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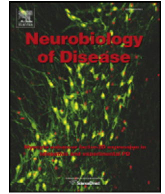

Inhibition of storage pathology in prenatal *CLN5*-deficient sheep neural cultures by lentiviral gene therapy

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ABSTRACT

The neuronal ceroid lipofuscinoses (NCLs, Batten disease) are inherited neurodegenerative lysosomal storage diseases caused by mutations in several different genes. Mutations in *CLN5* cause a variant late-infantile human disease and some cases of juvenile and adult clinical disease. NCLs also occur in animals, and a flock of New Zealand Borderdale sheep with a *CLN5* splice-site mutation has been developed for model studies. Dissociated mixed neural cells from CLN5-deficient foetal sheep brains contained no obvious storage bodies at plating but these accumulated rapidly in culture, mainly in microglial cells and also in neurons and astrocytes. Accumulation was very obvious after a week, as monitored by fluorescent microscopy and immunostaining for subunit c of mitochondrial ATP synthase. Photography at intervals revealed the dynamic nature of the cultures and a flow of storage bodies between cells, specifically the phagocytosis of storage-body containing cells by microglia and incorporation of the storage bodies into the host cells. No storage was observed in cultured control cells.
Transduction of cell cultures with a lentiviral vector expressing a C-terminal Myc tagged CLN5 resulted in secretion of post-translationally glycosylated and processed CLN5. Transduction of *CLN5*-deficient cultures with this construct rapidly reversed storage body accumulation, to less than half in only six days. These results show that storage body accumulation is reversible with enzyme correction and support the use of these cultures for testing of therapeutics prior to whole animal studies.

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Introduction

Batten disease (neuronal ceroid lipofuscinoses; NCLs) is a group of genetically distinct lysosomal storage diseases (www.ucl.ac.uk/ncl), characterised by the accumulation of specific proteins in fluorescent storage bodies in neurons and other cells (Palmer et al., 2013) and neurodegeneration leading to severe brain atrophy (Mole et al., 2011). Collectively these diseases, characterised clinically by blindness, seizures, dementia and movement disorders, form the most common neurodegenerative diseases of childhood, with a reported incidence of 1:12,500 (Rider and Rider, 1988). NCLs are autosomal recessive diseases with the exception of an autosomal dominant adult-onset form, and over 400 mutations have been described in more than a dozen genes (Kousi et al., 2012; Warrier et al., 2013). The products of these are diverse, but in general can be catalogued into two groups; those that encode membrane-associated proteins of the endoplasmic reticulum (*e.g. CLN6*) or endosome/lysosomes (*e.g. CLN3*), and those encoding soluble, secreted lysosomal proteins such as *CLN1*, *CLN2* and *CLN5*.

CLN5 is mutated in a variant late-infantile NCL, originally described as the Finnish-variant late infantile form, OMIM #256731 (Santavuori et al., 1991; Savukoski et al., 1998; Williams et al., 1994). Further *CLN5* mutations have been identified in patients diagnosed with juvenile and adult clinical disease, and in patients worldwide (Cannelli et al., 2007; Xin et al., 2010). Mutations include numerous missense mutations, almost all in highly conserved residues, nonsense mutations, and insertions or deletions (Fig. 1a; http://ucl.ac.uk/ncl/CLN5mutationtable.htm). Diseases caused by mutations in *CLN5* have also been diagnosed in a number of animals, including dogs, cattle and sheep, and a knock-out model developed in mice (Bond et al., 2013).

Abbreviations: AAV, adeno-associated virus; CIDR, controlled internal drug (progesterone) release device; DAPI, 4′,6-diamidino-2-phenylindole; div, days *in vitro*; Endo H, Endoglycosidase H; FBS, foetal bovine serum; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; LVMNDU3, lentiviral derived vector with a myeloid sarcoma virus U3 element; MAP, microtubule associated protein; MOI, multiplicity of infection; M6P, mannose-6-phosphate; M6PR, mannose-6-phosphate receptor; NCL, neuronal ceroid lipofuscinosis; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline, pH 7.2; PDI, protein disulphide isomerase; PNGase F, peptide-N-glycosidase F; PVDF, polyvinylidene fluoride; SDS, sodium dodecyl sulphate; TU, transducing units.

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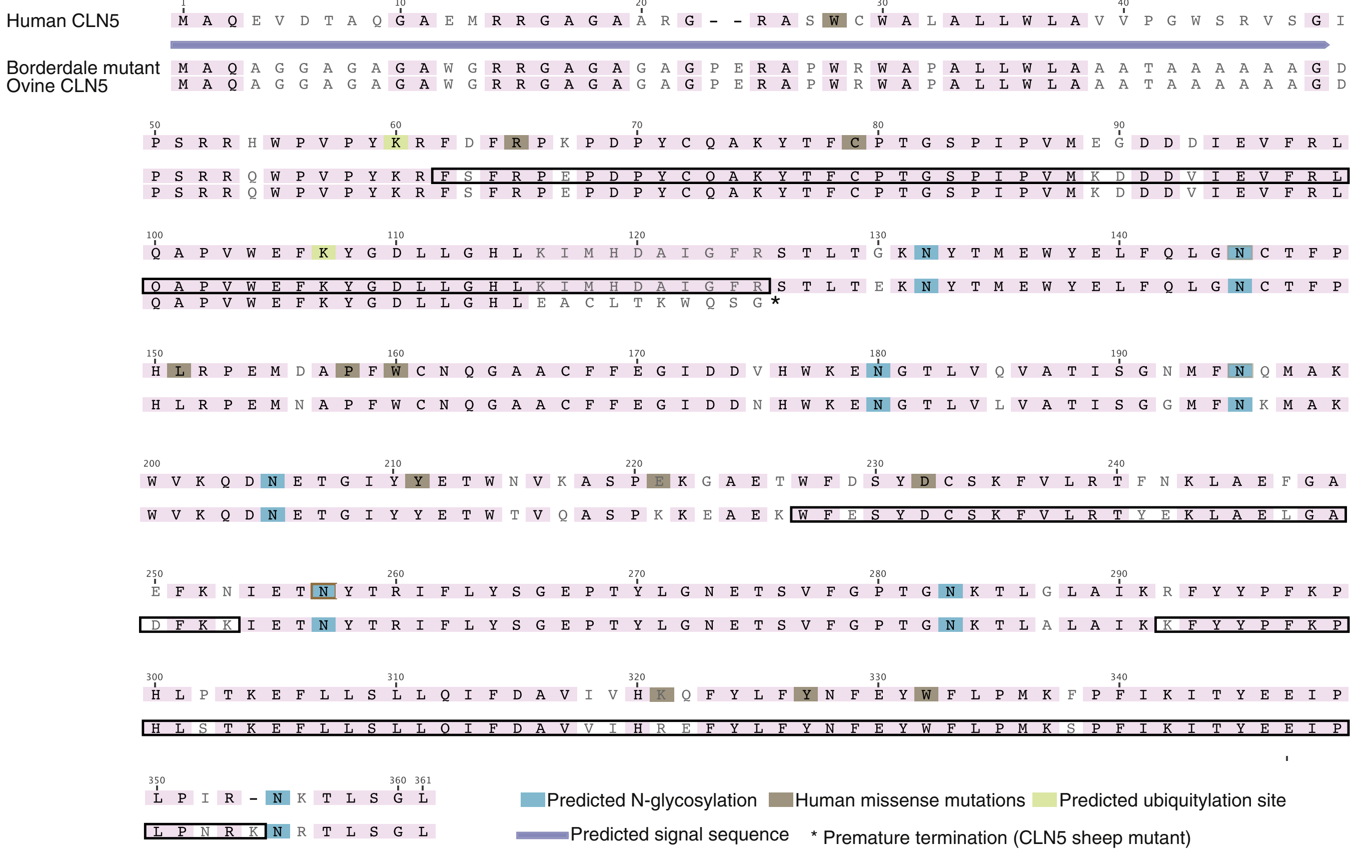

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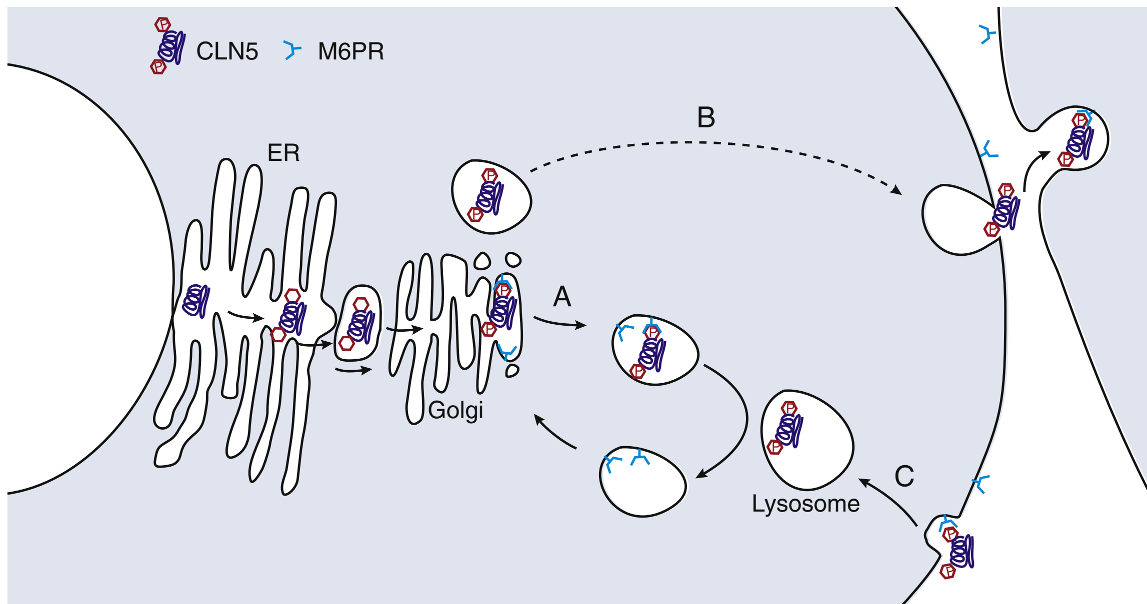

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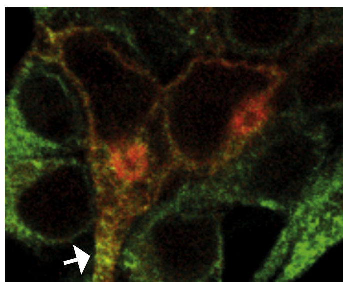

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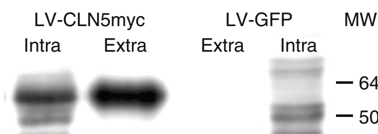

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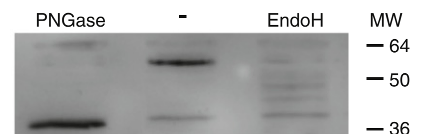

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