

## MUTANT BATTEN DISEASE PROTEIN SAYS “NO” TO UNSATURATED FATS

**CLN3P, the Batten’s Disease Protein, Is a Novel Palmitoyl-Protein  $\Delta$ -9 Desaturase** Narayan SB, Rakheja D, Tan L, Pastor JV, Bennett MJ. *Ann Neurol* 2006;60(5):570–577. **OBJECTIVE:** Batten’s disease, one of the most common recessively inherited, untreatable, neurodegenerative diseases of humans, is characterized by progressive neuronal loss and intraneuronal proteolipid storage. Although the gene for the disorder was cloned more than a decade ago, the function of the encoded protein, CLN3P, has not been defined thus far. **METHODS:** Sequence analysis using the Pfam server identified a low stringency match to a fatty acid desaturase domain in the N-terminal sequence of CLN3P. We developed a fatty acid desaturase assay based on measurement of desaturase products by gas chromatography/mass spectrometry. **RESULTS:** We show that CLN3P is a novel palmitoyl-protein  $\Delta$ -9 desaturase, which converts membrane-associated palmitoylated proteins to their respective palmitoleated derivatives. We have further demonstrated that this palmitoyl-protein  $\Delta$ -9 desaturase activity is deficient in *cln3*<sup>-/-</sup> mouse pancreas and is completely ablated in neuroblastoma cells by RNA inhibition. **INTERPRETATION:** We propose that palmitoyl-protein desaturation defines a new mechanism of proteolipid modification, and that deficiency of this process leads to the signs and symptoms of Batten’s disease.

## COMMENTARY

Although both clinicians and researchers now recognize epilepsy as a multifaceted central nervous system disease rather than simply an isolated seizure disorder (1), epileptologists give surprising little attention to progressive myoclonic epilepsy (PME) syndromes, neurodegenerative diseases that produce worsening neurological symptoms, as well as to myoclonic and tonic-clonic seizures (2). The neuronal ceroid lipofuscinoses (NCLs) comprise one subset of the PME syndromes, which in addition to myoclonic epilepsy, cause dementia, ataxia, early death, and except in the adult form, blindness. To date, NCL investigators have classified nine NCL subtypes, distinguished by age of onset, clinical features, pathology, and causative genes.

Juvenile NCL (JNCL), also called Batten disease, develops between ages 4 and 10 and typically starts with seizures along with rapid deterioration in vision, which is followed by motor, cognitive, and behavioral decline. Pathological features include neuronal death in the brain and retina; accumulation of the intracellular autofluorescent pigment, lipofuscin, and the presence of characteristic structures known as “fingerprint profiles,” with or without “curvilinear profiles,” identified via electron microscopy. The International Batten Disease Consortium cloned the Batten Disease gene, *CLN3*, in 1995 (3). The majority of patients possess a 1.02 kb deletion in both their *CLN3* genes, while the remainder possesses splice-donor site, frameshift, missense,

or nonsense mutations (<http://www.ucl.ac.uk/ncl/cln3.shtml>) either in both genes or in one gene in conjunction with the 1.02 kb deletion in the other gene (i.e., compound heterozygotes).

Persaud-Sawin and Boustany demonstrated that cells lacking wildtype CLN3 protein (CLN3P) undergo apoptotic and autophagic cell death (4). However, despite intensive investigation, the mechanisms by which CLN3P prevents cell death remain unknown. One hypothesis, based on studies of the yeast CLN3P ortholog, holds that CLN3P helps acidify the lysosome and thus, cells lacking functional CLN3P have abnormal lysosomal function and lysosomal amino acid transport (5,6). Another hypothesis, inspired by identification of a characteristic motif in CLN3P’s amino acid sequence, suggests that CLN3P functions to traffic sphingolipids from the Golgi to the plasma membrane and that deficient sphingolipid trafficking by mutant CLN3P impairs normal antiapoptotic signaling (7).

The results of the study by Narayan et al. suggest that CLN3P performs a previously unrecognized task and, moreover, that CLN3P represents a founding member of a new class of enzymes important for cell signaling. To discover possible CLN3P functions, Narayan et al. queried for proteins homologous to CLN3P within the Pfam database (<http://pfam.janelia.org/>), a database of protein families aligned by semimanual methods and thus is claimed by its curators to be especially sensitive to the identification of homologous proteins (8). The Pfam search revealed that CLN3P shared features with fatty acid desaturases, which are enzymes that insert double bonds at various positions within the long carbon chains of fatty acids. It must be emphasized that the Pfam search found only a very weak similarity between CLN3P and the fatty acid desaturase family;

this similarity localized primarily to a 14 amino acid segment of CLN3P, starting at phenylalanine 41.

Given the very weak structural similarity between CLN3P and the fatty acid desaturase family, it appears remarkable that this study found desaturase activity associated with CLN3P. Narayan et al. characterized CLN3P-associated desaturase activity using lysates from neuroblastoma cells stably overexpressing CLN3P; desaturase enzyme kinetics were determined by nicotinamide adenine dinucleotide spectrophotometry and reaction products were identified by gas chromatography and mass spectroscopy. These methods revealed three particularly important results concerning CLN3P-containing lysates: (i) preferred substrates consisted of 16 carbon fatty acids (palmitate) not conjugated to coenzyme A, but rather conjugated to either cysteine or the Ras protein; (ii) the desaturase inserted a single double bond between the ninth and tenth carbon atoms, as numbered from the carbonyl carbon (i.e., a  $\Delta$ -9 desaturase); and (iii) small inhibitory RNA “knock-down” of CLN3P mRNA substantially inhibited desaturase activity. In addition, Narayan et al. demonstrated that both pancreas and brain lysates obtained from *Cln3* knockout transgenic mice lacked substantial desaturase activity. These results certainly suggested that CLN3P participated in  $\Delta$ -9 desaturation of palmitoylated proteins. However, the experiments conducted by the investigators did not directly demonstrate that the CLN3P protein, itself, contained the catalytic active site. Despite the sequence homology of CLN3P and the fatty acid desaturase family, these experiments did not exclude the possibility that another, unidentified protein in the lysate performed the catalytic activity and that CLN3P simply acted as a necessary associated protein. In fact, although lymphoblasts express CLN3P (6), Narayan et al. were unable to measure  $\Delta$ -9 desaturase activity in lymphoblast lysates, a result that could suggest that a protein other than CL3P engaged in catalysis. Hopefully, future studies will characterize the  $\Delta$ -9 desaturase activity of purified CLN3P and define the catalytic active site.

How could the lack of CLN3P  $\Delta$ -9 desaturase activity result in neuronal death? While previously characterized  $\Delta$ -9 desaturases act upon coenzyme A conjugated fatty acids (9), this investigation showed that CLN3P-associated desaturase activity preferentially used palmitoylated proteins as substrates and thus,

in fact, defined a new enzyme class. As Narayan et al. discuss, palmitoylation functions as an important reversible posttranslational modification to target signaling molecules to specialized membrane domains, the so-called “lipid rafts” (10). Thus, it would be expected that alteration of the conjugated palmitate by the creation of the  $\Delta$ -9 double bond could modulate the targeting of these signaling molecules. Therefore, further insight into the role of CLN3P-associated desaturation activity may provide important clues not only to Batten disease pathogenesis but to cellular neurophysiology as well.

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## References

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