ABSTRACT

Objective: To critically re-evaluate cases diagnosed as adult neuronal ceroid lipofuscinosis (ANCL) in order to aid clinicopathologic diagnosis as a route to further gene discovery.

Methods: Through establishment of an international consortium we pooled 47 unsolved cases regarded by referring centers as ANCL. Clinical and neuropathologic experts within the Consortium established diagnostic criteria for ANCL based on the literature to assess each case. A panel of 3 neuropathologists independently reviewed source pathologic data. Cases were given a final clinicopathologic classification of definite ANCL, probable ANCL, possible ANCL, or not ANCL.

Results: Of the 47 cases, only 16 fulfilled the Consortium’s criteria of ANCL (5 definite, 2 probable, 9 possible). Definitive alternate diagnoses were made in 10, including Huntington disease, early-onset Alzheimer disease, Niemann-Pick disease, neuroserpinopathy, prion disease, and neurodegeneration with brain iron accumulation. Six cases had features suggesting an alternate diagnosis, but no specific condition was identified; in 15, the data were inadequate for classification. Misinterpretation of normal lipofuscin as abnormal storage material was the commonest cause of misdiagnosis.

Conclusions: Diagnosis of ANCL remains challenging; expert pathologic analysis and recent molecular genetic advances revealed misdiagnoses in >1/3 of cases. We now have a refined group of cases that will facilitate identification of new causative genes. Neurology® 2016;87:579-584

GLOSSARY

ANCL = adult-onset neuronal ceroid lipofuscinosis; CGP = custom gene panel; NCL = neuronal ceroid lipofuscinosis; WES = whole-exome sequencing.

Diagnosis of neurodegenerative diseases in younger adults is challenging due to the relative rarity of the problem, the heterogeneous causes, and the frequent absence of noninvasive diagnostic clues, raising the question of brain biopsy. The neuronal ceroid lipofuscinoses (NCLs) are a group of storage diseases presenting from infancy to adulthood. The stored material is largely made up of subunit c of mitochondrial adenosine triphosphate synthase or saposin proteins A and D—that is, of proteins so hydrophobic that they require special mechanisms for their breakdown and disposal.

Adult-onset NCL (ANCL) is a particularly demanding diagnostic problem. Childhood NCLs have well-characterized clinical patterns typically involving brain and eye, abundant storage in readily accessible peripheral tissues such as skin, and largely solved molecular genetic causes. In contrast, ANCL can present in a variety of ways with progressive myoclonus epilepsy, dementia, or motor disorders and shows limited storage in peripheral tissue. Stored material is usually only found in a subset of neurons, with the accumulation of age-related lipofuscin...
often confused with pathologic storage. Furthermore, molecular genetic characterization of ANCL is at an early stage. Hindered by the small number of cases, and the clinical and pathologic challenges, ANCL is poorly understood.

Kufs disease is the best-known form of ANCL; it differs from most childhood-onset forms because there is no retinal involvement, and the inheritance can be either recessive or dominant. It is widely accepted that the uncommon teenage-onset cases without retinal involvement are grouped with adult-onset cases. The clinical presentation is variable with 2 broad forms identified. Kufs type A presents with progressive myoclonus epilepsy, whereas Kufs type B presents with dementia and motor signs. Recessive mutations in CLN6 can be caused by recessive mutations in PPT1 (CLN1), CTSL, and CLN5. CLN5 (saposin protein D) material, i.e., subunit c of mitochondrial adenosine triphosphate synthase, saposin protein A, or saposin protein D. Finally, some patients with CLN3 mutations present with neurologic manifestations in adult life, on a background of visual failure in childhood.

Despite these important molecular discoveries, many reported cases that receive a diagnosis of ANCL remain unsolved. As ANCL is rare, we formed a consortium to combine putative unsolved cases from centers around the world and analyzed them as one large cohort. We aimed to refine the clinical spectrum of late-onset NCL by implementing an expert clinical and pathologic review process, followed by consensus diagnosis. Here we report our somewhat surprising results, in particular, the frequent misdiagnosis of a broad spectrum of neurologic disorders as Kufs disease. Our eventual aim is to discover the remaining molecular causes of ANCL.

**METHODS** Participants. We established the ANCL Gene Discovery Consortium encompassing researchers with clinical, neuropathologic, and molecular genetic expertise in ANCL from the United Kingdom, Europe, United States, Canada, and Australia. Consortium members pooled 47 unrelated, unsolved cases into a shared database. These cases had been referred to individual Consortium researchers for molecular genetic studies over a 20-year period because of diagnosis or putative diagnosis of ANCL. Clinopathologic support for an ANCL diagnosis varied considerably in these cases that remained unsolved despite study by a variety of molecular genetic and enzymatic assays over time. For familial cases, only the proband was included. No attempt was made to sequence all known NCL genes before entry into the study.

**Standard protocol approvals, registrations, and patient consents.** Local institutional review boards at each contributing site approved this research. Participants or their guardians provided informed consent.

**Diagnostic criteria.** We developed initial diagnostic criteria based on a combination of clinical and pathologic features. These criteria were based on review of the literature and the clinical experience of Consortium members.

**Clinical and pathologic review of cases.** Strenuous attempts were made to source detailed clinical data from treating clinicians. We deliberately used broad clinical criteria (table 1) that allowed for retinal involvement.

Neuropathologic diagnosis remains the gold standard for this group of disorders. A panel of 3 expert neuropathologists (J.F.S., S.C., G.W.A.) developed criteria to classify the pathologic data as definite, probable, or possible NCL (table 1). Images of the pathologic material, where available, were subsequently shared with each of the 3 neuropathologists. They provided independent opinions, which were then discussed, and a consensus on the classification reached.

**Overall classification of cases.** Each case was given a final classification of definite ANCL, probable ANCL, possible ANCL, or not ANCL based on clinical and pathologic data. This occurred via regular Consortium teleconferences where the clinical criteria and pathologic classification were integrated to determine an overall classification (table 2).

### Table 1 Adult-onset neuronal ceroid lipofuscinosis: Clinical and pathologic criteria

<table>
<thead>
<tr>
<th>Clinical criteria (all 4 criteria required)</th>
<th>Pathologic criteria</th>
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<tbody>
<tr>
<td>Age at onset between 12 and 60 years</td>
<td>Ultrastructural demonstration of one or more characteristic membrane-bound storage morphologies (granular osmiophilic deposits [GRODs], fingerprint profiles, curvilinear profiles, or rectilinear complexes) in more than one cell type, including but not limited to eccrine secretory and duct cells, endothelial cells, smooth and skeletal muscle cells, or neurons or their proximal axons. Fingerprint profiles in vascular smooth muscle and pericytes are excluded, since it has been shown that they accumulate there nonspecifically with aging.</td>
</tr>
<tr>
<td>Normal development and cognition prior to disease onset</td>
<td>Probable pathologic features</td>
</tr>
<tr>
<td>Presence of at least 2 of the following:</td>
<td>Ultrastructural demonstration of one or more characteristic membrane-bound storage morphologies (GRODs, fingerprint profiles, curvilinear profiles, or rectilinear complexes) in a single cell type AND Demonstration, in standard histologic preparations, of cytoplasmic inclusions with broad-range autofluorescence or Luxol fast blue positivity in well-differentiated sections in one or more of above cell types, to a degree significantly greater than expected for age, OR Demonstration, by standard immunohistochemistry, of characteristic cytoplasmic storage material, i.e., subunit c of mitochondrial adenosine triphosphate synthase, saposin protein A, or saposin protein D.</td>
</tr>
<tr>
<td>Seizures or myoclonus</td>
<td>Possible pathologic features (in absence of ultrastructural data)</td>
</tr>
<tr>
<td>Progressive cognitive decline</td>
<td>Demonstration, in standard histologic preparations, of cytoplasmic inclusions with broad-range autofluorescence or Luxol fast blue positivity in well-differentiated sections in one or more of the above cell types, to a degree significantly greater than expected for age</td>
</tr>
<tr>
<td>Ataxia</td>
<td></td>
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<tr>
<td>Pyramidal or extrapyramidal motor signs</td>
<td></td>
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<tr>
<td>Documented deterioration over more than 2 years</td>
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</tbody>
</table>

**Overall classification of cases.** Each case was given a final classification of definite ANCL, probable ANCL, possible ANCL, or not ANCL based on clinical and pathologic data. This occurred via regular Consortium teleconferences where the clinical criteria and pathologic classification were integrated to determine an overall classification (table 2).
Table 2  Adult-onset neuronal ceroid lipofuscinosis (ANCL) classification criteria incorporating both clinical and pathologic criteria (see table 1) for 47 putative cases

<table>
<thead>
<tr>
<th>ANCL classification</th>
<th>Clinicopathologic criteria</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite ANCL</td>
<td>All clinical criteria met plus definite pathology classification</td>
<td>5*</td>
</tr>
<tr>
<td>Probable ANCL</td>
<td>Atypical clinical presentation (e.g., late onset) plus definite pathology classification</td>
<td>2</td>
</tr>
<tr>
<td>Possible ANCL</td>
<td>Atypical clinical presentation plus probable pathology classification</td>
<td>9</td>
</tr>
<tr>
<td>Not ANCL</td>
<td>Clinical, pathologic, or molecular data strongly suggesting an alternate diagnosis</td>
<td>31</td>
</tr>
</tbody>
</table>

*One case had a compound heterozygous CLN6 mutation.

Molecular genetic studies. Screening for known ANCL genes (CLN6, CTSF, DNAJC5, GRN, and PPT1) was performed by both Sanger sequencing and high-throughput sequencing technologies in the majority of the 16 cases (detailed in table e-1 on the Neurology® Web site at Neurology.org). Genomic DNA was extracted from venous blood using standard methods. Briefly, for Sanger sequencing, coding exons and flanking intron regions including splice sites of the 3 genes were PCR amplified using primers designed for all isoforms with reference human gene transcripts (NCBI Gene; http://www.ncbi.nlm.nih.gov/; primers available on request). Amplification reactions were cycled on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA) and bidirectional sequencing was performed using BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing products were resolved using a 3730xl DNA Analyzer (Applied Biosystems). Sequence chromatograms were compared to published cDNA sequence with nucleotide changes detected using Codon Code Aligner (CodonCode Corporation, Dedham, MA).

A custom gene panel (CGP), designed to target coding regions (6.88 Mb) of 3,616 OMIM genes with known phenotypes including ANCL, and whole-exome sequencing (WES) was also performed for the majority (table e-1). DNA enrichment was performed according to the manufacturer’s protocol using either SeqCap EZ Choice Library (Roche Nimblegen, Madison, WI) for the CGP or Nimblegen SeqCap V3 (Roche Nimblegen) for WES. DNA sequencing was performed on the captured barcoded DNA libraries using either SOLID 4 System (Applied Biosystems) or...

Table 3  Summary of 10 cases with an initial diagnosis of adult-onset neuronal ceroid lipofuscinosis (ANCL) in which an alternate diagnosis was later confirmed

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical features</th>
<th>Initial ANCL diagnosis</th>
<th>Diagnostic reevaluation</th>
<th>Final diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC23</td>
<td>Onset mid-20s with motor and intellectual deterioration (dementia); no seizures; progressive ataxia but no distinct chorea; autosomal dominant family history</td>
<td>Clinical history consistent with ANCL; skin biopsy interpreted as showing fingerprint profiles</td>
<td>Pathology review: normal lipofuscin; clinical review suggested Huntington disease; genetic diagnosis: HTT expansion</td>
<td>Huntington disease</td>
</tr>
<tr>
<td>KC33</td>
<td>Onset 28 years with cognitive decline; seizure history unknown; deceased in 40s</td>
<td>Clinical history consistent with ANCL; brain biopsy interpreted as showing autofluorescent inclusions and GROD-like material</td>
<td>Pathology review: Normal lipofuscin; genetic diagnosis: NPC1 mutation</td>
<td>Niemann-Pick syndrome</td>
</tr>
<tr>
<td>KC51</td>
<td>Onset 34 years; dementia with frontal features, subtle facial dyskinesia, parkinsonian and mild cerebellar features including limb ataxia; no seizures; autosomal recessive family history</td>
<td>Clinical history consistent with ANCL; skin biopsy interpreted as showing fingerprint profiles in sweat glands</td>
<td>Pathology review: normal lipofuscin; genetic diagnosis: PLA2G6 p.G551S mutation</td>
<td>Neurodegeneration with brain iron accumulation</td>
</tr>
<tr>
<td>KC35</td>
<td>Onset 20s with progressive cognitive decline followed by tremor, myoclonus, and ataxia</td>
<td>Clinical history consistent with ANCL; skin biopsy interpreted as showing membrane-bound inclusions with granular material</td>
<td>Pathology review: images of source material not available; genetic diagnosis: c.19or12 mutation</td>
<td>Neurodegeneration with brain iron accumulation</td>
</tr>
<tr>
<td>KC10</td>
<td>Onset age 29 with rapidly progressing dementia, motor deterioration, and myoclonus</td>
<td>Clinical history consistent with ANCL; rectal biopsy identified electron-dense structure interpreted as fingerprints and curvilinear pattern consistent with NCL</td>
<td>Pathology review: images of source material not available; brain biopsy revealed malignant lymphocytes</td>
<td>Cerebral Lymphoma</td>
</tr>
<tr>
<td>KC24</td>
<td>Onset 22 years; myoclonus and tonic-clonic seizures; cognitive decline; personality change; unsteady gait from early 20s leading to wheelchair dependence; deceased at 26 years</td>
<td>Clinical history consistent with ANCL; postmortem brain biopsy interpreted as consistent with ceroid lipofuscinosis</td>
<td>Pathology review: extensive lesion of the thalamus consistent with &quot;familial&quot; fatal insomnia; immunohistochemistry: PrP999; genetic diagnosis: PRNP insertion c.154_177del13</td>
<td>Prion disease</td>
</tr>
<tr>
<td>KC29</td>
<td>Onset at 13 years with myoclonus; tonic-clonic seizures and absences at 15 years; progressive ataxia; wheelchair-bound at 25 years; late ophthalmoplegia; mild learning disability</td>
<td>Clinical history consistent with ANCL; skin and muscle biopsy negative</td>
<td>Pathology review: images of source material not available; genetic diagnosis: hypothesis free whole exome sequencing showed SACS p.P2798Q; p.T458I</td>
<td>Autosomal recessive spastic ataxia of Charlevoix-Saguenay</td>
</tr>
<tr>
<td>KCB</td>
<td>Onset at 33 years with gait ataxia and subsequent dementia, dystonia, and psychiatric symptoms; no seizures; autosomal dominant family history</td>
<td>Clinical history consistent with ANCL; premortem biopsies not done</td>
<td>Autopsy revealed classical Alzheimer disease; genetic diagnosis: PSEN1 p.S170F, CTD2 p.A587V</td>
<td>Early-onset familial Alzheimer disease</td>
</tr>
<tr>
<td>KC21</td>
<td>Onset at 13 years with behavioral changes (frontal dysfunction) and declining school performance; myoclonus with occasional tonic-clonic seizures and poor coordination</td>
<td>Clinical history consistent with ANCL; no pathology (biopsy not done)</td>
<td>Genetic diagnosis: hypothesis free whole exome sequencing showed SERRF1 p.G932E mutation</td>
<td>Neuroserpinopathy</td>
</tr>
<tr>
<td>KC7</td>
<td>Onset age 19 years; progressive disease with myoclonus, epilepsy, and ataxia</td>
<td>Clinical history consistent with ANCL; skin, muscle, and brain biopsy negative</td>
<td>Pathology review: images of source material not available; genetic diagnosis: MTND3 mutation</td>
<td>Leigh syndrome</td>
</tr>
</tbody>
</table>
Illumina HiSeq 1500. Data analysis was performed as previously described. The data were inspected for variations in known ANCL genes and the search for novel genes is ongoing. Molecular diagnosis of cases initially suspected of having ANCL, where other disorders were later established, were performed at a number of commercial and research laboratories using standard techniques.

RESULTS Of the 47 Consortium cases, 16 cases fulfilled the Consortium’s clinical and pathologic criteria for definite, probable, or possible ANCL disease (table 2). Of the 16 cases, 8 (2 definite) presented with seizures as a main feature, usually with a progressive myoclonus epilepsy (Type A Kufs). The remaining 8 cases (3 definite) presented with dementia and motor signs (Type B Kufs). None of the ANCL cases had retinal involvement. The mean age at onset was 35 years (median 35.5 years). There were 2 clusters; 6 had onset in the second decade of life and 10 in the fourth decade or later. Both Type A and Type B cases were represented in the 2 age clusters. Two cases fell outside of our arbitrary 12- to 60-years range for disease onset (onset at 10 and 62 years); these cases were considered clinically atypical and classified overall as possible ANCL (table 2).

A family history suggestive of dominant inheritance was found in 4/16 ANCL cases; in 12, there was no family history. DNAJC5 mutations were not found in the 4 dominant cases or the 10 other cases that had this gene sequenced. CLN6 and CTSF, known causes of recessive ANCL, were sequenced in 15 cases and 1 case had probable pathogenic compound heterozygous CLN6 variants identified. This patient was classified as definite ANCL with a Type A presentation. This leaves a subset of 15 genetically unsolved ANCL cases (4 definite).

Our criteria for ANCL were not met in 31/47 (66%) cases, including 2 cases previously published in detail as having ANCL. In 10 of these 31 cases, detailed review with further investigation established definitive alternate diagnoses (table 3). The main reason for misdiagnosis as ANCL was overinterpretation of normal age-related lipopigment as pathologic storage material (figure). Six cases had features suggesting that ANCL was not the correct diagnosis. Two of these had storage material without the characteristics of the lipopigment seen in NCL, but a specific diagnosis could not be made. Two had other neuropathologic abnormalities, again without leading to a specific diagnosis. In 2 cases, biopsy material reported as suggestive of NCL was considered nonspecific on review, and the clinical features were atypical. In the remaining 15 cases, the clinical and pathologic data were inadequate for classification.

DISCUSSION In this study of 47 cases considered to have ANCL, we found that the diagnosis could be supported in only 16 cases; of these, 9 were considered possible cases. Moreover, ANCL was confidently excluded in 16 cases, of which 10 had secure alternative diagnoses established. The classification criteria developed by the Consortium were useful in formalizing the approach to problematic cases and has highlighted the challenges and subtleties in diagnosing ANCL.

The pathologic diagnosis is challenging and is compounded by the relative rarity of these disorders such that most neuropathologists have very limited experience with the condition. In general, misdiagnosis occurred by overinterpretation of ultrastructural findings in biopsy samples, with failure to distinguish...
normal lipofuscin that accumulates with age, from abnormal lipopigment seen in NCL, which may be very limited in ANCL. In children, this distinction is easier, contrasting abundant storage in disease with minimal normal age-related pigment. Rather than relying on the amount of lipopigment, accurate diagnosis depends on analysis of the particular ultrastructural features. In all late-onset neuronal storage diseases, only a subset of neurons may be involved. This becomes important when it comes to electron microscopy on a brain biopsy. If the blocks have not been trimmed to include involved neurons, the study may be limited to normal neurons that inevitably contain lipofuscin, and confusion with ANCL may result. Importantly, the removal of these cases from the cohort helps simplify the apparent clinical and pathologic heterogeneity of ANCL. The clinical profile of our ANCL cases fit broadly into the previously described categories of presentation as progressive myoclonus epilepsy (Type A) and dementia with motor disturbances (Type B): the single case that was solved molecularly with a CLN6 mutation had a Type A presentation, as has been previously described. The degree of genetic heterogeneity of ANCL remains unclear. It is already known that contrary to the childhood forms, which are essentially all recessive disorders, the adult forms can have either recessive or dominant inheritance. While it is now possible to survey the whole exome (or genome) for genetic variation with high-throughput sequencing technology, determining which of the many thousand variants identified are pathogenic remains challenging. However, this is still a good option for treating clinicians and may avoid the need for brain biopsy should a plausible variant be found in one of the known ANCL genes or in a gene known to cause an alternative late-onset neurologic disease. The efficacy of genetic testing for diagnostic purposes will only improve as further ANCL genes are discovered. At this point in time, pathologic diagnosis remains the gold standard practice. Future research into the underlying genetic etiology of the unsolved ANCL will be supported by the removal of misdiagnosed cases that would hinder these important efforts. By pooling together unrelated ANCL cases that putatively may share the same causative gene, the genomic search space can be considerably narrowed. This ongoing work, resulting in a deeper understanding of the molecular genetic basis, will provide improved guidance for the accurate and early diagnosis of ANCL. We anticipate that the recently accepted nomenclature for the NCL will be expanded as new genes are identified.

AUTHOR CONTRIBUTIONS

Dr. Berkovic drafted/revised the manuscript for content, contributed to study concept/design, contributed to acquisition of the data, performed analysis/interpretation of the data, and provided study supervision and coordination. Dr. Stampfli revised the manuscript for content, contributed to study concept/design, contributed to acquisition of the data, performed analysis/interpretation of the data, and provided study coordination. Dr. Carpenter revised the manuscript for content and performed analysis/interpretation of the data. K.L. Oliver drafted/revised the manuscript for content, contributed to acquisition of the data, performed analysis/interpretation of the data, and provided study coordination. Dr. Sims revised the manuscript for content, contributed to study concept/design, contributed to acquisition of the data, and performed analysis/interpretation of the data. Dr. Carpenter revised the manuscript for content and performed analysis/interpretation of the data. Dr. Smith revised the manuscript for content and performed analysis/interpretation of the data. Dr. Cotman revised the manuscript for content, contributed to acquisition of the data, and performed analysis/interpretation of the data. Dr. Rudge revised the manuscript for content and performed analysis/interpretation of the data. Dr. Woolf revised the manuscript for content, contributed to study concept/design, contributed to acquisition of the data, performed analysis/interpretation of the data, and provided study coordination.

STUDY FUNDING

S.F.B. was supported by a National Health and Medical Research Council Program Grant (ID: 628952). S.K., I.J., and A.P. are funded by Charles University institutional programs P4/12/L13/3, UNCE 204011, and SYV/2016/260148 and by the project LG1604 NPU II from the Ministry of Education of the Czech Republic. This work was specifically supported by grant 15-28208A from the Ministry of Health of the Czech Republic and GA UK No. 269615 (I.J.) and No. 1402213 (A.P.). Instrumental support for exome and gene panel sequencing was provided by the Genomic facility in Motol University Hospital in Prague (OPPK.GZ.2.16/3.100/24022). S.E.M. received specific funding towards the work of the Rare NCL Gene Consortium and Kufi disease from the USA Batten Disease Support and Research Association. S.L.C. is funded by the National Institutes of Health: National Institute of Neurologic Diseases & Stroke (R01NS073813). M.C.-D. is supported by the Fonds de Recherche du Québec–Santé (FRQS).

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Diagnosis and misdiagnosis of adult neuronal ceroid lipofuscinosis (Kufs disease)


*Neurology* 2016;87:579-584 Published Online before print July 13, 2016
DOI 10.1212/WNL.0000000000002943

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