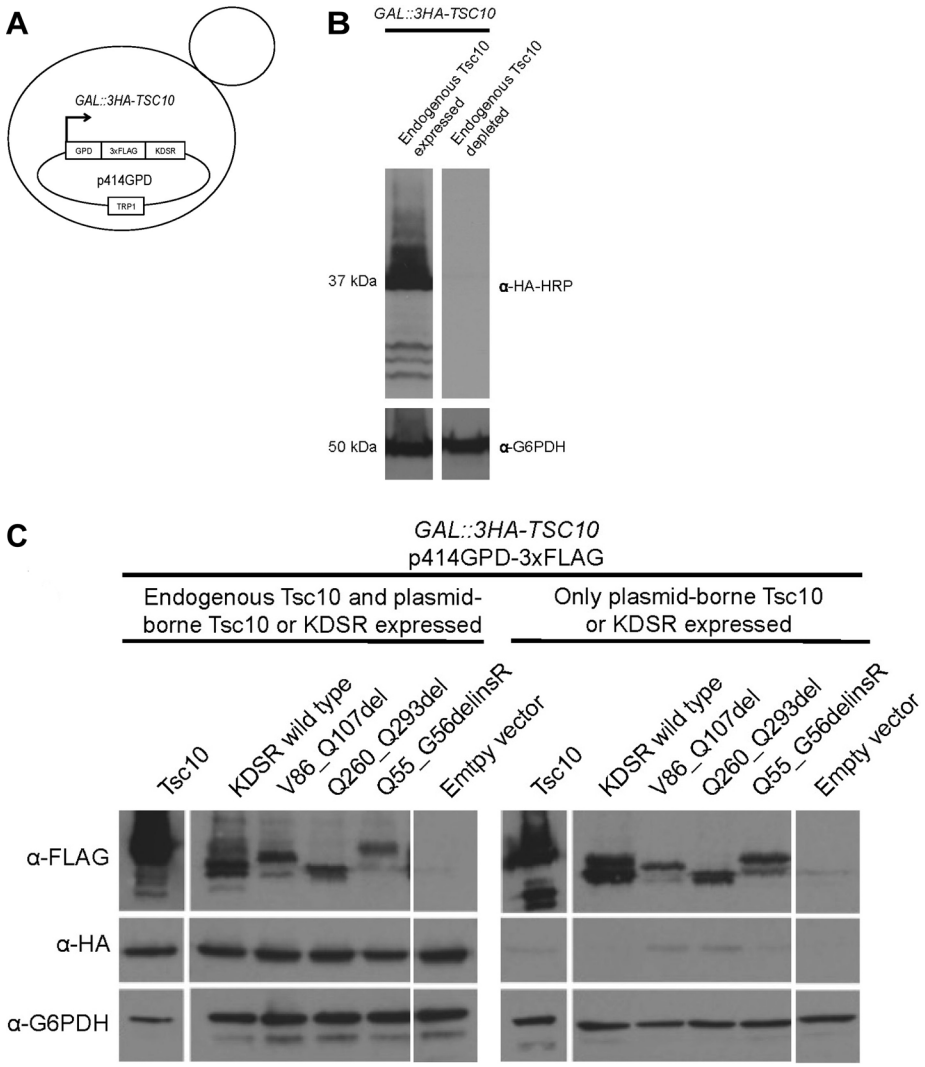
Screen captures from the Broad Institute Integrative Genomics Viewer (IGV) depict aligned reads from the genome sequence of Subject 1107 at 650 bp (top panel) and 5 kb (bottom panel) resolution. Aligned reads on chromosome 18 (arrows) are color-coded from the usual gray to indicate deviations from expected orientation (aqua and blue, top sub-panels) or insert size (dark red, bottom sub-panels), based on the reads' paired-ends. Reads within intron 2 of *KDSR* (left panels) and within intron 1 of *SERPINB11* (right panels) are shown (with clipping at panel borders). The presence of multiple reads with reversed orientation and a much greater than expected insert size with paired-ends at both of these locations is indicative of an inversion between the sites (g.63,361,789_63,707,612inv), which was confirmed by PCR (**Figure 1D-E**) and Sanger sequencing (**Figure S1E-F**).

Figure S3. Sequence alignment of KDSR orthologs



Completely conserved residues are shaded black; other residues conserved with human KDSR are shaded gray. Mutations in PSEK subjects are above the alignments in red (d: deletion). Protein domains are outlined as follows: YxxxK, N, and S residues that form the canonical catalytic triad (orange), putative TGxxxGxG NAD binding site (green), putative transmembrane domains (blue).

Figure S4. Immunoblotting validates depletion of endogenous yeast *TSC10* and expression of plasmid-borne *TSC10* and *KDSR* for yeast complementation studies

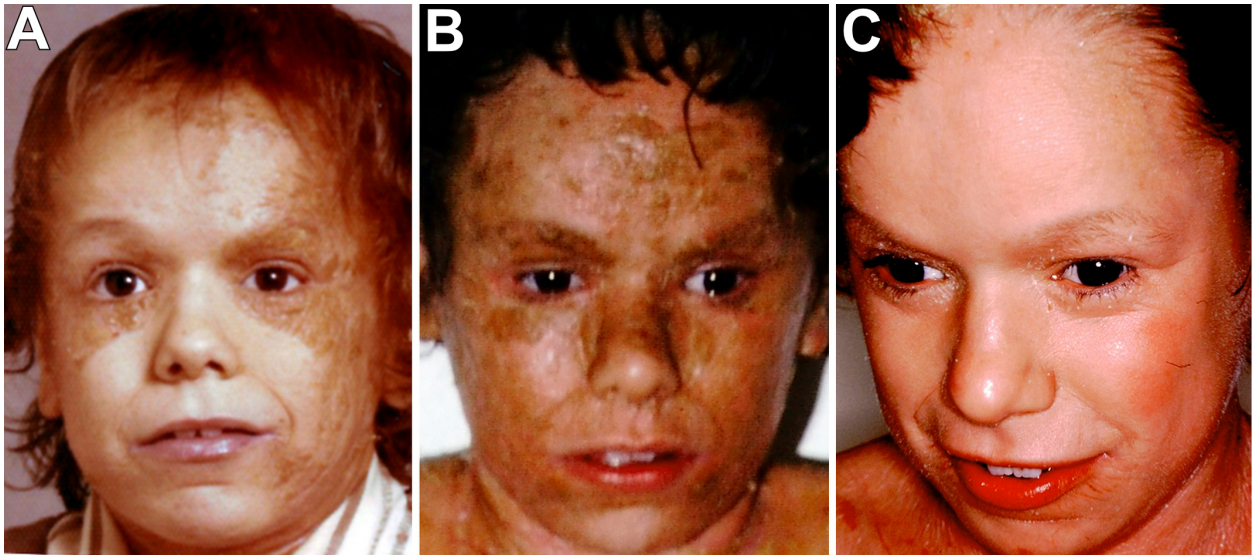


(**A**) Schematic depicting haploid yeast strains constructed to test the depletion of endogenous *TSC10* and complementation by yeast *TSC10*, wild-type *KDSR*, and mutant forms of *KDSR*. *TSC10* was placed under the control of a galactose-inducible and glucose-repressible promoter (*GAL*), and sequences encoding 3xHA tags were introduced. Wild-type and mutant genes were introduced via plasmids (p414GPD).

(**B**) Protein extracts before and after *TSC10* depletion were analyzed by Western blot with α-HA-HRP to detect tagged Tsc10 before and after depletion. Anti-G6PDH was used as a loading control.

(**C**) Western blots showing immunostaining for plasmid-borne Tsc10 or KDSR (α-FLAG), endogenous Tsc10 (α-HA), or loading control (α-G6PDH) in the presence and absence of endogenous yeast Tsc10. Left panels: Yeast were grown at 30 °C in SG/R-W (endogenous *TSC10* and plasmid-borne *TSC10* or *KDSR* expressed). Right panels: Yeast were first grown to mid-log phase in SG/R-W at 30 °C before being shifted to SD-W (only plasmid-borne *TSC10* or *KDSR* expressed) for six hours at 30 °C. The empty vector serves as a negative control.

Figure S5. Systemic retinoids improve skin phenotype



In two of our four subjects, systemic retinoids have been administered and have led to a marked improvement of hyperkeratosis.
(**A**) Subject 438 at five years of age shows prominent, well-demarcated plaques on the cheeks, temples and central forehead.
(**B**) At 9 years of age, these have extended to involve more of the face with sparing of the chin.
(**C**) After 6 months of isotretinoin at 1mg/kg/day, she has experienced near-complete clearing of facial hyperkeratosis.

Table S1. Exome sequence coverage

	Kindred 429			Kindred 101			Kindred 1107			Kindred 438		
	Proband	Mother	Father	Proband	Mother	Father	Proband	Mother	Father	Proband	Mother	Father
Number of reads	58M	76M	79M	73M	66M	128M	35M	39M	31M	73M	91M	97M
Mean coverage	50x	62x	67x	64x	58x	111x	29x	31x	27x	61x	74x	80x
Median coverage	42x	50x	52x	53x	48x	93x	24x	27x	23x	50x	58x	63x
Bases covered $\geq$ 8x	85%	87%	87%	86%	86%	89%	82%	95%	94%	86%	88%	88%
Bases covered $\geq$ 20x	74%	78%	78%	78%	76%	84%	59%	71%	62%	77%	80%	82%

Table S2. Exome sequence data at *KDSR* mutation sites

	Subject 429	Subject 101	Subject 1107	Subject 438
KDSR mutation 1				
DNA change	c.879G>A	c.879G>A	c.879G>A	c.557A>T
Protein change	p. Gln293Gln, p.Gln260_Gln293del	p. Gln293Gln, p.Gln260_Gln293del	p. Gln293Gln, p.Gln260_Gln293del	p.Tyr186Phe
Exon	9	9	9	6
Genomic location	chr18:63,335,257	chr18:63,335,257	chr18:63,335,257	chr18:63,350,940
Non-Ref/Total reads (proband)	22/59	34/82	20/42	36/63
Non-Ref/Total reads (mother)	38/81	0/52	0/35	32/59
Non-Ref/Total reads (father)	0/75	54/111	20/32	0/73
KDSR mutation 2				
DNA change	c.164_166delAAG	c.256-2A>C	none observed in exome	none observed in exome
Protein change	p.Gln55_Gly56delinsArg	p.Val86_Gln107del		
Exon	2	4		
Genomic location	chr18:63,362,811-813	chr18:63,355,565		
Non-Ref/Total reads (proband)	39/73	49/97		
Non-Ref/Total reads (mother)	0/158	42/81		
Non-Ref/Total reads (father)	71/160	0/164		

KDSR cDNA positions: RefSeq accession NM_002035.2.
KDSR protein positions: RefSeq accession NP_002026.1.
KDSR exon positions: RefSeq accession NG_028249.1.
Genomic location: DNA base(s), hg38 version of the human genome.
Non-Ref reads: The number of exome sequence reads with the non-reference (mutant) base(s).
Total reads: The total number of exome sequence reads.

Table S3. SNP rs62098681 in Subjects 1107 and 438

	Kindred 1107	Kindred 438
rs62098681		
Non-Ref/Total reads (proband)	0/1	1/1
Non-Ref/Total reads (mother)	2/5	0/0
Non-Ref/Total reads (father)	0/4	4/4

rs62098681: An intronic SNP 166 bp downstream from *KDSR* exon 1 (c.108+166C>T, hg38 position chr18:63,366,845) with a minor allele frequency of 0.01.
Non-Ref reads: The number of exome sequence reads with the non-reference (mutant) base(s).
Total reads: The total number of exome sequence reads.
Red lettering: Reads indicating that rs62098681 is present in the parent in which a damaging *KDSR* mutation was not revealed by exome sequencing.
Note: Given the low level of exome coverage at this site, Sanger sequencing was used to show that Subject 1107 and his mother, and Subject 438 and her father, are heterozygous for rs62098681.