The Neuronal Ceroid-Lipofuscinoses

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Abstract. The neuronal ceroid-lipofuscinoses (NCLs) collectively constitute the most common group of neurodegenerative diseases in childhood and usually share an autosomal recessive mode of inheritance. Despite varying ages of onset and clinical course characterized in most instances by progressive mental and motor deterioration, blindness, epileptic seizures, and premature death, all forms of NCL show unifying histopathological features. There is accumulation of autofluorescent, periodic acid-Schiff-, and Sudan black B-positive granules that are resistant to lipid solvents in the cytoplasm of most nerve cells and, to a lesser degree, of many other cell types. The storage process is associated with progressive and selective neuronal loss and gliosis with secondary white matter lesions. The ultrastructure of the storage deposits varies between different forms of NCL and, along with the age of onset, has provided the basis for the traditional classification of NCLs. Recent molecular genetic findings have established that defects in at least 7 different genes underlie the various forms of NCL. The purpose of this paper is to provide an overview of the NCLs, review recent molecular genetic and biochemical findings, and discuss their impact on our views on the classification and pathogenesis of these devastating brain disorders.

Key Words: Batten disease; Cell biology; Classification; Genetics; Neuronal ceroid-lipofuscinosis; Pathogenesis; Pathology.

INTRODUCTION

The neuronal ceroid-lipofuscinoses (NCLs) constitute the most common type of inherited neurodegenerative diseases in childhood (1). Their incidence in the US has been estimated at 1:12,500 (2) and they usually are autosomal recessive mode of inheritance. The age of onset varies from infancy to late adult. Most childhood forms are clinically characterized by progressive mental and motor deterioration, blindness, epileptic seizures, and premature death, while the rare adult-onset forms are dominated by dementia. In addition to the human forms of NCL, a number of NCLs have also been described in animals. Despite the varying ages of onset and clinical course, all forms of NCL share unifying pathomorphological features. There is accumulation of autofluorescent, periodic acid-Schiff- and Sudan black B-positive granules that are resistant to lipid solvents in the cytoplasm of most nerve cells and, to a lesser degree, of many other cell types. The storage process is associated with progressive and selective neuronal loss and gliosis with secondary white matter lesions. The ultrastructure of the storage deposits varies between different forms of NCL and has provided the basis for the traditional classification, along with the age of onset (3).

For most of the past century, NCLs had been conceived as lipidoses and grouped under the heading of the so-called amaurotic family idiocies (1, 3). However, studies of an ovine form of NCL in the late 1980s led to the seminal discovery that NCLs are, in fact, characterized by intraneuronal accumulation of proteins (4, 5). Since 1995, molecular genetic and biochemical analyses have led to the identification of 7 different human or ovine NCL-associated genes. Discoveries of further NCL genes are to be expected.

The purpose of the present paper is to provide an overview of this complex group of disorders, to review the recent molecular genetic and biochemical findings, and to discuss their impact on our views on the classification and pathogenesis of NCLs.

HISTORY AND CLASSIFICATION

The clinical features characteristic of juvenile NCL were first reported by Stengel in 1826, based on his observations of 4 Norwegian siblings with progressive blindness, epilepsy, cognitive decline, and motor dysfunction (6). Almost a century later, in 1903 and 1923, Batten, Spielmeyer, and Vogt independently demonstrated an intraneuronal storage process in juvenile patients with close clinical resemblance to those reported by Stengel (3). Patients with a similar intraneuronal storage process but with late infantile onset were described by Jänisky in 1908 and Bielschowsky in 1923, and patients with adult onset by Kufs in 1925 (3). Because of histochemical resemblance of their storage material to ceroid or lipofuscin, Zeman and Dyken (1) coined the term neuronal ceroid-lipofuscinosis in 1969 in order to separate the diseases described by Batten, Spielmeyer, Vogt, Jänisky, Bielschowsky, and Kufs from the gangliosidoses. An infantile NCL was later described by Santavuori et al (7) and Haltia et al (8, 9).

A subclassification of the NCLs emerged that is based on the age of onset and the ultrastructure of the storage material. Four main forms were recognized: infantile (INCL), late infantile (LINCL), juvenile (JNCL), and adult (ANCL) NCL. Recent molecular genetic and biochemical studies have revolutionized the classification.
The human NCLs are now classified into 8 main genetic forms (CLN1–8), based on the number of hitherto predicted gene loci (Table 1) (10, 11). This genetic classification will be followed in the present article where the different entities will be presented in their numerical order. It is important to note that different mutations in a single gene may result in different phenotypes, including varying ages at onset. Common mutations that predominate in a given form of NCL are usually associated with the classic clinical picture, while rare “private” mutations may produce a deviant phenotype.

**CLN1**

Patients with mutations of the CLN1 gene occur worldwide. CLN1 mutations can give rise to 4 main phenotypes with varying ages of onset: infantile NCL (INCL, Haltia-Santavuori disease, MIM 256730), and variant forms with late infantile, juvenile, or adult onset. The classic infantile form is by far the most common with close to 500 cases diagnosed worldwide. It is enriched in the Finnish population with an incidence of 1 in 20,000 and 500 cases diagnosed worldwide. It is important to note that different mutations in a single gene may result in different phenotypes, including varying ages at onset. Common mutations that predominate in a given form of NCL are usually associated with the classic clinical picture, while rare “private” mutations may produce a deviant phenotype.

**Clinical Features of INCL:** The affected children usually seem to develop normally until approximately 1 yr of age. However, the rate of head growth may begin to decrease by the age of 5 months. In addition to microcephaly, muscular hypotonia and clumsiness of fine motor control are further early signs. The development begins to slow during the second year of life. Hyperkinesias of the hands, myoclonic jerks, and seizures appear. There is progressive loss of motor abilities, truncal ataxia, and visual failure. Ophthalmological findings after 2 yr of age include brownish discoloration of the macula, retinal degeneration with involution of retinal vessels, and optic atrophy. By 3 yr of age the patients have lost their active movements and visual contact with the environment and they usually die at the age of 8 to 13 yr (7, 12). By magnetic resonance imaging, the first brain abnormalities, including hypointense thalami in T2-weighted images, may be detected at the age of 7 to 10 months, that is, before the first clinical symptoms are observed. After 3 to 4 yr of age, magnetic resonance imaging shows extreme cerebral and cerebellar atrophy with very high signal intensity in the white matter. The EEG becomes flat and the electroretinogram (ERG) extinguished by the age of 3 yr (12).

**Clinical Features of Variant Forms:** The variant forms with late infantile and juvenile onset clinically resemble the classic forms of LINCL and JNCL (see CLN3, vide infra), rather than INCL (11, 12). However, no vacuolated lymphocytes are seen in the variant cases, in contrast to classic JNCL. The characteristic granular osmiophilic storage cytosomes occur in all clinical forms of CLN1 (see below).

**Neuropathological and Biochemical Features:** INCL was originally identified on the basis of frontal brain biopsies carried out in order to establish the diagnosis in clinically unsolved cases of rapidly progressive encephalopathy (8). By the age of 1.5 yr, the cytoplasm of cerebral cortical neurons shows moderate granular deposits that are PAS- (Fig. 1D) and Sudan black B-positive and autofluorescent in paraffin sections. There is progressive neuronal loss, infiltration of the cortex by macrophages containing coarse PAS-positive and autofluorescent granules (Figs. 1E, 2A), and severe astrocytic hyperplasia and hypertrophy (Fig. 1F). Even the astrocytes harbor small granular storage deposits. By the age of 3 yr, almost all

<table>
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Abbreviations: INCL = infantile NCL; LINCL = late infantile NCL; vLINCL = variant late infantile NCL; JNCL = juvenile NCL; ANCL = adult NCL; GROD = granular osmiophilic deposits; CL = curvilinear profiles; FP = fingerprint bodies; RL = rectilinear complex; SAP = saposins; SCMAS = subunit c of the mitochondrial ATP synthase.
cortical neurons are lost and the cortex is dominated by numerous macrophages, often binucleate (8).

At autopsy, by about 10 yr of age, the strikingly atrophic brain (Fig. 1A) has a tough, rubbery consistency and weighs approximately 250 to 350 g. Its cut surfaces are grayish, without any clear demarcation between cortex and white matter. Both the cerebral (Fig. 1G) and cerebellar cortices (Fig. 1H) consist of a rim of hypertrophic astrocytes and are depleted of neurons, with few characteristic exceptions: a few giant cells of Betz (Fig. 1G), and a very occasional hippocampal CA1 pyramidal cell or Purkinje cell may still be seen. The glial centrum semiovale is almost devoid of axons and myelin sheaths, with the exception of a few myelinated axons derived from the precentral gyrus (Fig. 1J). The neurons of the basal ganglia and upper brainstem are still partly preserved but show characteristic intraneuronal storage. There is active neuronophagy and macrophagocytosis in these regions. In contrast, the spinal neurons are well preserved despite marked intraneuronal storage (Fig. 1I), as are the neurons of the spinal and autonomic ganglia (9). In the retina, the visual, bipolar, and ganglion cells are completely lost (Fig. 1K) with optic atrophy and gliosis (13). Small storage granules can be seen even in many extraneural cell types, including epithelial cells such as eccrine sweat glands or thyroid follicles, testes, skeletal, cardiac and smooth muscle cells, endothelial cells, and macrophages in lymphatic tissues (Fig. 1L). Such peripheral deposits are usually minor and not associated with tissue destruction (9).

The intraneuronal and peripheral cytoplasmic deposits show a characteristic electron microscopic appearance. They consist of membrane-bound conglomerates of roundish electron-dense globules (Fig. 3A) with a finely granular internal ultrastructure (8, 9), called granular osmiophilic deposits (GROD) (14). Enzyme histochemistry reveals acid phosphatase activity (8). The deposits show immunoreactivity for sphingolipid activator proteins A and D (Fig. 2B). Amino terminal sequence analysis and Western blotting of purified storage granules has identified sphingolipid activator proteins A and D as the major storage proteins (15).

Molecular Genetic Findings: The CLN1 gene on chromosome 1p32 (16) was identified by a positional candidate gene approach. It encodes palmitoyl protein thioesterase 1 (PPT1) (17). To date, almost 40 different PPT1 mutations are known. Almost all Finnish INCL patients are homozygous for the same missense mutation at Arg122Trp. By far the most common PPT1 mutation in the US population resulting in infantile onset is a premature stop codon at arginine 151. A common missense mutation, Thr75Pro, accounts for most of the variant juvenile cases with GROD in the US and for the cluster of such cases in Scotland. Other mutations (i.e. nonsense, missense, splice-site mutations, and small deletions) are infrequent (11, 12).

**CLN2**

Mutations of the CLN2 gene are known to cause almost all cases of classic late infantile NCL (cLINCL, Jànsky-Bielschowsky disease, MIM 204500). CLN2 probably has a worldwide distribution but seems to be more common in northern European populations than elsewhere. An incidence figure of 0.46 per 100,000 live births has been published from Germany (18), and the prevalence in Sweden, Norway, and Finland has been estimated at 0.6 to 0.7 per million inhabitants (19).

Clinical Features: The onset of major symptoms is usually heralded by seizures and occurs between 2 and 4 yr of life. The seizures may be partial, generalized tonic-clonic, or secondarily generalized and are soon followed by ataxia, myoclonus, and developmental regression, with patients becoming unable to walk or sit unsupported and losing speech. A gradual decline of vision leads to blindness by 5 or 6 yr. The patients usually die in middle childhood (11, 20). Findings at brain imaging are relatively nonspecific, but the neurophysiological findings are characteristic. The EEG shows an occipital photosensitive response using flash rates at 1 to 2 Hz. The ERG is diminished or extinguished and the visual evoked potentials (VEPs) grossly enhanced, as are the somatosensory evoked potentials (SEPs) (11, 20).

Neuropathological and Biochemical Features: At autopsy, the brain, particularly the cerebellum, is severely atrophic with brain weights of the order of 500 to 700 g (20, 21). On cut surfaces the cortex is thin and the ventricular system enlarged. By light microscopy there is severe, partially laminar, loss of cerebral cortical neurons, with activation of the micro- and macroglia. Even the cerebellar Purkinje cells and granule cells are markedly reduced in number. The cytoplasm of the remaining neuronal perikarya in the cerebral cortex and subcortical structures is slightly to moderately distended with storage granules. So-called megal neurites (i.e. spindle-shaped accumulations of storage granules in the proximal axonal segments of cortical neurons) are a characteristic feature, particularly in lamina III. So-called myoclonus bodies, or spheroids, may occur in the neurons of the basal ganglia and dentate nucleus (21). The neuronal loss is accompanied by a secondary loss of axons and myelin in the white matter. There is retinal atrophy, which is more severe in the peripheral than central part and begins at the photoreceptor layer (20, 21).

The storage granules are autofluorescent and stain with Luxol fast blue, PAS, and Sudan black B in paraffin sections. The granules are strongly immunoreactive for subunit c of the mitochondrial ATP synthase (SCMAS) (22) but also for SAP-A and SAP-D (23). Minor deposits of
Fig. 1. A–C: Generalized brain atrophy is a striking feature in all childhood forms of neuronal ceroid-lipofuscinosis (scale in millimeters). It is extreme in the infantile form CLN1 (brain weight 290 g) (A), severe in the Finnish variant late infantile form CLN5 (brain weight 650 g) (B), and less pronounced in the juvenile form CLN3 (brain weight 960 g) (C). D–L: Characteristic aspects of the pathology of the infantile form of CLN1, the most severe of the established forms of neuronal ceroid-lipofuscinosis. D: Periodic acid-Schiff (PAS)-positive storage material in the cytoplasm of cortical neurons of a 1.5-yr-old child. Biopsy, PAS stain, ×400. E: Total neuronal loss and infiltration of the cortex by numerous macrophages harboring PAS-positive granules in a 3-yr-old patient. Some
Fig. 2. A: The storage material in all forms of neuronal ceroid-lipofuscinosis shows autofluorescence in ultraviolet light, as seen here in cortical macrophages of a 3-yr-old patient with the infantile form CLN1. Biopsy, unstained section viewed in ultraviolet light, ×300. B: In the infantile form of neuronal ceroid-lipofuscinosis, saposins A and D constitute the main protein component of the storage material. Biopsy section of a 1.5-yr-old patient. Immunostain for saposin D, ×150. C: The autofluorescent eccentric intraneuronal storage material, as seen here in the Finnish variant late infantile neuronal ceroid-lipofuscinosis CLN5, forms moon-like semicircles. Autopsy, unstained section viewed in ultraviolet light, ×300. D: A section of the previous specimen shows immunoreactivity for subunit c of the mitochondrial ATP synthase, ×300. E: In several forms of neuronal ceroid-lipofuscinosis, particularly in the upper part of the cortical lamina III, the neurons may show accumulations of the storage material in the proximal axon (axonal spindles or megaleneurites). Immunostain for subunit c of the mitochondrial ATP synthase in a case of Finnish variant late infantile neuronal ceroid-lipofuscinosis, ×300. F–H: In all forms of the neuronal ceroid-lipofuscinoses, the abnormal storage granules in the cytoplasm of neurons stain positively with the Luxol fast blue (F), PAS (G), and Sudan black B (H) methods, as seen here in a patient with Northern epilepsy CLN8, ×1,000.

of the macrophages are binucleated. Biopsy, PAS stain, ×400. F: The cortex of a 10-yr-old patient consists of a dense network of hypertrophic astrocytes. Autopsy, Cajal stain, ×300. G: The precentral cortex of a 10-yr-old patient shows a few ballooned giant cells of Betz (arrows) while all other neurons have disappeared. The white matter (WM) in the lower left corner does not essentially differ from the cortex. Autopsy, PAS stain, ×50. H: The cerebellar cortex shows complete loss of both granular and Purkinje cells replaced by a rim of hypertrophic Bergmann astrocytes and occasional macrophages. Autopsy, PAS stain, ×50. I: The spinal anterior horn neurons are preserved but show eccentric cytoplasmatic accumulation of Luxol fast blue-positive material. Autopsy, Luxol fast blue-cresyl violet stain, ×200. J: White matter of the precentral gyrus showing a few preserved isolated myelinated axons, apparently derived from the remaining Betz cells. Autopsy, Luxol fast blue-cresyl violet stain, ×50. K: The neuroretina (between arrows) has been completely destroyed and replaced by gliotic scar tissue and occasional macrophages. Autopsy, PAS stain, ×100. L: Spleen tissue showing a group of large macrophages harboring PAS-positive storage material. Autopsy, PAS stain, ×200.
The ultrastructural appearances of the abnormal intraneuronal deposits vary between different forms of the neuronal ceroid-lipofuscinoses. Four basic types can be delineated: (A) granular osmiophilic deposits are characteristic of the infantile and other forms of CLN1, ×10,000; (B) curvilinear profiles are typical of classic late infantile neuronal ceroid-lipofuscinosis CLN2, ×20,000; (C) fingerprint bodies are the predominant type of intraneuronal inclusions in the juvenile form CLN3, ×30,000; and (D) many inclusions in the variant forms of late infantile neuronal ceroid-lipofuscinosis correspond to the rectilinear complex, ×15,000.

Similar granules also occur in astrocytes and in many other cell types throughout the body. The storage bodies have a fairly uniform ultrastructure in all cells involved. They are bound by a unit membrane and consist of accumulations of so-called curvilinear profiles (Fig. 3B), i.e. uniformly curved short thin lamellar stacks of alternating dark and light lines. Dimensions of the lines range from 1.9 to 2.4 nm (14). Granular or fingerprint components do not occur. SCMAS has been shown to be the major protein component of purified storage cytosomes (24).

**Molecular Genetics:** CLN2 was mapped to chromosome 11p15 by homozygosity mapping in consanguineous families (25), but the gene product and the gene were identified using a biochemical approach (26). The CLN2 gene encodes a ubiquitously expressed lysosomal protease with tripeptidyl-peptidase 1 activity (TPP1) (26–28). Over 40 different mutations of the TPP1 gene have been reported. Two mutations, IVS5-1G→C affecting splicing and the nonsense mutation R208X, are particularly common (29, 30).

**CLN3**

Juvenile NCL (JNCL, Batten-Spielmeyer-Vogt disease, MIM 304200) is the most common form of NCL worldwide and is usually caused by defects in the CLN3 gene. The incidence of JNCL varies in different countries, with the highest figures (up to 7 per 100,000 live births) having been reported from Scandinavia (19).

**Clinical Features of JNCL:** The first symptom is onset of progressive visual failure between 4 to 7 yr of age, leading to blindness within 2 to 10 yr. Funduscropy shows macular and retinal degeneration, optic atrophy, and pigment accumulation in the peripheral retina. Slowly progressive deterioration of short-term memory and other cognitive functions usually starts by 8 or 9 yr of age. Speech becomes dysarthric usually after the age of 15 yr. Seizures appear in most patients between 7 and 18 yr of age and are predominantly of the generalized tonic-clonic or the complex partial type. Many patients show signs of parkinsonism by their mid-teens (11, 31). The patients usually die in the third or fourth decade but patients with a more protracted course have been described (32). The EEG shows nonspecific abnormalities. ERG shows severe changes, and a reduced b-wave may be seen even at the earliest stage. VEPs are markedly reduced or abolished and SEPs often enhanced. After the age of 12 yr, neuroimaging usually shows progressive brain atrophy, mainly affecting the cerebral hemispheres. Vacuolated lymphocytes can be regularly demonstrated on peripheral blood films, a unique finding in NCL (11, 31).

**Neuropathological Features of JNCL:** At autopsy, the brain shows moderate generalized atrophy (Fig. 1C) with brain weight of approximately 800 to 1,000 g. On cut surfaces, the cortical ribbon is slightly reduced in thickness and may have a slightly brownish hue. Nigral pigmentation is reduced. The white matter has a relatively normal appearance but the ventricular system is slightly to moderately dilated. By histological examination there is variable neuronal depletion that may not be very obvious in routinely stained sections. By special techniques selective loss of neurons has been found in the cerebral cortical layers II and V, as well as in the corpus striatum and amygdala. In the cerebellar cortex there is severe loss of the granule cells while the Purkinje cells may be better preserved. The neuronal loss is associated with reactive astrocytic proliferation and hypertrophy and microglial activation. The remaining nerve cell perikarya are slightly to moderately distended by intracytoplasmic accumulation of strongly autofluorescent granular storage material. The entire neuroretina is usually largely destroyed.
and replaced by scar tissue, composed of proliferated Mueller cells and other astrocytes. There is migration of cells of the pigment epithelium through the subretinal space into the retina. Granular storage material also occurs in peripheral neurons and in many extraneural cell types, including the epithelial cells of the sweat glands, the endothelial cells, as well as smooth, skeletal, and cardiac muscle cells (31).

The intraneuronal storage granules have a pale yellow brown color in unstained sections and stain with Luxol fast blue, PAS, and Sudan black B. They show strong acid phosphatase activity and are immunoreactive for SCMAS and also for SAP A and SAP D (24). By electron microscopy, they consist of membrane-bound, electron-dense fingerprint bodies (Fig. 3C) composed of paired parallel dark lines of 7.6- to 9.6-nm width and the central lucent line ranging between 1 and 3 nm (14). The paired parallel dark lines are separated from each other by a lighter intervening layer of varying thickness. While fingerprint bodies are found in pure form in gastrointestinal intraneuronal storage bodies, they may be admixed with curvilinear or rectilinear profiles even within the same storage cytosome in endothelial and smooth muscle cells in rectal and skin biopsies and in the epithelial cells of the sweat glands of JNCL patients. The characteristic electron-dense storage inclusions may also be found within membrane-bound vacuoles in blood lymphocytes and in the epithelial cells of eccrine sweat glands (31). Biochemical analysis of purified JNCL storage granules has shown that SCMAS is the main storage protein (24).

Molecular Genetics: The CLN3 gene was initially linked to the haptoglobin locus on the long arm of chromosome 16 (33), and collaboration of 5 research groups finally led to the isolation of the gene on 16p12 by positional cloning (34). To date, over 30 CLN3 mutations have been reported, the most common being a 1.02-kb deletion leading to skipping of exons 7 and 8 and early truncation of the CLN3 protein (11, 31).

CLN4

The CLN4 locus corresponds to the hypothetic gene implicated in adult onset NCL (ANCL, Kufs/Parry disease, MIM 204300/162350). However, ANCL seems to be genetically heterogeneous (35), and although autosomal recessive inheritance is usually observed, several families with autosomal dominant inheritance have been reported. ANCL is a very rare condition, and the diagnosis should be accepted only after ultrastructural studies of affected tissues and careful exclusion of other alternatives.

Clinical Features: The clinical onset of the disease occurs, on the average, at 30 yr of age, with a range of 11 to 50 yr (35). The clinical phenotype is heterogeneous and 2 main forms can be delineated. Phenotype A is characterized by progressive myoclonus epilepsy associated with dementia, ataxia, and late pyramidal and extrapyramidal symptoms. With the progression of the disease the seizures may become intractable. Phenotype B initially shows behavioral problems, including depression and progressive dementia associated with motor problems such as dysarthria, ataxia, and extrapyramidal symptoms. In both phenotypes, vision is normal without evidence of pigmentary retinal degeneration. Some patients may display features of both main phenotypes. The course of the disease is slowly progressive and leads to death after an average duration of 12.5 yr. Neuroimaging usually demonstrates cortical brain atrophy (35, 36).

Neuropathological Features: There is mild to moderate cerebral atrophy with frontoparietal accentuation (37). At light microscopic examination of paraffin sections, cerebral cortical neurons show intracytoplasmic accumulation of autofluorescent granules stained with the Luxol fast blue, PAS, and Sudan black B methods. In addition to the neuronal perikarya, storage is also frequently seen in the proximal axonal segments (axon spindles). The granules are immunoreactive for SCMAS and also for SAP A and SAP D. The ultrastructural pattern is variable. The abnormal deposits frequently show a granular component either exclusively or in combination with fingerprint and curvilinear profiles (35–37).

The deeper cortical layers IIIc, V, and VI usually show the most pronounced enlargement of the neuronal perikarya, whereas axonal spindles are most conspicuous in layers IIIa and IIIb. Neuronal loss is variable with the stellate cells of layers II and III being severely affected. Even the cerebellar Purkinje cells may be considerably reduced in number. Storage is also observed in the neurons of the basal ganglia, thalamus, reticular formation of the brainstem, and spinal anterior horn neurons. Although retinal architecture is usually preserved at the light microscopic level, ultrastructural studies in some cases have revealed deposits of abnormal storage material in the ganglion cells. Biopsy and autopsy studies of other extracerebral tissues have given variable results without a consistent diagnostic pattern (35–37).

Molecular Genetic and Biochemical Findings: To date, no ANCL gene has been cloned or even mapped. Elevation in the levels of 2 mannose 6-phosphate glycoproteins in an individual with ANCL may indicate perturbation of lysosomal function (38).

CLN5

Mutations in the CLN5 gene result in the so-called Finnish variant LINCL (MIM 256731) (39), which is clinically and neuropathologically distinct from classic LINCL. This variant LINCL has so far been almost exclusively found in Finland.

Clinical Features: The Finnish vLINCL has its clinical onset between the ages of 4.5 and 6 yr, with slight motor clumsiness and muscular hypotonia followed by impaired
concentration, learning problems, and mental retardation. Visuomotor problems may be an early sign. Epileptic seizures are usually generalized and become manifest by the age of 7 to 8 yr, followed by ataxia and myoclonia between 7 and 10 yr of life. Athetosis may occur somewhat later. At an early stage, ophthalmological investigation reveals macular dystrophy and progressive optic atrophy is found after the age of 7 to 9 yr with functional blindness. The patients usually lose their walking ability by the age of approximately 10 yr and survive until 14 to 32 yr of age. Characteristic neurophysiological findings are observed by the age of 7 to 10 yr and include posterior spikes to low-frequency photic stimulation in the EEG, giant VEP and SEP, and abolished ERG. Neuroimaging shows early cerebellar involvement with severe cerebellar and cerebellar atrophy later. Vacuolated lymphocytes are not found (39, 40).

Neuropathological and Biochemical Features: There is severe generalized cerebral and extreme cerebellar atrophy at autopsy (Fig. 1B), with total brain weight of 450 to 650 g (41). In an early cortical biopsy the general cytoarchitecture is well preserved, but all neurons show moderate amounts of intracytoplasmic autofluorescent storage granules (Fig. 2C) positively stained with the Luxol fast blue, PAS, and Sudan black B methods. The granules are strongly immunoreactive for SCMAS (Fig. 2D) and also for SAP A and D (41). In older autopsy cases, the most intense storage is seen in the deeper cortical layers while the superficial part of layer III harbors numerous axonal spindles (Fig. 2E). There is progressive neuronal loss, partly in a laminar pattern and particularly involving layers III and V. The cerebellar Purkinje and granular cells are almost completely destroyed while most subcortical structures show moderate to pronounced storage but relatively modest neuronal loss. There is severe cortical astrocystosis and moderate to severe secondary loss of myelin in the white matter (41). The ultrastructure of the cerebral intraneuronal storage bodies (Fig. 3D) corresponds largely to the rectilinear complex (14). However, autonomic ganglion cells of the gut wall have shown cytosomes with pure fingerprint patterns. Storage granules are also found in many extraneural tissues (e.g., skin and rectal mucosa) where their ultrastructure corresponds to classic curvilinear profiles or to the rectilinear complex (39, 40). SCMAS was found to be the major protein species in isolated storage cytosomes (41).

Molecular Genetic Findings: The CLN5 gene is located on chromosome 13q22 (42) and was identified by positional cloning (43). To date, 4 mutations of the CLN5 gene have been identified (43, 44). The Finnish major mutation, a 2-bp deletion in exon 4, has been identified in over 90% of Finnish disease chromosomes.

CLN6
Defects in the CLN6 gene result in a variant form of LINCL, also called early juvenile NCL (Lake-Cavanagh) (MIM 601780) (45). Most patients with this disease are from Southern Europe, particularly Portugal, or of Indian or Pakistani extraction, including Romany people in the Czech Republic (46).

Clinical Features: The clinical features closely resemble those of classic LINCL. However, approximately one third of the patients have a slightly later onset and a somewhat more protracted course, with seizures, ataxia, and myoclonus as the leading symptoms. Even the neurophysiological characteristics resemble those found in classic LINCL. The EEG is abnormal, usually with a positive response to slow rate photic stimulation at early stages. The ERG becomes extinguished early on and a giant VEP may be seen before it becomes diminished or absent. The findings at neuroimaging are nonspecific. Vacuolated lymphocytes are not found (45–47).

Neuropathological Features: There is severe generalized brain atrophy, with the brain weights at autopsy ranging between 600 and 900 g. vLINCL is histologically characterized by ubiquitous intraneuronal storage of autofluorescent granules, which stain positively with Luxol fast blue, PAS, and Sudan black methods and are immunoreactive for SCMAS. There is cortical neuronal loss, most marked in the occipital lobe and in layer V. The granular layer of the cerebellum is depleted while part of the Purkinje cells may persist. As in classic LINCL, there are small storage deposits in many extraneural tissues. The ultrastructure of the storage cytosomes in cerebral neurons corresponds to a mixture of rectilinear complex and fingerprint patterns while intestinal neurons only show pure fingerprint profiles. The abnormal cytosomes in eccrine sweat gland epithelium, smooth muscle, and endothelial cells may contain both curvilinear, fingerprint, and rectilinear complex profiles (45–47).

Molecular Genetic Findings: The CLN6 gene on chromosome 15q21–23 was recently identified by positional cloning (48, 49). It encodes a novel protein with several predicted transmembrane domains. To date, 7 mutations have been reported, with E72X nonsense mutation in exon 3 occurring in several Costa Rican families.

CLN7 and CLN8
A defect of the CLN8 gene underlies Northern epilepsy (NE), also known as progressive epilepsy with mental retardation (50, 51). It is the most protracted form of human NCL (52, 53), to date only described in Finland. However, a number of patients with the so-called Turkish variant LINCL (54), originally thought to represent a distinct genetic locus designated CLN7, were recently linked to CLN8 (55).
TABLE 2
Spontaneous and Genetically Engineered Animal Models for Human Neuronal Ceroid-Lipofuscinoses (NCL)

<table>
<thead>
<tr>
<th>Model Animal</th>
<th>Animal disease/ modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital NCL? Sheep (Swedish Landrace)</td>
<td>Congenital ovine NCL with mutation of the cathepsin D gene (60, 61)</td>
</tr>
<tr>
<td>CLN1/INCL Mouse</td>
<td>Cathepsin D knockout (70, 71)</td>
</tr>
<tr>
<td>CLN3/JNCL Mouse</td>
<td>PPT1 knockout (66)</td>
</tr>
<tr>
<td>CLN6/vLINCL Mouse</td>
<td>CLN3 knockout (67–69)</td>
</tr>
<tr>
<td>CLN8/Northern epilepsy Sheep (New Hampshire)</td>
<td>Ovine NCL (63)</td>
</tr>
<tr>
<td>nclf mouse Mouse</td>
<td>nclf mouse (62)</td>
</tr>
<tr>
<td>mnd mouse Mouse</td>
<td>mnd mouse (57, 64, 65)</td>
</tr>
</tbody>
</table>

Clinical Features of NE: The clinical onset of Northern epilepsy occurs by the age of 5 to 10 yr with generalized tonic-clonic seizures. Some patients may also show complex partial seizures during the first few years. The frequency of the seizures increases towards puberty but decreases spontaneously during adulthood. Mental retardation becomes manifest 2 to 5 yr after the onset of epilepsy and slowly progresses. In young adulthood, the patients show progressive slowness and clumsiness in fine motor tasks, and balance problems become obvious after the age of 30. Some patients have shown diminished visual acuity without any observed ocular abnormality. At brain imaging, cortical atrophy has been seen in all patients over 40 yr of age but is rare before the age of 30 yr. EEG consistently shows slowing of the background activity. Rhythmic delta activity is abundant while specific sleep patterns can be missing. Epileptiform activity is frequently observed, but its amount is scant. No consistent VEP abnormalities have been reported and no vacuolated lymphocytes have been seen (50–52).

Neuropathological and Biochemical Features of NE: No specific macroscopic changes have been observed. The most striking histopathological abnormality is the presence of granular cytoplasmic storage material particularly within nerve cells but also, to a lesser extent, in many other cell types throughout the body. The storage granules are autofluorescent and Luxol fast blue-, PAS-, and Sudan black B-positive in paraffin sections (Fig. 2F–H) and immunoreactive for SCMAS. The amount of storage varies greatly between different neuronal populations, resulting in a strikingly distinct distribution pattern. The most prominent storage occurs in the hippocampal CA 2–4 sectors, while the CA1 sector and fascia dentata are almost spared. In the isocortex, the deep part of lamina III shows the most pronounced perikaryal ballooning of the large pyramidal cells while conspicuous axonal spindles are frequent in the upper parts of the same lamina. The cerebellar granule cells and Purkinje cells are relatively well preserved. The basal ganglia, thalamus, brainstem, and spinal cord show mostly slight to moderate intraneuronal storage. There is no or only slight observable neuronal loss, most evident in the hippocampal CA 2 sector where it is coupled with some astrocytic and microglial reaction. Limited electron microscopic studies of the storage material have shown membrane-bound, electron-dense cytosomes with structures resembling curvilinear profiles and occasional foci with a granular ultrastructure. Western blotting and N-terminal sequence analysis of purified storage granules indicates that SCMAS is the major storage protein (52, 53).

Turkish Variant LINCL: Only very limited clinical and morphological data are available on the Turkish variant LINCL patients with onset of symptoms in the late infantile age range. Seizures and poor motility are prominent early features while visual impairment may begin simultaneously or later. The subsequent course is characterized by motor and cognitive deterioration. Electron microscopic studies have shown rectilinear inclusions in a skeletal muscle biopsy, predominant curvilinear bodies with or without rectilinear profiles, and no fingerprint inclusions in rectal or skin biopsies (54).

Molecular Genetic Findings: The CLN8 gene is located on chromosome 8p23 (56) and encodes a novel 286 amino acid transmembrane protein (57). All Finnish patients share a missense mutation in codon 24 (R24G) (57).
where cathepsin D deficiency has not been excluded. Two animal models are available for CLN6: the nclf mouse (48, 49, 62) and ovine NCL in South Hampshire sheep (63). The so-called motor neuron degeneration (mnd) mouse is syntenic to CLN8 (57, 64). While the mnd mice show characteristic morphological features of NCL (65), their clinical manifestations are distinct from Northern epilepsy. The mice develop early spastic paraparesis and blindness but no epilepsy. A variety of further spontaneous animal forms of NCL have been described, however without molecular genetic characterization.

Genetically Engineered Models of NCL (Table 2)

Gupta et al (66) recently reported a knockout mouse model of INCL created by targeted disruptions of the PPT1 gene. The mice were viable and fertile but developed myoclonic jerking, epileptic seizures, spasticity, and progressive motor abnormalities leading to death by 10 months of age. There was prominent accumulation of autofluorescent storage material, neuronal loss, and apoptosis in the brains of these mice, providing the first animal model for CLN1. Also CLN3 knockout mouse models have been published (67–69). Despite characteristic intraneuronal storage these mice show no obvious clinical phenotype. Cathepsin D-deficient mice (70) show typical pathomorphological features of NCL and a severe clinical phenotype, including early seizures (71). Efforts to create nematode models of NCL are in progress.

PATHOGENETIC CONSIDERATIONS

The concept of NCL is based on a relatively uniform morphological phenotype, characterized by the accumulation in neurons and, to a much lesser extent, in many other cells of intracytoplasmic autofluorescent deposits with typical cytochemical properties and ultrastructural patterns. Despite ubiquitous storage, only the neurons of the CNS are selectively destroyed. The storage material is largely proteinaceous and, depending on the identity of its main protein component, the NCL can be divided into 2 broad categories, those storing SCMAS and those storing SAP (Table 1). High concentrations of SAP exclusively associate with GRODs, suggesting that the ultrastructure is determined by the stored protein. Immunoactivity of the intraneuronal deposits for amyloid beta has also been reported (72). However, the mechanisms of accumulation of these highly hydrophobic proteins and their relation, if any, to clinical symptomatology and neuronal death remain unsolved.

The morphological uniformity of the NCL contrasts with their newly discovered genetic heterogeneity. More than 115 mutations in at least 7 different genes (CLN1–3, CLN5–6, CLN8 and cathepsin D) underlie different forms of NCL in man and animals. The products of most of these genes are ubiquitously expressed and not neuron-specific. The CLN1 and CLN2 proteins, PPT1 (17) and TPP1 (26), are soluble lysosomal enzymes, as is cathepsin D, an aspartyl proteinase (61). In contrast, the predicted amino acid sequences of the products of the CLN3 and CLN5 genes suggest that they are integral transmembrane proteins, reported to have a lysosomal location in non-neuronal cells (73). However, mitochondrial (74), Golgi compartment (75), and nuclear and cytoplasmic (76) localizations have also been suggested for the CLN3 protein. The CLN8 gene also encodes a novel transmembrane protein (57). The CLN8 protein contains an ER retrieval signal and has been found both in the ER and the ER-Golgi intermediate compartment in non-neuronal cells (77).

PPT1 removes fatty acid groups from several S-acylated proteins such as oncogene H-ras (78). The function of TPP1, a serine protease, is to remove N-terminal tripeptides from substrates with free amino termini (79). In vitro, this enzyme has been reported to participate in the lysosomal degradation of SCMAS (80). However, the in vivo substrates of PPT1 and TPP1 are unknown. CLN3 as well as Btn 1, the yeast homolog of CLN3, may have a role in the regulation of vacuolar pH or vesicular trafficking (81–84). The exact function of the human CLN3 still remains elusive, and no data are yet available on the functions of the CLN5, CLN6, and CLN8 proteins. It is of interest in this context to note that altered lysosomal pH has been reported even in the fibroblasts of NCL patients (85).

The localization and functions of the NCL-associated proteins in neurons are not necessarily the same as in non-neuronal cells. In fact, there is growing evidence that at least PPT1 and CLN3 are involved in synaptic function. In mouse and rat brain, PPT1 expression is under developmental control and closely follows the temporal and spatial pattern of synaptogenesis (86, 87). In the mouse brain, PPT1 is enriched in the synaptosome and synaptic vesicle fractions (88) and colocalizes with presynaptic synaptophysin and the synaptic vesicle marker SV2 in transfected murine cortical neurons (89). Likewise, CLN3 is mainly found in the synaptosomes of presynaptic nerve terminals of mouse primary neurons (90).

Only limited information is available on the effects of the pathogenic mutations in the various forms of NCL. CLN1 is best studied in this respect and may serve as an example. The PPT1 enzyme has been crystallized (91) and its known 3-dimensional structure allows the establishment of phenotype-genotype correlations. Mutations associated with the severe infantile phenotype are located near the active site (Ser 155) and profoundly disturb the structure and function of the enzyme. In contrast, mutations causing late-onset forms of CLN1 are remote from the catalytic triad, leading only to limited changes in protein structure (91). The most common mutations causing the severe infantile phenotype, a premature stop-codon at
arginine 151 and the missense mutation Arg122Trp, result in a severely truncated or unstable protein that is degraded in the ER. In contrast, the missense mutation Thr75Pro leads to a milder juvenile phenotype (12). While all these mutations lead to deficient activity of the PPT1 enzyme, they may also have an effect on its intracellular trafficking, and this effect may differ between neurons and non-neuronal cells. In non-neural cell lines, mutant PPT1 polypeptides were retained in the ER while the wild-type protein was localized to the lysosomes (92). However, in primary neurons both wild type and mutant PPT1 polypeptides causing mild phenotypes were transported along the neurites and colocalized with synaptic vesicles. However, in primary neurons both wild type and mutant PPT1 polypeptides were retained in the ER while the wild-type protein was localized to the lysosomes (92).

The deranged metabolic pathways leading to neuronal degeneration and death in NCL remain to be elucidated. Both apoptotic (93, 94) and excitotoxic mechanisms have been proposed (95). It seems possible that the products of all NCL genes have either direct or indirect interactions, participating in the same trafficking pathway of vital importance for neurons (96). The recent advances in the field of molecular genetics presented above have exposed the striking genetic heterogeneity of NCL and form a solid basis for further studies. Identifying the molecular pathogenesis of these devastating neuronal disorders will likely deepen our understanding of the basic mechanism of neurodegeneration even in other more common diseases and aging.

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