Generation and Characterization of SULT4A1 Mutant Mouse Models

Patrick L. Garcia, Mohammed I. Hossain, Shaida A. Andrabi, and Charles N. Falany

Drug Metabolism and Disposition

Supplemental Methods

This work was conducted at the University of Alabama at Birmingham (UAB) Transgenic Facility and is described in detail (Challa et al., 2016). Pronuclear injections into zygotes obtained from C57Bl/6 mice were performed with a solution of sgRNAs (25 ng/µl each) and Cas9 capped mRNA (125 ng/µl). The sequence of the CRISPR/sgRNA for the Δ28 and Δ12 strains are as follows: Exon 1 (- strand) - 5' - GGTGACGATCCACACGTCGC (TGG) -3' <28 bp deletion> and Exon 3 (- strand) - 5' - AGGTGGCTCTTGATGAGGCG (GGG) -3' (12 bp deletion). Injected zygotes were implanted into pseudopregnant CD1 recipients. Tail biopsies from G0 pups were obtained upon weaning (3 weeks) and used to isolate genomic DNA. Tail genomic DNA was used as a PCR template to analyze sequence modifications. Preliminary assessment for the presence of indels was done by heteroduplex mobility assay (HMA). PCR amplicons showing heteroduplex mobility shifts were cloned into a plasmid vector (TOPO-TA cloning, Invitrogen) and individual colonies were processed for Sanger sequencing. Two female G0 animals carrying distinct mutations were bred with wildtype C57Bl/6 mice for germline transmission.

References

Challa AK, Boitet ER, Turner AN, Johnson LW, Kennedy D, Downs ER, Hymel KM, Gross AK, and Kesterson RA (2016) Novel Hypomorphic Alleles of the Mouse Tyrosinase Gene Induced by CRISPR-Cas9 Nucleases Cause Non-Albino Pigmentation Phenotypes. *PloS one* **11:**e0155812.

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Supplemental Videos 1 and 2. Δ28 and Δ12 homozygous mutant mice display severe neurological deficits. Day 17 old mice were video recorded for behavioral changes. As shown in the representative videos, both the Δ28 and Δ12 homozygous mice displayed seizures, lack of coordination, abnormal gait, and ataxia. Videos were edited for time and file size with Imovie'09 v8.06 Apple Inc., Cuppertino, CA) and then exported to Quicktime Player v10.4 (Apple Inc., Cuppertino, CA).

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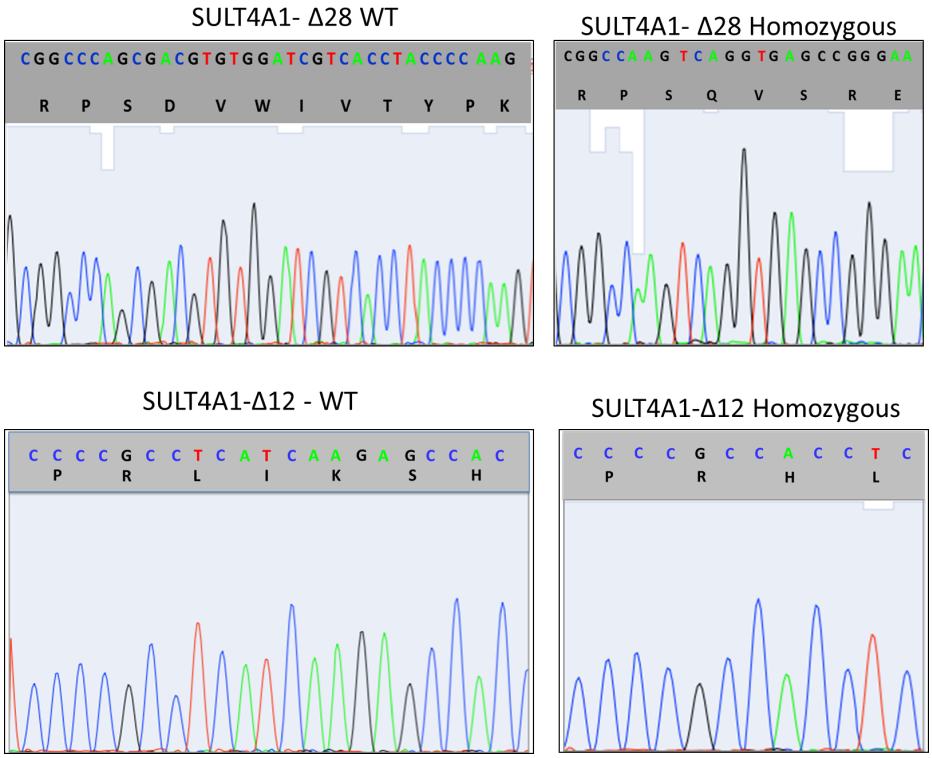

Supplemental Figure 1. Electropherogram tracings confirm mutational status of SULT4A1 was conserved in offspring of Δ28 and Δ12 founders. Genomic DNA was subjected to PCR amplification utilizing primer sets specific to the location of the Δ28 and Δ12 SULT4A1 mutations (**Materials and Methods**). Comparison of WT SULT4A1 and Δ28 homozygous mutant sequence. The Δ28 homozygous mutant possesses a frameshift mutation. Comparison of WT SULT4A1 and Δ12 homozygous mutant sequence. The electropherogram clearly displays the 4 AA deletion characteristic of the Δ12 strain.