Cell Survival in Corneal Endothelial Dystrophies
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OBJECTIVES/SPECIFIC AIMS: Purpose - The goal of this study is to understand how loss of the membrane protein SLC4A11 alters endothelial cell metabolism thereby producing Corneal Endothelial Dystrophy. Studies from our lab indicated that glutamine-dependent mitochondrial dysfunction is one of the outcomes of SLC4a11 loss. In the current study, we ask if autophagy and mitophagy pathways and the signaling pathways that regulate these processes are altered in SLC4a11 KO cells. METHODS/STUDY POPULATION: Methods - Immortalized mouse WT and SLC4a11 KO cell lines were incubated in DMEM with and without 0.5mM glutamine for 6 hours. In order to assess mitophagy, cells were stained using Lysotracker Red and Mitotracker Green. Co-localization co-efﬁciencies of red and green channels were obtained for at least 35 cells using Zeiss-Zen Pro software. Student's t-test was used to determine statistical signiﬁcance. For Western Blots, antibodies against LC3b, AMPK, pAMPK, and b-actin were used to examine autophagy. RESULTS/ANTICIPATED RESULTS: Results - In the presence of glutamine, the colocalization co-efﬁcient of Lysotracker Red and Mitotracker Green channels was signiﬁcantly increased in KO cells (0.74±0.18) relative to WT (0.58±0.20) with a p-value ≤0.0024. In the absence of glutamine, the colocalization co-efﬁcient was reversed, for KO cells 0.54±0.14 and for WT cells 0.77±0.16 with a p-value ≤0.0001, suggesting increased mitophagy by glutamine in KO cells. Western Blots indicated that glutamine increased autophagy ﬂux, as indicated by increased levels of LC3b following bafilomycin A treatment in KO cells. CONCLUSION/SIGNIFICANCE OF IMPACT: Conclusion and implications of these ﬁndings regarding the role of autophagy and mitophagy pathways in the pathogenesis of SLC4a11 KO cells need further investigations. Future studies will determine whether these processes regulate cell survival in mouse models of corneal endothelial dystrophies.

Characterizing the Neural Signature of Metabolic Syndrome
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OBJECTIVES/SPECIFIC AIMS: Our objective is to understand the influence of the features comprising metabolic syndrome (central obesity, raised fasting plasma glucose, triglycerides, blood pressure, and decreased HDL cholesterol) on brain structure in men and women. With the understanding that MetS is a strong predictor of metabolic syndrome variables and gray matter volume changes between brain regions comprising the neural signature of metabolic syndrome were identiﬁed, waist circumference, fasting plasma glucose, and triglycerides are the most reliable predictors of gray matter volume loss. The variance in gray matter volume of the neural signature of metabolic syndrome in men is more signiﬁcantly explained by waist circumference and triglycerides (when accounting for age) and in women is more signiﬁcantly explained by waist circumference and fasting plasma glucose (when accounting for age). A model of metabolic syndrome that emphasizes a risk of neurodegeneration should focus on waist circumference for both men and women and weigh the remaining variables accordingly by sex (triglycerides in men and fasting plasma glucose in women).

Chronic inflammation promotes intestinal macrophages to become modulators of the Notch pathway
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OBJECTIVES/SPECIFIC AIMS: The purpose of this research was to investigate how chronic inﬂammation promotes the generation of proinﬂammatory intestinal macrophages and if macrophages contribute to intestinal inﬂammation through Notch activation. METHODS/STUDY POPULATION: We utilized two animal models of chronic colitis, the chronic DSS-induced colitis mouse model and the spontaneous enterocolitis development in IL-10-deﬁcient mice to investigate the role of chronic inﬂammation in the generation of proinﬂammatory intestinal macrophages and its inﬂuence in Notch signaling. Bone marrow-derived monocytes were collected from each group and differentiated into macrophages (BMM) for gene and protein analysis. Ex vivo phenotypical and functional...