# Investigation of retina and brain development in a mouse model for Familial Dysautonomia

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August 4, 2016

**Montana INBRE Poster Session**

## ABSTRACT

Familial dysautonomia (FD) is a genetic disorder affecting the development and maintenance of the nervous system, and is prevalent in those of Ashkenazi Jewish descent. FD is caused by a point mutation in the gene called IKK complex-associated protein (*Ikbkap*), resulting in a decreased amount of the IKK complex-associated protein (IKAP). FD patients experience symptoms such as decreased sensitivity to pain or temperature, dysfunction of their autonomic nervous system, incoordination, hypotonia, various dysfunctions of the organs, and often die in early adulthood. In addition, FD patients experience progressive blindness due to the loss of retinal nerve fiber layer, which greatly affects their quality of life. In order to study the role of IKAP in the retina, the retina-specific *Ikbkap* conditional knockout (CKO) mouse model was developed using the Cre-lox system. The number of retinal ganglion cells, which make up the retinal nerve fiber, was counted in the CKO and littermate control retinas using immunohistochemistry.

We have generated another mouse model, known as the *Tuba1α-Cre+; Ikbkap*$^{flox/flox}$ mouse, in which *Ikbkap* is deleted in the CNS in order to further investigate the implications of FD on the CNS. Data from our lab has revealed evidence of behavioral alteration, a reduction in specific neuronal populations, reduction in spinal motor neuron innervation, and alteration in cortical morphology for mice with FD. Our data show that the *Tuba1α* mice have enlarged ventricles, a symptom occurring in other degenerative and developmental disorders, such as Alzheimer's and Huntington's diseases. To determine whether these CNS deficits are a consequence of disrupted development, we are investigating specific neuronal progenitor populations in the embryonic cortex.

**Methods:**

Retina

We generated the *Pax6-Cre ; Ikbkap*$^{LoxP/LoxP}$ mouse using Cre-lox recombination to investigate retinal degeneration associated with FD. Retinas from 3 month old mice were collected and analyzed using immunohistochemistry, during which the retinas were labeled with the Brn3 maker, specific to RGC. The neurons were counted and compared to wild type retinas.

Brain Development

We generated the *Tuba1α-Cre+ ; Ikbkap*$^{LoxP/LoxP}$ mouse model to study *Ikbkap* deletion in the CNS. The Cre recombinase has been engineered to be driven by the *Tuba1α* promoter, a gene that is only expressed in neurons. Homozygous *Ikbkap* conditional knockout mice were crossed with hemizygous Alpha-tubulin 1α-Cre (*Tuba1α-Cre*) mice. Embryonic (E17.5) brains were dissected, cryosectioned and stained with cresyl violet or fluorescently tagged antibodies.

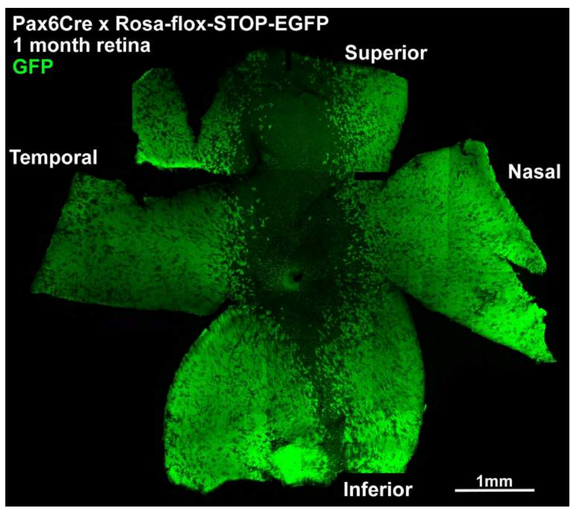


**Fig. 1 One month retina shows that Cre is expressed in the periphery of the retina.** Pax6Cre was crossed with Rosa-flox-STOP-EGFP (Cre reporter) in order to assess Cre expression. This image shows the distribution of Cre expressing cells in GFP. Cre is expressed more prevalently in the peripheral retina than in the central retina.

## RESULTS

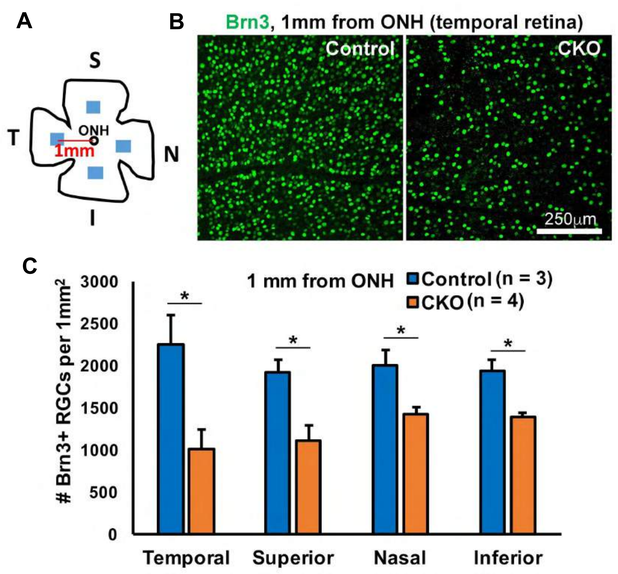


**Fig. 2 αPax6-Cre mice had decreased RGC counts 1 mm from the optic nerve head (ONH). A,** Retinal tissue was analyzed in four different regions, temporal, superior, nasal, and inferior, 1 mm from the ONH. **B,** The CKO temporal retina revealed decreased quantities of RGCs. **C,** Quantified results of RGC counts showed a significant decrease in neuronal populations of CKO retinas, in all regions.

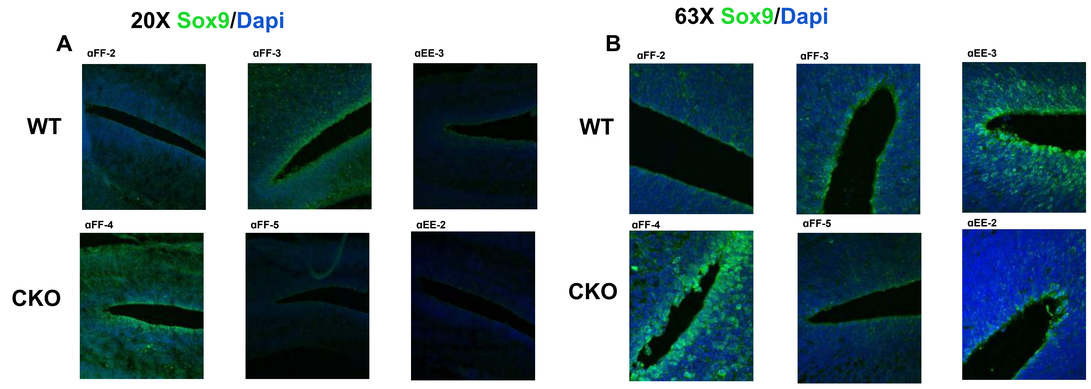


**Fig. 4 Immunolabeling of Sox9 (green), a marker of radial glial cells, and Dapi (blue) in the ventricular zone of E17.5 WT or CKO mice at 20X and 63X. There is no discernible difference between the WT and CKO mice, suggesting that the absence of *Ikbkap* does not influence the population numbers of radial glial cells. However, the inconsistent results may be due to variable phenotypes in the mutants or an uneven stain.**

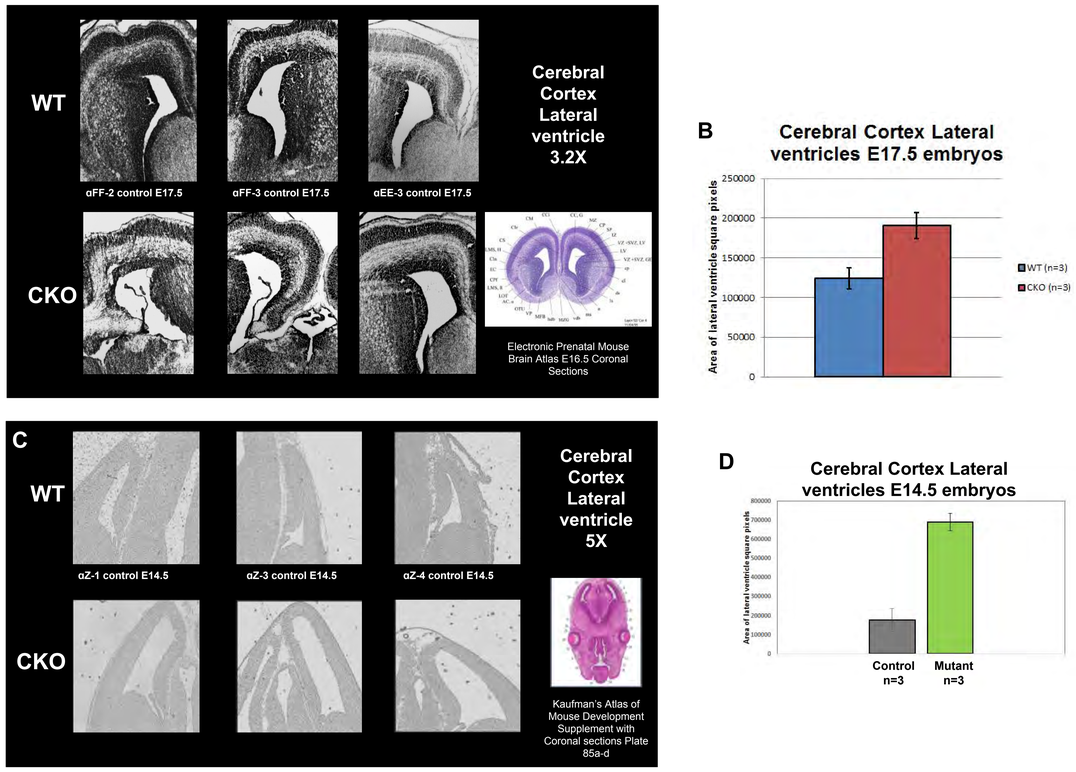


**Fig. 3 E17.5 *αTuba1* mice had enlarged lateral ventricles compared to WT mice. A,** Coronal sections of E17.5 WT and CKO mouse brain lateral ventricles at 3.2X. The ganglionic eminence was used as an indicator for the axial level at which the somatosensory cortex is located. The locations of the lateral ventricles and the ganglionic eminence are shown on the image taken from the Electronic Prenatal Mouse Brain Atlas. **B,** Average lateral ventricle areas of WT or CKO mice measured in square pixels. 124130.6 ± 12875.7 in WT and 190770.7 ± 16531.1 in CKO p-value = 0.0335. **C,** Coronal sections of E14.5 WT and CKO mouse brain lateral ventricles at 5X. The location of the lateral ventricles is shown on the image taken from Kaufman's Atlas of Mouse Development. **D,** Average Lateral ventricle areas of WT or CKO mice measured in square pixels. p-value = 0.0025

## CONCLUSION / DISCUSSION

### Retina

- In our experiments, we show that the lack of *Ikbkap* in the retina results in decreased numbers of retinal ganglion cells. These data recapitulate the FD visual phenotype. This is a useful model for testing potential therapeutics to prevent blindness in FD patients.
- Our next goal is to investigate the mechanisms causing the death of retinal ganglion cells and to test therapeutics for preventing the death of retinal ganglion cells in FD.

### Brain Development

- Our data show that without *Ikbkap* in the CNS, the lateral ventricles are enlarged in both E14.5 and E17.5 mice. The reason for this abnormality is unknown, however we will continue investigating plausible causes of ventricle enlargement, such as abnormal cilia, disrupted neurogenesis, or increased cell death.
- Although our preliminary experiments do not show a significant difference in radial glial cell numbers, we will continue to improve our methodology in order to achieve consistent results. We will also try to localize other specific cell populations, such as intermediate neuronal progenitors, mitotically active cells, and apoptotic cells.

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## ACKNOWLEDGEMENTS

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R01NS35714 and R01NS086796. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This research was also funded by a grant from the Familial Dysautonomia Foundation as well as Montana INBRE. Thank you to Alexis Thompson for her technical assistance throughout these experiments.