JORNADAS
'LIPOFUSCINOSIS NEURONAL CEROIDEA Y OTRAS ENFERMEDADES GENÉTICAS DE ATESORAMIENTO LISOSOMAL, DESDE LA CLÍNICA HASTA LOS ASPECTOS MOLECULARES'

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COMUNICACIONES

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35-YEARS CLINICAL EXPERIENCE WITH NCL DISORDERS

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NCL disorders are progressive brain diseases with an autosomal recessive inheritance in all childhood types. These occur worldwide, often one type dominating in a specific country. NCL disorders are enriched in Finland with about 430 diagnosed patients during the last 40 years. Initial symptoms and findings are discussed in the abstract, especially. Infantile, INCL (CLN1)The normal development can be well documented until six to twelve months, sometimes even later. After a period of a developmental stand still a rapid deterioration takes place. Most children with INCL have lost all abilities to move around and to speak by 2.5 years of age. Slowing down of the head growth and muscular hypotonia are additional early findings. MRI reveals hypointense thalami in T_2 weighted image by eight months of age before evident clinical findings. Classic late infantile, cLINCL (CLN2) Marked mental and motor decline combined with ataxia and/ or epilepsy (often drops) between 2 and 4 yrs are the early signs. Visual failure usually occurs a few yrs later. Photic spikes in the EEG (low rate) and giant VEP and SEP are characteristic findings, MRI is less typical. Finnish variant late infantile, vLINCLFin (CLN5). Attention deficit, visuomotor problems and slight clumsiness initiate by 3 to 6 yrs. These are followed by visual failure, mental and motor decline and ataxia. Clinical diagnosis is based on macular changes, abnormal ERG and marked cerebellar involvement in MRI/SPECT being evident by 6 yrs. Typical neurophysiological findings (see CLN2) become usually not evident before 8 yrs of age. Variant late infantile, vLINCL (CLN6). The onset varies between 18 mo to 8 yrs. The first symptoms and signs are dysarthria, ataxia, epilepsy and mental decline. Visual failure is an early sign in about half of the cases. Hypointense thalami in T_2 weighted MR-image and cerebellar atrophy are found. Typical neurophysiological findings (see CLN2) have also been reported. Juvenile, JNCL (CLN3) visual decline by 4-8yrs, macular and ERG abnormalities, vacuolated lymphocytes and normal MRI is a very typical combination. Mental decline, epilepsy, speech and motor impairment, behavioural and sleep problems initiate later. Northern Epilepsy Syndrome (CLN8) should be considered possible if a child with normal development showing no neurological abnormalities and no diagnostical MRI findings begins to decline mentally a few years after the onset of epilepsy by 9-13 yrs. Other less common types will be also discussed as the clinical pictures in more detailed.

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DIAGNOSIS AND TREATMENT OF THE NCLS

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The NCLs are disorders characterized by lipopigment storage, mostly in neurons but also in lymphocytes and other cells. Since neurons do not divide after birth they accumulate unmetabolizable substances. This may take years or decades to affect brain functions. The classic NCL types were mistakenly classified according to age of onset, into infantile, late infantile, juvenile and adult. Four new types are now known. NCLs are neurodegenerative disorders, acquired abilities are lost. Impaired functions lead to behaviour problems, alarm bells which ought to prompt a search for their cause. Making a diagnosis is a challenge amidst the number of new genetic diseases. History and physical examination remain the clinical mainstay. What are the concerns and when did they arise? Search for objective data, loss of ambulation or self-feeding, and unusual clues, looking through to the corner of the eyes. Focus on milestones, identify delay or regression. Inquire about attempts to explain problems away: we avoid facing what disturbs us. Proceed from the general to the particular. The Socratic dialogue is the key probe missed by questionnaires. Observe the child performing simple tasks. Regression suggests possible storage disease. Search for lipopigments. Lymphocyte inclusions are best identified by electron microscopy (EM). Skin or conjunctiva biopsies reveal many lysosomal storage disorders and other conditions such as neuroaxonal dystrophy. The NCL inclusions (granular, curvilinear or fingerprint) may be absent, missed or misinterpreted. Appropriate symptomatic treatment is available. Focus on the quality of life, use a few safe medications. Unusual side effects of common antiepileptic medications are known. Seizures may be misclassified, myoclonus and dyskinesia confused with myoclonic epilepsy. Parkinsonism and dyskinesia may be triggered by psychotropic drugs or metoclopramide. Antiparkinsonians may be unsatisfactory. Nissen fundoplication with pylorostomy prevents pneumonia. Clorazepate controls spasticity and myoclonus, trihexyphenidyl dystonia, excessive salivation and respiratory secretions.

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MOLECULAR DIAGNOSIS AND GENOTYPE-PHENOTYPE CORRELATIONS OF NEURONAL CEROID LIPOFUSCINOSES

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Neuronal ceroid lipofuscinoses (NCLs) are a group of progressive neurodegenerative disorders characterized by intra-lysosomal accu-

mulation of ceroid lipopigment. The NCLs consist of at least eight forms, for which six genes have been identified, and possibly more variants will be recognized. Although research studies are making progress for this group of disorders, the nature of the clinical process and the progression of the NCLs are yet unclear. Therefore, we have undertaken correlation studies of DNA mutations at genetic loci *CLN1-3* with NCL phenotypes among 517 subjects clinically affected by classical neuronal ceroid lipofuscinosis (NCL). We have noticed that the most common initial symptom leading to a clinical evaluation was developmental delay (30%) in NCL1, seizures (42.4%) in NCL2, and vision problems (53.5%) in NCL3. Eighty-two percent of NCL1 cases had GRODs or mixed-GROD containing EM profiles; 94% of NCL2 cases had curvilinear (CV) or mixed-CV–containing profiles; and 91% of NCL3 had fingerprint (FP) or mixed-FP–containing profiles. The mixed-type EM profile was found in approximately one-third of the NCL cases. DNA mutations within a specific *CLN* gene were further correlated with NCL phenotypes. Seizures were noticed to associate with common mutations 523G→A and 636C→T of CLN2 in NCL2 but not with common mutations 223G→A and 451C→T of CLN1 in NCL1. Vision loss was the initial symptom in all types of mutations in NCL3. Surprisingly, our data showed that the age of onset was atypical in 51.3% of NCL1 (infantile form) cases, 19.7% of NCL2 (late-infantile form) cases, and 42.8% of NCL3 (juvenile form) cases. Our data provide an overall picture regarding the clinical recognition of classical childhood NCLs. This may assist in the prediction and genetic identification of NCL1–3 via their characteristic features.

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MANAGEMENT OF CHILDREN WITH NCL DISORDERS

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Management includes, in addition to the drug therapy, rehabilitation of the patient and guidance and support of the whole family. Epilepsy: except for patients with NES all the other NCL types have myoclonic epilepsy with both partial and generalized seizures, i.e. AEDs with a broad spectrum are needed. During the early years monotherpy is efficient. However, later on a combination therapy is usually needed, in some cases even three AEDs at a late stage. Lamotrigine, valproate and benzodiazepines have been the most commonly used drugs in Finland. During the last years also topiramate is used to some extent. Irritability, muscle relaxation: baclophen and tizanidine are needed in most patients with NCL. In INCL from a very early stage, in LINCL later on and in JNCL depending on the symptoms and stage of ambulation. In INCL they are the best drugs for the intense irritability of the child. They are also helpful in sleep problems, myoclonia and atethosis. 15-30 mg baclophen is recommended during the early years, then increased to 60-80 mg and later on even as high as 200-300 mg. Tizanidine is also effective in dystonia. The dose and administration is very individual ranging from 1-2 mg in the evening to 0.25-0.5 mg 3-8 times a day at onset. Later on 10-20 mg and even 80-100 mg may be needed. In INCL and sometimes in other NCL types pains occur at an advanced stage. True painkillers including phentanyl patch are necessary. Extrapyramidal symptoms: L-dopa and bromokryptin has been found favorable especially in male patients with JNCL. The effect is of much shorter duration in female. Behavioral problems are common in JNCL. Sitolapram and atypical neuroleptics, mainly Risperidone have been used in Finland. Quetiapine was also found to be effective in some patients but not olanzapine. Benzodiazepines and Chloralhydrate and to some extent also Melatonin are recommended for insomnia. True circadian rhythm failure occurs only at a late stage. Rehabilitation includes physiotherpy in all NCL types, swimming and riding are also good. In JNCL occupational therapy and all kind normal sports are suitable but they may have to be modified. Ball games, goal ball, skiing and tandem cycling are among those to be recommended. A special computer program has been developed to support the communication and education of adolecents with JNCL. Guidance and support of the family: all help, official and unofficial, should be used to help the family carry on with life. There are many options. Adaptation training courses, NCL parents association activities, special NCL workers, 'support persons', interval visits outside home, all are important.

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THE DEVELOPMENT OF MOLECULAR DIAGNOSTIC ASSAYS FOR NEURONAL CEROID LIPOFUSCINOSES

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The neuronal ceroid lipofuscinoses (NCLs), are the most common autosomal recessive neurodegenerative disorders of childhood with an incidence of 1/25,000 births. NCLs are characterized by psychomotor deterioration and visual failure. Several phenotypic subtypes are classified on the basis of age of onset and clinical features. Eight causative genes (CLN1-8) for NCL have been mapped, of which six have been cloned (CLN 1,2,3,5,6 and 8). Although more than 100 mutations causing NCL have been reported, nine recurrent mutations account for disease in ~80% of the patients while 20% have unique mutations. Molecular testing of NCL will soon be clinically important not only for an accurate and early diagnosis of the disease but also to direct medical managment and therapy. We are developing and evaluating advanced molecular diagnostic tests for efficient detection of mutations in the CLN genes causing NCL. The test development has two stages. Initially a rapid single-nucleotide polymorphism (SNP) assay is being established to detect common recurrent mutations in CLN genes 1,2,3,5 and 8. In the second phase, a comprehensive screening method will be used to detect rare mutations in patients who do not carry the recurrent mutations tested in the SNP assay. This assay is based on directed DNA sequencing of the six cloned genes leading to NCL. This testing strategy will be used in a pilot study of NCL patients to define the spectrum of mutations causing NCL and to determine their frequencies. The molecular technologies developed is flexible and can easily be adaptable to future mutation testing of additional genes as their impact on NCL is determined. This test will provide cost-effective and comprehensive molecular testing for diagnosis in patients with NCL, carrier testing for the relatives of these patients, and prenatal diagnosis in families at high risk of having affected children.

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MOLECULAR GENETICS AND BIOLOGY OF *CLN6*

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We mapped the CLN6 gene by linkage analysis of consanguineous families and identified 14 (out of a total of 18) mutations in 26 families from nine countries. These mutations include missense, nonsense, small deletions or insertions and splice site changes. Ten mutations affect single amino acids, all conserved. Minor differences in the pattern of disease symptom evolution can be identified which may be associated with the country of origin and the disease causing mutation. One patient (from Argentina) with a more protracted disease progression was a compound heterozygote for a missense mutation and an unidentified mutation. Fifteen *CLN6* mutations have so far been reported in one or two families only, and families from the same

country do not all share the same mutation. There is no major founder mutation in *CLN6*. However, one mutation (E72X) is significantly more common in patients from Costa Rica than two other mutations present in that same population. In addition a 1bp insertion (c.316insC) is associated with families from Pakistan and I154del is associated with families originating from Portugal. A group of Roma Gypsy families from the Czech Republic share two disease associated haplotypes. All mutations are recorded in the NCL Mutation Database together with their country of origin for use in the development of rapid screening assays to confirm diagnosis and to facilitate carrier testing appropriate to a population. CLN6 is predicted to encode a 311 amino acid membrane protein. It has no homology with known proteins or functional domains although the sequence is highly conserved across vertebrate species. In HEK293 cells intracellular staining using polyclonal antisera raised against CLN6 is consistent with a location within the endoplasmic reticulum. The staining pattern is punctate suggesting that CLN6 may localise to distinct subcompartmental domains.

7.

THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE* AND THE NEMATODE WORM *CAENORHABDITIS ELEGANS* AS MODEL SYSTEMS FOR STUDYING THE BASIC BIOLOGY OF *CLN1* AND *CLN3*

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Two NCL genes, *CLN1* and *CLN3*, are conserved in eukaryotes down to yeast. We have used the fission yeast *Schizosaccharomyces pombe* and the nematode worm *Caenorhabditis elegans* as model systems for further investigation of these disease genes. A *S. pombe* strain deleted for *btn1,* the homologue of *CLN3*, is viable. *btn1*Δ cells are longer than the wild-type strain, have a high septation index and more cells are binucleate, suggesting that they are delayed in part of their cell cycle. In contrast to wild-type, *btn1*Δ loses viability in response to nitrogen starvation. *S. pombe* cells possess approximately 50 vacuoles (lysosomes) which fuse when they encounter environmental stresses, including immersion in water. Although *btn1*Δ vacuoles fuse, the response to starvation is different to wild-type as monitored by the size and number of vacuoles. Btn1 is targeted to the vacuolar membrane when expressed as an N-terminal GFP-Btn1 fusion protein. Targeted mutagenesis of Btn1 has reproduced some naturally occurring human mutations as well as altering conserved residues. The simple nematode *C. elegans* has a completely mapped nervous system, and is used as a model organism for the study of human neurological disorders. *C. elegans* has one homologue to *CLN1*, *CePpt-1*. RNA interference was used to mimic loss of functional *CePPT-1* resulting in a reproductive phenotype. However, since neurons are refractory to RNAi, a deletion knockout strain was obtained from the *C. elegans* Knockout Consortium. Despite the lack of functional PPT in the deleted worm lines, a striking neuronal phenotype was not observed, suggesting that a second enzyme may be compensating for the lack of *CePPT-1* in *CePpt-1* knockout animals. These results suggest that *S. pombe* and to some extent *C. elegans* will contribute to further our understanding of the cellular role of *CLN3* and *CLN1* and the pathology of Batten disease.

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DIAGNOSTIC ROLE OF NEUROPATHOLOGY IN THE NEURONAL CEROID-LIPOFUSCINOSES

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The advances in the molecular age have revolutionized the nosography of the neuronal ceroid-lipofuscinoses (NCL) having to date classified 8 different types of NCL with 6 different genes coding for two lysosomal enzymes and 4 transmembrane proteins, respectively. Thus, the NCL of many patients is now recognized by biochemical and genetic methods. However, availability of suitable laboratories, monetary costs or diagnostic limitations to only major mutations still leave sufficient room for neuropathology as a diagnostic technique, i.e. morphological investigation of cells (largely circulating lymphocytes, and, rarely in a pregnancy suspected of having CLN2 amniotic fluid cells) or tissues, foremost skin, but also rectum, conjunctiva, and skeletal muscle. The cells and tissues are studied by the electron microscopist –and at this time no other morphological technique has yet achieved electron microscopic significance– to identify the separate or combined appearance, in lipopigments, of three basic ultra-structural patterns: granular, curvilinear, and fingerprint. The granular pattern of lipopigments which accrue in the NCL almost ubiquitously and are the target of diagnostic neuropathology as typical of CLN1, curvilinear profiles of CLN2, the fingerprint profiles of CLN3, not infrequently combined with granular and curvilinear patterns in CLN5, CLN6, and CLN7, allow reliable genotype-morphophenotype correlations in CLN1, CLN2, not always in CLN3, but not among CLN5, CLN6 and CLN7/CLN8. Postmortem studies have over years declined and prenatal electron microscopic investigations have largely been replaced by biochemical and molecular ones. Thus, immunohistochemical, immunoelectron microscopic, in situ hybridisation techniques have hardly been applied to NCL-tissues, although absence of the enzyme protein tripeptidyl-peptidase1 has been demonstrated by immunohistochemistry as has the accumulation within lipopigments of saposins and subunit C of ATPsynthase proteins. These latter ones are, however, not mutant proteins. The study of different nerve cells classes in NCL brains is largely confined to animal models, especially mouse models. Here, however, neuropathology plays a major and significant role in elucidating pathogenetic mechanisms. The diagnostic and research in diagnostic tumor and muscle immunohistochemistry has not yet been extended to NCL neuropathology, chiefly because of lack of available antibodies, especially to CLN-related transmembrane proteins.

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NEURONAL CEROID LIPOFUSCINOSES (NCL) –THE PEDIATRIC APPROACH

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When confronted with a possible NCL disorder, a straightforward diagnostic strategy is required that takes into account the relative contributory value of clinical findings (age of onset, epilepsy, dementia, loss of vision through progressive retinopathy), electrophysiological studies, demonstration of lymphocyte vacuoles by light microscopy, enzymatic tests of palmitoylprotein thioesterase and tripetidylpeptidase (now possible in dry blood samples: Lukacs Z, et al. Clin Chem 2003; 49: 509-11), molecular genetic studies and electron microscopy. In the past, an NCL disorder was frequently recognized late in the course of the disease and after many frustrating investigations. The previous long way on a wrong track conferred some kind of relief on the definitive grim diagnosis because of the

clarity it brought about. Today the diagnosis is frequently made early in a healthy-looking child with apparently only a minor medical problem such as inadequate vision or seizures. This situation is difficult for discussing the problems and the dire prognosis of such a disease with the family. We will focus on an economical way to diagnosis and on selected problems of management of NCL disorders.

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QUANTITATIVE DESCRIPTION OF THE CLINICAL COURSE IN NEURODEGENERATIVE DISORDERS –THE EXAMPLE OF JUVENILE AND LATE INFANTILE NCL

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Sufficient knowledge of the natural course of a genetic disease is a prerequisite for considering a specific diagnosis and for selecting the adequate strategy towards it. Such knowledge is also indispensable for the evaluation of any proposed treatment modality in the still mostly incurable neurodegenerative disorders of childhood, such as the neuronal ceroid lipofuscinoses (NCLs). In the past, we developed a method for the quantitative description of the clinical course in 17 patients with juvenile NCL (Kohlschütter et al. Acta Paediatr Scand 1988; 77: 868-72). Recently we did the same for 26 patients with late infantile NCL and specified mutations of the *CLN2* gene (Steinfeld R, et al. Am J Med Genet 2002; 112: 347-54). We will present details on how to develop and use clinical scoring systems appropriate for specific neurodegenerative diseases of childhood and for covering prolonged periods of time. Our experience has been that the best scoring method for this purpose is one that relies on relatively simple information remembered with adequate precision by the parents of the sick child. How useful such methods will be for differentiating the variant forms of late infantile NCL and clinically similar disorders of childhood remains to be shown.

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NEURONAL CEROID LIPOFUSCINOSES (BATTEN DISEASE): ADVANCES AND PERSPECTIVES

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11A. FROM YEAST TO MICE: SOLVING THE MOLECULAR BASIS AND PATHOLOGY UNDERLYING JUVENILE BATTEN DISEASE

Mutations in the CLN3 gene are responsible for the neurodegenerative disorder Batten disease; however, the molecular basis of this disease remains unknown. In the first part of the presentation, utilization of a yeast model that exploits the fact that yeast have a protein identical to CLN3 in humans designated Btn1p will be described. This system has shown that the CLN3 protein is lysosomal/vacuolar and that disruption of its function alters the homeostatic environment of this organelle. Study of this homeostatic imbalance has lead to the discovery of a defect in transport of small molecules across the lysosomal membrane attributable to defects in CLN3/Btn1p. The second part of the presentation will focus on a mouse model for Batten disease, a cln3-knockout mouse. We have reported the presence of an autoantibody to glutamic acid decarboxylase (GAD65) in serum that we demonstrate associates with brain tissue. Furthermore, the cln3-knockout has decreased levels of GAD65 activity and accumulation of the enzyme substrate, glutamic acid. Finally, an autoantibody to GAD65 is also present in sera of 31 out of 31 individuals tested that have Batten disease.

11B. AN OVERVIEW OF CURRENT RESEARCH INTO THE NEURONAL CEROID LIPOFUSCINOSES

Mutations in CLN1, CLN2, CLN3, CLN5, CLN6 and CLN8 have been associated to infantile-, late-infantile-, juvenile-, Finnish-variant late infantile-, variant late infantile- and EPMR (epilepsy with mental retardation), respectively. Many biochemical and molecular genetic studies have been initiated to identify the function of the proteins encoded by the CLN-genes. CLN1 and CLN2 encode the soluble lysosomal membrane proteins, PPT1 and TPP1, respectively, that bear thiolesterase and proteolytic activities, respectively. CLN's 3, 5, 6 and 8 are novel transmembrane proteins. Progress in identifying the biochemical role of these proteins will be described, along with an overview of the Cln1-, Cln2-, Cln3-, Cln6- and Cln8 mouse models that serve as mammalian models for studying the effects of mutation in these genes. In addition an overview of the use of yeast, worms and flies which are widely used as genetic model systems to study the function of many gene products. Homologs to CLN1 and CLN3 which are associated to infantile- and juvenile-neuronal ceroid lipofuscinosis have been identified in yeast, worms and flies. Due to the utility of using these model systems, studies on CLN1 and CLN3 in these model systems is accelerating our progress in understanding the function of thee gene products.

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CHARACTERIZATION OF NEURONAL CEROID LIPOFUSCINOSIS IN VENEZUELAN PATIENTS

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Neuronal ceroid lipofuscinosis (NCL) represents a group of neurodegenerative disorders of genetic origin. Based on age at onset, clinical course, and ultrastructural morphology, three clinical forms of the disease in pediatric age groups have been described: infantile NCL (INCL), late infantile NCL (LINCL), and juvenile NCL (JNCL). Other variants, or atypical forms constitute approximately 20% of NCL cases in different population groups. Advances in genetics have allowed better characterization, diagnosis and classification of the disease. The author describes the clinical, neurophysiological, neuroimaging, and neuropathological features of patients with NCL studied at the Neuropediatric Service of the University Hospital of Maracaibo, during a ten-year period (1993-2003) All cases were classified as late infantile form of the disease (LINCL). Age at onset ranged from 2 to 5 years of age. In the majority of the patients initial symptoms were seizures, psychomotor retardation or developmental regression, accompanied by macular degeneration, and optic atrophy. EEG following low frequency photic stimulation displayed increased amplitude peaks in the occipital regions in 83% of the patients. Neuroimaging findings were characteristic of the late infantile form of NCL. 50% of the patients revealed thalamic hypointense lesions in T_2 weighted images. Ultraestructural exam of tissue obtained from the patients revealed curvilinear bodies in all cases. To the best of our knowledge, there is no epidemiological data related to NCL in Venezuela. The existence of clinical forms and variants in pediatric age groups is presumed. This maiden study might contribute to the knowledge of this group of disorders in our population, and stimulate further research.

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NEUROIMAGING IN LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS (LNCL)

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The neuronal ceroid lipofuscinoses (NCLs) represent one of the most frequent neurodegenerative diseases in children characterized by developmental regression, seizures, progressive visual loss, and premature death. Neuroimaging techniques especially magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (H-MRS) may yield relevant data for a better understanding of the symptoms and physiopathology of this disease. The most significant changes in MRI studies in all types of NCL are represented by progressive cerebral atrophy. In the classic late infantile type (LINCL) the atrophy is more pronounced in the infratentorial structures, particularly in the cerebellum, accompanied by high signal abnormalities in white matter on T_2-weighted images. In the variant subtypes of LINCL, MRI findings, especially in the early stages of the disease, may vary from normal to discernible areas of increased density in the periventricular white matter about the lateral ventricles, decreased intensity of signal in the thalami and putamina, and cerebellar atrophy. The most important findings with H-MRS consist in a reduction of N-acetyl aspartate in both grey and white mater, and increase of myoinositol, creatine and choline in white matter. The cerebral and cerebellar MRI and H-MRS findings and the link between the metabolic abnormalities and the duration of the ailment are discussed.

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CHILDHOOD NEURONAL CEROID-LIPOFUSCINOSES IN ARGENTINA: ELECTROCLINICAL FEATURES

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Neuronal ceroid lipofuscinosis (NCL) is the most common group of recessive neurogenetic disease in children and may occur as frequently as one in 12.500 births depending upon ethnicity, usually showing an autosomal recessive mode of inheritance. The human NCLs are now classified into 8 main genetic forms (CLN1-8), based on the number of hitherto predicted gene loci. It is important to note that different mutations in a single gene may result in different phenotypes, including varying ages at onset. In Argentina the most frequent phenotypes are the late infantile (LINCL) and juvenile forms (JNCL) (Taratuto, et al. Am J Med Genet 1995). Infantile NCL: the CLN1 gene on chromosome 1p32 was identified. Affected children usually seem to develop normally until approximately 1 year of age. However, the rate of head growth may begin to decrease by the age of 5 months. Muscular hypotonia and clumsiness of fine motor control are further early signs. During the second year of life, myoclonic jerks and seizures appear with progressive loss of motor abilities, truncal ataxia and visual failure. They usually die at 8 to 13 years of age. The MRI shows hypointense thalami in T_2-weighted images and extreme cerebral and cerebellar atrophy with high signal intensity in the white matter. Late infantile NCL: CLN2 was mapped to chromosome 11p15. The onset of major symptoms is usually heralded by seizures and occurs between 2 and 4 years of life. The seizures may be focal, generalized tonic-clonic, 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 years. The patients usually die in middle childhood. The brain-imaging is nonspecific, but the EEG shows an occipital photosensitive response using flash rates at 1 to 2 Hz. The ERG is diminished or extinguished and the potentials grossly enhanced, as are the somatosensory evoked potentials. Juvenile NCL: the CLN3 gene was initially linked to the hatoglobin locus on the long arm of chromosome 16p12. The first symptom is onset of progressive visual failure between 4 to 7 years of age, leading to blindness within 2 to 10 years. Slowly progressive deterioration of short-term memory and other cognitive functions usually starts by 8 or 9 years of age. Seizures appear afterwards. The patients usually die in the third or fourth decade of life. The EEG shows nonspecific abnormalities. ERG shows severe changes and a reduced β-wave may be seen even at the earliest stage. VEPs are markedly reduced or abolished and SEPs often enhanced. Neuroimaging usually shows progressive brain atrophy. Vacuolated lymphocytes can be regularly demonstrated on peripheral blood films, a unique finding in NCL.

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JUVENILE NCL: CONFIRMED BY ELECTRON MICROSCOPY, WITH MRI FOLLOW-UP

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The neuronal ceroid lipofuscinoses (NCL) are a group of progressive, autosomal –recessive, and finally fatal neurodegenerative diseases in children marked by lysosomal accumulation of disease– specific lipopigments in cerebral and extra-cerebral tissues and neuronal loss in the brain and the retina. The patient is a 13-year-old girl, daughter of unrelated healthy parents. Pregnancy and delivery were normal. Psychomotor development was normal until the age of 7 years, when her teacher noticed a progressive decrease in her attention level in addition to communication dysfunction and social withdrawal. A psychiatric condition was suggested. At the age of 8 she developed seizures. Neurological examination revealed an inattentive girl with poor fine motor skills. CT scan showed mild atrophy. The EEG showed pseudo periodic burst of high amplitude slow waves with posterior spikes at low frequency photic stimulation. She was prescribed Valproic acid. Two months later the myoclonic jerks became more frequent and clonazepan was added to the medication regime. At that time, she was completely mute. She continued to deteriorate, and by the age of 11 she fell due to ataxia. The brain MRI showed moderate atrophy. The ERG and the VEP were decreased at that time. Vacuolated lymphocytes were present and the ultrastructural examination of a skin biopsy showed vacuoles with predominance of fingerprint profiles. The ophthalmologic examination did not revealed macular degeneration. By the age of 13 she had myoclonies and motor disturbance with ataxia. The set of symptoms: myoclonic jerks, mental deterioration, ataxia, and progressive brain atrophy suggested NCL, and the definitive diagnosis was based on the skin biopsy, that revealed storage of the typical lipopigments.

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NCL IN CHILE: DESCRIPTION OF 13 CASES

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Purpose. To describe 13 cases with neuronal ceroid lipofuscinoses (NCL) detected in two neurology centers of our country.
Case reports. Early infantile: 1 case. Initiated at 1 year of age with myoclonic seizures, GTC seizures and spasms afterwards. Severe regression of developmental skills, microcephaly, bilateral optic atrophy, pyramidal syndrome. VEP were normal. Brain MRI: anterior periventricular and posterior subcortical calcification. Diagno-

sis: brain biopsy. Electronic microscopic (EM): giant lysosomes with curvilinear bodies. Late infantile neuronal ceroid lipofuscinoses: 8 cases. Onset of symptoms: between 18-36 months. Myoclonic seizures 5/8, atonic seizures 1/8, ataxia 8/8, coreathetosis 3/8, blindness 7/8, microcephaly 7/8, abnormal fundoscopy 6/8. All of them had progressive regression of psychomotor skills. VEP: abnormal in 2. Brain MRI: cerebellar atrophy: 8/8. Diagnosis: necropsy 4, brain biopsy 2, skin biopsy 2. EM: lysosomes with granular material and many curvilinear bodies with laminar appearance. Late infantil NCL variant: 2 cases. Onset of symptoms: between 4-4.9 years. All with previous normal development. Both of them started with GTC seizures. One of them presented also during the course atonic seizures, complex partial and photosensitive seizures refractory to treatment. The other patient presented ataxia and orofacial dyskinesias. Both patients had development skill regression, pyramidal syndrome. Abnormal fundoscopy in one. VEP: abnormal in 2. Abnormal ERG in one. Brain MRI: cortical and subcortical atrophy. Diagnosis: brain biopsy: 2. EM: great accumulation of lysosomes with curvilinear bodies, with a central middle line. Juvenile NCL: 2 cases. Both patients were normal until 6 and 9 years old. The onset was characterized in the first one with refractory partial epilepsy with evolution with atonic seizures. The other patient presented deterioration of cognitive and motor abilities with GTC seizures and ataxia afterwards. Fundoscopy was normal in both. VEP abnormal (1). Brain MRI: supra and infratentorial-cortico and subcortical atrophy. Diagnosis: brain biopsy: 1, skin biopsy: 1. EM: accumulation of lysosomes with curved laminar pattern in fingerprint.

Discussion. In both centers the most prevalent form was the late infantile NCL. The cerebral biopsy was utilized in prior years for the diagnosis, being replaced currently by the biopsy of skin. In our country is not possible genetic study yet.

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ULTRASTRUCTURAL ANALYSES IN A CASE SERIES OF PROGRESSIVE ENCEPHALOPATIES

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Ultrastructural analyses (UA) of the stored substances are often an essential tool to the diagnosis of several diseases, specially when a biochemical gold-standard is not ready for use –as happens with neuronal ceroid lipofuscinoses (NCLs), Lafora disease, and others–. UA are not available among us. When necessary, biopsies for UA were sent to other institutions, and for two reasons: (1) when patients presented with a typical clinical course of some of those already mentioned disorders ('good hypothesis' group); or (2) when patients presented with a diagnostic enigma ('bad hypothesis' group). We report the results of UA performed in our cases, in the last 7 years, and the rates of final diagnoses obtained with this method.

Patients and methods. all patients who shown progressive neurological deterioration, and whose IEM work-out gave normal results (including LSD, peroxisomal disorders, Wilson disease, aminoacidopathies and organic acidurias), were selected. When the parents agreed, a skin biopsy was collected and fixed with glutaraldehyde. Biopsy was then sent to UA.

Results. 37 patients, from 34 families, were examined. Before the UA results, 25/34 family cases had 'good hypotheses', whereas 9/34 had 'bad hypotheses'. 18 out of 25 'good hypotheses' group received some diagnosis after UA, while only one case out of 9 'bad hypotheses' group had a diagnosis. In the whole series, UA found 12 cases of NCLs, 4 cases of Lafora disease, one patient with neuroaxonal dystrophy and one patient with metachromatic leukodystrophy due to SAP 1 deficiency (the case found among 'bad hypotheses' group).

Discussion. These results suggest that the clinical evaluation is very efficient in selecting cases for UA (18/25 cases with 'good hypotheses'). The only positive diagnosis found among the 'bad hypotheses' group (1/9) was due to the fact that urine sulfatide analysis is not yet performed, among us. We concluded that (1) the clinical work-out, associated to a good IEM screening, is the best strategy for selecting cases to be sent to UA, specially in clinics like ours; and that (2) new biochemical assays must be implemented among us.

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THE COMMON DELETION OF CLN3 GENE AMONG BRAZILIAN PATIENTS WITH NCL

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Juvenile, neuronal ceroid lipofuscinosis (JNCL) is a neurodegenerative, autosomal recessive disorder characterised by progressive visual loss, seizures, mental and motor deterioration, and early death. The onset is usually between four and ten years of age. The diagnosis of JNCL rests on the clinical picture and on electron microscopy, which will show the presence of fingerprints or a mixed pattern of findings. The majority of JNCL patients (72%) are carriers of mutations in the CLN3 gene. More than 25 mutations are presently known to occur in CLN3 gene. The most common is a 1 kb deletion, which removes exons 7 and 8. This mutation is present in approximately 85% of all disease chromosomes in studied populations.

Objective. To present the preliminary results of the molecular study of the common deletion of CLN3 gene among a case series of patients with a suspected NCL.

Patients and methods. Seven unrelated patients were studied, with the following suspicions: 2 with a suspected JNCL; 2 with a suspected vJNCL/GROD; 2 with LINCL; and 1 with INCL. All of them started their clinical evaluation in 2003, so that not all individuals had a complete diagnostic work-out. After DNA extraction from peripheral blood, a PCR allele-specific, targeted directly to the presence of that deletion, was performed. Amplified fragments were observed in 1.5% agarose gel, stained with ethidium bromide.

Results. The two patients with a clinical and electron-microscopy (EM) picture of JNCL had normal results on PCR analysis, excluding the presence of the 1 kb deletion in the four (of three distinct origins) alleles. Surprisingly, one case with clinical and EM evidences of LINCL (age at onset: 2,5 years; and curvilinear bodies) carried one 1 kb deleted allele of CLN3 gene. This case had the CLN2 excluded, based on the enzymatic assay of tripeptidyl peptidase I.

Discussion. Three alleles of CLN3 gene of diverse origins, all suspected of carrying mutations, did not present the common deletion. These numbers, however, are still insufficient to give us an idea of the frequency of this mutation among Brazilian patients with JNCL. The unexpected finding of the same mutation in a child with a more severe clinical picture should be confirmed by further studies.

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REGIONAL STUDY OF NEURONAL CEROID LIPOFUSCINOSES IN ARGENTINA

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The NCLs are classified according to the current diagnostic consensus in 8 genetical types. We applied the international criteria of NCL: clinical, morphological, enzymological and genetic, to evaluate 35 patients belonging to 29 families at the Children's Hospital in Córdoba (1980-2003). Our algorithm included the data concerning MRI, EEG, evoked visual potentials, and electro retinograms; electron microscopy (EM) of skin and lymphocytes; palmitoyl-protein-thioesterase1 (PPT1) and tripeptidyl-peptidase-I (TPP-I) lysosomal enzyme assay in plasma, in leukocytes and in saliva. The molecular analysis is in progress.

Results. 6 patients, belonging to 4 families, were confirmed for NCL: 1) One male with juvenile phenotype had PPT1 deficiency (INCL). The scan of the CNL1 gene is in progress. 2) Two male siblings with juvenile phenotype had TPP-I deficiency (LINCL). The skin biopsy showed curvilinear bodies and fingerprint profiles (CB+FP) and one Q66X mutation was identified. 3) One male patient with juvenile phenotype was suspected for JNCL through the skin biopsy with FP profiles and vacuolated lymphocytes. 4) One female patient with juvenile onset was suspected for JNCL through the EM with FP profiles. These 2 last patients showed no enzyme deficiency, and any mutation in the CNL3 gene was detected by now. The remaining suspected subjects were rouled out enzymatically for INCL and LINCL. The data evidenced the heterogeneity of this group of patients. Through further studies, genotype-phenotype correlations of NCL may be expanded in our region.

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EVALUATION OF THE SCREENING OF PPT1 AND TPP-1 IN SALIVA IN THE INFANTILE (INCL) AND LATE INFANTILE (LINCL) NEURONAL CEROID LIPOFUSCINOSES

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We investigated if the PPT1 and TPP-I lysosomal enzyme assays in saliva could be contributory, easy and non-aggressive procedures to reach a fast diagnostic orientation in NCL suspected patients of all ages.

Methods. The methods were standardized and the referential range of both enzymatic activities in saliva were established for the normal Argentinean population. Deficient values were obtained in 1 INCL patient and in 2 LINCL patients, as well as in their obligate heterozygous mothers. We tested also the saliva of 4 subjects suffering other defined lysosomal diseases.

Results. 1) Both enzymes were expressed in saliva, a previously unknown fact; 2) The referential range of PPT1 in saliva for n = 44 control subjects was 78-398 (196.85 ± 72,76 nM/24 h/mg protein); 3) The referential range of TPP-I for n = 46 control subjects was 89-476 (213.58 ± 97.97 nM/24 h/mg protein); 4) PPT1 was deficient in the saliva of one INCL proband (0 nM/24 h/mg protein) and their mother had an intermediate value; 5) TPP-I was deficient in saliva of n = 2 LINCL siblings (0.6-2.05 nM/24 h/mg protein) and it was reduced in their mother; 6) One MPSIII type A proband had increased PPT-I value in saliva, reaching 906 nM/24 h/mg protein, while one MPS type IV and 2 Niemann Pick type A probands had values in the control's range. The PPT1 and TPP-I tests carried out in the saliva are suitable screening methods for INCL and LINCL. More studies are needed to evaluate the usefulness of these tests as secondary markers in other lysosomal storage diseases.