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. Colocalization co-efficients 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 flux and the signaling pathways that regulate 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. Concomitantly, there was an increase in pAMPK/AMPK and decreased HDL cholesterol (when accounting for age) and in women more significantly explained by waist circumference and fasting plasma glucose when accounting for age). What is of metabolic syndrome: METHODS/STUDY POPULATION: We conducted multiple linear regression analyses on a cross-sectional sample of 800 individuals from the Genetics of Brian Structure (GOBS) image archive (352 men and 448 women). GOBS is an offshoot of the San Antonio Heart Study involving an extended pedigree of Mexican Americans from the greater San Antonio area. Its goal is to localize, identify, and characterize genes/quantitative trait loci associated with variations in brain structure and function (Winkler, 2010). The archive has continuously added participants from approximately 40 families since 2006. Neuroanatomic (T1-weighted MRI scans obtained on a Siemens 3T scanner and processed using FSL), neurocognitive, and biometric phenotypes have been obtained for each subject (including blood lipids). Linear regressions were run using SPSS and incorporated biometric and gray matter volume values obtained from 800 GOBS participants. RESULTS/ANTICIPATED RESULTS: Linear regressions incorporating metabolic syndrome variables as dependent variables and gray matter volume from regions involved in the neural signature of metabolic syndrome as predictors show significant predictive patterns that are largely similar between men and women, with some differences. Another linear regression conducted with gray matter volume from the neural signature of metabolic syndrome as the dependent variable and metabolic syndrome variables as predictors shows that waist circumference and triglycerides are the greatest predictors of gray matter volume loss in men, and fasting plasma glucose and waist circumference are the greatest predictors of gray matter volume loss in women. DISCUSSION/SIGNIFICANCE OF IMPACT: Significant sex differences in the relationships between 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 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).