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Phenotypic Expression of Two Candidate Genes of Nonsyndromic Craniosynostosis in Danio rerio

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Phenotypic Expression of Two Candidate Genes of Nonsyndromic Craniosynostosis in *Danio rerio*

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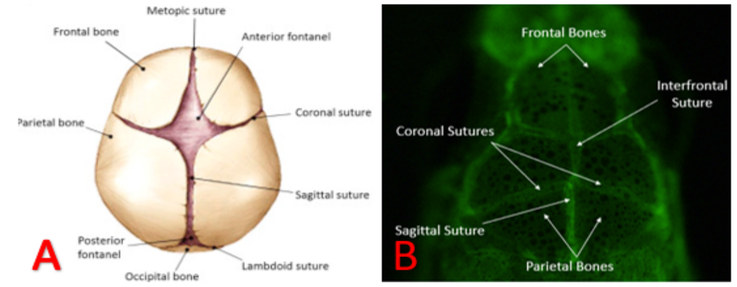
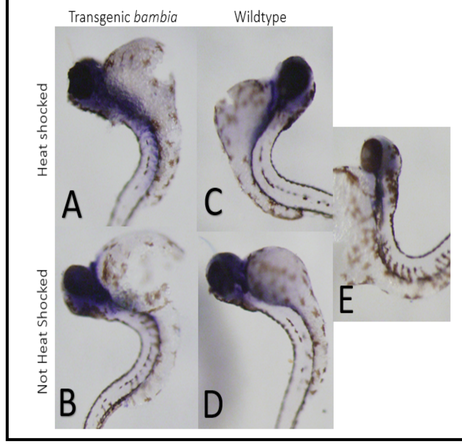
Background



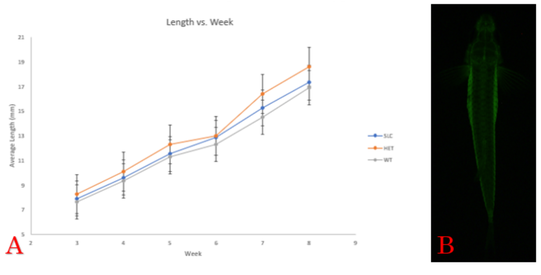
Craniosynostosis is the premature fusion of one or more sutures in the skull.The early fusion of a single or multiple suture can cause deformities in the head as well as neurological problems (Johnson et al. 2011). Nonsyndromic craniosynostosis is the premature fusion without the presence of other features (Heuzé et al. 2014). Humans (A) and zebrafish (B) have similar sutures of the skull, as seen in the figure, making zebrafish an effective model for this study. *SLC30A9* is a candidate gene for craniosynostosis as it was found in a father and daughter who both had craniosynostosis phenotypes (Hept 2017). *SLC30A9* codes for a protein, ZnT that transports zinc to maintain zinc homeostasis. The zebrafish in this study has a deletion mutation in *slc30a9* and is expected to cause the craniosynostosis phenotype. *BAMBI* is a candidate gene for craniosynostosis as it has been observed in individuals with craniosynostosis phenotypes (Rymer 2015). The BAMBI gene encodes for a pseudo receptor of TGF-β and inhibits the TGF-β signaling pathway. Overexpression of *BAMBI* is expected to display phenotypic characteristics of craniosynostosis in zebrafish based on previous research done by Zhou et al.

Wholemount *in situ* Hybridization of *bambia*



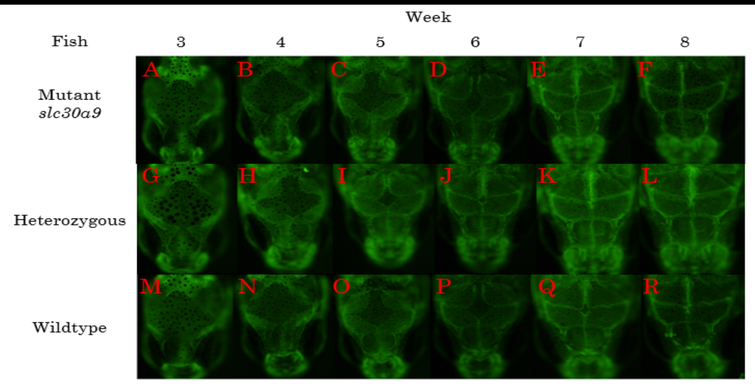
Purple staining displays expression of *bambia* in 74hpf zebrafish embryos. A: Heat shocked transgenic *bambia* embryo. B: Transgenic *bambia* embryo. C: Heat shocked wildtype embryo. D: Wildtype embryo. E: Wildtype embryo. A, B, C, and D, were hybridized with an antisense probe. E was hybridized with a sense probe; acting as the negative control.

Comparison of Length of *slc30a9*



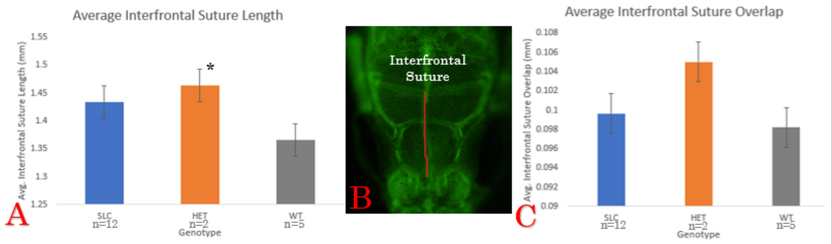
No significant difference found in the average length of *slc30a9* homozygous mutant, heterozygous, and wildtype fish.

Skull Development of *slc30a9*

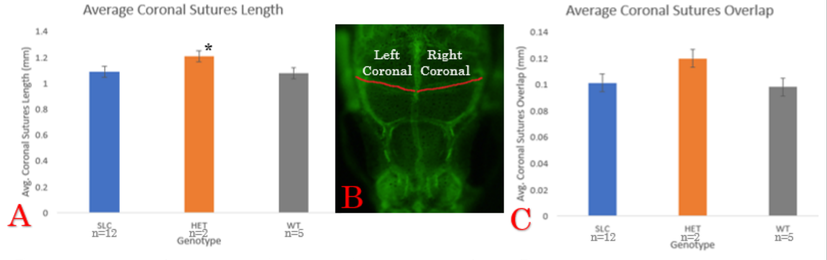


Panel A through F are *slc30a9* homozygous mutant fish from 3 to 8 weeks of age. Panel G through L are heterozygous for the mutant fish *slc30a9* allele from 3 to 8 weeks of age. Panel M through R are homozygous wildtype fish from 3 to 8 weeks of age. The images were taken under a green fluorescent light with an Olympus SZX12 Zeiss microscope.

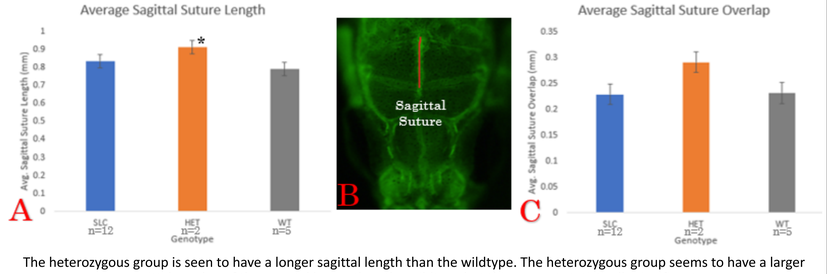
Comparison of Sutures of *slc30a9*



The heterozygous group is seen to have a longer interfrontal l length than the wildtype. The heterozygous group seems to have a larger interfrontal overlap of the frontal bones than the wildtype but this was not statistically significant.

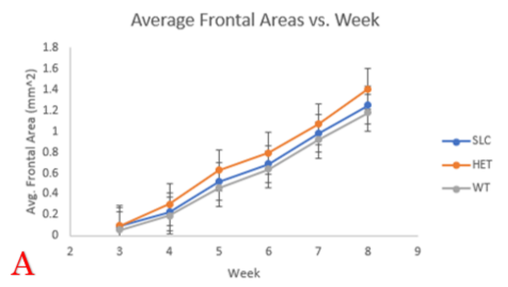


The heterozygous group is seen to have a longer coronal lengths than the wildtype. The heterozygous group seems to have a larger coronal overlaps of the parietal and frontal bones than the wildtype but this was not statistically significant.

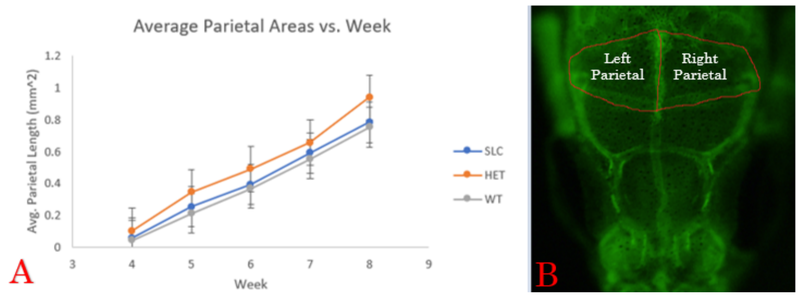


The heterozygous group is seen to have a longer sagittal length than the wildtype. The heterozygous group seems to have a larger sagittal overlap of the parietal bones than the wildtype but this was not statistically significant.

Comparison of Bone Area of *slc30a9*



There was no significant difference between all groups when comparing average frontal bone areas.



There was no significant difference between all groups when comparing average parietal bone areas.

Conclusion and Future Directions

The heterozygous *slc30a9* fish had larger measurements in the length. The heterozygous fish also seemed to have a larger overlap of bone over sutures but this was not shown to be statistically significant most likely because of the small sample size. This is most likely due to the mutation being a dominant negative mutation. This shows that the mutation in the gene may effect the growth of the skull as the length of each group of fish at 8 weeks showed no significant difference, but the overlap of every suture was larger in heterozygous fish indicating the premature fusion of sutures. The larger interfrontal suture length seen in *slc30a9* when compared to wildtype is interesting. Another 6 week study should be performed to confirm these results.

The overexpression of *bambia* in the heat shocked fish that is concentrated in the head shows that the gene could effect skull development. A 6 week study similar to that done with the *slc30a9* fish should be performed with heat shocking to further investigate this.

References

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