Functional and pathway analyses of transmembrane NCLs reveal novel neuron-specific roles of CLN3, CLN6, and CLN8

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A longstanding question in Batten research is whether the selective neuronal vulnerability is driven by neuron-specific roles for the causative proteins. Cellular localization of NCL proteins has been well defined separating NCLs into three broad categories including lysosomal, soluble, and transmembrane NCLs. However, the function of many of these proteins remains largely undercharacterized. To address this problem, CLN3, CLN6, and CLN8 BioID constructs were expressed in N2A cells, labeled candidate interactors were analyzed by mass spectrometry, and a list of shared interacting protein partners was compiled. Physical interactions (direct or indirect) were confirmed by coimmunoprecipitation and colocalization was confirmed with immunocytochemistry. Interestingly, interactomes for all three proteins were enriched for SNARE and tether proteins important for trafficking and binding of vesicles and neurotransmitter release. Early findings suggest that CLN3, CLN6, and CLN8 may serve an important role in the regulation of trafficking, as synaptic depletion of these SNAREs and tethers was observed across all three forms. Additional functional experiments confirm aberrant synaptic SNARE complex formation. Furthermore, neurotransmitter recycling and glutamate release assays were investigated, presenting additional evidence of functional roles of CLN3, CLN6, and CLN8 in synaptic processes. Collectively, these experiments identify novel neuron-specific roles of CLN3, CLN6 and CLN8, and may uncover potential therapeutic targets.