The Genetic Spectrum of Human Neuronal Ceroid-lipofuscinoses

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The neuronal ceroid lipofuscinoses (NCL), also known as Batten disease, are a group of inherited severe neurodegenerative disorders primarily affecting children. They are characterised by the accumulation of autofluorescent storage material in many cells. Children suffer from visual failure, seizures, progressive physical and mental decline and premature death, associated with the loss of cortical neurones. Six genes have been identified that cause human NCL (*CLN1, CLN2, CLN3, CLN5, CLN6, CLN8*), and approximately 150 mutations have been described. The majority of mutations result in a characteristic disease course for each gene. However, mutations associated with later disease onset or a more protracted disease course have also been described. At least seven common mutations exist, either with a world-wide distribution or associated with families from specific countries. All mutations are described in the NCL Mutation Database (*http://www.ucl.ac.uk/ncl*).

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INTRODUCTION

Within the last decade, and as a direct result of the great advances made in the field of molecular genetics and genome sequencing, 6 human genes have been identified that underlie the neurodegenerative disease neuronal ceroid lipofuscinosis (NCL) (Table 1). The NCLs are grouped together on the basis of a common characteristic feature associated with neurodegeneration and its consequences—namely the storage of autofluorescent material in many cells that resembles the lipopigments ceroid and lipofuscin that accumulate normally with age. Disease onset is usually in childhood, and can occur as early as the first few months of life or as late as adulthood. Children suffer visual failure leading to blindness, seizures and progressive mental and physical decline. These features are caused by the death of cortical neurones and result in early death. Each NCL gene is associated with a typical disease course defined by characteristics including the age of onset, the order in which symptoms appear and their severity, and a distinctive ultrastructural appearance of the storage material under electron microscopy.

The identified human genes causing NCL and their encoded proteins (CLN1, CLN2, CLN3, CLN5, CLN6, CLN8) have no features in common apart from the disease they cause. The NCLs can be subdivided according to a number of categories: disease in which subunit c of mitochondrial ATP synthase accumulates as the main protein component of the storage material (CLN2, CLN3, CLN5, CLN6, CLN8) or in which saposins A and D are the main protein component (CLN1); disease in which vacuolations are observed in lymphocytes (CLN3); disease in which defects in an enzyme are responsible (CLN1, CLN2); disease in which proteins located in the lysosome are responsible (CLN1, CLN2, CLN3, CLN5); disease in which the protein is predicted to be an integral membrane protein (CLN3, CLN6, CLN8); disease in which histopathology is granular (CLN1) or with a definite membrane-like pattern (CLN2, CLN3, CLN5, CLN6, CLN8). Now that at least some of the NCL genes have been identified the challenge is to discover the biological pathway or process that unites them. The common link is probably the functional integrity of the lysosome in that the NCLs are lysosomal storage disorders, and four of the human NCL genes (and one gene underlying an animal model, CTSD (58)) encode proteins that are located in the lysosome.

Whilst most mutations in the human NCL genes are associated with a classic disease phenotype, some mutations result in disease that is less severe or is protracted in its progression probably as a result of mutations that do not completely abolish the function of the encoded protein. NCL mutations are listed in the NCL Mutation database (*http://www.ucl.ac.uk/ncl/*) where each is fully cited, and are discussed in more detail below (Table 2).

This article will consider what is known about each gene in turn.

CLN1

CLN1 encodes the enzyme palmitoyl protein thioesterase 1 (PPT1), the function of which is discussed in more detail in an accompanying article. *CLN1* expression is ubiquitous and does not appear to be particularly elevated in any human tissue type (4). The crystal structure of the mature bovine enzyme, which is 95% identical to the human PPT1, has been determined (2) allowing a more complete understanding of the effect of mutations on PPT1 function. It is a monomeric, globular enzyme glycosylated at 3 sites (Asn197, Asn212 and Asn232) (2) and with a catalytic triad consisting of conserved residues Ser115, Asp233 and His289. Other conserved residues include some in the catalytic groove and those donating hydrogen bonds, such as Met41 and Gln116. PPT1 activity can be measured by simple enzyme assay enabling accurate diagnosis of NCL caused by mutations in *CLN1* (63).

Forty mutations have been reported in the *PPT1/CLN1* gene. These are distributed throughout the 9 exons of the gene and include 19 missense, 9 nonsense, 9 small deletions or insertions, and 3 mutations affecting splice sites. All mutations in *PPT1/CLN1* are associated with a granular appearance of the stored mate-

NCL disease	Gene	Gene location	Protein	Accession	MIM	Key references
Infantile, classic And later ages of onset up to adulthood	*CLN1*	1p32	Palmitoyl-protein thioesterase 1 (PPT1)	P50897 L42809	256730 (600722) 600680	(6, 22, 38, 42, 59, 61)
Late infantile classic And later ages of onset up to juvenile	*CLN2*	11p15	Tripeptidyl-peptidase I (TPPI)	AF017456	204500	(49, 52, 62)
Finnish variant late infantile	*CLN5*	13q22	407 amino acid protein	AF068227	256731	(24, 47, 48)
Variant late infantile (also referred to as Czech or Indian variant)	*CLN6*	15q21-q23	311 amino acid membrane protein	AK000568	601780	(9, 49, 50, 65)
Variant late infantile (also known as Turkish variant) ***Northern epilepsy***/Progressive epilepsy with mental retardation (EPMR)	*CLN8*	8p23	286 amino acid membrane protein	AI246377, AA80742, AA36822, AA51562, AA765593	- 600143	(37, 43, 44, 55, 64)
Juvenile, classic	*CLN3*	16p12	438 amino acid membrane protein	Q13286 U32680	204200	(5, 8, 19, 21)
MIM: Mendelian Inheritance in Man						

Table 1. NCL genes.

rial. Most missense mutations change residues conserved between some if not all species (H39Q, G42E, D79G, C96Y, G108R, G118D, R122W, Q177E, V181L, V181M, L219Q, L222P; T75P, F85del, Y109P, E184K, F225S, Y247H, G250V). Nonsense mutations or mutations causing frameshifts that result in premature truncation of PPT1 are predicted to lead to total loss of enzymatic activity (6) through deletion of His289. Other mutations are predicted to affect the geometry of the active site or the conformation of PPT1 (2). Decreased enzyme stability probably plays a more important role in determining the disease phenotype than decreased catalytic activity (7). Mutations associated with later onset of disease have residual activity (T75P, D79G, Q177E, G250V) (7). Mutations R122W, C45ins and F85del cause mutant PPT1 to be retained in the ER or Golgi, probably through incorrect folding (29, 46, 61). Mutations R151X and L10X are associated with an absence of mRNA probably due to nonsense-mediated RNA decay arising from mRNA instability caused by the location of the nonsense mutations close to 3′ splice junctions (16).

Several mutations are enriched in patients from certain countries, which facilitates DNA-based mutation and carrier testing. Mutations T75P and L10X are common in patients of Scottish or Irish origin (6, 38).

NCL gene	Number of mutations	Common mutation	Disease variation
CLN1/PPT	41	2 (R122W, R151X)	Great variation in age of onset
CLN2/TPP1	52	2 (IVS5 -1G>C, R208X)	Some variation
CLN3	31	1 (c.462-677del, 1 kb deletion)	Some variation
CLN5	4	1 (Y392X in Finland)	No variation
CLN6	18	None	Little variation
CLN8	5	None (except R24G for EPMR in Finland)	Great variation in phenotype

Table 2. Mutations in human NCL genes.

Common mutation R151X is found in numerous patients of different ethnic origins suggesting it is an older mutation. There is a founder effect for mutation R122W in Finland (61).

The clinical phenotype caused by mutations in PPT1 is highly variable. The majority of mutations (probably of the order of 50% of cases in the USA (6)) cause classical infantile NCL with an age of onset from 6 months. However some mutations are associated with later onset—either in the late infantile (2-4 years) or juvenile age range (>5 years) or in one instance adulthood. Mutations M1I, T75P, D79G, C96Y, Q177E, V181L, L219Q, L222P, Y247H. G250V, W296X have all been associated with onset in late infancy or the juvenile age range (6, 36, 38). Missense mutation G108R was associated with adult onset NCL in two siblings. Onset was in the third decade with psychiatric symptoms only followed by visual, verbal and cognitive decline, onset of cerebellar ataxia and support for walking (59). PPT1 activity was severely reduced in these patients to the upper part of the range associated with INCL patients. Clinical presentation was consistent in families with more than one affected child (6).

CLN2

CLN2 encodes a lysosomal enzyme tripeptidyl peptidase (TPP1), a member of a recently defined family of serine-carboxyl proteinases (69). CLN2 function is discussed in more detail in an accompanying article. It is produced as a precursor polypeptide that is finally processed to the mature enzyme in the lysosome and is glycosyated (10). The apparent Mr of the mature form is 46 kDa and the N terminus

of the mature form begins at residue L196 (31). The crystal structure of the related enzyme PSCP (*Pseudomonas* serine-carboxyl proteinase) has been resolved and its catalytic triad identified as Glu80, Asp84 and Ser287. Asp170 is also crucial to the catalytic activity of the enzyme (68, 69). The equivalent residues in human TPPI are Glu272, Asp276 and Ser495 with Asp360 also being crucial to the catalytic activity of the enzyme. CLN2 expression is widely expressed and is not particularly elevated in any human tissue type (4) although it reaches adult levels by the age of 2 in the cerebral cortex (27). TPP1 activity can be measured by simple enzyme assay enabling accurate diagnosis of NCL caused by mutations in *CLN2* (71).

Fifty-two mutations have now been defined in CLN2. Nineteen polymorphisms have been reported. The spectrum of mutations in CLN2 encompasses 7 small deletions, 2 small insertions, 29 missense mutations, 5 nonsense mutations and 9 mutations affecting splice sites or intronic sequence. Two mutations are particularly prevalent: IVS5-1G>C affecting a splice site in intron 5 and nonsense mutation R208X. In addition, several mutations, such as Q509X in Italy (57) and G284V in Canadian patients from Newfoundland (23), are more common in particular countries raising the possibility of DNA-based testing for carriers.

Mutations in *CLN2* result in undetectable levels of TPP1 activity. Missense mutations may directly affect the catalytic activity of TPP1 or may affect the structural integrity of the enzyme leading to abolition of enzymatic activity. In a patient heterozygous for a mutation affecting residue S475 (S475L) of the active site, the enzyme is processed normally but enzymatic activity is undetectable (10, 31). This is in contrast to patients homozygous for the common splice mutation or mutation R208X that have undetectable protein by western analysis (10).

At least 3 mutations are associated with later age of onset or a more protracted disease course: R447H (30, 53), S153P (3), R127Q (54) and also a mutation arising from insertion of intronic sequence (13). Mutations within the N terminus of the immature precursor protein may affect processing or stability of the protein preventing gain of enzymatic activity. Certainly it was not possible to detect residual enzyme activity in a patient with the predicted mutation R127Q (54). However the mutations causing predicted changes G77R and R127Q alter invariant residues at splice junctions so may in fact be affecting the splicing of *CLN2* rather than changing single amino acid residues. Mutation S153P should not affect splicing so is perhaps affecting the stability of immature TPPI. Predicted mutation Q422H may also be a splicing mutation rather than a missense mutation since it too is located at a splice junction and changes an invariant splice site residue. Q389E and R447H may affect the structural stability of mature TPP1 (30). Expression studies of protein containing mutation R447H demonstrated no residual TPP1 activity (30). Mutation N286S affects one of the 5 glycosylation sites in TPP1 and is associated with a slightly more protracted disease progression (54).

In a few instances patients carrying common mutations can be more protracted with onset in the juvenile age range (72). In one case homozygosity for R208X was associated with severe caudate atrophy and dystonia presenting at 4 months of age (51), but in this instance additional genetic or environmental causes may explain this unusual disease course.

CLN3

Most cases of NCL of juvenile onset are caused by mutations in *CLN3* which encodes a 438 glycosylated membrane protein of approximately 43 kDa. Its possible function is discussed in more detail in an acompanying article. CLN3 is a conserved protein with orthologous genes present in all eukaryotes down to yeasts suggesting it performs a fundamental cellular function. Its exact function is unknown although expression is highest in gastrointestinal and glandular/secretory tissues (4). In most cells CLN3 protein is located in the lysosome membrane but it may be present at additional locations in neurones (14, 20, 21, 34). CLN3 is predicted to be a transmembrane protein although its exact topology is not certain (19, 35).

Thirty-one mutations have been reported in CLN3. The spectrum of mutations in CLN3 encompasses 4 small deletions, 4 small insertions, 4 large deletions, 7 missense mutations, 7 nonsense mutations, 4 mutations affecting splice sites and 1 intron change. The most common mutation is a 1-kb deletion that is present on approximately 85% of disease chromosomes and therefore amenable to a DNA-based diagnostic test. Some mutations have been demonstrated to affect splicing of the mRNA (40, 41) and it is possible that some mis-spliced transcripts could encode proteins that retain partial function. All missense mutations affect residues conserved between diverse species. Residues L170, G187, V330, R334 are predicted to lie on the same topological face of CLN3, thus mutations affecting these residues may interfere with its binding to a cellular partner. Residues L101 and E295 are predicted to lie within a membrane and mutations affecting these residues may interfere with the topology of CLN3. Some mutations exert their effect because CLN3 does not reach the correct intracellular location but other mutations are not predicted to affect trafficking of CLN3 and must therefore directly impair the function of the CLN3 protein. It is clear that novel mutations remain to be defined since for several patients reported, a change in the coding region or flanking intronic sequence has not been found on their second disease chromosome. Mutations that affect the production or stability of the mRNA species may therefore exist. Patients with mutations in CLN3 have been found all over the world as well as in countries populated by emigration from Europe. The sharing of a common 1-kb deletion by the majority of CLN3 patients indicates their European ancestry (5, 39).

Four missense mutations are associated with an atypical disease course. E295K has been found in 3 patients and is associated with markedly protracted JNCL (28, 40, 67). A patient with R334H also had a more protracted disease course (28, 40) and patients with mutations L101P and L170P also had (different) atypical disease courses (40). In all cases of NCL caused by mutation in CLN3, visual failure has occurred by 10 years, suggesting the importance of CLN3 function in the eye. However in some instances seizures, motor dysfunction or mental regression were significantly delayed. Occasional atypical JNCL cases are seen including a case of granular deposits in some tissues in a patient homozygous for the 1-kb deletion (1).

The 1-kb deletion has been shown to encode a truncated CLN3 protein of ap-

proximately 24 kDa that is retained in the endoplasmic reticulum (ER), presumably because it lacks some critical trafficking motif or is recognized and retained as an aberrant protein (20). In contrast, mutations L101P, L170P and E295K that underlie protracted disease courses and missense mutations V330F and R334H which cause a classical disease course, do not abolish trafficking of the mutant CLN3 protein to the endosome-lysosome system (14); nor, in the case of mutation E295K, to vesicles containing the presynaptic marker synaptic vesicle protein 2 in neurons where presumably mutant CLN3 function is impaired but not absent (20). Studies of the CLN3 orthologous protein in *S. cerevisiae* have demonstrated that mutant proteins containing L170P and E295K do retain CLN3 activity whereas mutant proteins containing L101P, V330F, R334H and C435S have only partial CLN3 function, at odds with the protracted human disease course associated with L101P (14). In addition recent data using *S. pombe* as a model system showed that a mutation equivalent to disease mutation G187A prevented trafficking of a tagged protein orthologous to CLN3 beyond the ER (S Codlin and S Mole, unpublished data). Interestingly CLN3 with an engineered mutation in the putative C-terminal farnesylation motif (C435S) trafficked normally but was functionally impaired (14), although in *S. pombe* the nearest equivalent to this mutation did interfere with correct localisation (S Codlin and S Mole, unpublished data).

CLN5

CLN5 encodes a 407 amino acid glycosylated protein with an apparent molecular weight of 60 kDa which is predominantly located in lysosomes (18). It appears to be confined to vertebrates. Its expression increases during cortical neurogenesis (15) and it is synthesised as four precursor forms, all of which are targeted to the lysosome and most of which are predicted to be soluble (18, 60).

Four mutations have been described in *CLN5*. Two mutations are nonsense mutations (W75X, Y392X) predicted to result in truncated proteins. W75X results from a single base change and W392X from a 2-bp deletion. One mutation (c.669insC) is predicted to cause a translation frameshift resulting in a truncated protein. The fourth mutation (D279N) changes a charged aspartate residue conserved in the mouse *Cln5* orthologue to a polar but uncharged asparagine residue which must have a deleterious effect on protein activity since it causes as severe a phenotype as the truncating mutations. This missense change is located in a hydrophobic region (48). Most cases of NCL caused by mutations in *CLN5* are from Finland, but patients have also been described in Sweden (sharing mutations found in Finland) and one from The Netherlands (carrying a unique mutation) (17). One mutation is particularly common in Finland (Y392X). All mutations are associated with a classic disease course. There may be other patients with so-called "milder" mutations in *CLN5* that are not recognized as vLINCL.

Mutation Y392X has been reported to interfere with lysosomal targeting of overexpressed CLN5 (18) although in another study mutations Y392X and D279N did not prevent targeting of at least some overexpressed CLN5 polypeptide to the lysosome (60). If mutant CLN5 does not reach the lysosome then the disease is presumably caused by defective trafficking of CLN5 resulting in lack of functional protein in the lysosome. It is also possible that other proteins (such as CLN6 or CLN8 which are located earlier in the biosynthetic pathway) are involved in the trafficking of CLN5 to the lysosome, so that if these are defective in their function then a vLINCL phenotype similar to that caused by defective trafficking of CLN5 results. If mutant CLN5 does reach the lysosome then the mutation must be interfering with its function in this location. The trafficking of CLN5 and the effect of mutations on CLN5 location may be cell specific. It is not known what effects mutations in CLN5 have on its location in neurons.

CLN6

Mutations in the gene *CLN6* underlie variant LINCL. The gene is predicted to encode a 311 amino acid membrane protein (9, 65). CLN6 has no homology with known proteins or functional domains and is highly conserved across vertebrates.

Eighteen mutations have now been reported in CLN6. Ten mutations change or delete conserved amino acid residues. Five mutations plus one nonsense mutation are predicted to result in a translation frameshift and truncated proteins. Two mutations occur at splice sites and are predicted to cause aberrant splicing. One mutation (c.316dupC), an insertion of a single cytosine in a run of 6 cytosine residues, also occurs in nclf, the naturally occurring mouse model for CLN6. No major founder effect is evident for CLN6, however as expected patients that share the same mutation generally originate from the same country (50). For CLN6, diagnostic tests designed to detect specific mutations may be most appropriate for certain populations (eg, Costa Rica, Pakistan, Portugal).

All except one of the known mutations are associated with the classic disease course (50). One patient carrying Y221S on one chromosome and an as yet undefined second mutation has a more protracted disease progression (50). It is highly likely that so far undiscovered mutations in CLN6 could cause a milder disease that is not clinically diagnosed as vLINCL, although it would be expected that such cases will be characterized by the deposition of ceroid/lipofuscin-like material.

CLN8

CLN8 is a 286 amino acid predicted membrane protein (45) with an apparent molecular weight of 33 kDa (33). Its primary location appears to be in the endoplasmic reticulum (ER), but it shuttles between the ER and ER-Golgi intermediate complex (ERGIC) utilising a signal (KKRP) at its C terminus that serves to retrieve CLN8 from the ERGIC and return it to the ER (33). The exact function of CLN8 is unknown, however it is a member of the large TLC protein family that may contain lipid sensing domains (66). This raises the possibility that vLINCL is caused by defects in lipid biosynthesis or transport. A mouse model, mnd, exists that has a one base pair insertion in the orthologous mouse *Cln8* gene (45).

Five mutations have now been identified in the *CLN8* gene. A single bp deletion (c.88delG) is predicted to result in a frameshift after residue 29 resulting in a truncated CLN8 protein. Four mutations are missense changes (R24G, R204C, W263C) with one disease allele resulting in 2 different amino acid changes (L16M;T170M) (44). Two of the mutations change a residue to cysteine that may result in inappropriate disulphide bridge formation

in the mutant polypeptide. The R204C mutation affects an arginine residue that is absolutely conserved in the TLC family of proteins of which CLN8 is a member. The linked changes (L16M:T170M) could result in a methionine that can function as an alternative translation initiation codon. It is not known which of these mutations is functionally relevant.

Four mutations are associated with a typical vLINCL disease course (44), but one missense mutation (R24G) results in a very different and protracted disease course that was initially not recognised as NCL. This was the first reported mutation in *CLN8* and it causes Northern Epilepsy or progressive epilepsy with mental retardation (EPMR) (45). Thus, the change R24G is a mild mutation that causes a protracted disease whereas the other known mutations must be more deleterious at the protein level and abolish function of the CLN8 protein to the same extent. These 4 mutations are located within a region of the CLN8 protein homologous to the TLC family of proteins whereas R24 is located prior to the TLC region of homology (66) in a region of the protein with sequence unique to CLN8. T170 and R204 are located within predicted transmembrane domains and W263 is at the TLC C-terminal boundary, suggesting that mutations at these residues affect the TLC-specific function. A polymorphism 155V/A is located within a predicted transmembrane domain of the TLC region and affects a residue that is not conserved across the TLC family.

ARE THERE NCL GENES YET TO BE IDENTIFIED?

There are more NCL genes to be identified that may cause human disease. These include the human homologues of genes which carry mutations in animal models that share features with NCL-CTSD in sheep (58) and mice (25), PPT2 in mice (11, 12), the gene that underlies NCL in the English Setter dog (32) and possibly ClC-3 and ClC-7 chloride channels in mice (26, 70). Two assigned genes (*CLN4* and *CLN7*) have not yet been identified. *CLN4* was assigned to adult onset NCL, also known as Kufs disease or Parry disease. One mutation in *CLN1/PPT1* is known to delay onset of the disease until adulthood, therefore further cases of adult onset NCL may be caused by less severe mutations in the known NCL genes as well as by mutations in a novel gene(s). CLN7 was assigned to a group of consanguineous families from Turkey with variant late infantile NCL (37, 64). A subset of such families carry mutations in the *CLN8* gene (44) but the remaining families may still represent a novel locus (still termed *CLN7*). There are also cases of human NCL in which the gene has not yet been identified, for example within the subset of families with variant late infantile onset (44, 56)—whether these carry mutations in known or novel genes remains to be determined.

CONCLUSION

The identification of 6 human genes that cause NCL in recent years has allowed study of the biology of the NCL to begin. There is clearly much work to be done to elucidate the exact function of these genes, and to identify the cellular substrates and interacting partners. It is also clear that more genes remain to be identified in both human and animal disease. This will permit a more complete picture of the cellular processes that are interrupted in NCL disease, an essential prequisite to further therapeutic development.

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