## Abstract

The neuronal ceroid lipofuscinoses (NCLs) are a group of fatal genetically inherited neurodegenerative diseases. The hallmark is the lysosomal accumulation of lipofuscin-like material in multiple cell types. NCLs are caused by at least 8 mutant genes. INCL/*CLN1*, LINCL/*CLN2* and CoNCL/*CLN10* are caused by defects in soluble lysosomal enzymes, thiosterase, aminopeptidase and protease, respectively. vLINCL/*CLN3*, vLINCL/*CLN5*, vLINCL/*CLN6,* vLINCL/*CLN7* and vLINCL/*CLN8* are caused by defects in membrane-bound proteins whose function is currently unknown. Most variants manifest cell death, intracellular trafficking defects and dysregulated sphingolipid metabolism, suggesting that NCL proteins may interact along one pathway especially important for proper neuronal functioning.

One of the most recent described variants is the CLN6, which is caused by mutations in the *CLN6* gene. This highly conserved protein contains no signal peptide or asparagine linked glycosylation sites. The function of the CLN6 protein is unknown. Recently, the CLN6 protein has been located to various cellular compartments including endoplasmatic reticulum, Golgi, recycling endosomes and lipid rafts.

Aiming to get novel insights into the CLN6 cell location three polyclonal immunopurified peptide antibodies were produced and characterised. In cultured human fibroblasts, these antibodies were able to detect the endogenous protein in either SDS-PAGE/Western blot or immunofluorescence studies. Furthermore, data obtained by exposing the cells to pre immune serum or blocking peptide indicated that the labelling pattern was specific. The intracellular distribution of CLN6 in cultured human fibroblasts was investigated by double immunofluorescence microscopy. The wild type CLN6 showed partial co-localization in nucleus and along cell membrane in regions stained with phalloidin that specifically interacts with actin microfilaments. The mutation p.I154del in the *CLN6* gene did not interfere with CLN6 intracellular trafficking. Moreover, fibroblasts from CLN1, CLN2, CLN3 and CLN5 patients did not showed any severe miss localization of CLN6 protein.

The data here reported provided novel clues into the knowledge of CLN6 protein cell location. Altogether, data here reported along with previous findings suggest that the CLN6 protein may have a role in signal transduction from the membrane to the

cytosol, and then to the nucleus where can cause specific changes in gene expression. The molecular players of this cascade must be identified in the future.

Overall, the data reported in the present study will contribute to the design of future studies aiming to investigate the protein cell function and the disease pathology of CLN6 and other NCLs, as well as the mechanisms of neurodegeneration and aging.