Chick Yellow: A Spontaneous Mutation in Tryptophan 2,3-dioxygenase

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Mutation (allele) symbol: *Tdo*^{chky}
Mutation (allele) name: chick yellow
Current strain name: STOCK *Tdo*^{chky}/J
Stock #003148 (jaxmice.jax.org)

Abstract
The spontaneous, recessive mutation chick yellow (*chky*) has been genetically characterized and identified as a point mutation within the tryptophan 2,3-dioxygenase gene located on Chromosome 3. Whole exome sequencing with the Illumina HiSeq high-throughput sequencing platform and re-sequencing by the Sanger method was used to identify the mutation site.

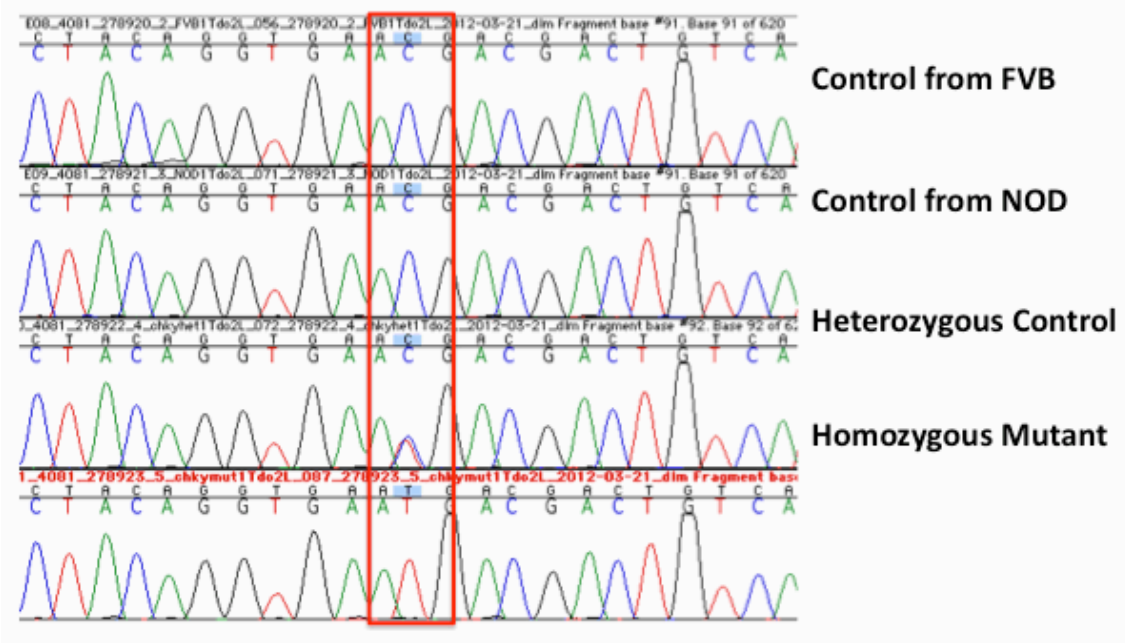
Genetic Analysis
Previous mapping data (see MGI reference J:149273) located *chky* on the distal (non-centromere end) of Chromosome 3.

Causative mutation identification
Mutation identification. Whole exome sequencing was used to identify candidate mutations in the mapped region, as described by Fairfield et al. (2011). Briefly, genomic DNA was prepared using Qiagen DNeasy Blood and Tissue kit (Qiagen, Santa Clarita, CA USA) or by phenol/chloroform extraction. DNA was enriched for coding sequence by hybridization-based capture using SeqCap EZ Mouse Exome SR (Roche NimbleGen). Post-hybridization amplification was completed via Illumina LMPCR protocol (Fairfield et al. 2011). The resulting enriched libraries were used in cluster formation on an Illumina cBot. Paired-end sequencing was done using the Illumina HiSeq. The sequencing data were analyzed using tools and workflows provided by GenomeQuest, including mapping (HS3), SNP calling, and annotation of variants (GenomeQuest). Analysis focused on novel variants that were not positioned in repetitive sequence and had expected allele ratios (>0.95 for homozygous variants and >0.2 for heterozygous variants), as well as sufficient locus coverage (at least 5X for homozygous variants and 10X for heterozygous variants). High priority was given to protein coding or splice variants within the mapped region, as well as unique variants that were not found in other exome data sets or in data generated by the Sanger Mouse Genomes Project (Keane et al. 2011; Mouse Genomes Project).

Validation. Candidate mutations were validated by PCR amplification and sequencing of DNA samples from 6 mutant mice, one wild-type sibling and one BALB/cJ control. Sequencing data were analyzed using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, MI, USA). Primers were designed using Primer3 software (Rozen and Skaletsky 2000).

Gene identification. Whole exome sequencing revealed a single nucleotide variant (SNV) or mismatch on Chromosome 3 at position 81,766,699 (NCBI Build 37). This C to T transition in the coding sequence at base pair 4,366 in exon 4 of the gene tryptophan

2,3-dioxygenase (*Tdo2*) (NM_019911), a gene that encodes an oxidoreductase enzyme in a tryptophan metabolism pathway. To validate the mutation primers were generated that produced a 339 base pair product spanning the predicted mutation [forward (TGTGAACTCCAGATCAGGGC) and reverse (GCCCAAGGCATGAGTAAAGA)]. The single SNV nominated for validation in the chky mutant correlated with the phenotype when samples of genomic DNA from mutant mice and unaffected animals were compared (see chromatograms below). This nonsense mutation is predicted to result in the introduction of a premature stop at amino acid 282 of tryptophan 2,3-dioxygenase.



Comparison of sequence chromatograms from wild type related controls, heterozygous control, and homozygous *chky* mutant sample.

Discussion

Tryptophan 2,3-doxygenase is the rate-limiting enzyme in the catabolism of tryptophan through the kynurenine pathway. Interesting, in an in vitro assay involving tyrosinase-catalyzed synthesis of eumelanin from tyrosine, two catabolites of kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, interfered with the kynurenine pathway and a reddish-brownish, water soluble pigment was produced (Soddu et al., 2003). It is conceivable that jn the total absence of tyrosinase, as is the case for albino mice, an alternate pigment is produced and deposited in the coat (i.e., the yellow pigment noted in the coat hair of *chky*/*chky* mice).

Because *TDO2* has been associated with several human psychiatric disorders, the mouse *Tdo2* gene was knocked out to gain insight into the role TDO2 plays in anxiety-related behaviors. In homozygous *Tdo2* knockout mice, plasma tryptophan are elevated as are 5-hydroxtindoleacetic acid and kynurenine (Kania et al., 2009). In addition, tryptophan catabolism seemed to be shunted more toward the pathway that leads to serotonin. Indeed, serotonin is elevated in the hippocampus and midbrain of these mice and they exhibit less anxiety in the elevated maze and open field tests.

Behavioral tests have not been conducted on homozygous *chky* mice nor have their trypophane/kynurenin pathway protein levels been measured. Likewise, it is not known if albino *Tdo2* knockout mice would display a yellowish colored pigment in their coats. The composition of the yellow pigment present in the hair of *chky*/*chky* mice awaits determination as does the question of whether this chemical is present in their urine.

Literature Cited

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