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Another disorder finds its gene

Siintola *et al.* describe for the first time three patients with a deficiency of the lysosomal aspartyl proteinase cathepsin D (CTSD). In one of these patients, the authors found a mutational inactivation of the cathepsin D gene (CTSD) that encodes CTSD. All the patients described had severe neurological abnormalities at birth including intractable seizures, spasticity, apnoea and microcephaly. This deficiency is proposed as the underlying cause of congenital neuronal ceroid lipofuscinosis (CNCL) (Humphreys *et al.*, 1985; Garborg *et al.*, 1987; Barohn *et al.*, 1992).

Originally described in a sheep model (Tyynela *et al.*, 2000, 2001) and further corroborated by findings in a canine model (Awano *et al.*, 2006), defects in CTSD were considered to be part of the neuronal ceroid lipofuscinoses (NCLs). The ovine model proved to be a feasible tool to study the disease. The affected sheep have a mutation in the active site of CTSD and newborn lambs suffer similar pathological and neurological consequences as seen in the human NCLs. The NCLs encompass a group of lysosomal storage diseases sharing similar clinical features (Goebel *et al.*, 1999; Herva *et al.*, 2000; Santavuori *et al.*, 2000; Weimer *et al.*, 2002; Mole, 2004). Described in the 19th and early 20th century as one disease with a variable age of onset, these diseases are now known to be caused by mutations in six different genes (CLN1–CLN3, CLN5, CLN6 and CLN8) located on different chromosomes (for a review see Mole, 2004). Adult NCL is expected to be caused by mutations in a gene not yet identified (CLN4). The worldwide incidence of the NCLs is 1 : 30 000 live births (Santavuori *et al.*, 2000).

The NCLs present with progressive loss of vision and neurodevelopmental decline, seizures, myoclonic jerks and premature death. The classification of the four major clinical forms is based on the age of onset: infantile (INCL/CLN1), late infantile (LINCL/CLN2), juvenile (JNCL/CLN3) and adult (Kufs disease or ANCL/CLN4). In addition the

NCLs also include three variants of the late infantile form (vLINCLs/CLN5, CLN6 and CLN8) and northern epilepsy (NE/CLN8) which is allelic with one of the vLINCLs (Ranta *et al.*, 2004). Now with the addition of CNCL due to mutation of CTSD the human NCLs currently consist of nine diseases due to mutation in eight genes, seven of which have been identified (Table 1). Little is known about ANCL as it encompasses a more diverse clinical presentation and evolution, and some cases initially classified as ANCL have been later re-classified as variants of lipidosis (Berkovic *et al.*, 1988; Goebel *et al.*, 1999). Although presumed to be caused by mutations in CLN4, lack of the ability to perform accurate diagnosis has been a limiting factor for the identification of this gene.

Now with the addition of CNCL due to mutation of CTSD, the human NCLs currently consist of nine diseases caused by mutations in seven genes, six of which have been identified (Table 1). The common pathological finding is the

Table 1 The Neuronal Ceroid Lipofuscinoses. Clinical classification, gene affected, and protein designation.

Disease	Chromosome location	Gene affected	Protein designation/function
CNCL	11p15.5	CTSD	Cathepsin D
INCL	1p32	CLN1	Palmitoyl protein thioesterase I
LINCL	11p15	CLN2	Tripeptidyl peptidase protein I
JNCL	16p12	CLN3	Unknown
ANCL	Unknown	CLN4?	Unknown
vfinLINCL	13q31–32	CLN5	Unknown
vLINCL	15q21–23	CLN6	Unknown
vturkLINCL	8p23	CLN8	Unknown
NE	8p23	CLN8	Unknown

presence of autofluorescent storage material in the lysosome, which varies in composition amongst the different NCLs (Palmer *et al.*, 1997; Tyynela *et al.*, 1997). Although not completely characterized, it is known that the deposits contained in INCL, LINCL and JNCL include saposins A and D (SAPs), various lipids and other proteins. However, in INCL there is no accumulation of the subunit c of mitochondrial ATP synthase, which has been found in LINCL and JNCL to be a predominant component of the storage material (Palmer *et al.*, 1992). In JNCL, accumulations also show variable amounts of amyloid beta peptide, dolichyl compounds and oligosaccharides. The mechanism(s) responsible for the accumulation of these materials is (are) not completely understood, and their heterogeneity suggests that more than one degradation pathway in the lysosome may be affected. Ultrastructural analyses of the storage material also reveal a different appearance depending on the disease (Palmer *et al.*, 1997; Tyynela *et al.*, 1997; Goebel *et al.*, 1999). Granular osmiophilic deposits (GRODs) are the hallmark for INCL. These aggregates are localized in neurons forming packed structures of nearly 5 μm in size, but can be found in other cell types as well. Curvilinear profiles (CP) are membrane bound bodies present in almost any cell, and are a hallmark of LINCL. Rectilinear complex and fingerprint bodies are present in lesser extent in JNCL and vLINCLs. However, the role that the storage material plays in the pathophysiology of the NCLs has been the focus of debate, and remains elusive.

To date, the only NCL genes whose encoded proteins have a known function are *CLN1* and *CLN2*. *CLN1* encodes palmitoyl protein thioesterase I (PPT1) and *CLN2* encodes the serine protease, tripeptidyl peptidase protein I (TPP1), both of which are soluble lysosomal enzymes (for a review see Lin *et al.*, 2001; Hofmann *et al.*, 2002).

The protein mutated in JNCL, denoted CLN3, is most likely a lysosomal transmembrane protein (for a review see Phillips *et al.*, 2005), which has been associated with different cellular pathways such as arginine transport into the lysosome (Kim *et al.*, 2003; Ramirez-Montealegre and Pearce, 2005), lysosomal vATPase activity and vacuolar pH regulation (Pearce *et al.*, 1999a,b; Ramirez-Montealegre and Pearce, 2005; Padilla-Lopez and Pearce, 2006), and apoptosis (Puranam *et al.*, 1999; Persaud-Sawin *et al.*, 2002). The Finnish variant for the late infantile form is caused by mutations in *CLN5*. It is likely a transmembrane protein, and localizes to the lysosome (Isosomppi *et al.*, 2002). The gene responsible for the vLINCL described in small populations of patients of Spanish, Romany or Portuguese origin was mapped to chromosome 15, and corresponds to *CLN6* (Sharp *et al.*, 1999). Naturally occurring animal models of this disease include the *nclf* mouse model (Bronson *et al.*, 1998) and the South Hampshire sheep model (Broom and Zhou, 2001). Interestingly, as opposed to most of the other CLN-proteins, CLN6 has been localized to the endoplasmic reticulum (Heine *et al.*, 2004; Mole *et al.*, 2004). The Turkish variant of LINCL

(vturkLINCL) has been recently identified as being caused by a mutation in *CLN8* (Ranta *et al.*, 2004), although it was originally classified as being due to mutation in a gene designated as *CLN7*. Northern epilepsy (NE) is caused by mutations in *CLN8* (Tahvanainen *et al.*, 1994). The function of CLN8 is not known; however, it has been localized to the endoplasmic reticulum (Lonka *et al.*, 2000).

CNCL is defined as a rare congenital condition characterized by severe microcephaly, spasticity, neonatal status epilepticus and death within the first days or weeks of life (Humphreys *et al.*, 1985; Garborg *et al.*, 1987; Barohn *et al.*, 1992). Based on clinical findings from reports of affected patients, CNCL was tentatively included as a variant NCL without genetic assignment (Goebel *et al.*, 1999), because evaluation of post-mortem tissue showed the presence of autofluorescent material with similar components to those found in INCL. Owing to the possibility of other severe neonatal conditions that could explain similar clinical signs, differential diagnosis is difficult and includes a large number of fatal metabolic diseases.

Using blood samples from a reported still-alive case of CNCL in a newborn of Pakistani origin, and based on their previous findings in the ovine model, Siintola *et al.* screened *CTSD* using samples obtained from the father and the affected newborn. This family had a previous history of two affected newborns clinically classified as CNCL. These two newborn boys died within the first days of life. Paraffin embedded samples from the two diseased newborns, and samples obtained from a fourth non-related case of British origin, were used for non-genetic analyses.

A search for mutations in the nine exons and adjacent intron/exon boundaries of *CTSD* revealed that the affected newborn had a homozygous single nucleotide duplication in exon 6 that alters the reading frame and causes a premature stop codon at position 255. Similar analysis of the father showed that he is a heterozygous carrier of the same mutation. Although not available for analysis, it is predicted that the mother carries a similar mutation due to consanguinity, as the parents are first cousins. As a consequence of the mutation, the *CTSD* mRNA is probably degraded by the nonsense-mediated mRNA decay system (Maquat, 2004). This likely accounts for the lack of detectable *CTSD* in the patient's tissues studied by immunohistochemistry. It is also possible that if the mRNA survives the normal decay controls, the truncated protein is degraded. The authors were able to express the truncated protein in model systems and found that it had no *CTSD* activity. Thus, it is almost certain that the *CTSD* mutation, and consequent lack of *CTSD* enzyme activity, accounts for the severe disease seen in the Pakistani patient. Further work will reveal if all cases of CNCL are due to mutations in *CTSD*. It is also possible that less damaging mutations in *CTSD*, that allow some residual enzyme activity, may cause CNCL with a later onset and milder presentation.

Neuropathological findings using paraffin embedded samples from the two Pakistani newborns and the British

patient showed absence of CTSD immunostaining, microglial activation, severe neuronal and white matter loss, and presence of storage deposits including SAPs, which were previously described in other CNCL patients. In the samples analysed, Siintola *et al.* report the absence of accumulation of the subunit c of mitochondrial ATP synthase. This finding was also reported in previous cases. The pathological reports presented in the article also resemble results previously obtained from the ovine model.

The findings of Siintola *et al.* strongly support the notion that CNCL is in fact another form of NCL. Their results also show that CNCL is caused by mutations in the gene encoding the lysosomal enzyme CTSD. Their approach nicely demonstrates the value of naturally occurring animal models for investigation of the NCLs. In this particular instance, the animal models pointed towards a likely candidate gene, which seems to be correct for the cases studied. In the absence of the animal models it would have taken much longer to identify the gene responsible for such a rare and hard to diagnose disease. The animal models will also very likely prove to be important in the elucidation of the normal functions of the proteins that are defective in other types of NCL. Furthermore, similar to INCL and LINCL in which lysosomal enzymes are lacking or defective, their findings suggest that common cellular pathways involving the proper lysosomal degradation of proteins and lipids are affected in the different NCLs. This could explain common features present in a set of diseases that are quite diverse with respect to the causative gene, composition of the storage material and severity (Table 1).

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