Investigating Suture Fusion of Bent Bone Dysplasia Syndrome Through Mouse Models

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Abstract

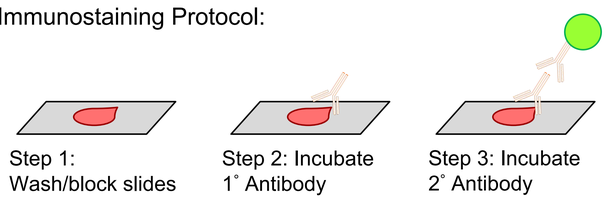
Fibroblast growth factor receptor 2 (FGFR2) is a receptor essential for correct bone development. The M391R mutation of this gene causes Bent Bone Dysplasia Syndrome (BBDS) which leads to bone defects such as craniosynostosis, or improper fusion of the skull bones. This disease is fatal in humans. Dr. Merrill's lab has created a mouse genetic model to assist in research of BBDS. Using the Cre-Lox system, the mutated gene can be selectively activated for study, allowing mice to carry the allele and survive its effects. We will visualize the skull to determine how it changes following activation of this mutation. There are many cellular changes that can be the cause of craniosynostosis. Two of these possible factors are differences in proliferation and apoptosis, which are known to cause premature fusion of cranial bones in other similar mutations. Proliferation is the reproduction of cells in an organism. Apoptosis is the controlled death of cells that are no longer useful in an organism. In this project, we will use immunofluorescence (IF) to measure expression of apoptosis related proteins. Antibodies are used to target a specific set of cells. A tag with a fluorescent dye is added that targets the antibody. We will also measure the number of proliferating cells with by injecting mice with a base analog, (EdU). We will then use a fluorescent microscope to image the results.

Objectives

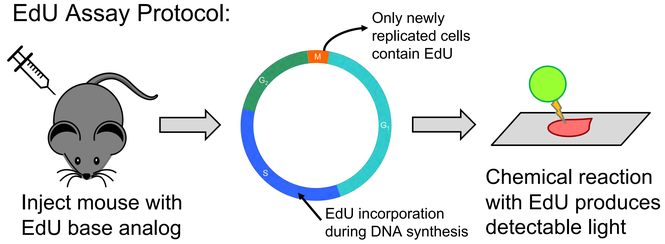
- One goal of this project is to either rule in or out that apoptosis and proliferation is a cause of the fusion of cranial bones in BBDS.
- Another goal of this project is to examine the phenotype of wild type, heterozygous, and homozygous mutant mice to find the difference in severity of the affected bones based on how many alleles they have.

Materials and Methods of IF and EDU Procedure

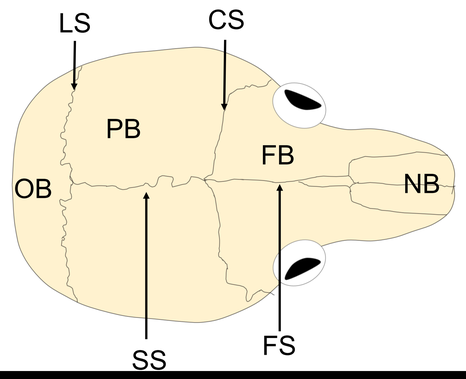
Immunostaining Protocol:



EdU Assay Protocol:



Map of Mouse Skull



Key

Bones:

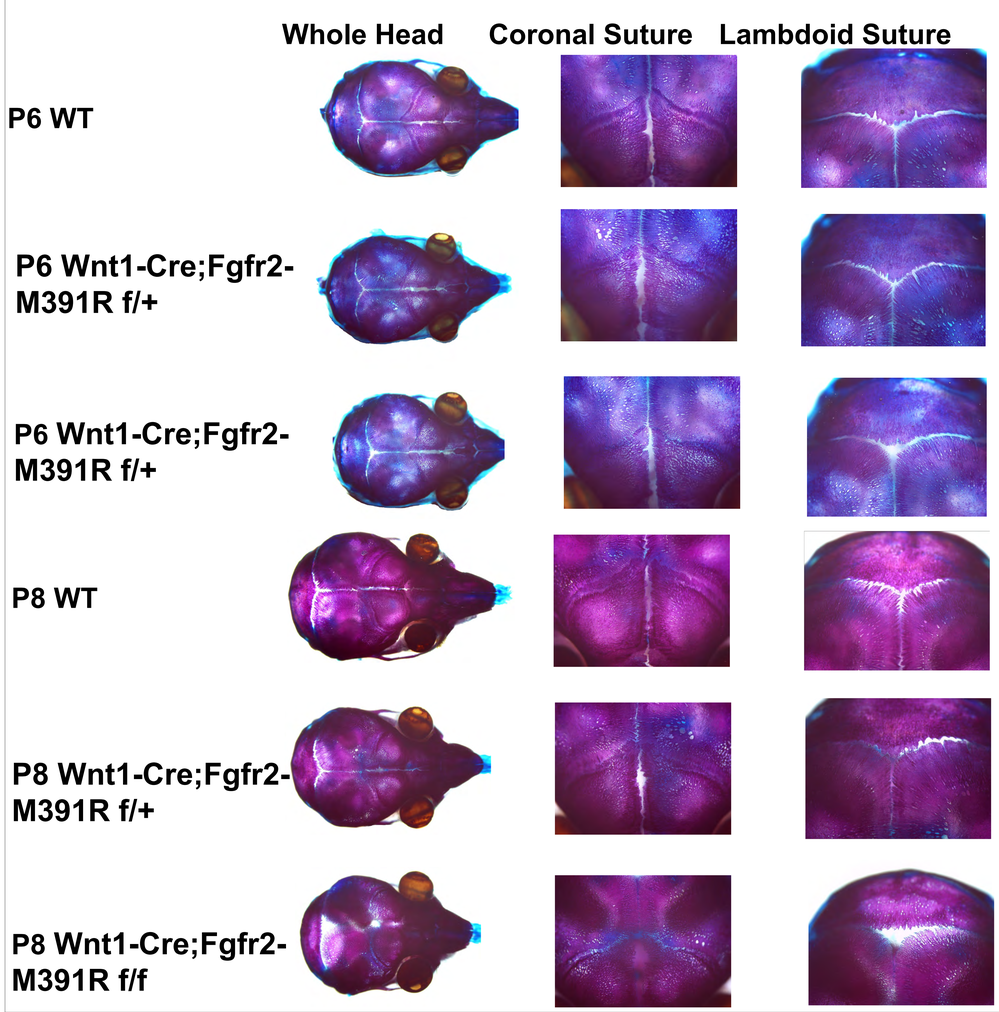
NB – Nasal Bones
FB – Frontal Bones
PB – Parietal Bones
OB – Occipital Bone

Sutures:

FS – Frontal Suture
CS – Coronal Suture
SS – Sagittal Suture
LS – Lambdoid Suture

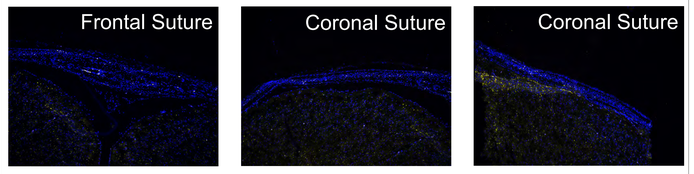
Comparing mice with 0, 1, and 2 copies of the M391R allele

Alizarin Red stains bone and Alcian Blue stains cartilage.

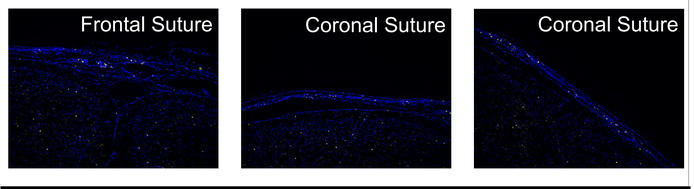


Results of Proliferation Assay

P6 WT +EdU

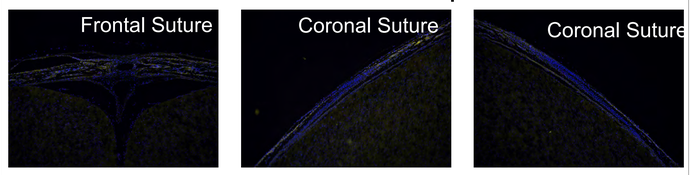


P6 W1M3f+ +EdU

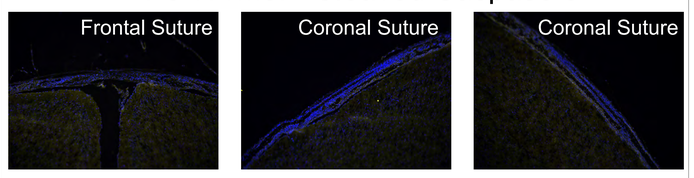


Results of Apoptosis IF

P8 WT Cleaved Caspase 3



P8 W1M3f+ Cleaved Caspase 3



Summary

- Through the course of this project, we have learned that the phenotype differs in the lambdoid suture between wild type, heterozygous, and homozygous mutant mice.
- We have also learned that both proliferation and apoptosis are not changed when the mutation is active.
- There are other processes related to suture fusion that might be affected by the M391R mutation that will be studied in future projects.

Acknowledgements

bridge.usc.edu/bugs