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Analyzing the Effects of PLD3 on APP Processing

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Alzheimer's disease is a form of dementia that affects 30 million worldwide. Neuropathologically, AD is defined as neuronal cell death accompanied by amyloid plaque accumulation and neurofibrillary tangle formation within the brain. Current models of AD have several limitations: animal models incompletely simulate the development of AD; cells from deceased AD patients are difficult to isolate and manipulate; and immortalized cells do not display typical neuronal phenotypes observed in human AD brains. Human induced pluripotent stem cells—stem cells created from adult somatic cells—provide a solution to this problem. IPSC technology facilitates in vitro derivation of human neurons to study neurodegenerative diseases like AD. The goal of this study was to use human iPSC to examine a gene that increases risk for developing AD. IPSC-derived cortical neurons were used to investigate the effects of the A442A variant of the phospholipase D3 (PLD3) gene on PLD3 and amyloid precursor protein (APP) metabolism. Sequential APP cleaving generates amyloid beta peptides, some of which accumulate to form the amyloid plaques that characterize AD. PLD3 has no known function but has been found to be a risk factor for AD. Human iPSC were generated by reprogramming dermal fibroblasts from PLD3 risk variant carriers and non-carriers using non-integrating Sendai virus. To ensure the faithful reprogramming of patient derived human dermal fibroblasts into iPSCs, we measured Oct4, TRA, Sox 2, Nanog, and SSEA4 expression using immunocytochemistry. Validated iPSC were then differentiated into cortical neurons, the main cell type affected in AD, and PLD3 and APP metabolism were measured using immunoblotting. APP levels were statistically similar in both lines; however, we found that PLD3, glycosylated and non-glycosylated forms, was higher in cells carrying the PLD3 A442A variant than in control cells. Together, these findings suggest that the AD risk variant, PLD3 A442A, alters PLD3 accumulation in iPSC-derived neurons.