PROGRAMMED LECTURES

L - 1

GENE THERAPY COMES OF AGE: FROM INVESTIGATIONAL AGENT TO THERAPEUTIC PRODUCT

HIGH KA.
Howard Hughes Medical Institute, William H. Bennett Professor of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Center for Cellular and Molecular Therapeutics at The Children's Hospital of Philadelphia, Room 5064, Colket Translational Research Building, 3501 Civic Center Blvd, Philadelphia, PA 19104, USA

The disease burden of inherited disorders is high, accounting for as many as 50% of admissions to children's hospitals, and a substantial number of admissions to adult hospitals as well. Yet for many of these disorders, treatment options are limited. The goal of gene therapies is to expand therapeutic options for his group of patients, but this seemingly straightforward concept has proven challenging to reduce to practice. Laboratory and small-scale clinical studies have, after two decades of clinical investigation, resulted in clear-cut therapeutic successes in a few well-defined diseases settings, and, in the case of lipoprotein lipase deficiency, have led to the first licensed gene therapy product for a genetic disease. *In vivo* gene transfer with AAV vectors has led to sustained clinical improvements in the setting of inherited retinal degenerative diseases, and of hemophilia B as well. Studies are underway for many other disorders. Some specific obstacles to clinical efficacy had been anticipated based on animal studies, but others were elucidated only through clinical studies that highlighted problems not predicted by pre-clinical studies. Resulting laboratory studies enabled solutions that have led to the current generation of successful clinical studies. A nuanced understanding of the interaction of AAV vectors with the human immune response, and with other homeostatic mechanisms, has allowed investigators to harness the power of gene identification and gene delivery vectors, to provide long-lasting therapeutic outcomes for previously incurable diseases.

L - 2

EUROPEAN POLICY IN THE FIELD OF RARE DISEASES TO IMPROVE PATIENT CARE AND BOOST RESEARCH

AYMÉ S., RODWELL C.
INSERM, US14, Orphanet, Paris, France

In 2009 the European Council of Ministers adopted a Recommendation on an action in the field of rare diseases. This Recommendation encouraged countries to define and implement a plan or a strategy for rare diseases by the end of 2013 and requested that the European Commission establish an EU Committee of Experts in the field of Rare Diseases (EUCERD) as a forum of stakeholders to propose areas of action at European level. There is now enough evidence to judge the outcome of Recommendation which can be qualified as positive despite the economic crisis. The activity in the field is dynamic and impressive. The publication of the annual report on the *State of Art of Rare Disease Activities in Europe* by the EUCERD Scientific Secretariat is testament to the many achievements of the Committee. The EUCERD has adopted five sets of recommendations, including a set of quality criteria for centres of expertise for rare diseases at national level. All countries will have a plan or a strategy for rare diseases by the end of 2014. The recent developments in genomics now translate into more diagnostic tests for rare diseases. So far almost 3 000 rare diseases can be tested in one of the EU countries. Targeted funding for rare diseases has also produced its effects, with more transnational cooperation which has translated into the important decision to establish an international consortium to fund research, the IRDiRC. The Consortium will allow more ambitious goals to be set and achieved faster and it will ease the mobilisation of the critical mass of expertise and resources whilst avoiding overlaps in research. The Regulation on Orphan Medicinal Products is still producing positive effects with currently over 80 products with a marketing authorisation in the EU and many more in development. The Orphanet database became a Joint Action between all European countries, showing the great degree of willingness to provide unified, high-quality information to all citizens. Last but not the least, patient organisations are increasingly better organised in order to make their voice heard. Not only is EURORDIS the voice of patients in Europe, but umbrella organisations have been established in most European countries. Despite all these achievements, the forecast is less than bright as health budgets are shrinking all over Europe and this may impact on patients' access to innovations, a situation for which alternative solutions have to be identified jointly by all stakeholders.

L - 3

COMMON TERMINOLOGIES AND NOMENCLATURES FOR REGISTERING RARE DISEASES DATA: STATE OF CURRENT INTERNATIONAL INITIATIVES

AYMÉ S., RATH A.
INSERM,US14, Orphanet, Paris, France

The community requires stable standardised terminologies in order to achieve interoperability between databases intended for clinical research that include phenotype descriptions. This is crucial in interpreting genomic rearrangements as well as future high-throughput sequence data. Phenome description requires wording to define entities (diseases and syndromes) and wording to define the elements constituting these entities (signs and symptoms). Current multiplicity of medical terminologies in use impose the need for a reference tool in which all these data are integrated in a normalised fashion in order to render them available for health information system and for research. Since 1997, Orphanet maintains a multilingual database of rare diseases (RD), based on the literature and on expert advice and manually curated, comprised of a nosology (classification of RD), relationships (genes-diseases, epidemiological data, orphan drugs) and cross-references with other terminologies (MeSH, SNOMED CT, UMLS), databases (OMIM) or classifications (ICD10) in use. These data are freely available for download at the OrphaDataplatform (www.orphadat.org) and available as an ontology of Rare Diseases, which provides a robust and consistent modellisation of data and their semantic relationships, as well as interoperability standards with other scientific resources in use both in research and in public health. The Orphanet Rare Diseases Ontology will be available in BioPortal (http://bioportal.bioontology.org) and in OrphaData and is updated monthly. The Orphanet nomenclature is proposed by the IRDiRC as the recommended ontology to define rare clinical entities. The Human Phenotype Ontology (HPO) aims to provide a standardized vocabulary of phenotypic abnormalities encountered in human disease. The HPO was initially developed using information from Online Mendelian Inheritance in Man (OMIM), which is a hugely important data resource in the field of human

genetics and beyond. The HPO is currently being developed using information from OMIM and the medical literature and contains approximately 10,000 terms. Over 50,000 annotations to hereditary diseases are available for download or can be browsed using the PhenExplorer. The HPO can be used for clinical diagnostics in human genetics (Phenomizer), bioinformatics research on the relationships between human phenotypic abnormalities and cellular and biochemical networks, for mapping between human and model organism phenotypes, and for providing a standardized vocabulary for clinical databases, among many other things. It is recognized by the IRDiRC as the recommended ontology to use to code human phenomes.

L - 4

THE GIVING FORTH OF SMALL SHARP SOUNDS: THE ORPHAN (RARE?) DISEASES. A PUBLIC-HEALTH AND RESEARCH PRIORITY. THE ROLES OF PRIMARY CARE AND REFERENCE CENTRES

DODELSON DE KREMER R.
Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Ministerio de Salud Provincia de Córdoba, Hospital de Niños de la Santísima Trinidad, Córdoba, Argentina

Public awareness about difficulties of patients with Orphan Diseases (ODs) was first raised by the report of the National Commission of ODs of the US government in 1989. Since then, 25 years later, Comission´s hearings with hundreds of stakeholders highlighted issues affected patients´ care, such as little information, drawbacks of providing adequate health insure and mainly, almost without exceptions, these patients face with diagnostic delays and limited availability of effective treatments. The true burden of ODs is difficult to estimate, but ODs are no so rare, at least among our community. Since epidemiological data for most of these diseases in general and particularly, in Inherited Metabolic Diseases our own work's field is lacking, in this issue, its will consider our experiences of a diagnostic service of NCLs and the wide spectrum of subtypes of Lysosomal Storage Disorders (LSDs) recognised in a single Argentinean center. A key challenge in genomics is to understand the phenotypic consequence of genomic variation; the challenge is no longer to generate DNA sequence data, but to interpret them. The analysis of phenotype abnormalities provides a translational bridge from genoma-scale biology to a disease-centered view of human pathobiology. Human Phenotype Ontology (HPO), available at http://www.human-phenotype-ontology.org, provides the terms from ontologies with logical definitions for anatomy, cell types, function, embryology, pathology and other domains; an aspect that must be adopted. Who are the players and which are the irresolute main tasks? Bio-medical genetic education and establishement of the statement for Reference Centres joined to information how to set up these centres.

L - 5

GENE THERAPY FOR THE CNS MANIFESTATIONS OF THE LYSOSOMAL STORAGE DISORDERS

CRYSTAL RG.
Department of Genetic Medicine, Weill Medical College of Cornell University, New York, USA

The blood brain barrier, mediated by tight junctions of CNS capillary endothelium, represents a major challenge in delivering therapies for CNS disorders. To circumvent this barrier, we have developed a variety of gene transfer strategies where therapeutic genes are delivered directly to the CNS using adeno-associated virus (AAV) gene transfer vectors. These strategies have been successful in treating experimental animal models of the CNS manifestations of lysosomal storage diseases, and are being used in an ongoing clinical trial of late infantile neuronal ceroid lipofuscinosis. As part of this clinical study, we have developed new MRI and cerebral spinal fluid quantitative biomarkers to assess the progression of the disease state.

L - 6

TOLEROGENIC CIRCUITS MEDIATED BY GALECTIN-GLYCAN INTERACTIONS IN THE RESOLUTION OF AUTOIMMUNE INFLAMMATION

RABINOVICH G.
Laboratorio de Inmunopatología, Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina

Recent efforts toward decoding the glycosylation signature of immune cell processes have revealed dramatic changes in N- and O-glycan structures during T cell activation and differentiation. These alterations have also been detected during the course of DC differentiation and maturation, suggesting that protein-glycan interactions may have a decisive role in the control of immune cell responsiveness and tolerance. The responsibility of interpreting these glycosylation changes is assigned in part to endogenous glycan-binding proteins. Galectin-1, an endogenous lectin which recognizes multiple galactose-β1-4-N-acetylglucosamine (LacNAc) units present on the branches of N- or O-linked glycans, elicits a broad spectrum of anti-inflammatory and immunomodulatory effects. Blockade of galectin-1 expression in tumor tissue results in heightened T cell-mediated tumor rejection and increased secretion of T helper type-1 (TH1) cytokines. Moreover, galectin-1-deficient (Lgals1-/-) mice exhibit augmented TH1 and TH-17 responses and are considerably more susceptible to immune-mediated fetal rejection and autoimmune disease than their wild-type counterparts. Yet, the mechanistic bases underlying these anti-inflammatory effects are still uncertain. We will discuss in this session the identification of a circuit linking galectin-1 signaling, generation of tolerogenic dendritic cells and expansion of regulatory T cells which contributes to the resolution of autoimmune inflammation and modulates T cell responses in autoimmune settings. This immunoregulatory circuit also operates at the level of central nervous system during autoimmune neuroinflammation and involves a cascade of molecular events and a network of cells including astrocytes, classically-activated microglia and neurons. Strategies to manipulate this circuit in either direction (stimulation or blockade) may be capable of influencing immune tolerance versus activation, a critical decision with broad therapeutic implications in immunopathology.

ORAL PRESENTATIONS

O - 1

PERSPECTIVES ON NCL GENETICS: GENES, MUTATIONS AND GENOTYPE-PHENOTYPE CORRELATIONS

MOLE S. E.[1-3].
[1]MRC Laboratory for Molecular Cell Biology; [2]UCL Institute of Child Health; [3]Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK

Over 400 mutations in thirteen genes cause NCL disease, listed in the NCL Mutation Database (http://www.ucl.ac.uk/ncl). These genes are CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, CLN7/MFSD8, CLN8, CLN10/CTSD, CLN13/CTSF, with mutations in CLN11/GRN, CLN12/ATP13A2 and CLN14/KCTD7 described in single families. A further eight genes cause NCL-like disease in animals. There is a characteristic disease phenotype known for most NCL genes that is associated with complete loss of gene function, and for some 'milder' mutations that underlie disease that is more protracted or of later onset. The late infantile group of NCLs can arise from loss-of-function mutations in several different genes as well as milder mutations in others. The genetic basis of adult NCL is being revealed, with some cases carrying mild mutations in genes that usually cause NCL in childhood and others in genes that cause onset only in adulthood. There are several mutations in NCL genes that cause distinct disease phenotypes. Families and animals diagnosed with NCL for whom the genetic basis remains elusive are benefitting from the recent advances in DNA sequencing technologies that provide the means to identify their genetic basis. These advances have led to a new nomenclature for NCL that is gene-based

O - 2

PERSPECTIVES ON NCL CELL BIOLOGY: AN UP-TO-DATE OVERVIEW OF THE FUNCTIONS OF THE NCL PROTEINS

COTMAN S. L.[1]
[1]Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge St., Boston, MA 01778, USA

In the past two decades, significant genetic efforts have led to the identification of most NCL-related genes, and the molecular pathways of the encoded proteins leading to lysosomal dysfunction are now coming into focus. The NCL proteins include several lysosomal enzymes and a number of membrane proteins primarily residing within the endosomal-lysosomal compartment but also within the endoplasmic reticulum. Emerging areas of research focus spanning more than one subtype of NCL include dysregulation of biometal homeostasis, palmitoylation, autophagy, and post-Golgi lipid and protein trafficking, and some NCL proteins may function in concert with one another in these processes. An increasingly improved understanding of the complex interplay of the NCL cell biological pathways should help guide rationale therapeutic development in the coming years.

O - 3

THE IMPLICATION OF CALNUC IN NEURONAL CEROID LIPOFUSCINOSIS PATHOGENESIS

LARKIN H.[1], THÉRIAULT C.[1], COSTANTINO S.[2], LAVOIE C.[1]
[1]Department of Pharmacology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada; [2]Centre de Recherche de l'Hôpital Maisonneuve-Rosemont, Université de Montréal, Montréal, Canada

The efficient intracellular transport of lysosomal enzymes is important as their improper delivery lead to lysosomal dysfunction that gives rise to neurodegenerative diseases such as the neuronal ceroid lipofuscinoses (NCL). Lysosomal enzyme transport is carried out by lysosomal receptors that traffic between the Golgi and the endosomes. Ceroid Lipofuscinosis Neuronal protein-5 (CLN5), a protein involved in late-infantile variants of NCL, has been recently implicated in the retrograde transport of lysosomal receptor. We recently demonstrated that Calnuc, an ubiquitous Ca2+ binding protein, participates in this trafficking pathway. Interestingly both proteins regulate the endosomal recruitment of retromers, key proteins of the endosomal sorting machinery, through an action on the small G protein Rab7. The overall objective of this study is to investigate the role of Calnuc on the regulation of CLN5 and its potential implication in NCL. Biochemical studies indicated that Calnuc interacts directly with CLN5. Furthermore, depletion of Calnuc by small interfering RNA decreased the cellular levels of CLN5. Interestingly, confocal and electron microscopy analysis of Calnuc knockdown cells exhibit the characteristic lysosomal features of NCL including accumulation of enlarged lysosomes containing two classical markers of NCL (autofluorescent material and subunit C of the mitochondrial ATP synthase complex), the presence of curvilinear and autophagic vacuoles. These evidences support a potential implication of Calnuc in NCL pathologies. We are currently looking for mutations or level variations of Calnuc in NCL tissues or cell lines. Identification of new functional causative proteins may help clarify the pathogenesis and may point to new therapeutic strategies.

O - 4

CELL-SPECIFIC EXPRESSION OF PALMITOYL-PROTEIN THIOESTERASE-1: UTILIZING A NOVEL METHOD TO STUDY INFANTILE BATTEN'S DISEASE

SHYNG C., MACAULEY S. L., SANDS M. S.[1]
[1]Washington University School of Medicine, St. Louis, USA

Background: Infantile NCL (INCL) is caused by a deficiency in the soluble lysosomal enzyme palmitoyl-protein thioesterase-1 (PPT1). Determining the role of specific cell types in the pathogenesis of INCL using established transgenic approaches is confounded by cross-correction. Therefore, we have created a chimeric version of PPT1 containing the transmembrane domain of LAMP1 in order to prevent cross-correction.

Aims: Determine the role of specific cell types in the pathogenesis of INCL. Material and Methods: We linked the C-terminus membrane-spanning domain of LAMP1 to PPT1 and included a lox-flanked transcriptional stop cassette. We generated transgenic mice harboring the PPT1-LAMP1 transgene and Cre recombinase driven by the synapsin-1, GFAP, and ubiquitin-C promoters. Results: PPT1-LAMP1 retained enzymatic activity in vitro and was sequestered in the cell. Upon in vivo expression of Cre recombinase from each promoter: 1) PPT1 activity was restored indicating the removal of the transcriptional stop cassette, 2) there was no PPT1 activity in the serum indicating no aberrant secretion of PPT1-LAMP1, and 3) there was a reduction in autofluorescent accumulation in the brain. Conclusion: Taken together, these preliminary data strongly suggests that the lox-STOP-lox PPT1-LAMP1 transgenic mouse is a viable model system to study cell-specific expression of PPT1.

O - 5

USING INDUCED PLURIPOTENT STEM CELLS (IPSCS) TO MODEL EARLY DISEASE MECHANISMS OF CLN1 DISEASE

UUSI-RAUVA K.[1,2], KYTTÄLÄ A.[2], BLOM T.[2], LEHESJOKI A. E.[1,3,4], JALANKO A.[1,2].
[1]Folkhälsan Institute of Genetics, Biomedicum Helsinki, P.O.Box 63 (Haartmaninkatu 8), Helsinki 00014, Finland; [2]National Institute for Health and Welfare (THL), Public Genomics Unit, Biomedicum Helsinki, Helsinki, Finland and FIMM, Institute for Molecular Medicine Finland, Biomedicum Helsinki, Helsinki, Finland; [3]Neuroscience Center, University of Helsinki, Helsinki, Finland; [4]Research Program's Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland

Although previous studies have provided important information on the mechanisms of CLN1 disease, particularly in mice, the models used so far have not been phenotypically and/or functionally comparable to affected human neuronal cells. In the current study, induced pluripotent stem cell (iPSC) technology was utilized to generate characterized iPSC and iPSC-derived human neuronal models for CLN1 disease, classic infantile and CLN1 disease, late infantile representing three disease-associated mutations in four patients of Finnish or Italian origin. Control and CLN1 patient-derived iPSCs were differentiated into neuronal cultures, and both control/patient-derived iPSCs and neuronal cultures were analysed for early anomalies by gene expression microarray profiling. Most interesting findings in gene expression analysis were selected for validation at a functional level. Furthermore, microscopy analysis was carried out to dissect disturbances in basic intracellular features of affected iPSCs and neurons. This study reveals early common abnormalities, including defective neuronal migration, of genotypically different affected neurons but also provides clues to differential disturbances of distinct intracellular metabolic pathways that may influence the course of CLN1 disease. Furthermore, the study provides important information on the usefulness of different model systems to study CLN1 disease.

O - 6

A DICTYOSTELIUM MODEL FOR NEURONAL CEROID LIPOFUSCINOSES (BATTEN DISEASE)

CHAVAN A., ANNESLEY S., FISHER P.[1]
[1]La Trobe University, Bundoora campus, 3086, Melbourne, Australia

Neuronal ceroid lipofuscinoses (NCL) is a neurodegenerative disorder whose major symptoms include neurodegeneration, retinopathy, dementia and premature death. The various forms of NCL arise from mutations at any of 14 loci (CLN 1-14) encoding one of several lysosomal or endoplasmic reticulum proteins. To unravel molecular mechanisms of pathogenesis in NCL caused by CLN5 and CLN7 mutations, we used the eukaryotic haploid cell model, D. discoideum, as its unique life cycle allows study of different cell signalling pathways. CLN7 has been hypothesized to function as a lysosomal transport protein and we found direct evidence for this in the form of altered lysosomal uptake of Neutral Red (NR) in our mutants. In vitro lysosomal uptake assays showed that NR

uptake could be inhibited, presumably competitively, by a variety but not all potential substrates and was independent of ionophores suggesting its passive transport. Knockdown and overexpression approaches caused defects in cell growth, endocytosis, autophagic cell death, lysosomal volume and osmoregulation in CLN7 mutants. CLN5 mutants similarly showed abnormalities in cell growth, endocytosis and lysosomal pH regulation. Our results suggest that simulating NCL in Dictyostelium by altering CLN5 and CLN7 protein expression affects cell signaling pathways that regulate vesicle trafficking in autophagy and endocytosis.

O - 7

LOSS OF CLN3 FUNCTION IN THE SOCIAL AMOEBA DICTYOSTELIUM DISCOIDEUM CAUSES PLEIOTROPIC EFFECTS THAT ARE RESCUED BY HUMAN CLN3

HUBER R. J., MYRE M. A., COTMAN S. L.[1]
[1]Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

The social amoeba Dictyostelium discoideum is emerging as a significant model organism for the study of neurological disease. It is particularly suited for investigation of trafficking, lysosomal function, cell-cell communication, and chemotaxis. We therefore generated a new Dictyostelium model for JNCL research. In single cells, Dictyostelium Cln3 (DdCln3) localizes to endomembrane compartments with clear enrichment on the contractile vacuole. Importantly, heterologous expression of human CLN3 (hCLN3) shows the same intracellular localization. cln3- cells proliferate faster concomitant with a significant alteration in the secretion and proteolysis of the autocrine proliferation repressor, AprA. During early multicellular development, cln3 deficiency alters cAMP chemotaxis which results in ~30% more aggregation territories compared to parental cells. Development of cln3- cells is precocious and cln3- slugs display defects in migration. These phenotypes can be rescued by expression of DdCln3 or hCLN3 in cln3- cells. Together, these data suggest that CLN3 functions in regulating cell-cell and cell-environment communication. Current work is focused on investigating the mechanisms by which DdCln3 exerts its effect on these processes. The ability of hCLN3 to restore abnormalities in Dictyostelium cln3- cells provides proof-of-concept that use of Dictyostelium will complement ongoing JNCL research, with the potential to identify CLN3 function and translational therapeutic approaches.

O - 8

EXPLOITING YEAST TO HIGHLIGHT NEW THERAPEUTIC STRATEGIES IN CLN3 DISEASE

BOND M.[1], VIEIRA M.[1], FREEMAN J.[1], MAROTTA D.[1], BROWN R.[1], RUSSELL C.[2], KETTELER R.[1], MOLE S. E.[1,3,4]
[1]MRC Laboratory for Molecular Cell Biology; [2]The Royal Veterinary College, Royal College Street, London NW1 0TU, UK; [3]Institute of Child Health; [4]Department of Genetics, Evolution and Environment, University College of London, London WC1E 6BT, UK

Juvenile CLN3 disease presents many challenges to therapeutic development. It is caused by mutations in CLN3, which encodes a multi-pass membrane protein localised to the lysosome and Golgi apparatus. CLN3 has been associated with a number of cellular processes, including lysosomal function, trafficking, and cytoskeletal organisation, however the function of CLN3 is still unknown. This gap in understanding demonstrates the complexity of the problem, highlighting the need for a simplified model of disease. Fission yeast represents an ideal system for the study of CLN3 disease, as it contains a single non-essential orthologue of CLN3 (btn1). We present data demonstrating that btn1 displays a significant interaction with the conserved Tor signalling pathways, defects in which mimic many aspects of CLN3 disease. Our data highlight interplay between the Tor kinases and btn1, including a rescue of many aspects of the loss of btn1 upon manipulation of Tor pathway function. Such data provide an insight into the dysfunctional changes key to CLN3 disease, as well as new avenues for therapeutic development. Further, we have utilised the yeast model to identify new small molecule therapies. Using this approach, we have identified compounds that reverse disease phenotypes in yeast, mammalian cells and a zebrafish model.

O - 9

ANALYSIS OF THE IMMUNE PHENOTYPE IN CLN3ΔEX7/8 MICE

SCHMIDTKE C.[1], MAKRYPIDI G.[1], OTOMO T.[1], MITTRÜCKER H. W.[2], BRAULKE T.[1], SCHULZ A.[1]
[1]Department of Biochemistry, Children's Hospital; [2]Department of Immunology, University Medical Centre Hamburg-Eppendorf, Germany

CLN3 disease is caused by mutations in the CLN3 gene, encoding a lysosomal membrane protein of unknown function. Besides profound neurological impairment, recent studies suggested that CLN3 disease also displays alterations in the immune system. However, the relationship between lysosomal dysfunction and immune system modulation still remains unclear. To assess the link between functional impairment of lysosomes and possible immune phenotype abnormalities in CLN3 pathology, we performed biochemical and in vivo functional assays on immune cell populations of Cln3Δex7/8 mice. First, an increase of the lysosomal membrane protein Lamp1 was observed in T and B cells, indicating an increase of number or size of lysosomes. Interestingly, predominantly T cells but not B cells showed accumulation of lysosomal storage material under electron microscopy. Protein levels of soluble lysosomal proteases such as cathepsins D and B were unchanged in T and B cells but were decreased in peritoneal macrophages confirmed by delayed processing of endocytosed DQ-BSA. Ex-vivo stimulation of T cells resulted in an increase in TNF-α and IFN-γ both in naïve Cln3Δex7/8 mice and more strikingly after infection of mice with a murine Listeria monocytogenes strain. These results deliver new insights into the relationship between lysosomal dysfunction and modulation of the immune system in CLN3 disease.

O - 10

CRMP2/CLN6/KLC4 COMPLEX IN MEDIATING DISTAL NEURONAL TRANSPORT

KOH S.Y., CAIN J., MAGEE H., HENSLEY K.,WEIMER J.[1]
[1]Sanford Research Center,Sioux Falls, USA

Studies from our laboratory have identified a novel complex containing the ER-associated CLN6, whose mutation results in a variant late infantile NCL (vLINCL), the collapsin response mediator protein 2 (CRMP2), and the kinesin motor protein, KLC4. Acting through a network of protein interactions, CRMP2 regulates axonal/dendritic specification and extension during neurodevelopment and contributes to maintenance/regeneration in the mature brain. We hypothesize that the CRMP2/CLN6/KLC4 (CCK) complex utilizes CLN6 as a "molecular tag" on ER-vesicles for segregation of cargo to distal sites in dendrites and axons. Disruption of this signaling complex could contribute to the pathogenesis of vLINCL through altered neuronal process outgrowth and maintenance. We will present recent findings from our lab on how the CCK complex contributes to ER-vesicle transport as well as early events in neuronal differentiation. Moreover, we will discuss efforts to stabilize CRMP2-associated complexes, independent of CLN6 rescue, as a mechanism to ameliorate the neurological deficits in a pre-clinical NCL mouse model.

O - 11

THE ADULT NCL GENE DISCOVERY CONSORTIUM

KMOCH S.[1], STAROPOLI J.[2], OLIVER K. L.[3], COTMAN S. L.[4], HILDEBRAND M.[3], DAMIANO J.[3], BAHLO M.[5], SMITH K. R.[5],JEDLIČKOVÁ I.[1], STRÁNECKÝ V.[1], SIMS K.[4], COSSETTE P.[6], CARPENTER S.[7], ANDERSON G.[8], MOLE S. E.[8], BERKOVIC S. F.[3]
[1]Institute for Inherited Metabolic Disorders, Prague, Czech Republic; [2]Biogen Idec, Cambridge, USA; [3]University of Melbourne, Melbourne, Australia; [4]Massachusets General Hospital and Harvard Medical School, Boston, USA; [5]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; [6]Centre Hospitalier de

L´Universite de Montréal, Montréal, Canada; [7]Centro Hospitalar de São João, Porto, Portugal; [8]University College London, UK

To facilitate diagnosis and enhance research in unsolved adult-onset forms of Neuronal Ceroid Lipofuscinosis (ANCL) we established a Consortium involving groups from the UK, Europe, USA, Canada and Australia. So far we have pooled 48 familial and sporadic unsolved cases into a shared database. According to established diagnostic criteria each case is given a classification of 'definite', 'probable', 'possible' or 'not' ANCL based on the available clinical data. Of the 48 cases, detailed review with further investigation established an alternate diagnosis in eight. These comprise single cases of Huntington disease, Leigh Syndrome, Alzheimer disease, cerebral lymphoma, ARSACS, NBIA, neuroserpinopathy, and Niemann-Pick disease. Additionally, two cases were re-classified as childhood or juvenile NCL. Nine of the remaining 38 cases fulfil our criteria of ANCL (4 definite; 2 probable; 3 possible). Seven cases have features suggesting an alternate diagnosis, but no specific condition was identified, in five the data were inadequate for classification and 17 cases remain under evaluation. Of the nine cases, 3 demonstrate autosomal dominant inheritance, one case is autosomal recessive and 5 cases are sporadic. Molecular analysis has identified CLN6 in one case, known genes have been excluded in the remainder and they are subjected to comprehensive genomic analysis.

O - 12

ASSESSING THE CONTRIBUTION OF GLIAL DYSFUNCTION TO NCL PATHOGENESIS

COOPER J. D., PARVIAINEN L., DIHANICH S., LANGE J., ANDERSON G., VAN TRIGT L.,WILLIAMS B. P.[1]
[1]King's College London, Institute of Psychiatry, Psychology and Neuroscience, London, UK

In all NCLs, localized glial activation is an early event that accurately predicts where neurons subsequently die. We have explored whether the biology of glia, which would normally express the deficient proteins, is compromised and possible subsequent effects upon neurons. In juvenile NCL (JNCL) we have found basic defects in both Cln3 deficient astrocytes and microglia, which failed to transform morphologically and displayed an altered protein secretion profile upon stimulation. These defects were far more pronounced in astrocytes, in which cytoskeletal abnormalities, impaired calcium signalling and reduced glutamate clearance were observed. Importantly, using a co-culture system, Cln3 deficient glia were shown to negatively impact the health of both Cln3 deficient and wildtype neurons, with mutant neurons being the most severely affected. These data reveal that JNCL astrocytes are functionally compromised, and together with microglia, may play an active role in the neurodegeneration observed in JNCL. Performing similar studies in glial cells isolated from mice that model infantile and late infantile NCL is also revealing a range of glial phenotypes, different to those seen in JNCL. These data raise the possibility that glia should also be considered as therapeutic targets in these disorders.

O - 13

NEW ANIMAL MODELS FOR THE STUDY OF BATTEN DISEASE

PEARCE D. A.[1]
[1]Sanford Children's Health Research Center, Sanford Research, Department of Pediatrics, Sanford School of Medicine of the University of South Dakota, 2301 E. 60th st. North, Sioux Falls, SD, 57104-0589, USA

Neuronal ceroidlipofuscinoses (NCLs), also known as Batten disease, are a group of autosomal recessive neurodegenerative disorders in children characterized by the progressive onset of seizures, blindness, motor and cognitive decline and premature death. Patients with mutations in *CLN1* primarily manifest with infantile NCL (INCL). *CLN1* encodes a lysosomal enzyme, palmitoyl-protein thioesterase 1 (PPT1). Nonsense mutations in *CLN1* account for 52.3% of all disease causing alleles in INCL, the most common of which is the p.R151X mutation. Patients with mutations in CLN2 primarily manifest with late-infantile NCL (LINCL). CLN2 encodes the lysosomal serine protease tripeptidyl peptidase (TPP1). Nonsense mutations in CLN2 account for 33.8% of all disease causing alleles in LINCL, the most common of which is the p.R208X mutation. We have created mouse models that harbor the CLN1 p.R151X and CLN2 p.208X mutations as a means to have murine models that like patients retain a residual activity of PPT1 and TPP1, respectively. These models can be used to test strategies that would enhance existing enzyme activity through mechanisms that might stabilize mRNA or protein and thus increase enzyme activity. In addition, there is also a need for large a large animal model for juvenile Batten disease. A porcine model for CLN3 will be discussed.

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THERAPEUTIC TARGETING OF NEUROINFLAMMATORY PATHWAYS IN JUVENILE BATTEN DISEASE

KIELIAN T.[1]
[1]University of Nebraska Medical Center, Omaha, NE, USA

Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), or Juvenile Batten Disease, is a fatal lysosomal storage disease caused by an autosomal recessive mutation in the CLN3 gene. JNCL typically presents in children between the ages of 5-10 years, initiating as blindness and progressing to seizures, motor loss, and cognitive decline, with a decreased life expectancy into the late teens or early twenties. Activated microglia and astrocytes are observed in the brains of JNCL patients, as well as associated mouse models, and predict regions that will undergo neurodegeneration. Our laboratory has been actively investigating the functional implications of glial activation during JNCL with the hypothesis that aberrant glial responses contribute to neuronal loss during later stages of disease. In agreement with this premise, we have found that CLN3Δex7/8 microglia exist in a primed state, producing exaggerated levels of numerous proinflammatory mediators in response to danger-associated molecular patterns (DAMPs) present in the diseased brain. In addition, we have discovered a progressive decline in astrocyte health during JNCL progression typified by the reduced expression of molecules critical for glutamate homeostasis. These changes in microglial and astrocyte function have been linked to exaggerated phosphodiesterase-4 (PDE4) activity, resulting in lower cAMP levels, which also decline in the brains of CLN3Δex7/8 mice with advancing age. Targeting these dysfunctional glial responses with select PDE4 inhibitors over a 6 month period has shown promise in delaying disease progression, as revealed by improvements in motor deficits in CLN3Δex7/8 mice. Defining mechanisms of glial dysfunction using the CLN3Δex7/8 mouse model will provide a foundation for understanding JNCL pathogenesis and the development of new therapeutic strategies.

O - 15

SIALOADHESIN-POSITIVE MICROGLIA/MACROPHAGES PROMOTE NEUROINFLAMMATION AND AXONAL PERTURBATION IN THE RETINOTECTAL SYSTEM OF TWO MOUSE MODELS OF NEURONAL CEROID LIPOFUSCINOSIS

GROH J.[1], STADLER D.[1], CROCKER P.[2], MARTINI R.[1]
[1]Department of Neurology, Developmental Neurobiology, University of Wuerzburg, Germany; [2]Wellcome Trust Biocentre, School of Life Sciences, University of Dundee, Dundee, UK

Neurodegeneration in CLN diseases usually afflicts the visual system and is accompanied by inflammation. We have previously shown that CD8+ T-lymphocytes contribute to axonal perturbation and neuron loss in Ppt1-/- mice, a model of CLN1. We now investigated the role of microglia/macrophage-like cells in Ppt1-/- mice and Cln3-/- mice, a model of CLN3. We characterised microglia/macrophages in the retinotectal system and observed increased expression of markers for proinflammatory "M1-like" activation and antigen presentation at distinct ages. Microglia in both models upregulated sialoadhesin (Sn), a cell recognition molecule implicated in macrophage-T-cell interaction.

To analyse its pathogenic impact, we crossbred both models with Sn-deficient mice and scored neuronal integrity using im-

munohistochemistry, electron microscopy and optical coherence tomography. Importantly, retinotectal pathology was significantly reduced in the absence of Sn. Ppt1-/-Sn-/- mice also presented with an ameliorated clinical phenotype and extended survival. Sialoadhesin-deficiency attenuated the numbers of "M1-like" microglia/macrophages, the expression of proinflammatory cytokines and increased the numbers of CD8+CD122+ regulatory T-lymphocytes. These observations suggest that Sn-positive microglia/macrophages contribute to neurodegeneration in Ppt1-/- and Cln3-/- mice by stimulating inflammation and CD8+CD122- T-lymphocytes and by inhibition of regulatory CD8+CD122+ T-cells. These studies provide insights into CLN pathogenesis and may guide in designing immuno-regulatory treatment strategies.

O - 16

IDENTIFICATION OF COMPOUNDS THAT IMPROVE AUTOPHAGIC VESICLE TURN-OVER IN JUVENILE NCL BY HIGH CONTENT SCREENING

PETCHERSKI A.[1,2], CHANDRACHUD U.[2], ZHAO W. N.[2], HAGGARTY S. J.[2], RUONALA M. O.[1†], COTMAN S. L.[2†]

[1]NeuroToponomics Group, Center for Membrane Proteomics, Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany; [2]Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA USA; [†]These authors contributed equally to this study

Drug discovery for hereditary lysosomal storage disorders has been slow to emerge due to the financial risk taken by the pharmaceutical industry in developing treatments for a relatively small patient population. However, academic researchers are increasingly conducting high-throughput screening (HTS) in order to accelerate both novel target identification and drug discovery. Here, we report a new HTS encompassing ~ 2000 compounds with known bioactivity, most of which are FDA-approved drugs, in the knock-in cell culture model of Juvenile Neuronal Ceroid-Lipofuscinosis (CbCln3Δex7/8, JNCL, CLN3). Based on the established defect in autophagy in the knock-in mouse model and the corresponding cerebellar granule neuron precursor cells, we further optimized a recently established automated microscopy-based, high content screening assay probing for clearance of GFP-LC3 positive autophagic vesicles. From the screen, 42 distinct compounds were found to reduce the GFP-LC3 load by at least 50%. Compounds with known human safety profiles potentially appropriate for chronic treatment were further analyzed for concentration and incubation-time dependency effects on GFP-LC3 clearance and cell viability. Subsequently, we focused on three leading compounds that primarily affect functionality of ion channels and receptors. These compounds partially improved autophagic flux, cellular viability, and corrected lysosomal storage. These studies underline the potential of this cell model as a platform for drug screening.

O - 17

DEFINING OF THE NATURE AND PROGRESSION OF SPINAL CORD AND BRAINSTEM PATHOLOGY IN PPT1-/- MICE

NELVAGAL H. R.[1], DMYTRUS J., SRI S.[1], DEARBORN J.[2], SANDS M. S.[2], COOPER J. D.[1]

[1]The Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK; [2]Department of Medicine, Washington University Medical School, St. Louis, USA

The characterization of Ppt1 null mutant mice (Ppt1-/-) has provided a wealth of data about the relationship between glial activation and neuron loss within the forebrain. Although forebrain directed AAV-mediated gene therapy significantly prolongs life span, these mice nevertheless still die prematurely. These data prompted us to investigate whether other brain regions that were not therapeutically targeted may also contain significant neuropathology. We focused our analysis upon the brainstem and spinal cord, which have not been characterized in any detail in any form of NCL. Our findings reveal that there is indeed an extensive and progressive neurodegenerative process that occurs in the brain stem and spinal cord, which is evidenced by profound glial activation and neuron loss. This appears to affect both motor and sensory pathways, and displays a rostro-caudal gradient. These neuropathological changes worsen with time, but are untouched by therapeutic approaches that target the forebrain. These data not only define the extent and nature of brain stem and spinal cord pathology, but also reveal these brain regions as important targets for future therapeutic approaches.

O - 18

A COUPLED GENE/ER THERAPY EFFICIENTLY CORRECTS THE BRAIN PATHOLOGY IN MPS-IIIA MICE

SORRENTINO N. C.[1], D'ORSI L.[1], DE LEONIBUS E.[1], BALLABIO A.[1,2], FRALDI A.[1]

[1]Telethon Institute of Genetics and Medicine, Via P. Castellino 111, 80131 Naples, Italy; [2]Department of Pediatrics, Federico II University, Via S. Pansini 5, 80131 Naples, Italy

Mucopolysaccharidoses (MPS) type IIIA is a lysosomal storage disorder (LSD) caused by mutations in the sulfamidase gene. The brain represents the principal target of pathological lesions in this disorder but no effective therapies exist for the treatment of brain pathology. We generated an engineered sulfamidase capable to be highly secreted and to cross the blood brain barrier (BBB). The engineered sulfamidase contains the low-density lipoprotein receptor (LDLR)-binding domain of apoliproprotein B (ApoB-BD), which confers to the modified enzyme the capability to cross the BBB by LDLR-mediated transcytosis. The chimeric sulfamidase also contains an alternative signal peptide (sp) belonging to the iduronate sulfatase (IDS), a highly secreted protein, which allows the modified enzyme to be efficiently secreted from the liver.AAV serotype 8 vectors were used as vehicle for the systemic delivery and liver targeting of the chimeric sulfamidase (hIDSsp-SGSHflag-ApoB-BD). We demonstrated that a single intravenous injection of these vectors in adult MPS-IIIA mice converted the liver into a factory organ for the sustained release of the modified sulfamidase in the blood stream of injected animals. We detected a significant increase in the sulfamidase activity into the brain of injected MPS-IIIA mice, thus indicating that modified sulfamidase was able to efficiently cross the BBB. Moreover, an overall amelioration of brain pathology (including lysosomal storage, inflammation and autophagic stress) together with an improvement of behavioural abnormalities was observed in treated animals. These data provide a proof of principle that modifying sulfamidase with domains that increase secretion efficiency and allow BBB transcytosis has promising therapeutic potential for the design of a low-invasive strategy to treat the brain pathology in MPS-IIIA.

In conclusion, our strategy will open new perspectives for clinical gene and enzyme replacement therapeutic protocols for MPS-IIIA as well as for other neurodegenerative LSDs caused by hydrolase defects.

O - 19

NPC DISEASE: UNEXPECTED LINKS TO RARE AND COMMON DISEASES

PLATT F.[1]

[1]Dept. Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK

Niemann-Pick disease type C (NPC) is a complex lysosomal lipid storage disorder that occurs at an estimated frequency of 1:120,000 live births. Unusually, it can be caused by defects in two independent genes (NPC1 and NPC2) and the proteins they encode are believed to function in a common cellular pathway or share a common molecular target. The biochemical and cellular features of NPC disease include the accumulation of several classes of lipids (cholesterol, sphingomyelin, spingosine and glycosphingolipids) and a block in late endosome-lysosome fusion that is the result of a unique acidic store calcium defect[1]. The details of the functions of the NPC cellular pathway remain to be fully elucidated in healthy cells. An open question is whether the NPC cellular pathway is involved in other human diseases and whether therapies developed for treating NPC patients may

[1]Lloyd-Evans, et al. Nature Medicine 2008; 14: 1247-55.

have utility in apparently unrelated diseases. In this presentation I will review current knowledge of links between NPC and other diseases and discuss the scientific and therapeutic implications of these unanticipated findings.

O - 20

FACTS AND FALLACIES ABOUT STORAGE, NEUROINFLAMMATION AND PATHOGENESIS IN THE NCLS

PALMER D. N.[1]

[1]Faculty of Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand

Searching for "the answer" has dominated NCL congresses since the 1970s, when peroxidase deficiencies were proposed. These aligned with descriptions of lipidoses and the polymerised peroxidised complexes postulated to be the core of ceroid and lipofuscin, so called lipopigments similar to the characteristic NCL storage bodies. However the supporting enzymology was appalling. Careful analytical studies showed no evidence of lipid peroxidation and the storage bodies to be mostly protein, dominantly subunit c of mitochondrial ATP synthase in most forms. No analytical evidence for a peroxidative origin of lipofuscin has emerged either, but dogmas often trump reason and we are constantly bedevilled by consequent misunderstandings and errors. Discovering "the gene" was the next quest. Discoveries of several genes linked to different forms were huge advances, but none revealed the biochemical linkages apparent in many storage diseases. Cell biology studies have indicated a bewildering array of defects around intracellular trafficking and processing, and of gene regulation interactions. These need annotation and decisions on which model systems are relevant to what. Animal studies indicate a primary role for neuroinflammation in pathogenesis, which also has an important physiological component, evidenced by the specificity of neurodegeneration to particular areas of the brain. Finding "the answer" clearly requires co-ordinated evaluations of findings from different approaches and models.

O - 21

PROTEOMIC ANALYSIS OF THE PALMITOYL PROTEIN THIOESTERASE 1 INTERACTOME IN SHSY5Y HUMAN NEUROBLASTOMA CELLS

SCIFO E.[1,2*], SZWAJDA A.[3], SOLIYMANI R.[1], DĘBSKI J.[4], UUSI-RAUVA K.[5,6], DADLEZ M.[4], GINGRAS A. C.[7],TYYNELÄ J.[1],JALANKO A.[6,8], BAUMANN M. H.[1], LALOWSKI M.[1,5*]

[1]Meilahti Clinical Proteomics Core Facility, Institute of Biomedicine / Biochemistry and Developmental Biology; [2]Doctoral Program Brain & Mind; [3]Institute for Molecular Medicine (FIMM), University of Helsinki, Helsinki, Finland; [4]Mass Spectrometry Laboratory, Department of Biophysics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [5]Folkhälsan Institute of Genetics, Helsinki, Finland; [6]National Institute for Health and Welfare Helsinki, Finland; [7]Centre for Systems Biology, Samuel Lunenfeld Research Institute at Mount Sinai Hospital, Toronto, and Department of Molecular Genetics, University of Toronto, Ontario, Canada; [8]Public Health Genomics Unit, Department of Chronic Disease Prevention

Neuronal ceroidlipofuscinoses (NCL) are a group of inherited progressive childhood disorders with a variable age of onset. They are characterized by early accumulation of autofluorescent storage material in lysosomes of neurons or other cells and degeneration of cortical neurons. Mutations in the CLN1 gene that encodes Palmitoyl protein thioesterase 1 (PPT1) or CLN1, cause Infantile NCL (INCL, MIM#256730). PPT1 removes long fatty acid chains such as palmitate from modified cysteine residues of proteins. In this study, we utilised single step affinity purification coupled to mass spectrometry (AP-MS) to unravel the in vivo substrates of human PPT1 in the brain. Protein complexes were isolated from PPT1 expressing human SH-SY5Y-PPT1-CTAP-Puro stable cells, subjected to filter assisted sample preparation (FASP) and analysed on both SynaptG2-S (Waters) and Q Exactive™ Hybrid Quadrupole-Orbitrap (Thermo Scientific) mass spectrometers. Following label free quantitation of the MS data by TransOmics and SAINT, respectively, 14 and 16 PPT1 interacting proteins (IP) were identified from the MS runs on two different instruments. Identified PPT1 IP included neurodegenerative disease causative proteins, as well as pyruvate dehydrogenase and mitochondrial ATP synthase complexes. In subsequent experiments, a subset of the PPT1 IP will be validated in co-immunoprecipitation and immunofluorescence co-localization assays. Our proteomic analysis confirms previously suggested roles of PPT1 in axon guidance and lipid metabolism, yet implicates the enzyme in putative new roles, including: involvement in neuronal migration and dopamine receptor mediated signalling pathway.

O - 22

LYSOSOMAL MEMBRANE PERMEABILITY AND EVIDENCE FOR LYSOPHAGY FAILURE IN THE CNS OF NCL DISEASE MODELS

MICSENYI M. C., WALKLEY S. U.[1]

[1]Albert Einstein College of Medicine, Bronx, New York, USA

Lysosomal membrane permeability (LMP) is a pathophysiological event that initiates cell death through release of lysosomal cathepsins in the cell cytosol. Recent studies have reported that LMP typically induces autophagy – a mechanism termed lysophagy. Lysophagy selectively targets damaged lysosomes for engulfment by nascent autophagosomes, in an effort to redeliver this material back to intact lysosomes. We recently reported that LMP occurs in neurons of the late-infantile NCL mouse model (Cln2-/-). Notably, we found that LMP stimulates formation of intraneuronal protein aggregates in brain regions susceptible to neurodegeneration. Our studies identified that LMP induces a novel response by autophagy adapter proteins including p62, to sequester released lysosomal storage material as cytosolic aggregates. Importantly, this response occurs in the absence of further induction of autophagy to potentially clear these aggregates. Given our findings, we predicted that LMP is a common feature of the NCLs. In the CNS of the juvenile NCL mouse model (Cln3Δex7/8) we identified that p62 decorates the periphery of storage lysosomes, strongly suggesting a response to LMP. Again, we observed no changes in autophagy induction to potentially clear these aggregates. These findings suggest LMP is a novel NCL disease mechanism, and that failure of lysophagy contributes to disease pathogenesis.

O - 23

PROGRESS IN THE MOLECULAR DISSECTION OF NEUROINFLAMMATION IN OVINE BATTEN DISEASE

CHEN J. Z., BARRY L. A., MITCHELL N. L., PALMER D. N.[1]

[1]Lincoln University, Christchurch, New Zealand

The regionally specific neuroinflammation preceding neurodegeneration in CLN6 ovine Batten disease was not inhibited by chronic minocycline treatment, indicating a need to understand the neuroinflammatory cascade in Batten disease to identify druggable targets. Neuroinflammatory modulators (TNF-α, TGF-β, IL-1β, IL-10, NF-κB, MnSOD, iNOS, JAK2, SOCS3, TrkB and BDNF) were investigated by qPCR on different brain regions after 2, 6, 9, 18 and 24 months of disease development. MnSOD and iNOS expression was also studied by immunohistochemistry on perfusion-fixed brain sections. Different inflammatory mediators were expressed differently in affected animals. Both pro- (TNF-α and IL-1β)and anti- inflammatory cytokines (TGF-β and IL-10) were up-regulated between 4-7 fold and suppression of cytokine signalling mediated by SOCS3 was activated dramatically, 5 -74 fold, at the initiation of neurodegeneration at 4-6 months of age, prior to clinical disease and cortical atrophy. NF-κB and JAK2 activation followed whereas the oxidative responsive genes MnSOD and iNOS were not activated. TrkB expression increased at advanced disease while BDNF expression remained unchanged. The results show an uncontrolled neuroinflammatory pathway mediated by irregular cytokine signalling and the futility of therapies targeting oxidative stress. A similar study of CLN5 ovine Batten disease underway will indicate the generality of these findings.

O - 24

LKE IMPROVES DISEASE OUTCOMES IN THE CLN6NCLF MOUSE MODEL OF VLINCL

MAGEE H., LAUFMANN R.,NELSON T., BAACK S.,KOH S. Y., O'TOOLE R., HENSLEY K., WEIMER J.[1]

[1]Sanford Research, Sioux Falls, SD; University of South Dakota, Vermillion, SD, USA

The variant late infantile (vLINCL) form of NCL results from mutations in the Cln6 genes, and is associated with visual, cognitive and motor decline, seizures, and premature death. Pathologically, this disease is characterized by neuronal loss, glial activation, and retinal degeneration. CLN6 is a transmembrane ER protein that binds collapsin response mediator protein 2 (CRMP2), which regulates axonal growth, guidance and trafficking in neurons. CLN6 and CRMP2 also partner with kinesin light chain 4 (KLC4) to form the CCK complex which we hypothesize to be involved in selective trafficking of ER vesicles. To identify potential therapeutic compounds for NCLs, we have focused on the CLN6/CRMP2/KLC4 complex and identified compounds that specifically interact with CRMP2, based on the prediction that stabilizing CRMP2 associated complexes that are disrupted in NCLs will improve disease outcomes. Lanthionine ketiminine ethyl ester (LKE) is a cell permeable, neuroprotective brain metabolite that targets CRMP2 to stabilize its protein interactions. LKE was screened in Cln6nclf mice, and resulted in improved survival, motor and cognitive function, and visual acuity, and attenuated glial activation. Ongoing studies including screening LKE in the Cln3-/- mouse model of JNCL to determine if LKE has therapeutic effects in other NCL models.

O - 25

A DOMINANT-NEGATIVE DISEASE MECHANISM LINKS HUMAN CSPA MUTATIONS TO ABERRANT LYSOSOMAL PALMITOYLATION IN ADULT NEURONAL CEROID LIPOFUSCINOSIS

HENDERSON M. X.[1,2,3], WIRAK G.[1,2], ZHANG Y. Q.[1,2], GINSBURG S. D.[4], DOLZHANSKAYA N.[5], STAROPOLI J. F.[5,6,7], NIJSSEN P. C. G.[8], ROTH A. F.[9], DAVIS N. G.[9], DAWSON G.[10], VELINOV M.[11,12], CHANDRA S. S.[1,2,3,7]

[1]Program in Cellular Neuroscience, Neurodegeneration and Repair Yale University, New Haven, CT; [2]Department of Neurology Yale University, New Haven, CT; [3]Interdepartmental Neuroscience Program, Yale University, New Haven, CT; [4]Nathan Kline Institute, Orangeburg, NY; [5]Center for Human Genetics Research, Department of Neurology, Massachusetts General Hospital, Boston, MA; [6]Department of Pathology, Massachusetts General Hospital, Boston, MA; [7]Department of Molecular Cell and Developmental Biology, Yale University, New Haven, CT, USA; [8]Department of Neurology, St. Elisabeth Hospital, 5022, Tilburg, Netherlands; [9]Department of Pharmacology, Wayne State University, Detroit, MI 48201; [10]Department of Pediatrics, University of Chicago, IL; [11]Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY; [12]Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA

Dominant mutations in DNAJC5 encoding cysteine string protein α (CSPα) cause adult neuronal ceroid lipofuscinosis (ANCL; CLN4B). To elucidate why mutations in CSPα, a synaptic vesicle protein, result in a lysosomal disease, we examined patient brain tissue. Our investigation revealed that CSPα levels are decreased, while the INCL protein palmitoyl-protein thioesterase (PPT1; CLN1) was specifically and substantially increased. Intriguingly, PPT1 depalmitoylation enzyme activity did not increase proportional to protein levels, leading to overall diminished specific activity of PPT1 in these brains. As CSPα is a heavily palmitoylated protein, we tested if CSPα is a protein substrate for PPT1. Our results showed that CSPα:PPT1 indeed have a substrate:enzyme relationship, and depalmityoylation controls CSPα protein levels. Next, we purified total palmitoylated proteins from control and patient brains and compared the 'palmitomes' by unbiased, quantitative proteomics. We discovered that the majority of the palmitoylated protein changes in ANCL are lysosomal enzymes. Our findings establish for the first time a functional link between infantile and adult forms of NCL and also give us insights into the nature of lysosomal dysfunction in ANCL.

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WHAT IS NEURONAL CEROID-LIPOFUSCINOSIS (NCL)?

GOEBEL H. H.[1]

[1]Depts. of NeuropathologyJohannes Gutenberg, University Mainz and Charite, Berlin, Germany

In view of the recent numerical surge in genetically different forms of NCL (CLN1-CLN14) and the expectation of further forms, a discussion of nosological criteria which constitute NCL appears appropriate. Hence, apart from morphological features, i.e. loss of cortical neurons and widely spread intra- and extracerebral accumulation of lipopigments with certain ultrastructural patterns, clinicians will outline the spectra of childhood and adult NCL, including possible variants. Moreover, as there exists a multitude of spontaneous animal models of NCL, their inclusion in this discussion may be helpful.

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CHILDHOOD NEURONAL CEROID LIPOFUSCINOSIS

SIMONATI A.[1], SANTORELLI F. M.[2]

[1]University of Verona Medical School, Department of Neurological Sciences and Movement-Neurology (Child Neurology) and Neuropathology, Verona, Italy; [2]IRCCS Fondazione Stella Maris-Molecular Medicine, Pisa, Italy

Neuronal Ceroidlipofuscinoses (NCL) are the most common group of neurodegenerative disorders associated with lysosomal storage. Thirteen disease-related genes have been identified. Most NCL start in childhood. Major clinical symptoms are seizures, amaurosis, motor function impairment, cognitive decline and behavioural problems; the length of survival is related to the specific NCL type. Phenotypic variability can be observed within each form. Three NCL (CLN1, CLN10, CLN14), start within the second year, show a rapid clinical progression (the full clinical picture being evident within one-two years after onset), and an early fatal outcome. Most NCL are clinically evident in preschool ages. CLN2 shows relative homogeneous phenotype and features of disease progression; three forms (CLN6, CLN7, CLN8) may have similar onset and early phases of disease, whereas the features of progression and ages at outcome are variable. Notably CLN6 may also start in adolescence or young adulthood. In CLN5 early cognitive regression and delayed seizure onset are common. Early behavioural disturbances and progressive amaurosis are the hallmark of CLN3, whose onset occurs at school age and progression lasts into adulthood. Increased knowledge of the different phenotypes of childhood NCL is helpful to improve the quality of care necessary to cope with these untreatable diseases.

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THE CLINICAL SPECTRUM OF ADULT NEURONAL CEROID LIPOFUSCINOSIS DISORDERS (ANCL) KUFS DISEASE

SIMS K. B.[1].

[1]Director, NCL Disorders Clinic, Massachusetts General Hospital. Professor of Neurology, Harvard Medical School, USA

The adult-onset neuronal ceroid lipofuscinosis disorders (ANCL), often referred to as Kufs disase, are a group of both autosomal recessive (AR) and autosomal dominant (AD) neurodegenerative genetic diseases characterized by a mix of seizures, movement abnormalities and dementia. Unlike the other NCL disorders, vision is almost always spared. Although clinically characterized and recognized in familial cases for many years, only recently have etiologic genes been identified for those that predominantly have later-onset including: CLN4 [DNAJC5; AR, Kufs, Parry type]; CLN11 [GRN]; CLN12 [ATP13A2]; CLN13 [CTSF] and CLN14 [KCTD7]. These genes have been associated with clinical non-NCL disorders and their identification in associated with NCL pathology and clinical phenotype raises interesting and important questions about the overlapping cellular pathobiology. Other of the NCL genes, recognized predominantly in onset at much younger ages, have also been recognized to in some cases appear in late-juvenile or adult years. These include most prominently CLN6 and less frequently CLN1, CLN5, CLN3. Despite extensive work

by many researchers, including the international Kufs Consortium, some families/probands with Kuf-like clinical features and/or pathologic findings, remain undiagnosed and are the focus of continuing study. Phenotypic expression of these disorders will be discussed.

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WHAT ARE NCLS IN ANIMAL MODELS?
PALMER D. N.[1].
[1]Lincoln University, New Zealand and Batten Animal Research Network (BARN), New Zeland

Naturally occurring forms of the neuronal ceroid lipofuscinoses (NCLs, Batten disease) have been diagnosed in a number of domestic production and companion animals. Studies of various forms in dogs and later sheep have been unusually informative, often leading dissections of the disease process ahead of understanding of the human conditions. Being able to take deliberately bred animals before end-stage disease has allowed an understanding of the development of pathology not possible in humans. Thorough veterinary pathology investigations led to a set of criteria for disease to be regarded as NCLs. These include inherited disease, developing blindness with retinal atrophy, neurodegeneration and brain atrophy, characteristic neurological behaviours and the accumulation of fluorescent ceroid or lipofuscin-like storage bodies in brain but also in many other tissues throughout the animals. Veterinarians often regard the diseases as generalised rather than neuronal as a consequence. Biochemical investigations showed the storage bodies to be composed of subunit c of ATP synthase in most cases. Causative mutations were found in genes homologous to those causing human disease, and vice versa. Many invaluable mouse homologs have been created or discovered over the last two decades. Unfortunately some lack sufficient pathological detail to ensure that they are really NCLs.

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MEMBRANE PERTURBATION AND SMALL MOLECULE DRUG INTERVENTION FOR JNCL
DAVIDSON B. L.[1,2], SCHULTZ M.S.[1], TECEDOR L.[1], STEIN C.[1]
[1]Department of Internal Medicine, University of Iowa, Iowa City, IA; [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA

The juvenile form of Batten disease (JNCL) results from recessive inheritance of mutations in CLN3. CLN3 is a multi-pass transmembrane protein localized to various intracellular compartments. While its molecular function remains obscure, combined findings suggest a primary role for CLN3 in membrane traffic; cells from JNCL patients or mouse models are defective in endocytosis, post-Golgi traffic, autophagosome maturation, and cell migration pathways. As multiple phenotypes involve actin-driven steps we wondered whether Cdc42, a small GTPase that regulates actin polymerization, was affected by CLN3 loss. We discovered that the active (GTP-loaded) form of Cdc42 is elevated in endothelial cells from CLN3 deficient mouse brain. Consistent with Cdc42 misregulation, we found increased filopodia, defective fluid phase endocytosis, and impaired cell migration in CLN3-null endothelial cells. We investigated players proximal to Cdc42 activation; ARF1 is known to recruit ARHGAP21 to the plasma membrane, while ARHGAP21 is a GTPase-activating protein that directly facilitates Cdc42 cycling. Interestingly CLN3 null cells showed a decline in GTP-loaded ARF1 and a dramatic reduction in plasma membrane-localized ARHGAP21. We speculate that CLN3 has a central effect on membrane microdomains, which indirectly impacts the ARF1-Cdc42 signaling cascade. The ARF1-Cdc42 pathway presents a potential target for developing JNCL therapeutics, as does modulation of membrane microdomain properties.

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NEGATIVELY CHARGED QUANTUM DOTS DELIVER TRIPEPTIDYLPEPTIDASE-1 TO NEURONS: IN A MODEL OF CLN2 BATTEN DISEASE
WALTERS R.[1], KRAIG R. P.[1,2], MEDINTZ I.[3], DELEHANTY J. B.[3], DAWSON P. E.[4], DAWSON G.[1,5]
[1]Committee on Neurobiology, University of Chicago, Chicago, IL 60637, USA; [2]Department of Neurology, University of Chicago, Chicago, IL 60637, USA; [3]Center for Bio/Molecular Science and Engineering, Code 6900, U.S. Naval Research Laboratory, Washington, DC 20375, USA; [4]Scripps Research Institute, La Jolla, CA 92037, USA; [5]Departments of Pediatrics, Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA

Storage diseases such as Battens disease involve the central nervous system and enzyme replacement therapy involves crossing of the blood brain barrier. To overcome this we have designed 6nm semi-conductor nanoparticles ("quantum dots" or "QDs") solubilized by a dihydrolipoic acid-based chemical coat allowing Zn to bind His6-tagged cell-penetrating peptides and a His6, FLAG-tagged lysosomal hydrolase. (tripeptidylpeptidase-1) (TPP1). The extracellular and intracellular fate of QDs can be followed because of their robust 625nm fluorescence, their resistance to photobleaching, and their universal distribution throughout the brain when injected into the ventricle. We initially demonstrated the efficacy of the system by attaching both the peptide and green fluorescent protein (GFP) to the QD and showed that uptake into neurons in hippocampal slices only occurred if GFP was linked to the QD. By altering the charge of the QD coating through selective replacement of neutral hydroxyl groups with negatively charged carboxyls to generate novel zwitterionic-like particles or negatively charged particles, we significantly increased the neuronal uptake and trafficking of these dots with little to no uptake by glia. By degrading the extracellular matrix with Chondroitinase ABC (an enzyme which digests negatively charged chondroitin sulfate, a major component of the glial extracellular matrix) we could show increased trafficking anduptake of QDs into oligodendrocytes as well as neurons but had a lesser effect on astrocytes or microglia. Recombinant human TPP1 containing His and FLAG tags was prepared by overexpression in HEK-293 cells and retained activity when coupled to QDs. Initial studies on hippocampal slices suggest that it has excellent distribution and stability and is ready for testing on animal models of CLN2.

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GENE THERAPY FOR LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS IN LARGE ANIMAL MODELS
TECEDORL.[1], KATZ M. L.[2], CHENY. H.[1], YOUNGW.[2], COATESJ. R.[2], MCBRIDE J. L.[3], DAVIDSON B. L.[1,4]
[1]Center for Cell and Molecular Therapy, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Ophtalmology, Patholbiology, Neurosciences Mason Eye Institute, University of Missouri School of Medicine; [3]Oregon Health and Science University; [4]The Children's Hospital of Philadelphia, USA

Late infantile Neuronal Ceroid Lipofuscinosis (LINCL) is a childhood neurodegenerative disease that appears between 2-4 years old and progresses with visual, motor, and mental decline, with a life expectancy less than two decades. LINCL is caused by deficiency in the soluble lysosomal enzyme TPP1 as a result of mutations in the CLN2 gene. TPP1 is a mannose-6-phophate decorated enzyme which makes it amenable to a cross-correction approach by gene therapy. In this study, we investigated the therapeutic effects of TPP1 gene transfer directed to the ependymal cells lining the brain ventricles of a TPP1 deficient dog model. Adeno-associated virus (AAV) was used as vector for introduction of the canine CLN2 gene. High levels of recombinant TPP1 were detected in cerebrospinal fluid (CSF) as early as 5 days post-injection, and reached up to 1000-fold normal in the first 10 days. Although CSF TPP1 declined thereafter, supra-normal levels were maintained for many months. Clinical signs measured over more than one year showed remarkable therapeutic effect. Symptomatological progression was significantly delayed as determined by longitudinal scores on several motor and learning tasks, and lifespan was increased in treated compared to untreated LINCL dogs. At sacrifice, parenchymal levels of TPP1 were assayed in multiple regions across the brain and were found to be elevated. We additionally examined AAV-mediated gene transfer of human TPP1 to ependymal of non-human primate brain. TPP1 levels significantly above normal were detected in the in CSF and brainstem, tested

at 30 days after gene transfer, and no adverse reactions were detected. Importantly, the ependymal remained intact in treated dogs and nonhuman primates, indicating the safety and tolerability of the approach. Our findings are encouraging and suggest that this strategy holds promise for translation to LINCL patients.

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ANTISENSE OLIGONUCLEOTIDES FOR THE TREATMENT OF JUVENILE NEURONAL CEROID LIPOFUSCINOSIS

JODELKA F. M.[1], HINRICH A. J.[1], RUIZ M.[2], HAVENS M. A.[1], WEIMER J. M.[3],MAGEE H.[3], RIGO F.[4], DUELLI D. M.[1], HASTINGS M. L.[1]

[1]Department of Cell Biology and Anatomy, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL USA; [2]Department of Biology, Lake Forest College, Lake Forest, IL USA; [3]Sanford Research Center, Sioux Falls, SD USA; [4]Isis Pharmaceuticals, Carlsbad, CA USA

Juvenile neuronal ceroid lipofuscinosis (JNCL), or Batten disease, is an autosomal recessive neurodegenerative disease caused by mutations in the CLN3 gene. This fatal disease has an onset at five-eight years of age with symptoms including vision loss, progressive motor function decline, seizures, and loss of cognitive function. Currently, there are no treatments for the disease. The function of the CLN3 protein is not well understood, but it is implicated in membrane trafficking, phospholipid distribution, and response to oxidative stress. Most cases of Batten disease are caused by a deletion of exons 7 and 8 (CLN3Δ78), which causes a frameshift and a premature stop codon in exon 9. We have developed an antisense oligonucleotide that targets CLN3 splicing to restore the reading frame of the CLN3Δ78 mRNA. This ASO efficiently restores CLN3 reading frame in patient cells in culture. Mice with the CLN3Δ78 mutation have motor deficits by two months of age. Treatment of CLN3Δ78 mice with a single neonatal ICV injection of the ASO restores the CLN3Δ78 reading frame and improves motor coordination in the mice. Our results suggest that ASO-mediated reading-frame correction may be a promising therapeutic approach for Batten disease.

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PRECLINICAL INTRATHECAL ENZYME REPLACEMENT THERAPY FOR PPT1-DEFICIENT NCL

LU J. Y.[1], WANG L.[1], COOPER J.[2], HOFMANN S. L.[1]

[1]University of Texas Southwestern Medical Center, Dallas, Texas; [2]King's College, London, England

The infantile form of NCL and most late onset cases with granular osmiophilic deposits (GROD) are caused by deficiency in a soluble lysosomal enzyme, palmitoyl-protein thioesterase-1 (PPT1), which functions to hydrolyze fatty acids from cysteine residues of S-fatty acylated proteins. A PPT1 deficient mouse model recapitulates the major features of the disorder, showing neurological signs by 5 months of age and death at about 7 months of age. Cohorts of 15-20 mice were injected at 6 weeks of age with 80 microliters of human recombinant PPT1 administered daily for three days via lumbar puncture in the L5-L6 lumbar space using a 30g needle and infused at a rate of 10 microliters per min over 8 min using a Harvard pump. Three dose levels (2.6, 5.3 and 10.6 mg/ml) and vehicle were tested, and concurrent uninjected PPT1 knockout and control mice were also enrolled. Rotorod performance and survival are the primary endpoints. The treatment was generally well tolerated. The decline in motor performance was delayed at least one month in all treatment groups, and a dose response was observed. A subset of mice will be sacrificed at seven months for pathological examination. Preliminary findings suggest that enzyme replacement therapy delivered to the cerebrospinal fluid may be useful in the treatment of PPT1 deficient NCL.

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NCL RESOURCE – A GATEWAY FOR BATTEN DISEASE

MOLE S. E.[1,2,3].

[1]MRC Laboratory for Molecular Cell Biology;[2]Institute of Child Health;[3]Department of Genetics, Evolution and Environment, University College of London, London WC1E 6BT, UK

The NCL Resource web site (home page http://www.ucl.ac.uk/ncl) serves as a global gateway for clinicians, families, researchers and those offering professional support, who have an interest in or are affected by Batten disease or who wish to find out more. Information can be accessed via four main routes - Clinicians, Families, Researchers, Professional Support. The Clinical route describes Batten disease and includes details on diagnosis and diagnostic services. The Family route also describes Batten disease and lists support groups. The Research route includes the international NCL Mutation Database, established in 1998, and other useful information. The mutation database has been expanded to list gene data by case as well as by genetic variation. The Professional Support route includes details of coordinated initiatives to support those affected by Batten disease. Much of the content relies on active clinicians, researchers and diagnostic labs from around the world, and is accessed from all over the world., with half the visits from the UK or USA.

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NCL REGISTRY AND DATABASE

PEARCE D. A.[1]

[1]Sanford Children's Health Research Center, Sanford Research 2301 E. 60th St. North, Sioux Falls SD, 57104-0589, USA

Background: At the Children's Health Research Center at Sanford Research an international rare disease patient registry named Coordination for Rare Diseases at Sanford (CoRDS) has been established to advance rare disease research. CoRDS houses data on individuals with a rare disease diagnosis and those awaiting diagnosis. The mission is to accelerate research by offering researchers a resource of de-identified data and a mechanism by which eligible registry participants may be contacted about research opportunities. Methods: Key components to operating CoRDS include data collection, data management and data dissemination. An advisory board provides oversight and reviews applications. Patient Advocacy Groups (PAGs) supporting rare disease patients partner with CoRDS to utilize the general registry or to customize a registry for their disease of interest. Results: CoRDS currently has 1539 fully enrolled participants representing 271 unique rare diseases. To date, CoRDS has partnered with over 40 PAGs and has created 7 disease-specific registries, including one for Batten disease. Discussion: Collecting and collating rare disease data offers the opportunity to perform a comparative analysis to better understand and treat these diseases. Many treatments are symptomatic, thus treatment strategies for one disease may be beneficial in application to other diseases with similar clinical profiles. Establishing a registry like CoRDS has potential to accelerate the timeline of rare disease research efforts.

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MGH NCL CLINICAL DATABASE AND BIOREPOSITORY

SIMS K. B.[1]

[1]Massachusetts General Hospital, Neurology Department, Center for Human Genetic Research, Harvard Medical School, Boston, MA, USA

The MGH Neurogenetics DNA Diagnostic Lab has done clinical molecular testing for NCL disorders since 1994 starting with CLN3 and added others as they have been identified. Through this clinical service we tested over 3000 cases for diagnosis or carrier analysis and have identified molecular etiology in a total of ~450 probands: CLN1(12%), CLN2(30%),CLN3(41%),CLN5(4%),CLN6(10%); CLN4; CLN7; CLN8; CLN10; CLN11; CLN12; CLN13; CLN14 each <1%. For many cases we also collected cross-sectional clinical information, gathered to facilitate testing triage strategies. To better understand the natural history of the NCL we established a IRB-approved Clinical Database and Biorepository in 1999 [upgraded to relational database, 2007]. We have compiled ~2927 patients/family members in this resource (clinical data ~20% cases) which is linked to both IRB-Biorepository and historical DNA lab sample collection. Patient materials, including DNA, plasma, lymphoblast cell lines, fibroblasts, and in some cases autopsy materials, are an invaluable resource for iPS cell generation and other

biologic research. Beyond the goal of offering clinical testing and service to professionals and families for all known NCL disorders, we work with both MGH-CHGR and international researchers to facilitate transfer of patient materials for research study including gene discovery. We would like to extend the longitudinal clinical data collection and expand to include formal assessment scales. We are committed to making this resource, both clinical data and patient materials, available to the entire NCL research community.

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EPILEPSY IN NCL, THOUGHTS ON PATHOPHYSIOLOGY

GLYKYS J.[1]

[1]Massachusetts General Hospital, Boston, USA

The neuronal ceroid lipofuscinosis (NCL) are the most common neurodegenerative disorders of childhood. These inherited disorders generally manifest with different temporal combination of blindness, seizures, progressive dementia and motor failure. Epilepsy in NCL is treated with different combinations of anticonvulsive drugs (ACD). There are multiple ACD in the market and some of the newer ones have novel mechanisms of action. Yet, there is no rationale for choosing any specific ACD in NCL. The pathophysiology of epileptogenesis is poorly understood in NCL and underscores the urgent need for a better rational in choosing an anticonvulsive medication to manage seizures in this condition. Clinical trials comparing several ACD in NCL populations are not feasible at this time. Research in NCL animal models expressing hyperexcitability or epileptiform activity will allow a better understanding of the mechanism(s) of hyperexcitability/epileptogenesis and most importantly, will provide the basis for a rational selection of ACD for NCL patients.

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THE NATURAL HISTORY OF LATE INFANTILE CLN2 DISEASE: STRIKING HOMOGENEITY OF CLINICAL PROGRESSION IN TWO INDEPENDENTLY OBTAINED LARGE CLINICAL COHORTS

SCHULZ A.[1], NICKEL M.[1], DOWNS M.[2], MEZEY J.[3], LANDY H.[4], SONDHI D.[3], JACOBY D.[4], WITTES J.[2], CRYSTAL R.[3], KOHLS-CHÜTTER A.[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, DE; [2]StatisticsCollaborative Inc., Washington DC, USA; [3]Weill Cornell Medical College, New York, NY, USA; [4]BioMarin Pharmaceutical Inc., Novato, CA, USA

Late infantile CLN2 disease (CLN2) is a characterized by progressive psychomotor and language decline. A disease-specific rating scale was developed 10 years ago and now used in an expanded cohort of 29 genetically confirmed patients. Data obtained in this study are used as natural history control data in a phase 1 study for intraventricular enzyme replacement therapy in CLN2 disease. Objectives: This new study focused on (i) first symptoms to support early diagnosis, (ii) prospective longitudinal data acquisition covering a period of 26 years, and (iii) quantification of rate of decline as a means to measure disease progression. Results: (1) Early symptoms of CLN2 comprise delayed language acquisition and seizures (73% of patients; median age of onset 37 months). (2) Disease progression was measured longitudinally by the sums of 3-point motor and language subscales of the Hamburg-LINCL score. Onset of neurological decline occurred at a median of 39 months of age. Onset of symptoms leads to a rapid, progressive clinical decline with a linearized mean rate of decline of 2.2 units/year (SD±1.1). Slowly progressing patients were uncommon and mostly related to unusual genotypes. The age-specific level of functioning was similar in an independent dataset of 62 observations in 43 patients from the Weill Cornell CLN2 cohort. Conclusion: This analysis of CLN2 natural history shows high homogeneity in the population across time and geography. The data underscore the rapid decline in this disease, and therefore the importance of early diagnosis for potential therapies.

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MOVEMENT DISORDERS IN CHILDHOOD NCL

NARDOCCI N.[1]

[1]Pediatric Neuroscience Department, Fondazione IRCCS Istituto Neurologico "C Besta" Milano, Italy

The term Movement Disorders (MDs) includes a series of different abnormal movements characterized by excess (dystonia, chorea, myoclonus, tremor,tic) or reduction of movement (hypo/akinesia). MDs are frequently described among the clinical features of the different forms of childhood and adult onset Neuronal Ceroidolipofuscinoses (NCL) and may represent the most severe and disabling motor symptoms. However little is known regarding the types and the prevalence of the different MDs, the results of the symptomatic treatment and the biological basis of the motor disorder. The analysis of the literature and the result of a retrospective study on a series of Italian patients suggest that cortical and subcortical myoclonus, dystonia and hypo-akinetic syndrome are the most frequent MD observed in childhood-onset NCL. Myoclonus may be rarely the symptom-onset but the MDs usually appear during the course of the disease. Generalized and segmentary myoclonus and generalized dystonia with rapid development of fixed postures appear to be most frequent in Late-infantile onset; hypo-akinetic syndrome has been reported in the late infantile and juvenile one; a particular syndrome called status dystonicus may be a severe and life threatening occurrence during the course of the NCLs. No correlation between genetic status and MDs phenomenology has been documented so far. Therapeutical algorithms for the different MDs including status dystonicus will be discussed.

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BEHAVIOURAL AND COGNITIVE ISSUES IN JNCL: WHAT ARE THE SYMPTOMS, HOW DO WE MEASURE THEM, AND HOW DO WE MANAGE THEM?

ADAMS H. R., THE UNIVERSITY OF ROCHESTER BATTEN CENTER[1]

[1]University of Rochester, Rochester New York, USA

Juvenile neuronal ceroid lipofuscinosis (JNCL; Batten Disease) is an inherited, childhood-onset neurodegenerative disease with many challenging and evolving symptoms, including progressive vision loss, motor and cognitive decline, behavioral and psychiatric problems, and seizures. As with all NCLs, standardized and consistent evaluation of neurocognitive/behavioral symptoms is challenging because of the disease progression and sensory and motor impairments. However, these neurocognitive/behavioral symptoms impact daily function and quality of life, and as such, are important to understand and address to reduce disease burden. Finally, evidence-based guidelines for clinical management of these symptoms is lacking. In this talk we will review the challenges of evaluating and managing neurocognitive/behavioral symptoms in children with Batten disease, introduce possible solutions and related emerging data, and propose additional new methods to improve our understanding of the clinical phenomenology of Batten disease. While the focus will be on research results from juvenile NCL, we will also consider the application of these methods to other NCLs and more broadly, to other neurodegenerative diseases and inborn errors of metabolism as well.

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TRANSLATIONAL RESEARCH EXPERIENCE IN ARGENTINA: THE STUDY PROGRAM FOR NEURONAL CEROID LIPOFUSCINOSES (NCL)

GUELBERT N.[1], CISMONDI I. A.[1,2], PESAOLA F.[1,3], PONS P.[4], ALONSO G.[1], DODELSON DE KREMER R.[1], OLLER-RAMÍREZ A. M.[1], SIMS K.[5], KOHAN R.[1,2], NOHER DE HALAC I.[1,3]

[1]Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Children´s Hospital- Province of Cordoba, Department of Medical Sciences, Universidad Nacional de Cordoba; [2]Department of Dentistry, Universidad Nacional de Cordoba; [3]National Research Council- CONICET; [4]Electronic Microscopy Center,Department of Medical Sciences, Universidad Nacional de Cordoba, Argentina; [5]MGH-Neurogenetics, Masachussetts General Hospital, Boston, MASS, USA

Background: The Argentinean Neuronal Ceroid Liprofuscinoses Research Program was initiated more than a decade ago. The aim was to overcome misdiagnoses and underdiagnoses in the region. Subjects: 216 NCL suspected patients from 8 different countries and their direct family members. Methods: Clinical assessment, enzyme testing, electron microscopy, DNA screening. Results and discussion: 1) the study confirmed NCL disease in 100 individuals. Phenotypic studies comprised epileptic seizures and movement disorders, ophthalmology, neurophysiology, image analysis, rating scales, enzyme testing, and electron microscopy; 2) DNA screening and validation of mutations in genes CLN1, CLN2, CLN3, CLN5, CLN6, CLN7 and CLN8: definition of subtypes, new/known mutations and polymorphisms; 3) Progress of the epidemiological picture in Latin America; 4) NCL-like pathology studies in progress; 5) Genomic screening: the known mutation Chr.2, exon 13 p.Arg604His in the gene Sodium channel, voltage-gated, type 1, alpha subunit (SCN1A) in heterozygosis was found in one patient, segregating from his mother; 6) Experimental neurobiology of CLN8. Concluding remarks: translational research is highly efficient to overcome misdiagnoses/underdiagnoses. The study of "orphan diseases" in a public administrated hospital should be adopted by the health systems, it impacts the family's life quality, the collection of epidemiological data, and triggers research advances.

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EXPERIENCE IN THE MANAGEMENT OF THE ARTIFICIAL NUTRITION IN CHILDHOOD NEURODEGENERATIVE DISEASES IN LATIN AMERICA

RIGA C., CRESPO D.[1]

[1]Servicios de Gastroenterología y Nutrición, Hospital de Niños de la Santísima Trinidad, Pucará y Ferroviarios, Córdoba, Argentina

Children with neurodevelopmental disorders may show a variety of physical and intellectual limitations along development. In the last decade in our region it has been a longer survival and a lower requirement for admissions due to clinical complications. This change in the natural history of the disease responds to a multiplicity of causes, being the most optimal nutritional management one of the factors involved. Objectives of nutritional management: the main objectives of the nutritional care in these complex patients are: 1. preventing clinical complications of malnutrition, reducing the frequency of hospitalizations and infections, and 2. facilitating the management and care by their parents with a positive effect on the family relationships. Food management: Oral: changing food consistency. Enterally: options being bolus or continuous, full time or night; tubes nasogastric, nasojejunal or gastrostomy.

Gastrostomy: the percutaneous endoscopic gastrostomy has changed the management of children requiring long-term nutritional support or having difficulty with oral feeding, favoring dietary management and improving social relationships and quality of life. It is the best surgical technique for performing a gastrostomy, safe, minimally invasive and cost-effective. As compared with conventional gastrostomy, it shortens the surgical time and the risk.

Conclusions: • Patients with complex degenerative brain disease must be managed by a multidisciplinary team. • The type and route of feeding depend on motor skills, feeding difficulties and whether the patient is ambulatory or hospitalized. • Nutrition can be the focus of an ethical dilemma in cases where it is the only therapy that sustains the life of the patient. In this situation the indication of nutritional support should be discussed by the medical team, family, caregivers and the community.

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ARTIFICIAL NUTRITION IN LATER STAGES OF DEGENERATIVE BRAIN DISEASE IN CHILDREN. QUESTIONS AND ANSWERS ABOUT ARTIFICIAL NUTRITION (BASED ON CONCLUSIONS OF THE INTERDISCIPLINARY MEETING- HAMBURG 2012)

KOHLSCHÜTTER A.[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, DE, Germany

Questions regarding artificial nutrition in children with degenerative brain disease were discussed at an interdisciplinary meeting in Hamburg in August 2012. The following points were felt to be important: **Basic questions and aims**. Professionals caring for such patients are in need of principles that are applicable in practice when the question of tube feeding comes up. Clear concepts should create a feeling of safety in caregivers and avoid traumatic irritations through the interfering of persons not directly related to the field. **Start of artificial nutrition**. Before starting artificial alimentation (through PEG[1] or otherwise), an unambiguous medical indication must be formulated and put down in writing with simple clear words. Such a drastic medical measure as artificial nutrition should never be started as a quasi-automatism. An indication undoubtedly exists when swallowing difficulties have become tormenting and the patient's general condition is otherwise still relatively good. The indication is much less clear in far advanced stages of cerebral decline, as improvement of the quality of life through artificial nutrition may not be convincing at this stage. Quality of life, however, is a very variable notion that only can be assessed *regarding the individual situation*. As a rule this is *only* possible for parents and for physicians thoroughly acquainted with the medical history. **Termination of artificial nutrition**. There can be good reasons for termination, such as recognition (by parents and a physician experienced with the patient) that continuation of artificial nutrition will be without benefit to the patient and will prolong life unnaturally[2]. In this situation, parents, who are the only ones responsible for their child's welfare, may be under psychological pressure towards continuation of artificial nutrition. The frequent use of industrially produced formulas adds to the risk of influence by commercial interest. **Family counseling**. These deliberations should be touched upon and be adequately documented already at the very first consultation where questions of nutrition come up. **Documentation**. An adequate possibility is using a DNR (Do Not Resuscitate) form and make corresponding entries there. Such entries should be signed by a professional; parents should sign only when it appears psychologically justified. **Inclusion of outsiders**. In a standard case, the simple documentation mentioned is sufficient, and inclusion of outsiders not acquainted with the individual case (ethical committee, lawyers, judges) is not necessary. Inclusion of outsiders is advisable in situations with dissension between parents, between parents and physician, or when there is uncertainty about the custody of the patient. In such cases, persons working in the institution where the child is primarily being cared for are preferred consultants. **Legislation**. Compared to the legislation for adults, the legislation for minors is insufficient in some countries at present. When such deficiencies can be corrected is unforeseeable. Clear and simple maxims, however, should be formulated right now.

[1]Percutaneous endoscopic gastrostomy

[2]As a stop of alimentation will lead to malnutrition and dehydration after variable intervals, the question was raised how death certificates should be completed correctly (in respect to natural vs. unnatural death). It was suggested that a wording such as "dehydration, secondary to disease" may be adequate in such cases and would be compatible with natural death.

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LATINAMERICAN PERSPECTIVES ON ARTIFICIAL NUTRITION IN CHILDREN WITH DEGENERATIVE BRAIN DISEASE. ETHICAL CHALLENGES IN DECISION-MAKING

TORRES J. M.[1]

[1]Hospital Privado de Cordoba, Cordoba, Argentina

Decision-making in patients with degenerative brain disease, regarding artificial nutrition, focuses mainly when resuming seems hopeless (futility), taking into account it does not contribute to the well being of the child. Since the mid 1970's, it has been strongly questioned that decision-making on the interruptions of vital support should be exclusively the authority of the medical doctor or team. In Latin America also, this paternalistic attitude (called hard paternalism) has lost its validity, with exceptions in regions where the lack of empowerment in the citizens (lack or weak abilities for vulnerable situations), prevents them from self-governing decision-making. If the paternalistic model has lost validity, this does not

mean that other models have been installed and can determine how to reach a reasonable and ethical decision-making. Several "methods" have been highly criticized for avoiding this empowerment that allows a lesser asymmetric relationship between the patient and the medical doctor. First: "Objective approach" is when the medical doctor presents the parents with the different choices, pros and cons, with no recommendations what so ever, leaving the decision-making exclusively to the parents. Second: "Broad shoulders", where medical doctors intervene by making suggestions or attempt to influence by arguing that the responsibilities lay under the medical doctor's shoulders more than in the parents; reaching a sort of soft paternalism. Third: "Shared deniability approach", where the decisions are taken without a frank dialogue and without a clarification process over the principles that are at stake (for example: Sanctity vs. Quality of Life), and the dilemmas aren't solved, they are "dissolved" as a result of not determining who is responsible for such decisions. Perhaps the most consensual criteria in all latitudes is: "shared decision-making", where through a deliberation process, meaning a dialogue in which a good disposition for rational clarification is involved, and where communications are not only an exchange of information on certain opinions, but also a "trusting" settlement, a principle that is only achieved more or less after a long period of time and that is built daily (chronic diseases allow this so called "time"). The decision to cancel food supplies is emotionally disturbing, that is why such trust is a strong necessity, which is created not only from intelligence but also from the empathy feelings that are established between the medical doctor/s and the parents. When conflicts are unsurpassed, the most legitimate instance in Latin America are not the judicial authorities but the Ethics Hospital's Committee instead, these moral debate extended forums, issue suggestions that only ease the shared decision-making in an ethical way and with a higher technical quality. We could not expect for more, nor less.

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BIOETHICAL DILEMMAS IN THE CARE OF CHILDREN WITH DEGENERATIVE BRAIN DISEASE

PENCHASZADEH V.[1]

[1]Universidad Nacional de La Matanza, Buenos Aires, Argentina

The management, care and prevention of degenerative brain disease with autosomal recessive inheritance present numerous ethical challenges. The topics to discuss include the care of the child affected with a progressively deteriorating condition without effective treatment and where the goal is to prevent suffering and assure the best possible quality of life as deterioration advances; artificial feeding is often offered at some point, and strict ethical guidelines have to be followed for initiating and terminating it, in which every effort should be made to follow the will of the parents, provided that it is not futile nor it prolongs suffering unnecessarily. Given that care should include the whole family, issues to address are the management of guilt feelings of parents, who are carriers of the recessive gene; the management of mental health issues and "survivor's guilt" in healthy siblings; and ethical issues surrounding genetic counseling to the family, including the option of prenatal or pre-implantation diagnosis to avoid further affected children. On the latter, the ethical principle of personal autonomy of the parents in reproductive decision making should be honored, as well as the principle of justice assuring equity in the access of genetic technologies.

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FINDINGS FROM OVINE MODELS AND BARN (BATTEN ANIMAL RESEARCH NETWORK)

PALMER D. N.[1]

[1]Faculty of Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand; Batten Animal Research Network (BARN)

Naturally occurring Batten disease in large animals has played a central role in our understanding of these diseases, beginning with a "generalised lipidosis" in English Setters in the 1950's. Subsequent studies on large animal models revealed many insights into the NCLs and clarified some fundamental misconceptions. Studies on CLN6 affected South Hampshire sheep established that these diseases are not lipidoses, lipid peroxidation is not involved in the pathogenesis and the storage bodies are unrelated to either ceroid or lipofuscin as classically described. These diseases are lysosomal proteinoses with storage of subunit c of mitochondrial ATP synthase dominant in most forms. Further studies revealed that the development of brain atrophy is regional, is preceded by neuroinflammation, and unrelated to storage body accumulation which is generalised. There is intercellular communication of corrective factors and a physiological component to pathogenesis, unlikely to be accessible by cell biology studies. We in BARN concentrate three naturally occurring ovine models, another CLN6 in Australian Merinos and a CLN5 in Borderdales, and mouse comparisons. While sheep reproduction is slower and gene transfer technologies less advanced than in mice, the sheep brain size, anatomy and organisation and the course of the ovine diseases are more comparable to humans.

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GENE THERAPY USING ADENO-ASSOCIATED VIRUS SEROTYPE 9 IN THE SHEEP BRAIN

MITCHELL N. L.[1,2], BARRELL G. K.[1], WELLBY M.[1], WICKY H. E.[2,3], PALMER D. N.[1,2], HUGHES S. M.[2,3]

[1]Faculty of Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand; [2]Batten Animal Research Network (BARN); [3]Department of Biochemistry, Brain Health Research Centre, University of Otago, Dunedin, New Zealand

Large animal models are vital in the validation of gene therapies, acting in a translational capacity for human studies. Sheep are particularly advantageous in this role, possessing a human-like gyrencephalic brain of comparable size and physiology. Intracerebroventricular and intraparenchymal deliveries of recombinant adeno-associated virus serotype 9 (rAAV9), encoding green fluorescent protein (GFP), were examined in naïve, healthy juvenile sheep and transgene expression analysed by immunohistochemistry 30 days later. Following intraventricular delivery, transgene distribution was observed throughout the CNS, including the prefrontal and occipital cortices, and all along the spinal cord. Robust GFP expression after intraparenchymal infusion was localised to the injection sites but transduced cells were also observed in many distal regions, including the Purkinje cells of the cerebellum and projection neurons of the frontal association and motor cortices, probably from retrograde axonal transport. Co-immunolabeling with GFP and cell-type specific markers indicated dominantly neurotropic transduction. The neuronal tropism by both delivery methods and extensive protein expression in the sheep CNS provided proof of principle for the use of rAAV9 to deliver corrective genes to ovine models. Gene therapy trials of rAAV9 vectors containing corrective CLN5 and CLN6 injected into sheep affected with CLN5 and CLN6 Batten disease are underway.

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NEW ANTIBODIES PREDICT AN INTERACTION BETWEEN CLN5 AND CLN6

HUGHES S. M.[1,2], PALMER D. N.[2,3], SCHODERBOECK L.[1,2], MITCHELL N. L.[2,3], MCINTYRE K.[1,2], HASKELL R. E.[4], ANDERSON R. D. [4], WICKY H. E.[1,2], XU J. B. [2,3]

[1]Department of Biochemistry, Brain Health Research Centre, University of Otago, Dunedin, New Zealand; [2]Batten Animal Research Network (BARN); [3]Faculty of Agriculture and Life Science, Lincoln University, Canterbury, New Zealand; [4]Viraquest Inc. Iowa City, IA, USA

The CLN5 and CLN6 genes encode proteins associated with two variant late-infantile NCLs in humans. Sheep forms for these diseases have been extensively studied, but the lack of reliable antibodies has been a long standing problem. This has been overcome using the ovine CLN5 and CLN6 coding sequences each cloned into an adenovirus shuttle vector and packaged into adenoviral particles. Antibodies were generated by single injections of CLN5 or CLN6 expressing adenovirus into New Zealand white rabbits. Sera collected from rabbits 50 days post-injection

contained antibodies which specifically recognised the appropriate overexpressed proteins by immunocytochemistry and on western blots. The CLN5 antibodies also detected endogenous protein expression in normal sheep brains. CLN5 was abundant in the hippocampus and also detected in the cerebral cortex, cerebellum and occasional neurons in the striatum. Antibody specificity was shown by a lack of immunoreactvity in CLN5-/- brain tissue, and intermediate levels of staining in CLN5+/- animals. CLN5 expression was also attenuated in brain tissue from sheep affected by two different CLN6 mutations, to be between that observed in CLN5+/- and CLN5-/- sheep, suggesting a cross regulation of the expression of these two proteins. Work is continuing to understand the mechanism underlying this interaction.

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SYNAPTIC PATHOLOGY IN CLN6 OVINE NEURONAL CEROID LIPOFUSCINOSIS
NEVERMAN N. J.[1,2], PALMER D. N.[2,3], HUGHES S. M.[1,2]
[1]Department of Biochemistry, Brain Health Research Centre, University of Otago, Dunedin, New Zealand; [2]Batten Animal Research Network (BARN); [3]Faculty of Agriculture and Life Science, Lincoln University, Canterbury, New Zealand

Naturally occurring forms of Batten disease (neuronal ceroid lipofuscinosis, NCL) in three breeds of sheep have been extensively studied: CLN6 in New Zealand South Hampshires and Australian Merinos, and CLN5 in New Zealand Borderdales. Established flocks provide excellent large animal models to study the pathology and potential treatment strategies in both forms of NCL. Primary neural cell cultures from fetal South Hampshire sheep (CLN6-/- and CLN6+/- control) were studied to identify pathological features at the neuronal synapse. We have previously shown defects in lysosomal acidity in CLN6-/- neural cultures which led us to investigate the functionality and integrity of CLN6-/- synapses. Excitation-induced synaptic endocytosis of both 10,000 and 40,000 molecular weight dextrans was significantly impaired (10,000 p=0.001, 40,000 p=0.0024, unpaired t-test). Expression of the key pre-synaptic vesicle protein, synaptophysin, was also significantly reduced in CLN6-/- neurons (p=0.0069, unpaired t-test) and not trafficked to the synapse. Together, these pathologies suggest major consequences for neuronal function in affected sheep, and indicate possible target sites for therapeutic correction.

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RNA-SEQUENCING AND DIFFERENTIAL EXPRESSION ANALYSIS IN THE CLN6 MERINO SHEEP MODEL
CHANG C. T. [1], HOUWELING P. J.[2], TAMMEN I.[1,3]
[1]Faculty of Veterinary Science, The University of Sydney, Australia; [2]Institute for Neuroscience and Muscle Research, Children's Hospital at Westmead, Australia; [3]Batten Animal Research Network (BARN)

Despite our improved understanding of the genetic cause of Neuronal Ceroid Lipofuscinoses (NCLs), the disease pathomechanism for this group of inherited neurodegenerative diseases is still not well understood, with various biological processes proposed to have either a primary or secondary role in the development of disease. In order to identify the progressive molecular changes that underlie the development of CLN6 disease we investigated gene expression profiles in the frontal and occipital cortex of normal and CLN6 affected Merino sheep at both early and late stage disease using next generation sequencing technology (RNA-seq). Gene ontology analysis showed differential gene expression patterns associated with the induction of inflammation, cell surface receptor linked signal transduction, cellular and metal ion transport, tissue remodeling (activation and inactivation of genes associated with actin, myotubules, cell-adhesion) and apoptosis. Our data supports the diverse molecular changes suggested by various studies, and highlights that inflammatory mechanisms play an important role in the progression of NCLs. Characterization of these early molecular changes in the CLN6 Merino sheep may also lead to the identification of novel biomarkers.

O - 52
THE RATIONALE FOR DIRECT CNS DELIVERY OF rhTPP1 FOR CLN2 DISEASE
JACOBY D.[1], O'NEILL C. H.[1], VUILLEMENOT B.[1], KENNEDY D.[1], TSURUDA L.[1], KATZ M.[2], COATES J.[2]
[1]BioMarin Pharmaceutical Inc., San Rafael, CA, USA; [2]University of Missouri, Columbia, MO, USA

CLN2 disease (late infantile NCL) is a rare lysosomal storage disorder that results from deleterious mutations in the gene encoding TPP1 peptidase. CLN2 is manifest as a primary pediatric neurodegenerative disorder, in which children that are developmentally normal until about 3 years of age rapidly deteriorate, characterized by epilepsy, dementia, gait and motor impairment, bulbar dysfunction, visual loss and ultimately death. BMN 190 is rhTPP1 being developed for intracerebroventricular (ICV) delivery to treat children with CLN2 disease. The pharmacodynamic activity of BMN 190 was assessed in two disease models, tpp1 KO mice and TPP1-null homozygous dachshunds. In these models, BMN 190 led to sustained reduction of pathologic accumulation of storage material and widespread expression of TPP1 activity in the CNS. The dachshund model exhibits a similar disorder, and the disease progression, cognitive function, histopathology and quantitative MRI were substantively attenuated with treatment. Comparison of intrathecal and ICV biodistribution of BMN 190 showed efficient uptake and TPP1 throughout the CNS by both routes, but that ICV was superior in delivery to deeper CNS tissue. Nonclinical toxicology studies of ICV BMN 190 led to no observed limit of dosing and a clinical study of ICV rhTPP1 has been initiated in CLN2-affected children.

POSTER PRESENTATIONS

P - 1
EVIDENCE FOR IMPAIRED GLIAL BIOLOGY IN INFANTILE NCL
LANGE J., COOPER J., WILLIAMS B., ANDERSON G.[1]
[1]King's College London, Institute of Psychiatry, UK

In all forms of NCL localized microglial and astrocyte activation is an accurate predictor of subsequent neuronal loss. This suggests that glial cells may play an important role in NCL pathogenesis, and since these cells also carry the disease causing mutation, their function may also be compromised. Given the close functional relationship between neurons and glia, it is possible that glial dysfunction may impact neurons. In vitro data reveals that juvenile NCL microglia and astrocytes both have functional defects, and have a detrimental impact upon neuron health. We are now exploring whether a similar situation exists in Infantile NCL. Our studies reveal that primary astrocytes cultured from Ppt1-/- mice, a well characterised model of INCL, exhibit a more activated, process bearing morphology in culture than did wild type astrocytes, as well as impaired survival. Similarly, primary Ppt1-/- microglial cultures also exhibit a more activated phenotype, however seem to transform more slowly in response to stimulation than WT microglia. We are currently expanding our functional analysis of INCL glia and investigating their impact on neuronal health and survival.

P - 2
DISRUPTION OF THE A-SERIES GANGLIOSIDE METABOLISM IN THE NEURODEGENERATIVE STORAGE DISEASE JNCL
SOMOGYI A.[1,*,†], PETCHERSKI A.[1,2,†], COTMAN S. L.[2], RUONALA M. O.[1]
[1]NeuroToponomics Group, Center for Membrane Proteomics, Goethe University Frankfurt am Main, Frankfurt, Germany; [2]Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [†]These authors contributed equally to this study.

Gangliosides are glycosphingolipids based on an oligoglycosylceramide backbone. Defects in their metabolism play a crucial role

in many lysosomal storage disorders; however, the contribution of gangliosides to NCL pathology is largely unknown. Approximately 10% of the total lipid content of neuronal membranes and 20-25% of their outer layer are composed of gangliosides. Particularly, a-series gangliosides have been shown to be involved in the development and sustenance of the brain, where they are essential for neurite outgrowth and cell survival. In this study, we analyzed central enzymes and metabolites of the a-series ganglioside pathway in a JNCL cell model, which accurately represents the mutation found in 85% of the patients. The amount of the 'prototype' ganglioside GM1 in CbCln3Δex7/8 cells was shown to be reduced due to an upregulation and enhanced action of -galactosidase. This effect was further amplified by a higher LBPA concentration in the lysosomes of CbCln3Δex7/8 cells. In addition, the trafficking of GM1 is impaired in this cell line resulting in retention of the ganglioside within the trans-Golgi network, where it colocalizes with CLN3. These findings suggest a role for CLN3 in ganglioside trafficking, subsequently altering membrane composition and its regulatory functions.

P - 3

THE HOMODIMERIZATION ABILITY OBSERVED IN THE CLN3 PROTEIN IS LOST IN THE MOST COMMON MUTATNT CLN3 FORM

RAMOS MORENO J. M. , RUONALA M. O.[1]

[1]Center for Membrane Proteomics, Goethe-University, Frankfurt am Main, Germany

Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is caused by mutations in the CLN3 gene, which encodes the CLN3 protein. Up to now, information about the structure, localization and function of this protein remains diffuse. In this study we approached the modus operandi of the human CLN3 protein by investigating the possibility of oligomerization in cells using spectroscopic methods. Förster resonance energy transfer coupled to fluorescence lifetime image microscopy (FLIM-FRET) demonstrated the close proximity between two CLN3 molecules. The bimolecular fluorescence complementation (BiFC) assay supported this finding and showed that the full-length protein is capable of homodimerization, while the most common mutant form arriving from the deletion of exons 7 and 8 is not. The BiFC assay also displayed the capability of the mutant CLN3 protein to form dimers with the full-length protein. These findings present the oligomerization ability of the full-length CLN3 protein as a possible prerequisite for its proper functioning and lay ground for better understanding the cellular role of CLN3.

P - 4

CLN5 DEFICIENCY RESULTS IN ALTERATIONS IN THE ACTIVATION OF AUTOPHAGY

BUDDEN T.[1,2], DE SILVA B.[3,4], ADAMS J.[1], LEE S. Y.[1,2,4]

[1]Division of Biology; [2]Molecular, Cellular, Developmental Biology Program, Kansas State University, Manhattan, Kansas, USA; [3]Department of Biochemistry and Molecular Biophysics; [4]Biochemistry and Molecular Biophysics Graduate Group, Kansas State University, Manhattan, Kansas, USA

CLN5 is one of several proteins that when mutated result in the lysosomal storage disorder Neuronal Ceroid Lipofuscinosis (NCL). CLN5 is a soluble lysosomal protein that has no known function at this time. We have identified a link between the activation of autophagy and CLN5 deficiency. The autophagy-lysosomal protein degradation system is one of the major pathways the cell uses to degrade intracellular material and recycle cellular building blocks. It was recently shown that several other CLN proteins affect the relative level of autophagy, indicating a potential link between the autophagy pathway and the NCLs. By knocking down endogenous CLN5, we showed that, upon stress induction, cells responded with higher levels of autophagy activation. Consistent with this, there is a higher level of the autophagy marker protein LC3-II in CLN5 patient cells. Induction of autophagy through different means also showed higher LC3-II levels compared to control, though patterns differed in the type of autophagy induced. In summary, we discovered that the autophagy pathway is altered in CLN5 deficient cells, indicating a potential role for CLN5 in autophagy. Further analyses of the autophagy pathway will shed light on where CLN5 is acting and the mechanism by which defective CLN5 causes NCL.

P - 5

A NOVEL CLN8 MISSENSE MUTATION UNDERLIES VARIANT LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS IN SOUTH AMERICA

PESAOLA F.[1,2], CISMONDI I. A.[1,3], GUELBERT N.[1], KOHAN R.[1,3], CARABELOS M. N.[1], ALONSO G.[1], PONS P.[4], OLLER-RAMIREZ A. M.[1], NOHER DE HALAC I.[1,2]

[1]Centro de Estudio de las Metabolopatías Congénitas,CEMECO, Children's Hospital- Province of Cordoba, Department of Medical Sciences, Universidad Nacional de Cordoba; [2]National Research Council- CONICET, Buenos Aires, Argentina; [3]Department of Dentistry, Universidad Nacional de Cordoba; [4]Electronic Microscopy Center, Department of Medical Sciences, Universidad Nacional de Cordoba, Cordoba, Argentina

Introduction: The mutated CLN8 is known to be associated with Progressive Epilepsy with Mental Retardation (EPMR) and variant Late Infantile (vLI) phenotypes, both forms of Neuronal Ceroid Lipofuscinosis. The CLN8 protein function remain unknown but it was suggested its participation on sphingolipids biosynthesis pathway. Aim: To characterize genetically CLN8 in Latin America. Methods: Fifteen individuals with CLN8 suspicion were tested by PCR, DNA sequencing and in silica analysis. Results and discussion: The previously registered c.685C>G change, assumed as the disease causing mutation in 2 subjects, is a frequent polymorphism in the Argentinean population. The c.1A>G mutation was found in heterozygous state in 1/15 vLI individuals. This mutation affects the translation by losing the first methionine, producing downstream a new initiation codon, a frameshift and a premature stop codon. The severity of the vLI phenotype is compatible with the predicted effect of the mutation on the protein. The search of a second mutation in non-coding or intronic regions is in progress. Conclusion: The c.1A>G mutation in CLN8 is the first confirmed mutation associated to a vLI phenotype in Latin America. Project: neuronal in vitro studies to shed light on the role of CLN8 protein related to sphingolipids for a Doctoral Thesis.

P - 6

A NOVEL CTSF/CLN13 MUTATION IN AUTOSOMAL RECESSIVE KUFS DISEASE TYPE B

SANTORELLI F. M.[1], DI FABIO R.[2], MESCHINI M. C.[1,3], PESTILLO L.[4], DOCCINI S.[1], PEZZINI F.[5], MORO F.[1],SIMONATI A.[5]

[1]IRCCS Stella Maris, Pisa, Italy; [2]Sapienza University of Rome, Latina, Italy;[3]Baylor College of Medicine, Houston, TX, USA; [4]ASL Fondi, Latina, Italy; [5]University of Verona, Italy

Background: Neuronal ceroid lipofuscinosis (NCL) has different forms, of which Kufs disease (KD) is the least frequent and the most difficult to diagnose. Aims: To describe the neurological features of 3 patients belonging to an Italian kindred with autosomal-recessive Kufs disease type B. Patients and Methods: A 23-year-old woman presented clusters of tonic-clonic seizures. After seven years, she developed a progressive cognitive impairment, evolving in frontotemporal dementia. Five additional cases (4F, 1M; mean age at onset 31.9±21.1 years, range 8-67 years) were detected in the same pedigree originating from Fondi, Lazio, Italy. The clinical picture consisted in generalized seizures at onset, followed by cognitive deterioration after a variable period of time (range 12-69 years). Results: We identified a c.213+1G>C homozygous mutation in CTSF , encoding cathepsin F. The mutation segregated with the affected phenotype. cDNA analyses in peripheral cells showed skipping of exon 1 predictably resulting in a shorter protein with a truncated N-terminus domain. Discussion: A novel mutation cropping the CTSF protein may account for Kufs disease type B. In this family, the mutation caused an identical neurological picture in all the patients, characterized by generalized epilepsy, followed by dementia.

P - 7

THE NOVEL ROLE OF SYNAPTIC PROTEIN (CSPα) IN LYSOSOME FUNCTION AND ANCL PATHOLOGY

BENITEZ B. A., SANDS M. S.[1-3]
Departments of [1]Medicine, [2]Genetics, and [3]Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, MO, USA

Background: Our group was one of the first to identify mutations in DNAJC5/CSPα gene as the cause for Autosomal dominant adult onset neuronal ceroid lipofuscinosis (AD-ANCL). However, the role that mutant CSPα plays in ceroid production, clearance, and autofluorescent storage material (AFSM) accumulation is poorly understood. Aims: Determine how CSPα mutants contribute to AD-ANCL pathology. Material (Patients) and Methods: AFSM accumulation was characterized and the levels manipulated in fibroblasts from CSPα-p.L115R carriers. Results: Cultured fibroblasts from human CSPα-p.L115R carriers exhibit a two-fold increase in AFSM compared to controls. CSPα co-localizes with lysosomal markers in fibroblasts from CSPα-p.L115R carriers, wild-type primary hippocampal neurons and fibroblasts from CSPα hemizygous mice. Mutant CSPα displays an exclusive punctate cytoplasmic pattern, forms aggregates in the lysosome and increases the levels of de-palmitoylated protein. Four different brain regions and cerebellum from AD-ANCL patients show a significantly increase in the levels of lysosomal markers and secondary elevation of three lysosomal enzymes. Agents that decrease lysosome function or increase autophagy significantly increase or decrease the levels of AFSM accumulation, respectively. Conclusion/Discussion: Functional in vitro data combined with findings from AD-ANCL brain samples strongly suggest that CSPα plays a role in the lysosome. Mutant CSPα affects intracellular trafficking, subcellular location and palmitoylation status.

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MODELLING LYSOSOMAL STORAGE DISORDERS: USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO INVESTIGATE SHARED ETIOLOGIES OF NEURONAL CEROID LIPOFUSCINOSES AND PARKINSON'S DISEASE

HILLJE A. L., SCHWAMBORN J. C.[1]
[1]Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, 4362 Esch-sur-Alzette, Luxembourg,

Human induced pluripotent stem cells (hiPSCs) offer enormous potential to establish disease models for various neurodegenerative diseases to study cellular processes that lead to degeneration. The CRISPR/Cas9 system allows the precise modification of any genomic sequence and thereby realizes the full potential of hiPSCs for disease modelling. Mutations in the ATP13A2 (PARK9) gene are linked to juvenile onset neuronal ceroid lipofuscinoses (NCL) in humans. Interestingly, mutations in the same gene cause the Kufor-Rakeb syndrome (KRS), a juvenile onset form of Parkinson's disease (PD). High frequency of Parkinsonism signs in NCL suggest that NCL and PD might share common etiological features. Here, we use the CRISPR/Cas9 system to introduce the NCL causing mutation M810R as well as the KRS associated mutation G504R into hiPSCs. We study how the different mutations affect cellular phenotypes and components of the lysosomal pathway. In addition, we monitor changes in transcriptomics, proteomics and metabolomics that eventually lead to the disease. Finally, we aim to build a comprehensive map combining cellular phenotypes with '-omics' data for each mutation which enables us to investigate potential overlap in etiologies of NCL and KFR and allow a better understanding of how different mutations in the same gene cause different diseases.

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NCL DISEASE MODELLING IN HUMAN NEURAL CELLS USING GENOME EDITING TECHNOLOGY

ANDERSON G. W., BROOKS H., WILLIAMS B. P., COOPER J. D.[1]
[1]King's College London, Institute of Psychiatry, Psychology and Neuroscience, London, UK

Previous work using primary neuronal and glial cultures derived from NCL transgenic mice has allowed us to identify robust, disease-associated cellular phenotypes. These could feasibly be used for drug screening, but a human cell-based model would clearly be more powerful. We have extensively characterised a human, cortically-derived neural progenitor cell line (CTX0E16) and are employing it to introduce specific genetic mutations to mimic various forms of NCL. We are using Zinc Finger Nucleases (ZFNs) to reproduce the common 966 bp deletion of exons 7 and 8 of CLN3, responsible for most JNCL cases, and TALE Nucleases (TALENs) to reproduce the most common CLN1 disease (INCL), and CLN2 disease (LINCL) causing mutations. The benefit of this approach is that once the "diseased cells" have been generated, the unmodified CTX0E16 cells represent an ideal phenotypic control. In parallel, we are also characterising patient-derived iPSCs to validate disease-associated cellular phenotypes identified in genome-edited CTX0E16 cells. Using this approach we seek to create cell lines to model various forms of NCL, with a view to their use as the basis of drug screens to identify novel therapies for these diseases.

P - 10

DEFINING THE ONSET AND PROGRESSION OF NEUROPATHOLOGICAL CHANGES IN MOUSE MODELS OF LINCL

BROOKS H., VAN TRIGT L., NELVAGAL H., MICSENYI M., WALKLEY S. U., COOPER J. D.[1]
[1]King's College London, Institute of Psychiatry, Psychology and Neuroscience, London, UK; Albert Einstein College of Medicine, Bronx, NY, USA

The Tpp1-targeted mouse recapitulates many of the pathological and clinical features of classic late infantile NCL. We have previously defined the end-stage pathology of these mice on an immune-deficient NOD-SCID background. Given the influence of the immune system in other forms of NCL, we decided to compare the phenotype of these immune-deficient NOD-SCID Tpp1-/- mice, to that seen in Tpp1 deficient mice with an intact immune system. We are currently analyzing fixed tissue from Tpp1 deficient mice at different stages of disease progression (3, 6, 9 and 14 weeks of age). Our analysis so far reveals pronounced and progressive neuron loss in the somatosensory and visual regions of the cortex and in the corresponding thalamic relay nuclei. High levels of astrocytosis and microglial activation were present in the same regions where neuron loss was most pronounced, and these phenotypes clearly extend into the cerebellum and individual nuclei within the brainstem and spinal cord. This analysis is providing detailed information about the relationship between glial activation and neuron loss, and the influence of the immune system upon these pathologies. These data also provide detail landmarks for evaluating the efficacy of therapeutic interventions.

P - 11

GENOME WIDE STRATEGIES IN THE S.POMBE MODEL FOR JUVENILE CLN3 DISEASE

BROWN R.[1], BOND M.[1], RALLIS C.[2], JEFFARES D.[2], BÄHLER J.[2], MOLE S. E.[1-3]
[1]MRC Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, UK; [2]Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK; [3]UCL Institute of Child Health, University College London, London WC1E 6BT, UK

We use the fission yeast Schizosaccharomyces pombe as a model for juvenile CLN3 disease. Since the function of CLN3 remains unknown, non-hypothesis based, genome-wide strategies to identify the pathways Btn1p (the CLN3 orthologue) influences may be particularly instructive about its molecular function. Synthetic genetic array (SGA) analysis is an established technique to find positive (suppressors) and negative (synthetic lethal/sick) genetic interactions between a gene of interest, and other annotated coding sequences. Using this approach, we have revealed that btn1 interacts with various stress response genes. These include components of the tor kinase pathways, two MAPK stress

response pathways, and various genes regulated by these pathways. This suggests deregulated stress response is involved in disease pathogenesis. These hits are currently being validated, with a focus on finding potential therapeutic options. We are also developing a transposon-based mutagenesis system in a strain lacking btn1. Transposon insertion sites and abundance in a pool of competitively grown mutants are identified by Illumina sequencing. Insertion abundance is then used as a proxy for mutant fitness. This information will be used to identify genomic regions that, when disrupted, worsen or improve the phenotype of cells lacking btn1. This approach will allow genetic interactions between btn1 and non-coding elements to also be identified, and is therefore potentially more powerful than traditional SGAs.

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CELL-BASED STUDIES USING GENETICALLY ACCURATE MODELS OF JUVENILE NCL IMPLICATE CLN3 FUNCTION AT THE INTERSECTION OF CALCIUM HOMEOSTASIS AND AUTOPHAGY PATHWAY REGULATION

CHANDRACHUD U.[1], WALKER M.[2], SIMAS A.[1], HEETVELD S.[1], PETCHERSKI A.[1,3],WOLF P.[1], ZHAO W. N.[1], HAGGARTY S. J.[1], LLOYD-EVANS E.[2], COTMAN S. L.[1]

[1]Center for Human Genetic Research, Massachusetts General Hospital/ Harvard Medical School, Boston, MA, USA; [2]School of Biosciences, Cardiff University, Cardiff, UK; [3]NeuroToponomics Group, Center for Membrane Proteomics, Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany

Using a GFP-LC3 cell-based screening assay to identify autophagy modifiers in a genetically accurate JNCL murine cell model, we observed a heightened sensitivity of mutant cells to thapsigargin. Thapsigargin blocks SERCA, an endoplasmic reticulum (ER) calcium pump. To determine whether the heightened sensitivity of the Cln3 mutant cells to thapsigargin was mediated through disruption of calcium homeostasis, and to gain mechanistic insight, we further analyzed calcium and late steps in autophagy. Calcium chelation reversed thapsigargin's effect on autophagosome accumulation. Further, calcium measurements revealed abnormalities in intracellular calcium homeostasis, particularly related to ER and lysosomes. We also observed elevated p62/SQSTM1 aggregates co-localized with GFP-LC3 in mutant cells, which worsened with thapsigargin treatment, suggesting autophagosome-lysosome flux is deficient with both Cln3 mutation and thapsigargin treatment. Moreover, blocking autophagosome-lysosome fusion with bafilomycin sensitized wildtype cells to thapsigargin, mimicking the effect of Cln3 mutation. Finally, JNCL patient iPSC-derived neural progenitor cells similarly displayed heightened sensitivity to thapsigargin. These studies validate use of our genetically accurate murine cell model for study of JNCL pathogenesis, and highlight a role for CLN3 in regulating calcium-mediated autophagy pathway flux. Further analysis of this pathway holds promise for developing novel therapeutics for the treatment of JNCL.

P - 13

AUTOANTIBODY IDENTIFICATION IN BATTEN DISEASE

TIMM D., MAGEE H., KOH S. Y., O'TOOLE R., CAIN J., WEIMER J.[1]

[1]Sanford Research, Sioux Falls, SD, USA

Expression of autoantibodies is associated with a number of neurological diseases with many varying epitopes or protein targets. Frequently, these autoantibodies target proteins essential to the function, development, and survival of the neuron. Here we identify a new autoantibody associated with NCLs against CRMP2, a protein whose neuronal functions include axon/dendrite differentiation, neurite outgrowth, and neuronal polarity. These antibodies are present in both JNCL and vLINCL (Cln6 variant) patient serum samples as well as the serum of the Cln3 Δ7/8 and Cln6nclf mouse models representing juvenile and variant late infantile NCL, respectively. The presence of these autoantibodies in the Cln6nclf mouse model and vLINCL patients is the first indication of an autoimmune component in this form of NCL. Future studies are aimed at revealing the role of this particular antibody in the pathogenesis of NCLs.

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CHARACTERIZATION OF DEFECTIVE SYNAPTIC TRANSMISSION IN A MOUSE MODEL OF JNCL

RAMIREZ-MONTEALEGRE D.[1,2], KAPUR J.[1], GOODKIN H. P.[1,2]

[1]Department of Neurology; [2]Division of Child Neurology,University of Virginia, Charlottesville, VA, USA

Juvenile Neuronal Ceroid Lipfuscinosis (JNCL) is one of the most common lysosomal autosomal recessive neurodegenerative disease of childhood. Clinical features of the disease include central vision loss, behavioral problems, parkinsonism, cognitive decline, seizures and premature death. The disease is caused by mutations in Cln3, a gene that codifies for a protein with unknown function that has been localized to the synaptic cleft and has been involved in pH homeostasis, transport of small molecules, cell survival, properties of membrane microdomains and protein transport. Previous reports have shown that different animal models of Cln3 display abnormal metabolism of dopamine, decreased levels of GABA, increased glutamate levels and are more prone to excitotoxicity. However, the underlying mechanisms triggering these defects have never been studied in detail. Using patch clamp techniques, we have found abnormalities in neurotransmission in brain slices and hippocampal cultures of a mouse model of Cln3, that present early in life. Our results suggest that Cln3 may be involved in packing and transport of neurotransmitters.

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ANALYSIS OF A KNOCKOUT MOUSE MODEL FOR CLN7 DISEASE

BRANDENSTEIN L.[1], DAMME M.[2], JANKOWIAK W.[3], SCHWEIZER M.[4], BARTSCH U.[3], STORCH S.[1]

[1]Department of Biochemistry, Children's Hospital, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; [2]Biochemical Institute, Christian-Albrechts University, 24098 Kiel, Germany; [3]Department of Ophthalmology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; [4]Center for Molecular Neurobiology Hamburg, ZMNH, University Medical Center Hamburg, Germany

CLN7 disease is a lysosomal storage disease caused by mutations in the MFSD8 gene coding for the polytopic lysosomal membrane glycoprotein CLN7. We have generated a knockout mouse model for CLN7 disease to study neuropathology and lysosomal biogenesis in Cln7-deficient tissues and cells. Cln7-deficient mice were viable and fertile but had a higher mortality with an average life span of 9-10 months. Neuropathological analyses in the brain revealed widespread autofluorescent material, storage of subunit c of mitochondrial ATP synthase and increased CD68 and GFAP immunostaining in different brain regions at 9 months of age. Lysosomal storage material with lamellar bodies and heterogeneous content was detected by EM in Cln7-deficient neurons in the brain. An early degeneration of photoreceptors in the retina starting at the age of 1 month was observed. Western blot analyses revealed increased levels of soluble lysosomal proteins and lysosomal membrane proteins. Enzymatic activity of β-hexosaminidase in different Cln7-deficient tissues was increased. Biosynthetic sorting and processing of cathepsin D and Z in primary Cln7-deficient MEFs was not changed indicating that lysosomal pH is not altered. The data indicate that loss of the putative lysosomal transporter CLN7 in mice leads to an NCL-like neuropathology in the brain and in the retina thus allowing to study CLN7-specific mechanisms in the Mfsd8 knockout mice.

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THE IMPORTANCE OF MONITORED FOLLOW UP FOR DIAGNOSIS: COCKAYNE SYNDROME

ILARI R., DENZLER I., KAMINKER C., GIORDANO L.[1]

[1]Policlínica Bancaria, Hospital Italiano de Buenos Aires, Argentina

Introduction: Cockayne Syndrome (CS) is a hereditary defect of transcription coupled nucleotide excision repair, the mechanism which prevents DNA damage caused by UV radiation. It is a multisystemic, degenerative and progressive autosomal recessive

disease.The incidence is about 1:200000. Molecular and clinical diagnosis can be made. Objective: To describe the importance of periodic monitoring of the phenotype to make the diagnosis in a neurodegenerative disease. Methods: Review of medical records during 15 years of two kids born from non consanguineus parents. The male was on follow up since 19 month old and his younger sister from 18 month old. Results: Both kindred presented microcephalus, mental retardation, failure to thrive,ataxia, dystonic and spastic tetraparesis, retinopathy, progressive hearing loss,photosensitivity, skin and dental anomalies and abnormal neuroimaging. Metabolic tests to study mitochondrial disease and congenital glycosilation defects were negative. They developed the characteristic phenotype over the time. Conclusion: Molecular genetic testing allow diagnostic certainty. However in rare diseases clinical follow up acquires maximum hyerarchy to guide supplementary exams especially in cases where the phenotype is expanding through the time.

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CYSTIC FIBROSIS. MOLECULAR CHARACTERIZATION OF 381 ARGENTINEAN CYSTIC FIBROSIS PATIENTS AND HETEROZYGOTES

OLLER RAMÍREZ A. M.[1], BOTELLI M.[2], MUGNAINI J.[1], GUELBERT N.[1], DODELSON DE KREMER R.[1]

[1]Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Hospital de Niños; [2]Fundación para el Bienestar del Niño, Programa de Asistencia a la Fibrosis Quística, Córdoba, Argentina

Background: The Cystic Fibrosis (CF) is an autosomal recessive disorder caused for mutations in the transmembrane conductance regulator gene (CFTR). It has been identified more than 1900 variations (www.genet.sickkids.on.ca/cftr). Aims: 1) To establish the spectrum and frequency of mutations in CFTR gene of Argentinean CF. 2) To analyze patients with Late Diagnosis and CFTR-related disorders. 3) Detect carriers. Patients: It was diagnosed by clinical and 2 positive tests 138 CF, 8 by molecular analysis. Total: 146 CF. 235 relative were analyzed. Methods: An exhaustive screening of CFTR gene was performed by techniques high sensitivity. Results: 38 mutations were detected (10 more than 1%). Percent detection of mutated alleles was 87.4%. 22 people were classified according to the sweat chloride values in Positive Values (60 mmol/L or>) Intermediate values (40-59 mmol/L), and normal values (<40 mmol/L). 8 mild mutations were detected. Of the 235 people studied 156 carriers were identified. Conclusions: The positive tests confirm CF. Identify mild mutations in the 3 CF groups explain the late diagnosis. Recognize 2 mutations in atypical phenotypes (intermediate or normal sweat values) reaffirm the importance of molecular analysis. Furthermore, this information is crucial for genetic counseling and the application of specific molecular therapies. Grants: Fundación para el Bienestar del Niño, Programa de Asistencia a la FQ, SECYT, PICT 2010.

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UREA CYCLE DEFECTS: BIOCHEMICAL AND MOLECULAR FINDINGS IN ARGENTINIAN PATIENTS

SILVERA-RUIZ S. M.[1], ANGARONI C. J.[1], ARRANZ J. A.[2], HÄBERLE J.[3], DODELSON DE KREMER R.[1], LARÓVERE L. E.[1]

[1]CEMECO, Hospital de Niños Córdoba, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina; [2]Unitat Metab, Hospital Vall d´Hebron, Barcelona, Spain; [3]Metabolism and Molecular Pediatrics, University Children's Hospital Zurich, Zürich, Switzerland

Urea cycle disorders (UCD) encompass several enzyme deficiencies with a wide clinical spectrum from asymptomatic to severe, mostly with cerebral damage. Objective: to communicate the autochthonous experience in the study of UCD. The diagnostic protocol included metabolites analysis by HPLC, genetic analysis, and computational validation. We recognised: i) Ornithine transcarbamylase deficiency, 12 patients: 2 males with neonatal onset (OTC mutations: delExon2-10, c.533C>T), 4 males with late onset (c.216+1G>A, c.386G>A, c.622G>A, c.829C>T), 6 females (delExon2-10, c.533C>T, c.452T>G, c.540+1G>A, dupExon1-9/delExon10), mSNCs were validated with bioinformatic tools and correlated the fenotype/clinical data with their genotype; ii) Argininosuccinate synthetase deficiency, Citrullinemia type I (CTLN1): one compound heterozygote c.79T>C/c.847G>A patient and 16 newborn patients (c.1168G>A/c.1168G>A) from 10 unrelated families from San Luis Province. This mutation was studied on their relatives and 172 healthy volunteers. The carrier frequency in that population was 4.1% and the estimated incidence of CTLN1 1:2,427; iii) Argininosuccinate lyase, 1 patient (c.587A>G/c.587A>G) who died during neonatal period. Our experience remarks: a) a high morbi-mortality at least in our region, despite an early diagnosis and prompt treatment, b) OTC heterozygotes showed severe manifestations and mostly early onset, c) due the high CTLN1 incidence in a risk population, we recommend a preconception carrier screening.

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BIOCHEMICAL AND CLINICAL CHARACTERISTIC OF CEREBRAL CREATINE DEFICIENCY. FIRST CLINICAL RESEARCH IN ARGENTINA

MARTÍNEZ L. D.[1,2], BEZARD M.[2], GUELBERT N.[2], BECERRA A.[2], DODELSON DE KREMER R.[2]

[1]Cátedra de Biología Celular "B", Facultad de Odontología, Universidad Nacional de Córdoba; [2]Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Hospital de Niños, Córdoba, Argentina.

Introduction: Cerebral creatine deficiency (CCD) represents a newly group of inborn errors related to alterations of creatine (Cr) metabolism: deficiencies in a) arginine:glycine amidino transferase (dAGAT), b) guanidinoacetate methyltransferase (dGAMT) and c) the Cr transporter (dCRTR). The clinical phenotype of CCD is variable, associating nonspecific mental retardation, developmental delay/regression, epilepsy, extrapiramidal disorders and autistic behavior. The aim is to report a prospective biochemical screening method for CCD in mentally retarded boys and girls recruited at the CEMECO. Methodology: Over a period of 24 months, children referred to the CEMECO with unexplained mild to severe mental retardation were prospectively screened for CCD. Cr metabolism was evaluated using Cr/creatinine and guanidinoacetate/creatinine ratio in urine. Results and discussion: 85 children were eligible to participate: 45 boys (53 %) and 40 girls (47 %). Some were severely retarded and others had mild to moderate mental retardation. Four boys from four unrelated families had elevated Cr/creatinine ratio, suggesting a Cr transporter defect. These results indicate biochemical data approaching possible CCD. Conclusion: Experience indicates that the extraordinary clinical heterogeneity and biochemical complexity require research programmed within a specific protocol, justified by the serious neurological compromise involved and the possible therapeutic management in dAGAT and dGAMT.

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CHITOTRIOSIDASE ACTIVITY AND 24-BP DUPLICATION ON CHIT1 GENE ANALYSIS IN ARGENTINEAN PATIENTS WITH LYSOSOMAL STORAGE DISEASES (LSDs). AN UNDESCRIBED FINDING IN NEURONAL CEROID LIPOFUSCINOSIS TYPE CLN2

GINER AYALA A., ANGARONI C., PASCHINI CAPRA A., GUELBERT N., DODELSON DE KREMER R.[1]

[1]Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, Universidad Nacional de Cordoba, Hospital de Niños, Cordoba, Argentina

Background: A previous study investigated plasma chitotriosidase (ChT) activity in inherited metabolic diseases. Subsequently, the frequency of the 24-bp duplication in the CHIT1 gene was established in the Argentinean population. This polymorphism produces ChT deficiency in homozygosity, a limitation of this biomarker. Objective: we investigated ChT 24-bp duplication genotype

and plasma activity in autochthonous patients with different LSDs. Methods: plasma ChT activities and ChT genotypes were determined by fluorogenic and PCR methods. Patients and Results: The genotypes normal homozygous (wt/wt), 24bp-duplication homozygous (d/d) and heterozygous (wt/d) and ChT activities (mean±SD, nmol/h/ml) were, normal controls: wt/wt (26 ±12) n=52; wt/d (l4±9) n=45; d/d (0,1±0,3) n=3. Gaucher disease type 1/3: wt/wt (14955±10369) n=11; wt/d (4471±4479) n=6; d/d (0) n=1. Niemann-Pick (NP): NP-A, wt/wt (685±50) n= 2; NP-B, wt/d (2440) n= 1; NP-C, wt/d (140) n= 1. Cholesteryl Ester Storage Disease: wt/wt (267) n=1. GM1 Gangliosidosis: (520±426) n=4. CLN2: wt/wt (288±126) n=2. Conclusions: The 24bp-duplication frequency in the LSDs studied in our centre was 22%. In heterozygous subjects, the ChT activity decreased by approximately 50%. An unprecedented observation was increased ChT activity in two brothers with CLN2. This work contributes to the confirmation of ChT as a high presumption biomarker of certain LSDs, extending to NCL.

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CLASSICAL HOMOCYSTINURIA IN ARGENTINEAN PATIENTS, FOCUS ON AN ATYPICAL RESPONSE ON BETAINE SUPPLEMENTATION: IN SEARCH OF PUTATIVE PATHOGENIC MECHANISM

GROSSO C. L., ANGARONI C. J., DODELSON DE KREMER R.[1]
[1]Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Hospital de Niños, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, Universidad Nacional de Cordoba,Cordoba, Argentina

Classical Homocystinuria (HCU) is caused by deficiency of Cystathionine ß-synthase and is characterized by multiple connective tissue disturbances, mental retardation and mainly, thromboembolic complications. Treatment for pyridoxine non-responsive HCU involves lowering homocysteine levels with a methionine-restricted diet and betaine supplementation. Our study series identified 8 individuals belonging to 7 non related families. The exact diagnosis was established by clinical, biochemical and molecular assessments. This report considers 3 non-responsive HCU, who showed a lack, partial or total, response to betaine. It is known that Betaine-homocysteine S-methyltransferase (BHMT) catalyzes the synthesis of methionine using homocysteine and betaine as methyl donor. We investigated the BHMT gene for possible mutations and yet known polymorphism: G199S, R239Q, Q406H. The 8 exons of BHMT gene and its flanking regions, amplified by PCR and genomic sequencing, did not show genomic changes nor polymorphism in the 3 patients. Recent advances in HCU mice with the co-administration of taurine with betaine, showed relatively sharp threshold-effect between hyperhomocysteinemia and thrombotic risk. It was suggested that this new therapeutic approach will have a strong antioxidant effect on BHMT with an improvement of therapeutic effect in this disease. This focus is currently in progress in addition to molecular analysis of promoter of BHMT gene.

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BEHAVIORAL CHARACTERIZATION OF CLN3ΔEX7/8 KNOCK-IN MICE

LANGIU M., RAGUZ N., SOMOGYI A,. RUONALA M. O.[1]
[1]Center for Membrane Proteomics, Goethe University Frankfurt am Main, Germany

The Juvenile form of Neuronal Ceroid Lipofuscinosis (JNCL) is an inherited pediatric disease that has its onset around 5 – 7 years of age with vision loss, continuing with motoric and cognitive dysfunctions, and leading to premature death. The aim of this study was to investigate how well the Cln3Δex7/8 knock-in mouse model replicates the cognitive and motor impairments present in the patients. 5-month-old Cln3Δex7/8 knock-in male mice were tested with different cognitive and motor assays not requiring intact visual skills. With the use of the rotating platform and open field tests no deficit of the motor activity was detected. However, from the novel object recognition and social recognition tests emerged a mild cognitive impairment. In addition, the social recognition test highlighted the tendency of the Cln3Δex7/8 knock-in mice to avoid an unfamiliar conspecific, suggesting the presence of anxiety. This study indicates that tests measuring cognitive functions could be used when evaluating the effects of therapy approaches in mice.

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ARE YOU USING THE RIGHT MOUSE MODEL? COMPARISON OF NEUROLOGICAL PHENOTYPES IN THE CLN3-KNOCKOUT AND CLN3DELTAEX7/8-KNOCK-IN MOUSE MODELS ON TWO DIFFERENT GENETIC BACKGROUNDS

KOVÁCS A. D., PEARCE D. A.[1]
[1]Sanford Children's Health Research Center, Sanford Research, Sioux Falls, South Dakota, USA

Mutations in the CLN3 gene cause juvenile CLN3 disease also known as juvenile Batten disease. The most common disease-causing mutation of CLN3 is a 1.02 kb deletion, CLN3Deltaex7/8, theoretically resulting in a truncated protein. Evidence suggests, however, that the truncated CLN3 is unlikely to be expressed, and thus, CLN3Dex7/8 is a null mutation. Accordingly, the behavioral phenotypes and their progression in the Cln3-knockout (Cln3-/-) and Cln3Dex7/8-knock-in mouse models of juvenile Batten disease should be very similar. To test this we compared the exploratory activity, motor function, and depressive-like behavior of 1-, 3- and 6-month-old Cln3-/- and Cln3Deltaex7/8-knock-in mice on two different genetic backgrounds (129S6/SvEv and C57BL/6J). Though in many cases the behavior of Cln3-/- and Cln3Deltaex7/8 mice was similar, we found genetic background-, gender- and age-dependent differences between the two mouse models. We also observed large differences in the behavior of the 129S6/SvEv and C57BL/6J wild type strains, which highlights the strong influence the genetic background can have on the disease phenotypes of transgenic mice. Our study demonstrates the importance of testing mouse models on different genetic backgrounds and comparing males and females in order to find the best disease model for therapeutic studies.

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OBSERVATIONS ON ALTERED BEHAVIOUR AND GROWTH IN THE AUSTRALIAN CLN6 MERINO SHEEP MODEL

CRONIN G. M.[1], BEGANOVIC D. F.[1], SUTTON A. L.[1], PALMER D. J.[1], THOMSON P. C.[1], TAMMEN I.[1,2]
[1]Faculty of Veterinary Science, The University of Sydney, Australia; [2]Batten Animal Research Network (BARN)

Neuronal Ceroid Lipofuscinoses (NCL) occur in a variety of non-human species including sheep, which are recognised as valuable animal models. This experiment investigated the progressive postural, behavioural and liveweight changes in NCL-affected lambs, to establish practical, non-invasive biomarkers of disease progression for future preclinical trials in the Merino sheep model. A flock of eight lambs (homozygous affected (n = 4), carriers (n = 2) and homozygous normal (n = 2)) was studied, with the observer blind to the disorder status. Lambs were observed in 11 observation weeks, at 3-5 week intervals between 26-60 weeks of age. Direct observation and accelerometer measurements during 72 h periods, were used to quantify lamb posture and behaviour. Behavioural reactions of the lambs to visual and auditory stimuli were recorded and lamb weights were measured. NCL-affected lambs gained less weight as the experiment progressed. Other statistically significant Genotype × Age interactions were found for walking behaviour, a composite variable of key behaviours identified in a principal components analysis, responsiveness to visual and auditory stimuli, steps taken per 24 h and grazing behaviour. A number of behavioural changes identified in the experiment could form the basis for a protocol for monitoring and evaluation of disease progression.

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PILOT INVESTIGATION OF POSSIBLE CARDIAC INVOLVEMENT IN NEURONAL CEROID-LIPOFUSCINOSIS IN THE CLN6 MERINO SHEEP MODEL

TAMMEN I.[1,2], PIRIPI S.[1], BEIJERINK N. J.[1]
[1]Faculty of Veterinary Science, The University of Sydney, Australia; [2]Batten Animal Research Network (BARN)

Various studies have reported cardiac abnormalities such as repolarization disturbances, ventricular hypertrophy, and sinus node dysfunction in patients with various forms of NCL. The pilot study described here aimed to investigate if any cardiac pathology could be identified ante mortem in the Australian CLN6 Merino sheep model. Six-lead electrocardiograms (ECG) were conducted on 6 affected and 5 clinical normal sheep at approximately 13 and 20 month of age. Echocardiograms were conducted in the same sheep at 20 month of age. Analysis of the ECG (heart rate, P-wave duration, P-wave amplitude, PQ-time, QRS-duration, Heart axis, QT-time, T-wave duration and T-wave amplitude) and echocardiographic (interventricular septum thickness in diastole and systole; left ventricular internal diameter in diastole and systole; left ventricular free wall thickness in diastole and systole; end diastolic/systolic volume; stroke volume; ejection fraction, fractional shortening; aortic root diameter; left atrial diameter; aortic peak velocity; pulmonic peak velocity) data did not demonstrate any abnormalities between diseased and clinically normal sheep. Heart tissue will be collected at post mortem for further investigation. Any cardiac pathology identified in the Merino CLN6 model can inform future systematic studies in this model that aim to better understand the role of cardiac pathology in NCL disease.

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COMBINATION THERAPIES FOR JUVENILE BATTEN DISEASE

TARCZYLUK M. A., SALZLECHNER C., SMITH D., LIM M. J., PEARCE D. A.[2], PLATT F. M.[3], WILLIAMS B. P., COOPER J. D.[1]
[1]The Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK; Evelina Children's Hospital, London, UK; [2]Sanford Children's Health Research Center, Sioux Falls, USA; [3]Department of Pharmacology, University of Oxford, Oxford, UK

As the function of CLN3 remains unclear, experimental therapies for juvenile NCL (JNCL) are currently limited to targeting downstream pathomechanisms. We have recently reported that ameliorating the adaptive immune response with the immunosuppressant drug mycophenolate mofetil (MMF) provides beneficial effects in Cln3-/- mice, and MMF is currently evaluated in a Phase II clinical trial (NCT01399047). We have investigated whether targeting the innate immune response with ibuprofen and/or minocycline in combination with a neuroprotective agent lamotrigine improves the beneficial effects of MMF alone. Drugs were administered daily to pre-symptomatic (3 months) and symptomatic (6 months) Cln3-/- mice over a 3 month period. The combination of ibuprofen and lamotrigine had a greater beneficial impact in both cohorts of mice upon behavioural measures than either of these drugs alone, or MMF. These treatments are currently being evaluated over an extended period to confirm sustained benefit, with the impact of these therapies upon JNCL neuropathology currently being assessed. These data have major therapeutic implications since both Lamotrigine and Ibuprofen are already commonly used in children and have a favourable safety profile (even in JNCL patients), thus could be moved quickly into clinical testing.

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HUMAN SERUM ALBUMIN-BASED NANOPARTICLE-MEDIATED IN VITRO GENE DELIVERY

LANGIU M.[1], DADPARVAR M.[1,2], KREUTER J.[2], RUONALA M. O.[1]
[1]Center for Membrane Proteomics, Goethe University Frankfurt am Main, Germany; [2]Institute of Pharmaceutical Technology, Goethe University Frankfurt am Main, Germany

A large number of neurodegenerative diseases are recognized to be caused by a singular genetic defect, resulting in the production of functionally modified proteins. Enzyme replacement and gene therapy are considered as the new frontier of treatment of neurodegenerative disease. One of the major problems in the delivery of genetic material into the brain is the blood-brain-barrier, which selectively blocks the passage of gene delivery vehicles. The aim of this study was to optimize a non-viral gene delivery system in vitro, which is known for its ability to reach the brain after intravenous injection. DNA-polyetylenimine coated HSA nanoparticles were characterized and tested for their ability to be taken up and degraded by cerebellar granular cells in vitro and their feasibility as gene delivery system was assessed. Physically, the DNA-PEI HSA nanoparticles are small in size and have a spherical form. Functionally, they are able to enter cerebellar granular cells and been degraded, and they induce the expression of the firefly luciferase in vitro. These results put the basis for an in vivo adaptation of the HSA nanoparticle delivery system.

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GLOBAL AAV9-MEDIATED CNS GENE TRANSFER AS A TREATMENT STRATEGY FOR INCL AND LINCL

NAGABHUSHAN KALBURGI S., ROZENBERG A., SLONIOWSKI S., GRAY S. J.[1]
[1]Gene Therapy Center, University of North Carolina, USA

Several forms of Batten Disease, including those caused by loss-of-function mutations in PPT1 (306 aa) and TTP1 (563 aa), are amenable to gene replacement therapy. The efficacy and translation of gene therapy for INCL and LINCL has been limited by the extent to which a transgene can be widely delivered to the entire CNS in large animals and humans. Adeno-associated virus serotype 9 (AAV9) vectors are capable of widespread dose-responsive CNS gene transfer after either intravenous or intra-CSF administration, which has been validated in murine, porcine, feline, canine, and non-human primate models. Key to the success of this approach is the use of self-complementary (sc) AAV vectors, which are approximately 20- to 50- fold more efficient for gene delivery compared to traditional single-stranded (ss) AAV vectors. However, the packaging capacity of scAAV will only accommodate ~2.1 kb of foreign DNA. We have developed and validated strong but compact expression cassette amenable to packaging the TPP1 and PPT1 genes. We are currently testing these scAAV9 vectors in the murine models for INCL and LINCL, using both IV and intra-CSF delivery approaches. The supporting data for this gene transfer platform will be presented, along with the current state of the efficacy studies.

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ENGINEERING TPP1 VARIANTS WITH LONGER HALF-LIVES FOR ENZYME REPLACEMENT THERAPY IN LINCL

KUBER N., NANDA V., LOBEL P.[1]
[1]Center of Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ, USA

Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL) is a lysosome storage disorder caused by the deficiency of a lysosomal protease, Tri-Peptidyl Peptidase I (TPP1). LINCL belongs to the group of Neuronal Ceroid Lipofuscinosis (NCL) and shares a common causality with other prevalent lysosomal storage disorders resulting from specific lysosomal enzyme deficiencies. In LINCL, neurons and photoreceptors are the primary cells affected. LINCL has a poor prognosis and currently there is no cure for this fatal disease. A breakthrough in treatment for LINCL will provide a template for development of therapies for similar lysosomal disorders. Enzyme replacement therapy (ERT) could be effective if sufficient TPP1 activity could be restored in all affected cell types including the brain. However, delivering therapeutic amounts of TPP1 or any large molecule therapeutic to the brain is a formidable challenge. We have been working to engineer TPP1 into a more effective drug molecule, concentrating on two areas. First, we are trying to create variant TPP1 molecules that have an increased half-life in the lysosome, thus essentially increasing the activity over time for any TPP1 that gets properly delivered to neuronal lysosomes. Second, we are attempting to create TPP1 conjugates that can cross the blood-brain barrier.

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EX VIVO GENE THERAPY AS A DIRECTED THERAPY FOR PROGRESSIVE RETINAL DEGENERATION IN A CANINE MODEL OF CLN2

TRACY C. J.[1], YOUNG W.[2], PEARCE J. W.[2], COATES J. R.[2], KATZ M. L.[3]
[1]University of Missouri Graduate School, Columbia, MO, USA; [2]University of Missouri College of Veterinary Medicine, Columbia,

MO, USA; [3]University of Missouri School of Medicine, Columbia, MO, USA

CLN2 results from the lack of the soluble lysosomal enzyme tripeptidyl peptidase-1 (TPP1). Gene and enzyme replacement therapies directed toward the central nervous system are currently in clinical trials for treating CLN2. Significant preservation of neurological functions has been observed with these treatments, but the treatments do not prevent retinal degeneration or vision loss. We are using our CLN2 canine model to assess the potential for a similar sustained delivery of TPP1 to the retina as a means of preventing blindness in this disease. In our approach, autologous bone marrow-derived stem cells are transduced with a DNA construct that directs high levels of TPP1 protein production. The transduced cells are implanted into the eyes of affected dogs prior to significant retinal degeneration. The dogs are monitored to determine whether the treatment delays retinal degeneration and loss of retinal function. Preliminary studies indicate that this treatment results in substantial delays in retinal degeneration and in the progression of functional impairment of the retina. It appears that this ex vivo gene therapy approach will have the potential of delaying or preventing vision loss in children who are exhibiting neurological benefits from gene or enzyme replacement therapies directed to the central nervous system.

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EVALUATING READ THROUGH COMPOUNDS FOR THE TREATMENT OF INCL AND LINCL CAUSED BY NONSENSE MUTATIONS

GERAETS R. D., PEARCE D. A.[1]
[1]Sanford Research, Sioux Falls, South Dakota, USA

A variety of genetic disorders are caused by nonsense mutations which result in premature termination codons (PTCs). These PTCs can be recognized by the ribosome as non-termination codons utilizing read through compounds, thus leading to full-length proteins. Currently, read through compounds are being used in clinical trials for the treatment of genetic disorders that stem from nonsense mutations. Based on genetic information, it is known that some cases of INCL and LINCL result from nonsense mutations. PTC-124, RTC-13, and other read through compounds are being screened against patient-derived,immortalized lymphoblasts and point-mutant mouse models in anticipation of identifying potential therapies for INCL and LINCL that result from nonsense mutations.

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RESULTS OF HIGH-THROUGHPUT DRUG SCREEN AND VALIDATION FOR INCL AND LINCL

GERAETS R. D., PEARCE D. A.[1]
[1]Sanford Research, Sioux Falls, South Dakota, USA

Neuronal ceroid lipofuscinoses (NCLs) are a group of rare neurodegenerative lysosomal storage disorders. Due to the lack of definitive therapeutics, NCLs are only treated symptomatically and eventually lead to an early demise. Current emerging therapies for NCLs consist of gene therapy, enzyme replacement therapy, stem cell therapy, immunomodulators, small molecule therapy, etc. Both INCL and LINCL are caused by an enzyme deficiency and thus both diseases can potentially be treated with enzyme enhancing therapies. To identify enzyme enhancing compounds, a high throughput drug screen (HTS) was performed utilizing patient-derived, immortalized lymphoblasts. Preliminary results identified fifteen potential PPT-1 enhancing compounds and seventeen potential TPP-1 enhancing compounds. All identified target compounds are being processed through an extensive secondary validation.

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EXPLORING THE POTENTIAL OF AAV9-MEDIATED GENE THERAPY FOR JUVENILE NCL

PEARSE Y., CUKA S., MOLE S. E., KLEINE-HOLTHAUS S. M., SMITH A. J., ALI R. R.[1],COOPER J. D.[2]
[1]King's College London, Institute of Psychiatry, Psychology and Neuroscience, London, UK; [2]Institute of Child Health, Institute of Ophthalmology, University College London, London, UK

The potential use of gene therapy as a strategy to treat juvenile CLN3 disease (JNCL) presents a number of challenges. Because CLN3 is a transmembrane protein, transduced cells cannot secrete the protein to cross-correct neighbouring cells, and the possibility remains that CLN3 overexpression may be toxic to cells. Nevertheless, recent studies with AAVrh.10 mediated delivery of human CLN3 have shown positive effects in partially alleviating some aspects of disease pathology. Therefore we opted to test the use of AAV9 vectors to drive the expression of either human CLN3 or mouse Cln3. Testing these vectors in primary neuron and glial cultures confirmed their ability to transduce different cell types, but this appears to result in some cell death at the highest multiplicity of infection (MOI) values, compared to vectors expressing eGFP alone. Injection of these vectors in vivo results in localized inflammation around the injection track, but this declines over time with no vector-associated inflammation or overt neuronal loss detected 3 months after injection. We are now assessing the longer-term impact of delivering human CLN3 or mouse Cln3 upon the well-defined neuropathology of Cln3 (delta ex1-6) and Cln3 (delta ex7-8) mice.

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RETINAL PHENOTYPE IN CLN6NCLF MICE, A MODEL FOR EARLY VISION LOSS IN TRANSMEMBRANE NEURONAL CEROID LIPOFUSCINOSES (NCL), A TARGET FOR AAV-MEDIATED GENE THERAPY

KLEINE HOLTHAUS S. M.[1,2], LUHMANN U. F.[1], BAINBRIDGE J. W.[1], SMITH A. J.[1], MOLE S. E.[2], ALI R. R.[1]
[1]Department of Genetics, Institute of Ophthalmology, University College London; [2]MRC Laboratory for Molecular Cell Biology, University College London,UCL Institute of Ophthalmology, Department of Genetics, 11-43 Bath Street, London, EC1V9EL., UK

A major obstacle to developing therapies for NCL is the challenge to deliver agents to the brain. Adeno-associated virus (AAV) mediated gene therapies have been used for eye diseases to restore the expression of proteins and improve retinal morphology and function. We hypothesize that an AAV mediated gene therapy could preserve eyesight in NCL and may pave the way towards more widespread therapeutic treatments. The Cln6nclf mouse is a model for NCL that presents with early vision loss before the onset of severe neurological symptoms. These mice harbour a mutant Cln6 gene leading to a short-lived protein. To determine the time window for treatment, we investigated the retinal phenotype in Cln6nclf animals. Histological and functional alterations occur as early as 2 and 3 weeks ultimately resulting in dramatic retinal thinning – mainly due to photoreceptor loss - and reduction of photoreceptor functioning. Aiming to correct for the degeneration, we performed subretinal injections in postnatal Cln6nclf mice using a range of titers of AAV2/8 vectors carrying Cln6. Our preliminary data shows that high expression levels of Cln6 are detrimental to retinal function and morphology. Currently, we are assessing whether lower expression levels of Cln6 in photoreceptor cells are therapeutic in mutant mice.

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LATE TALKERS IN LATE INFANTILE CLN2 DISEASE - RED FLAGS FOR AN EARLY DIAGNOSIS

NICKEL M., SCHULZ A., KOHLSCHÜTTER A.[1]
[1]University Medical Center Hamburg-Eppendorf, Childrens Hospital, Hamburg, Germany

Problem. Late-infantile CLN2 disease is caused by a deficiency of the lysosomal enzyme TPP1 and is characterized by rapid psychomotor decline and epilepsy. Although the disease is presently incurable, early diagnosis is desirable. In our patients diagnosis was usually delayed two or more years after the onset of striking symptoms (epilepsy and ataxia). We were looking for possible forerunners of the overt manifestation of the disease. Method. As an abnormal language development has occasionally been reported, we reviewed systematically the early development of 38 patients, focussing on language acquisition. Results. Two thirds of patients never achieved language capacities in the nor-

mal range for age. Most of these children had been classified as "late talkers". Conclusion. "Late talkers" have an increased risk to be presymptomatic CLN2 patients. The availability of a simple enzymatic dry blood spot test helps clarifying a suspicion of this severe genetic disease.

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CLN5 DISEASE: PHENOTYPIC FEATURES AND MOLECULAR CHARACTERIZATION

SIMONATI A.[1], BATTINI R.[2], NARDOCCI N.[3], WILLIAMS R. W.[4], COZZOLINO M. R.[5], GUERRA S.[1], GARAVAGLIA B.[3], MORO F.[2], PEZZINI F.[1], SANTORELLI F. M.[2]

[1]Dept Neurological Sciences and Movement, University of Verona School of Medicine, Verona; [2]IRCCS Stella Maris, Pisa; [3]IRCCS Istituto Neurologico C Besta, Milano; [4]Children's Neurosciences Centre, St Thomas's Hospital, London; [5]Dept Pediatria, Università Federico II, Napoli, Italy and United Kingdom

CLN5 disease (MIM#256731) is a rare form of Neuronal Ceroid Lipofuscinosis (NCL) with worldwide distribution. Disease onset occurs in early childhood, but it can be during adolescence or adulthood. CLN5 gene, on 13q22.3, encodes a glycosylated, soluble non-enzymatic protein of the lysosomal matrix. Ten patients of both sexes, belonging to three ethnic groups, were diagnosed according to standardized procedures, and scoring of the disease course was assessed. Mean age at onset was 4,6 years; patients' ages at latest observations were 11-22 years. Earliest symptoms refer to impaired cognition and behavioural problems in eight children. Seizures or visual decline occurred as a late event in eight children. Full clinical picture was reached 1,6-9,6 years after onset. Twelve ultrastructural examinations (eight skin biopsies and four lymphocyte pellets) were performed, and FPP (100%) and CVB (>50%) identified. Eight mutations (on twenty alleles) were detected, including nucleotide substitutions and insdels. Rapid rate of disease progression was seen in two children, who carried homozygous nonsense mutations, predicting truncated pCLN5. The clinical features at onset, such as behavioural symptoms and delayed cognitive development (mainly of language), and delayed seizures seem to be peculiar to this NCL form. Most severe mutations seem to account for phenotype severity.

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EXPANDING THE PHENOTYPE OF NCL TYPE 6: A NOT SO RARE CAUSE OF NEURONAL CEROID LIPOFUCINOSIS IN BRAZIL

LOURENCO C. M.[1], SOBREIRA C.[1], SANTORELLI F. M.[2], SIMONATI A.[3], MARQUES JR W.[1]

[1]Neurogenetics Division, Clinics Hospital of Ribeirao Preto, Faculty of Medicine of Ribeirao Preto, University of São Paulo, Brazil; [2]IRCCS Stella Maris-Molecular Medicine Unit, Pisa, Italy; [3]Department of Neurological, Neuropsychological, Morphological, Motor Sciences-Sections of Neurology (Child Neurology and Psychiatry) and Neuropathology, University of Verona, Piazzale LA Scuro 1, Verona, 37134, Italy

Background: The neuronal ceroid-lipofuscinoses (NCLs) are a group of inherited, neurodegenerative, characterized by progressive intellectual and motor deterioration, seizures, and early death. Clinical phenotypes have been characterized traditionally according to the age of onset and order of appearance of clinical features. Aims: To present the clinical features of Brazilian patients with NCL type 6, identifying the spectrum of mutations and to discuss possible genotype-phenotype relations. Patients and Methods: A cohort of 20 Brazilian NCL patients was evaluated based upon on clinical features following ultrastructural investigation of peripheral tissues. NCL type 6 molecular confirmation was obtained during the diagnostic procedure or retrospectively. Results: Seven patients had a confirmed NCL type 6 molecular diagnosis. All patients showed as first manifestation cerebellar ataxia followed by dementia; visual loss was present only in 3 patients. No clear-cut genotype-phenotype correlations were observed, but relatively intra-familial variability was present in one of the families with 2 affected sibs. Ultrastructural findings were suggestive of an impaired lysosomal function and ruled out the commonest NCL types. Conclusion/Discussion: Most patients with CLN6 mutations have the variant late-infantile NCL (v-LINCL) phenotype. Our cohort of patients expands the clinical presentation features of such rare group of NCLs.

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TELEMEDICINE FOR COGNITIVE ASSESSMENT IN BATTEN DISEASE

ADAMS H. R., RAGBEER S., THE UNIVERSITY OF ROCHESTER BATTEN STUDY GROUP[1]

[1]University of Rochester Medical Center, Rochester, NY 14642, USA

Background: Juvenile NCL is a rare, inherited, childhood-onset neurodegenerative disease. Because of its rarity and wide geographic dispersal, and the limited number of expert clinicians available, affected children and their families often travel great distances to access specialist care. Methods: We evaluated the burden of travel for families to be seen at the University of Rochester Batten Center (URBC), and piloted the feasibility and reliability of telemedicine to conduct remote neuropsychological assessment. Three children with genetically confirmed Juvenile NCL (CLN3 disease) and one healthy sibling completed remote assessment. Each child was present in one room, with testing administered and/or scored remotely by an expert clinician in another room. Main results: The average travel distance to the URBC was 1,000 miles (range: 181 to 2,634 miles). Inter-scorer agreement for remote evaluations was high (from 77.8% to 100.% agreement) on all but one of the neuropsychological tests. Conclusion: Remote cognitive assessment is feasible, demonstrates initial reliability, and has the potential to reduce travel, time, and cost burden to families for both clinical assessments and participation in clinical research.

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CHALLENGE IN THE TREATMENT OF STATUS EPILEPTICUS IN CHILDREN WITH NEURONAL CEROID LIPOFUSCINOSIS

KRAVLJANAC R.[1]

[1]Institute for Mother and Child Healthcare of Serbia, Belgrade, Serbia

Aims: Evaluation of clinical features of status epilepticus (SE) and response to the treatment in children with neuronal ceroid lipofuscinosis (NCL). Method: The study included NCL patients diagnosed by enzyme, genetic and histopathologic analyses from 1991-2013. SE was defined as continuous seizures, or intermittent seizures without recovery of consciousness between seizures lasted >30 minutes. Refractory SE was defined as seizures lasted >60 minutes, while superrefractory if SE continues >24 h after the onset of anaesthetic therapy. All patients were treated by same SE protocol: initially by benzodiazepines, if failed by phenobarbital, phenytoin, midazolam/thiopental infusion, and levetiracetam. Results: All included (20) patients suffered epilepsy. Five patients experienced 11 SE: refractory (5), superrefractory (6) including 4 episodes of epilepsia partialis continua (EPC). SE treatment with phenobarbital (20mg/kg iv) and midazolam continuous infusion (0.2-0.8mg/kg/h) were partially effective with significant side effects (prolonged coma and respiratory impairment). Levetiracetam (60mg/kg iv) was effective in superrefractory SE, but one girl with CLN1 developed severe bradicardia. Methylprednisolon infusion (500mg/m²) was helpful in the EPC case. Conclusion: SE in NCL patients is usually resistant to antiepileptic drugs with common side effects. Very careful drug dosage and application are advocated, suggesting "etiological approach" to SE treatment in NCL patients.

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MGH CENTER FOR HUMAN GENETIC RESEARCH CLINICAL DATABASE AND BIOREPOSITORY: 15 YEARS OF INTEGRATIVE, COLLABORATIVE NCL RESEARCH

SIMAS A. M., STAROPOLI J. F., CHEN T., SLATE N., METTERVILLE D., GLYKYS J. C., COTMAN S. L., SIMS K. B.[1]
[1]Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge St, Boston, MA, 02114, USA

To facilitate improved NCL natural history data, we developed a relational database comprised of NCL patients and family members using Filemaker Pro software. This searchable database links patient clinical information to samples. The majority of affected individuals in our database have been molecularly diagnosed with mutations in one of the known NCL genes including PPT1 (8%), TPP1 (20%),CLN3 (23%), DNAJC5 (4%), CLN5 (4%), CLN6 (2%), CLN8 (2%), GRN (5%) and KCTD7 (1%). 6% of the enrolled have a single pathologic mutation identified and remaining 25% are molecularly undefined, enrolled because an NCL disorder was strongly suggested by clinical or lab data. These cases are important materials for ongoing NCL gene identification research, including whole exome sequencing and bioinformatic analyses. Included in the linked biorepository are fibroblast (from >80 individuals) and lymphoblastoid (from >170 individuals) lines, as well as a smaller number of autopsy and other tissues. Our biorepository also allows us to generate induced pluripotent stem cells (iPSCs) from consented participants who have donated skin samples. The establishment of this unique resource is facilitating genotype-specific disease pathogenesis research for early stage drug discovery and testing. As we incorporate longitudinal data, this integrated clinical database and biorepository we expect will allow us and other researchers to elucidate NCL clinical and pathobiologic mechanisms.

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PARENTS' NEEDS FOR WEB BASED INFORMATION ON RARE CONDITIONS: WHAT PARENTS TELL US

NICHOLL H., TRACEY C., BEGLEY T., LYNCH A., KING C., HEFFERNAN T., KEARY E.[1]
[1]School of Nursing and Midwifery, Trinity College Dublin & The Saoirse Foundation,Castledrum, Castlemaine Co Kerry, Ireland

Background: The use of the web by parents of children with rare diseases for information, advice and support is increasing. However searching the web can be an overwhelming, frustrating and disappointing experience as literally thousands of links may be returned to web pages that contain irrelevant and incomprehensible material. In Ireland no dedicated website exists to assist these parents. Aims: The aim was to identify parent's web-based information needs and to make recommendations for the content of a culturally appropriate web based resource. Methods: This descriptive exploratory study received ethical approval from Trinity College Dublin. An audio recorded focus group interview was conducted with parents (n=8) of children with rare disorders from all parts of the country. Results: Data were analysed and five themes were identified. These related to needing accurate information at the point of diagnosis, the need for peer support and reassurance from other parents, difficulties managing conflicting information, accessing support groups and the 'ideal' web site. Conclusion/Discussion: Parents identified their specific needs and made recommendations for a web-based information related to their children. The findings from this study were used in the development a larger national study of parents (n=1000) of children with rare diseases in Ireland.