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Mass Spectrometric Lipidomic Analyses of Human Aqueous Humor

Abstract: Primary Open Angle Glaucoma, the leading cause for peripheral blindness in the United States, has an etiology not completely understood but highly related to elevated levels of intraocular pressure. Presumably, the plasma membrane, mainly composed of phospholipids, of the cells experience imperative structural and functional changes during the glaucoma neuropathy. Previous studies were unable to adequately investigate ocular lipidomics because of low lipid yield and sub-par identification and quantification methods until the recent advent of mass spectrometry. The objective of the project is to understand how to use mass spectrometry to characterize 4 classes of phospholipids in terms of quantity and identity utilizing human aqueous humor (AH) samples. Human AH samples were obtained during surgery from human control donors, and split into 1ul, 2ul, 4ul, 6ul, and 8ul aliquots after a modified Bilgh and Dyer lipid extraction process. Extracted lipids were dried and resuspended in liquid chromatography-mass spectrometry (LC-MS) grade Acetonitrile: Isopropanol (1:1) (v/v) and analyzed for specific phospholipids [phosphocholine (PCs), phosphoserine (PSs), phosphoethanolamine (PEs), and phosphoinositols (PIs)] utilizing a TSQ Quantum Access Max triple quadrupole mass spectrometric instrument. Class-specific lipid standards will be added during scans for quantification purposes. Ratiometric quantification will be done utilizing In-House Macros in the MATLAB software. The spectrum of each sample for each specific phospholipid is analyzed through MZmine 2.10 and LipidSearch 4.0 to provide specific identification and structure characterization. Further studies will include an investigation into the comparison of MZmine 2.10 and

LipidSearch 4.0 and an investigation into the comparison of the TSQ Quantum Access Max and the orbitrap Q-Exactive mass spectrometers, thus creating a standard in the reliability for future research on lipids in ocular tissue.