The neuronal ceroid-lipofuscinoses (NCLs) [1,2] form a special group within the inherited lysosomal storage disorders. They are also collectively called Batten disease, according to the British neurologist and neuropathologist Frederick Batten (1865–1918), an early pioneer of the field, who contributed to the identification of two forms of NCL (see Section 2). The NCLs have a worldwide distribution, but their incidence rates may vary from 1:67000 in Italy and Germany to 1:14000 in Iceland, and their prevalence rates from 1:1,000000 in some regions to 1:100000 in the Scandinavian countries [3]. A genetic founder effect is well documented in some populations [3]. In addition to 14 genetically distinct human NCLs so-far identified (Table 1), numerous spontaneous forms of NCL have been discovered in domestic and laboratory animals. Most human NCLs show an autosomal recessive mode of inheritance, and may have variable ages of onset such as congenital, infantile, late infantile, juvenile, adult or even late adult onset according to the severity of mutation. The clinical characteristics of most childhood forms include progressive loss of vision as well as mental and motor deterioration, epileptic seizures and premature death, while the rarer adult-onset forms are dominated by dementia.

Despite the molecular genetic and clinical differences, all forms of NCL share unifying pathomorphological features. Autofluorescent, electron-dense, periodic acid-Schiff (PAS)- and Sudan black B-positive granules, resistant to lipid solvents, accumulate in the cytoplasm of most nerve cells and, to a lesser extent, in many other cell types. In the brain, this storage process is associated with selective destruction and loss of neurons in the brain and retina. The present paper outlines nearly 200 years of clinical, neuropathological, biochemical and molecular genetic research, gradually leading, since 1995, to the identification of 13 different genes and over 360 mutations that underlie these devastating brain disorders and form the basis of a new classification system. These genes are evidently of vital importance for the normal development and maintenance of cerebral neurons. Elucidation of their functions and interactions in health and disease is a prerequisite for the identification of possible therapeutic targets, but may also further our understanding of the basic mechanisms of neurodegeneration and ageing. An account is also given of the development of international cooperation and free access electronic resources facilitating NCL research. This article is part of a Special Issue entitled: The Neuronal Cereoid Lipofuscinoses or Batten Disease.

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possible therapeutic targets, but may also further our understanding of the basic mechanisms of neurodegeneration and ageing.

2. Early clinical and neuropathological studies of the NCLs

The first clinical description of patients who may have suffered from NCL [4] was published in 1826 by Dr. Otto Christian Stengel, a general practitioner at the small copper-mining community of Roros in South-Eastern Norway. During his long career he had observed a "singular illness" affecting four children of a local family. The parents were apparently healthy. By the age of 6 years, after unremarkable early development, the sight of two sons and two daughters began to deteriorate. Within years, the disease led to blindness, progressive mental deterioration, loss of speech, and epileptic seizures. The two oldest siblings had died by the age of 20 and 21 years [4]. No autopsies were performed but, in retrospect, the clinical features are compatible with CLN3 disease, classic juvenile. Unfortunately, Stengel's report, written in Norwegian, remained unnoticed by the scientific community until Nissen focused attention to its significance in the 1950s [5].

By the end of the 19th century the American neurologist Sachs formulated the influential concept of "amaurotic family idiocy", based on his observations in a set of siblings of rapidly progressive loss of vision and severe mental retardation of infantile onset [6]. At autopsy, accumulation of material of lipid nature was observed within markedly ballooned nerve cells of the brain. This disease, subsequently known as Sachs form was easily extractable, the membrane cytoplasmic bodies described in the infantile form (Sachs) as GM2-ganglioside. Findings were later confirmed by Klenk [20] who demonstrated an increased cerebral ganglioside concentration in the infantile form (Sachs) but not in the juvenile type (Spielmyer–Sjögren). Klenk's findings were later confirmed by Svennerholm [21] in 1962 who finally identified the major storage material in the infantile form (Sachs) as GM2-ganglioside.

The final blow to the unitarian view came, however, through the histochemical and electron microscopic studies of Zeman and his collaborators in the 1960s. They showed [22] that the intraneuronal storage cytosomes in the late infantile (Janský–Bielschowsky) and juvenile (Spielmyer–Sjögren) forms of "amaurotic idiocy" radically differed from the membraneous cytoplasmic bodies described in the infantile form (Sachs) by Terry and Korey [23]. While the storage material in the infantile form was easily extractable, the autofluorescent and electron-dense storage cytosomes in the late infantile (Janský–Bielschowsky) and juvenile (Spielmyer–Sjögren) forms were largely resistant to lipid solvents, and showed characteristic from those reported by Sachs. However, inspired by the superficial clinical similarities (familial occurrence, progressive loss of vision, and psychomotor retardation) and the newly introduced unifying pathological concept of intraneuronal "therasaurismosis" [16,17] or "storage", all these cases were gradually grouped together. They were simply considered to represent variants of "amaurotic family idiocy" of either infantile (Tay–Sachs), late infantile (Janský–Bielschowsky) or juvenile (Spielmyer–Sjögren) onset. In 1925 Kufs published his first report on adult onset mental deterioration with similar intraneuronal storage but without evident loss of vision.

3. The NCL concept and clinico-pathological classification of the NCLs

In the 1930s two studies were published which began to undermine the validity of the prevailing unitarian view of the "amaurotic family idiocies". Sjögren [19] carried out extensive clinical and genealogical studies of patients with "juvenile amaurotic idiocy" and their families in Southern Sweden. Based on statistical analyses of about 4500 members of affected families he concluded that "juvenile amaurotic idiocy" showed, with high probability, a "monohybrid recessive inheritance", and was genetically distinct from "infantile amaurotic idiocy" (Tay–Sachs disease). A further argument against the unitarian view was provided by Klenk [20] who demonstrated an increased cerebral ganglioside concentration in the infantile form but not in the juvenile type (Spielmyer–Sjögren). Klenk's findings were later confirmed by Svennerholm [21] in 1962 who finally identified the major storage material in the infantile form (Tay–Sachs) as GM2-ganglioside.

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curvilinear (Jansky–Bielschowsky) or predominantly fingerprint (Spielmeyer–Sjögren) ultrastructural patterns [22,24]. These characteristics and the distribution of the storage materials bore a striking resemblance to the autofluorescent “lipopigments” ceroid or lipofuscin. Based on this resemblance, in 1969 Zeman and Dyken proposed the new term neuronal ceroid-lipofuscinosis (NCL) [1], in order to clearly distinguish the late infantile and juvenile forms of “amaurotic family idiocy” and the histochemically similar adult onset cases reported by Kufs from Tay–Sachs disease and other gangliosidoses.

A new infantile type of NCL was soon described by Haltia and Santavuori and their collaborators [25–28]. This disease was characterised by autofluorescent electron-dense storage cytosomes with a finely granular ultrastructure (granular osmiophilic deposits or GRODs). Furthermore, the first animal form of NCL was reported in English setter dogs by Koppang [29].

In 1976 Zeman [30] proposed a classification of the NCLs, essentially based on the age of clinical onset and the ultrastructure of the storage cytosomes. He distinguished four different human and one canine form of NCL. The NCLs thus included the human infantile (Haltia–Santavuori), late infantile (Jansky–Bielschowsky), juvenile (Spielmeyer–Sjögren), and adult (Kufs) types, and the canine form in English setters described by Koppang.

4. Expansion of the NCL family

In his classification in 1976 Zeman [30] had not taken into consideration the occasional cases of congenital “amaurotic family idiocy,” reported since 1941 [31–33]. Furthermore, since the end of the 1970s, a number of “atypical” or “variant” cases of NCL were reported, many of them with a late infantile or early juvenile onset. These included what were described as “early juvenile,” “Czech” or “Indian” [34,35], “Finnish” [36–38], and “Turkish” [39] variant late infantile forms of NCL. Interestingly, even “Northern epilepsy” or progressive epilepsy with mental retardation [40] later turned out to be a member of the NCL category at neuropathological analysis [41,42], although it had originally been conceived as an inherited childhood epilepsy syndrome with relatively mild mental retardation. While autosomal recessive inheritance is the rule in the various forms of NCL, there were also a few reports of autosomal dominant adult onset NCL [43,44].

In addition to the expansion of the category of human NCLs, an increasing number of spontaneous forms of NCL were discovered in domestic and laboratory animals. Various forms of NCL were reported in many breeds of dogs [see 45], cats [46–49], sheep [50–52], goats [53], cattle [54,55], horses [56], and mice [57,58].

5. Biochemical studies of the NCLs

As indicated before, by the end of the 1930s Klenk [20] had biochemically distinguished “juvenile amaurotic idiocy” (Spielmeyer–Sjögren) from “infantile amaurotic idiocy” (Tay–Sachs), demonstrating cerebral ganglioside accumulation only in the latter, a finding confirmed by Svennerholm in 1962 [21]. In the 1970s and 1980s progress was hampered by the fallible peroxidase hypothesis (see Section 7).

Important new information was first gained by biochemical analyses of the South Hampshire sheep model of NCL by the end of the 1980s. Palmer et al. [59] were able to solubilise the ovine storage material, showing that it was composed of discrete chemical species, i.e. phospholipids, neutral lipids, dolichols and proteins. The major component of the isolated storage cytosomes was low-molecular-weight protein [59], soon identified as subunit c of mitochondrial ATP synthase [60,61]. Subsequently, this mitochondrial protein was also found to be the predominant constituent of the storage cytosomes in most other human and animal forms of NCL [62]. However, corresponding protein chemical analyses of isolated storage bodies in the human infantile NCL disclosed sphingolipid activator proteins (saposins) A and D as their main components [63]. Later, accumulation of sphingolipid activator proteins was also observed in the miniature Schnauzer dog model of NCL [64], congenital ovine NCL [65], congenital human NCL [66], and the autosomal dominant form of adult human NCL or Parry disease [44]. The various human and animal forms of NCL can thus be grouped into two main categories: those predominantly storing subunit c of the mitochondrial ATP synthase, and those accumulating sphingolipid activator proteins A and D. Interestingly, the ultrastructure of the storage cytosomes seems to be related to their main protein component. Accumulation of sphingolipid activator proteins has invariably been associated with GRODs, while storage of subunit c of mitochondrial ATP synthase has been linked with membrane-like structures of curvilinear, rectilinear or fingerprint patterns. It is worthy of note that mutations in genes encoding SCMAS and SAPs have not yet been identified in the NCLs.

6. Molecular genetic studies and genetics-based classifications of the NCLs

The juvenile Spilmyeier–Sjögren type of NCL was linked to the haptoglobin locus on the long arm of chromosome 16 in 1989 [67]. However, the first NCL gene, responsible for the infantile Haltia–Santavuori form of NCL, was identified by a positional candidate gene approach in 1995 in Leena Peltonen’s laboratory [68]. This gene encodes palmitoyl protein thioesterase 1 (PPT1), a soluble lysosomal enzyme. Later in the very same year, the combined effort of an international consortium of five research groups led to the isolation, by positional cloning, of the gene underlying the juvenile Spilmyeier–Sjögren type of NCL [69]. This novel gene encodes a membrane protein [70]. Since 1995 more than ten further genes (see Table 1) and a continuously increasing number of mutations (>360) [71] have been linked with membrane-like structures of curvilinear, rectilinear or fingerprint patterns. It is worthy of note that mutations in genes encoding SCMAS and SAPs have not yet been identified in the NCLs.

Zeman’s classification of the NCLs in 1976 [30] was based on their clinical and neuropathological phenotypes, mainly on the age of onset of the clinical manifestations and ultrastructure of the storage cytosomes. The advances in molecular genetics made this old classification problematic. Different mutations in a given gene may give rise to varying phenotypes, including widely different ages of onset. For example, mutations of the CLN1 gene may result in disease with an infantile, late-infantile, juvenile, or even adult onset, depending on the exact type and location of the mutation in relation to the active site of the gene product. Consequently, a new purely genetic classification of the NCLs evolved, based on verified or predicted gene loci [73]. At the moment, the human NCLs are classified into 14 genetic forms from CLN1 to CLN14 (Table 1) [74,75].

More recently, an international group of physicians and scientists proposed and evaluated a new diagnostic classification system and nomenclature of the NCLs to provide a definition for NCL subtypes that is universally understood and of value for clinicians responsible for diagnosis and treatment, research scientists, patients and families [76–78]. This axilary system derives from the primary genetic defect and takes into account varying phenotype, e.g. “CLN1 disease, infantile”, or “CLN1 disease, adult” (Table 1).
7. Hypotheses on the pathogenesis of the NCLs

The first pathogenetic hypothesis on the NCLs was put forward in 1975 [79], after their separation from Tay-Sachs disease and other gangliosidoses. It was inspired by the physicochemical resemblance of the storage materials to the “wear and tear lipopigments” lipofuscin or its pathological counterpart ceroid [1]. Lipofuscin granules had been first described in the cytoplasm of nerve cells by Hannover in 1843 [80], and Hueck [81] had concluded that “das fettehaltige Abnutzungsmaterial” might form by oxidation of fatty acids. Zeman and Rider now hypothesised that the basic defect in the NCLs was formation of pathological “lipopigments”, possibly due to an increased rate of peroxidation of polyunsaturated fatty acids [79]. However, despite numerous experimental and clinical studies, including treatment of patients with various antioxidants, no conclusive evidence could be presented in favour of this idea.

Since 1995, molecular genetic studies have identified over 360 mutations in 13 different genes underlying the various established human forms of NCL [71]. The NCL genes are evidently of vital importance for the normal development and maintenance of cerebral neurons. Four of these genes code for soluble lysosomal enzymes, whereas the products of several of the remaining genes are putative transmembrane proteins with still largely unknown functions (Table 1). This impressive set of new molecular data provides a solid foundation, and the numerous available spontaneous or genetically manipulated models [2] efficient tools, for studies aiming at filling the gap between the primary genomic defects and the final neuropathological and clinical manifestations of the NCLs.

Interestingly, the striking molecular genetic heterogeneity of the NCLs is in sharp contrast to their remarkably uniform morphological phenotype, the very basis of the whole NCL concept [82,83]. All forms of NCL share at least two essential features: 1) accumulation in the lysosomes of nerve cells and, to a lesser extent, of many other cell types, of autofluorescent, electron-dense, PAS- and Sudan black B-positive material containing subunit c of mitochondrial ATP synthase and/or sphingolipid activator proteins A and D, and 2) progressive and selective loss of neurons, particularly in the cerebral and cerebellar cortex and, less constantly, in the retina, leading to mental, motor and visual deterioration. Any valid theory on the pathogenesis of the NCLs must account for these two phenomena and explain them at the molecular level [82,83]. Elucidation of the pathogenetic mechanisms of the NCLs may not only indicate molecular targets for rational therapies but also deepen our understanding of ageing and neurodegenerative pathways in general. It should not be forgotten that the time-dependent accumulation of lipofuscin in lysosomes of postmitotic cells is considered the most consistent and phylogenetically constant morphological change of ageing [84].

8. International collaboration and electronic resources in NCL research

The remarkable acceleration of research on the NCLs during the past few decades would not have been possible without extensive international collaboration between clinicians, neuropathologists, biochemists, geneticists, and molecular biologists. This collaboration has been greatly facilitated by the support of a number of private and public initiatives and organisations [see 85].

The Children’s Brain Diseases Foundation, established by Dr. J. Alfred Rider, San Francisco, USA, sponsored a series of six “round table” conferences on the pathogenesis of the NCLs in 1969–1975 [79]. These meetings were followed in 1980 by the first International Symposium on Human and Animal Models of Cereoid-Lipofuscinosis in Ræros, Norway [86], and since 1987, by the International Conferences/Congresses on the NCLs, held at 2–3-year intervals in the USA or different European countries. A European Concerted Action “Molecular, pathological and clinical investigations of neuronal ceroid-lipofuscinoses (NCL) — new strategies for prevention, diagnosis, classification and treatment” (ECA-NCL) was initiated and coordinated by Professor Hans H. Goebel of Mainz, Germany, as part of the BIOMED 2 programme of the European Union. ECA-NCL integrated the NCL-related work of 25 European research groups, starting a series of regular meetings in Helsinki in 1996. ECA-NCL established the European clinical and tissue registries and produced the first edition of the handbook “The Neuronal Ceroid Lipofuscinoses (Batten Disease)” [73].

As mentioned before, the accelerating pace of discovery of new NCL-related genes and mutations prompted the establishment, in 1998, of an electronic NCL Mutation Database, maintained by Dr. Sara Mole at University College London. The database is now part of the “NCL Resource — a gateway for Batten disease” (http://www.ucl.ac.uk/ncl/index.shtml), providing up-to-date information on the NCLs for clinicians, families and researchers on a free access basis. The most recent developments include the establishment of the “Rare NCL Gene Consortium”, coordinated by Dr. Sara Mole with participants from Europe and the USA, and a web-based NCL Registry, spearheaded by Professor Alfred Kohlschütter and Dr. Angela Schultz, Hamburg, Germany. In the USA the longstanding Batten Disease Support and Research Association supports similar activities, including DNA and cell banks. There are also disease-rating scales for juvenile and late infantile NCL, long established in Europe and more recently in the USA.

The active collaboration of the patients families and their organisations with the NCL research community has been invaluable in promoting both research and the rapid and efficient transfer of the newest scientific information to daily diagnostic and therapeutic practice for the benefit of the affected individuals. These increasingly professional family-led organisations have been leading the way in coordinating family support within their country as well as directing the funding of research, and several are now working together as the Batten Disease International Alliance.

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