The neuronal ceroid lipofuscinoses

Wiesława Grajkowska¹, Elżbieta Czarnowska¹, Tomasz Kmiec², Maciej Pronicki¹

¹Department of Pathology
²Department of Neurology and Epileptology
The Children's Memorial Health Institute
Warsaw, Poland

Abstract

The neuronal lipofuscinoses (NCLs) are described as a complex of inherited neurodegenerative disorders associated with intralysosomal accumulation of lipopigment in neuronal and extraneuronal cells. Morphologic pathology in NCL is marked by two processes: degeneration and loss of neuronal cells of central nervous system, resulting in atrophy of the cerebrum and cerebellum and widespread accumulation of autofluorescent lysosomal lipopigments of varying ultrastructure, the demonstration of which allows diagnostic recognition of NCL disease. Ultrastructural examinations of affected tissues may disclose 5 different patterns of lipopigments: usual lipofuscin, fingerprint deposits, granular profiles, curvilinear bodies, and microtubular aggregates, which may be found in conjunctiva, liver, lymphocytes, skin, full thickness rectal biopsies material. Deposits characteristic for NCL are ultrastructurally observed also in trophoblastic cells and amniotic fluid cells. Ultrastructural studies of biopsy tissues in order to identify pattern of pigment remains the gold standard to identify NCL, together with clinical aspects and respective gene defects. Advances in molecular genetic techniques have allowed to identification of defective genes and their protein products in some NCL clinical forms.

Key words: clinical characteristics • lipopigments • lysosomal disorders • neurodegenerative diseases neuronal ceroid lipofuscinoses • ultrastructural characteristics

Introduction

The childhood disorders, characterized by progressive myoclonic epilepsy, psychomotor deterioration with different motor disturbances, visual failure, familial occurrence and the intralysosomal accumulation of the autofluorescent lipopigment, lipofuscin (from Greek lipos - fat; from Latin fuscus - dusky) and ceroid (from Latin cera - wax) in neuronal and other cellular components of various tissues are described as the neuronal ceroid lipofuscinoses (NCLs). NCLs are a heterogeneous group of inherited neurodegenerative diseases that display an autosomal recessive pattern of inheritance, however occasionally dominant forms also occur [1, 2]. Forms of the NCLs were first described in Norway by Stengel in 1826 and in 1903 by Batten, Spielmeyer and Vogt. The NCLs occur worldwide, but the gene frequency appears to be greater in the Northern European population. The disorders are relatively rare and an incidence is of 0.46 in West Germany and 0.36 in Italy per 100 000 live births. The infantile NCL and late infantile NCL occur predominantly in Finland and form a part of the Finnish disease heritage [18].

Neurons in the central nervous system are the most severely affected, thus causing interrupted development of the brain and death [8, 16, 17].

NCL classification

An important criterion for the classification of NCL disorders is the age of onset of the clinical symptoms and signs. In 1989 Dyken proposed a classification of NCL on the ground of the age, clinical symptoms, and ultrastructural aspects of the lipopigments, identifying 4 main and 6 minor types of NCL [5]. To these latter, 3 more minor types have been recently added (Table 1). The main childhood subtypes are in-
fantile (INCL, Haberg-Santavuori-Haltia disease, McKusick 256730), late infantile (LINCL, Jansky-Bielschowsky disease, early-onset Batten’s disease, McKusick 204500), and juvenile (JNCL, late onset Batten’s disease, Batten-Spielmeyer-Vogt disease, McKusick 204200). A fourth type, adult type, adult NCL (Kufs’ disease, McKusick 204300), is typically a disease of adult [18].

Genetics

Recently a new genetic classification (CLN1-CLN8) is proposed (Table 2) [2, 8, 11]. In fact, 8 genes underlie the NCL syndromes, of which 5 have been isolated and mutations characterized: CLN1 (Infantile NCL, Haberg-Santavuori-Haltia disease), CLN2 (Late infantile NCL, Bielschowsky), CLN3 (JuvenileNCL, Batten dis.), CLN5 (Late infantile, Finnish variant dis.), and CLN8 (Protracted juvenile northern epilepsy/EPMR) [1, 11, 14]. Genetic mutation and chromosome localization in adult NCL are not known [14].

Clinical features

Clinical course of NCLs and symptoms vary with each person [17]. Following is an outline of the most typical symptomatology: visual impairment progressing to complete blindness, seizures, which may be frequent and difficult to control, decline in cognitive function, personality and behavioral changes, loss of communication skills, loss of motor skills and increasing spasticity, facial grimacing and abnormal body movements, a general, progressive deterioration to a vegetative state. Other symptoms that may develop include slowing of head growth with age, poor circulation in lower extremities, with legs and feet often cold and bluish-red in color, decreased body fat and muscle mass, curvature of the spine, hyperventilation and/or breath-holding spells, teeth-grinding, and constipation.

Infantile neuronal ceroidlipofuscinosis (INCL)

INCL clinical signs are presented between 8 and 18 months of age. Affected children fail to thrive and have abnormally small heads. Also typical are short, sharp muscle contractions called myoclonic jerks. Initial signs of this disorder include delayed psychomotor development with progressive deterioration, other motor disorders, or seizures. The infantile form has the most rapid progression. Death occurs at between 3 and 10 years [1].

Late infantile NCL (LINCL)

The symptoms of classic LINCL appear between ages two and four years. All affected children present three stages of progressive disease with typical clinical symptoms. The major symptoms are seizures average at 3.2 years, usually herald the onset of
further signs. Partial, generalized tonic-clonic or secondary
generalized, absences may also occur. Myoclonus started at
3.7 yr, speech regression is observed at 4.8 yr, ataxia at 3.8 yr,
visual failure increased at 4.2 yr, blindness is confirmed at 5.6
yr of age. Children generally become chair bound between
4 and 6 years and death occurs in middle childhood [16].

Juvenile NCL (JNCL)

Clinical signs of JNCL are presented between 4 and 10 years of
age with progressive visual impairment caused by a character-
istic retinitis pigmentosa. Most children become blind over 2-
6 years. Dementia becomes evident in the second decade of
life starting with compulsive speaking and characteristic stam-
mer. Spastic tetraplegia leads to death in the late teens [16].

Adult NCL

The disease starts mostly at age of 30 years, however later
onset of clinical symptoms has been also described [4]. Clinici-
al presentation includes a progressive myoclonal epilepsy
with dementia, ataxia and late pyramidal and extrapyramidal
features. The vision is normal. Some patients present photo-
sensitivity. In literature are also reported cases of adult NCL
type at paediatric age [17].

Pathogenesis

In NCLs, a specific lysosomal defect can gradually lead to a
general lysosomal dysfunction, either by disruption of an es-
ternal metabolic route or by mechanical filling of the lysoso-
mes due to accumulation of lipofuscin. Lysosomal proteins
are known to have a tendency to compensate a defective
enzyme with an increased activity of the others, as the ex-
ample of NCL diseases shows. Six forms of NCL. (CLN2,
CLN3, CLN4, CLN5, CLN6, CLN8) accumulate subunit c of
mitochondrial ATP synthase as a component of the storage
lipopigment [13, 15, 18]. CLN2 is associated with a defi-
cency of lysosomal pepstatin-insensitive peptidase (PPPI).
CLN3 is believed to be associated with mutations of a lyso-
somal membrane protein of unknown function [15].

Three lysosomal enzymes are responsible for three
early-onset forms of NCLs: PPT1 in infantile NCL (INCL,
CLN1), tripeptidyl peptidase 1 (TPP1) also called pepstatin-
insensitive protease in late-infantile NCL (LINCL, CLN2),
and cathepsin D in congenital ovine NCL [15,18]. The mu-
tations of catalytic site of PPT1, substrate binding, or folding
result in inactive enzyme and lead to severe clinical pheno-
types. TPP1 is an exopeptidase that cleaves N-terminal tripeptides from peptides or proteins with a preference for the
N-terminal GlyProX sequence. Cathepsin D is a well-known
aspartyl protease. Activity of PPT1 and TPP1 can be mea-
sured in white blood cells. JNCL is caused by the deficiency
of transmembrane protein of lysosomal membrane, what is of
unknown function and with no diagnostic assay [3, 18].

Ultrastructural features

NCL is generally included with the lysosomal storage disor-
ders because the lipopigment inclusions are bound by the
lysosomal membrane [13]. With electron microscopy it is
possible to distinguish 5 different type of osmiophilic lipi-
pigments: usual lipofuscin, fingerprint deposits, curvi-
linear bodies, granular profiles (GRODs) and microtubular
aggregates [2,3]. The abnormal deposits result from a short-
age of enzymes normally responsible for the breakdown of
lipopigments [16].

The GRODs are deposits composed of conglomerates
of spherical globules of 100-500 nm in diameter, which can
be thickly or loosely bound [12]. Occasionally single or paired
membranous bodies occurred more frequently in the late infantiile forms and GRODs in infantile cases. In adult NCL type a granular
component is frequently seen, however both fingerprint and
curvilinear profiles also observed [2,3].

The accumulation of deposits is not restricted to nervous
system but is systemic, and may be observed also in peripheral
blood lymphocytes, epithelial cells, fibroblasts, endothelium,
myocytes, Schwann cells, eccrine sweat gland epithelial cells,
and trophoblastic cells. The most explored for diagnosis of NCL
were conjunctiva, rectum and liver biopsy material [7].

Ultrastructural examination of a pellet of lymphocytes
is sufficient to identify the deposits, however there must be
examined not less than 100 cells. It must be pointed that
ultrastructural analysis of blood lymphocytes can be often
false negative. Ultrastructurally characteristic deposits in lymphocytes include GRODs in NCL1, curvilinear bodies in
CLN2, fingerprint-pattern in CLN3 and single inclusions with a
condensed fingerprint pattern in CLN6 [2,3].

In conjunctiva deposits are typically seen in fibroblasts,
endothelial cells and nerves. It is reliable tissue for diagnosis
of all type of NCL.

In rectal samples, the neurons of submucosal plexuses
storage GRODs in CLN1, curvilinear bodies in CLN2, and
fingerprint inclusions in CLN3, CLN5 and CLN6 [17].

The diagnostic of skin biopsy material is reliable if it
is taken enough deep to include sweat glands. The secretory
eccrine epithelial cells are the most important for diagnosis
exhibiting NCL deposits, which are not founded in the seba-
ceous gland and apocrine glands.
In skeletal muscle biopsy material are found GRODs of CLN1 and heavy condensed curvilinear bodies of CLN2. Deposits of fingerprints are not presented and due to that reason the muscle biopsy is not recommended for NCL diagnosis.

Prenatal diagnosis of NCLs is possible in amniotic fluid cells (fetal lymphocytes and fetal skin at later gestational age) or chorionic villus samples by a combination of ultrastructural, biochemical and/or molecular analysis [6, 10]. Under electron microscopy were found deposits characteristic for CLN1, CLN3 [3].

**Summary**

At present the diagnosis of NCL is a complicated process requiring a complete clinical evaluation, laboratory testing, and periodic reevaluations. These may include: ultrastructural studies of blood or skin, or conjunctival biopsy, biochemical studies of blood and fibroblast cultures for enzyme studies (PPT1, TPP1), molecular genetic studies of blood or fibroblast cultures for identification of the gene mutation, electrophysiological studies of the brain, including EEG, ERG, and visual evoked response, neuroradiological studies including MRI and CT of the head, prenatal diagnosis in amniotic fluid cultures or chorionic villus samples by a combination of biochemical and/or molecular analysis [6, 7, 14, 15].

Although considerable progress in understanding and diagnosis of NCL has been achieved recently, the metabolic basis of the NCL disorders has remained unexplained. Still must be clarify why lipopigments accumulate. An understanding of the initial biochemical defect is essential for diagnostic purposes and possibly specific therapeutic strategies [9, 13].

Presently, there is not known treatment or cure of NCLs. The seizures can be reduced or controlled with anticonvulsant drugs. Physical and occupational therapy may help patients retain function as long as possible. Some reports have described a slowing of the disease progression in children with JNCL who treated with vitamins C and E and with diets low in vitamin A.
Tabele 2

Genetic classifications and molecular features of NCL

<table>
<thead>
<tr>
<th>Clinical forms</th>
<th>Genetic type</th>
<th>Chromosome location</th>
<th>Gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infantile NCL (Harberg-Santavuori-Haltia)</td>
<td>CLN1</td>
<td>1p32</td>
<td>Lysosomal palmitoyl protein thioesterase</td>
</tr>
<tr>
<td>Late infantile NCL (Bielschowsky)</td>
<td>CLN2</td>
<td>11p15</td>
<td>Lysosomal peptain-insensitive peptidase</td>
</tr>
<tr>
<td>Juvenile NCL (Batten)</td>
<td>CLN3</td>
<td>16p12</td>
<td>Lysosomal transmembrane CLN3 protein</td>
</tr>
<tr>
<td>Adult NCL (Kufs)</td>
<td>CLN4</td>
<td>Not know</td>
<td>Not know</td>
</tr>
<tr>
<td>Late infantile (Finnish variant)</td>
<td>CLN5</td>
<td>13q31-32</td>
<td>Lysosomal transmembrane CLN5 protein</td>
</tr>
<tr>
<td>Late infantile (Indian variant)</td>
<td>CLN6</td>
<td>15q21-23</td>
<td>Not know</td>
</tr>
<tr>
<td>Late infantile (Turkish variant)</td>
<td>CLN7</td>
<td>Not know</td>
<td>Not know</td>
</tr>
<tr>
<td>Northern epilepsy/EPMR</td>
<td>CLN8</td>
<td>8p23</td>
<td>Membrane protein</td>
</tr>
</tbody>
</table>

References