

Proceedings of the Third Joint Symposium on Neuroacanthocytosis and Neurodegeneration with Brain Iron Accumulation: From Benchside to Bedside

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Neuroacanthocytosis – Chorea-acanthocytosis

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Progress on Red Cells in NA Syndromes: Focus on ChAc

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Erythrocyte structure and function depend upon interactions between proteins in and associated with the membrane, centered around band 3. This protein harbours physiological removal signals and, as a main component of the links between the cytoskeleton and the lipid bilayer, plays a central role in deformability. Also, as a binding partner of hemoglobin and key glycolysis enzymes, band 3 is the link between the oxygen-dependent trade-off between ATP production and NADPH-mediated protection against oxidative damage. Phosphorylation of band 3 by Lyn and Syk kinases is involved in the regulation of these processes.

The presence of acanthocytes in the blood of patients with chorea-acanthocytosis (ChAc) and other NA syndromes provides the rationale for attempts to identify the molecular causes and consequences of acanthocytosis. The results of this approach indicate that a disturbance of the interaction between band 3 and other membrane-cytoskeleton proteins is at the center of the causes of the acanthocyte shape. The pathway leading to this disturbance includes abnormal membrane recruitment of and band 3 phosphorylation by Lyn kinase. In most patients, the presence of acanthocytes does not have clinical consequences, but various in vitro analyses show functional alterations. These alterations are not restricted to the acanthocytes in ChAc patients, but are also present in patients

with other NA and NBIA syndromes. A morphological, functional and molecular comparison of the acanthocytes and non-acanthocytes of the same patient, and of erythrocytes of various NA and NBIA patients, provides additional possibilities to identify the neuropathological mechanisms of these disorders.

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A Vps13-Cdc31 Complex is Directly Required for TGN to Late Endosome Vesicular Transport and TGN Homotypic Fusion

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The yeast Vps13 protein (Vps13p) is the prototype of a family of molecules that includes human VPS13A, for which loss of function results in chorea-acanthocytosis. The VPS13 gene was first identified for its requirement in retrograde transport from the late endosome to the Golgi in yeast. Further genetic, cell biological and biochemical studies identified Vps13p as a 357 kDa cytosolic protein implicated in regulating forward transport from the trans Golgi network (TGN) to the late endosome and required for a late stage in sporulation (Brickner, J.H. & Fuller, R.S. 1997. *J. Cell Biol.* 139:23-36). Using cell-free fusion and transport assays (Brickner et al. 2001. *J. Cell Biol.* 155:969-978; Blanchette, J.M., et al., 2004. *J. Biol. Chem.* 279:48767-73), we find that Vps13p is directly required for TGN homotypic fusion and TGN to late endosome vesicular transport. Extracts from cells with deletions or

temperature-sensitive mutations in VPS13 are defective in these fusion and transport reactions but are complemented by Vps13p that has been purified to near homogeneity from yeast cells. Soluble, purified Vps13p is monomeric and is in complex with the small calmodulin-like protein, Cdc31p (yeast centrin). Cdc31p is required for both cell-free reactions. Under reaction conditions, purified Vps13p binds to yeast membranes in an ATP-stimulated fashion. The binding of Vps13p to phosphorylated phospholipids and a low resolution electron microscopic structure for the protein derived from negative stained (uranyl formate) samples will be discussed.

This work has been supported in part by the Protein Folding Disease Fast Forward Initiative, the Advocacy for Neuroacanthocytosis Patients, the Endowment for the Basic Sciences at the University of Michigan Medical School and NIH RO1 grant GM50915 (RSF).

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Organelle Trafficking is Severely Impaired in Human Neurons Derived from Chorea-acanthocytosis Patients

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Chorea-acanthocytosis (ChAc) is an autosomal recessive inherited disease caused by loss-of-function mutations in the VPS13A gene, which encodes for chorein protein. The yeast homolog of VPS13A functions in vesicle and mitochondrial trafficking. These processes are aberrantly regulated in Huntington disease (HD), which is phenotypically very similar to ChAc, and suggests that they play an important role in ChAc pathogenesis. Therefore, this study focuses on vesicle and mitochondria trafficking. Live imaging of neurons differentiated from induced pluripotent stem cells (iPSCs) revealed a decreased lysosome count in neurites and reduced motility of mitochondria. Mitochondria were abnormally shortened, reminiscent of vesicles. Characterization of patient mitochondria by respirometry showed diminished function. It is possible that the disease progression of HD and ChAc share a common underlying mechanism. We are currently investigating genes identified as hallmarks of trafficking impairment in HD using qPCR and cytoskeleton dynamics via immunocytochemistry and Western blot.

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EMINA 2 & Friends: Dissecting the Molecular Pathophysiology of Chorea-acanthocytosis

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Introduction: The European Multidisciplinary Initiative on Neuroacanthocytosis (EMINA) has provided detailed clinical characterization of the different NA syndromes and collected valuable brain and muscle samples of chorea-acanthocytosis (ChAc) patients. ChAc is caused by loss-of-function mutations within the gene VPS13A encoding a protein of unknown function named chorein. In contrast to other types of neurodegeneration, ChAc shows no abnormal protein inclusions. Within the EMINA initiative, we recently found two signaling kinases to be involved in ChAc pathogenesis, but the exact pathophysiology remains enigmatic.

Overall goal: The overall goal of the EMINA-2 consortium is to dissect the molecular pathophysiology of ChAc caused by mutations in the VPS13A gene as a model for NA syndromes to provide novel drug targets for causal therapies.

Experimental approach: To use patient material, patient-specific induced pluripotent stem (iPS) cell models and animal models (VPS13A^{-/-} mice, Drosophila models) of ChAc for studying the effects of VPS13A mutations on intracellular signalling pathways and cytoskeleton formation. The EMINA-2 consortium now combines the clinical excellence of EMINA including the characterized patient material (blood, brain and muscle samples) with basic cell and molecular biology as well as animal model research to characterize the molecular events leading to neurodegeneration in ChAc.

Significance: The results will contribute to an understanding of the molecular pathophysiology of ChAc as a model not only for NA syndromes but also for inclusion body-independent neurodegenerative diseases of the basal ganglia. This knowledge will help to generate novel causal treatment approaches in ChAc and eventually other neurodegenerative diseases of the basal ganglia.

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Case Report: Chorea-acanthocytosis in Iran: Focus upon Therapies

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Chorea-acanthocytosis (ChAc) is a progressive multisystem neurodegenerative disorder which manifests with several devastating symptoms. The complex nature of motor and psychological disorders on one hand, and the rarity of the disease on the other, makes physicians and researchers unable to reach a definite solution for patients' sufferings. Thus, most of our knowledge in management of this disease is based

on small case series and personal experiences. Among the wide range of complications in our ChAc patients, oral problems have been very challenging to tackle. Dysarthria and dysphagia are two major problems that affect most patients. The first one disturbs social relationships, and the second one puts patients at high risk of aspiration pneumonia. In addition, dysphagia reduces food intake and the ensuing malnutrition leaves patients vulnerable to infectious and metabolic disorders. Bruxism, not uncommon in ChAc, causes early tooth flattening and decay. Lip- and tongue-biting, habitual or dystonic in nature, is not only painful and deteriorate pre-existing dysphagia, but may result in serious oral cavity infection. Finally, drooling is both embarrassing and negatively affects speech. Control of these complications with speech therapy, mouth guards, anti-cholinergic drugs, and botulinum toxin injection into salivary glands and tongue muscles are at best modestly effective. Overtreatment worsens speech and swallowing and can be dangerous. Frequent visits, gradual dose adjustments, and trials of different treatment methods are necessary factors to achieve acceptable treatment results. In this presentation, I review and share our experiences in controlling the above symptoms in Iranian patients.

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In ChAc Red Cells the Abnormally Activated Lyn Affects Ankyrin Multiprotein Complexes and is Inhibited by Dasatinib

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Chorea-acanthocytosis (ChAc) is a hereditary neurodegenerative disorder, one of the neuroacanthocytosis syndromes (NA). One of the hallmarks of NA is the presence of circulating acanthocytes, generation of which is still under investigation. Recently, we reported increased Tyrosine phosphorylation state of the red blood cell (RBC) membrane proteins from ChAc patients, related to abnormal activation of Lyn, an Src family kinase (SFKs) (Blood 118; 5652; 2011). In the context of international collaboration, we further characterized Lyn signaling pathway in RBC from ChAc patients. In ChAc RBCs, we found a weakness of ankyrin-based multiprotein complex bridging the membrane to the cytoskeleton, contributing to the generation of acanthocytes. We then evaluated the state of Lyn (active-inactive) in the cytoplasmic fraction from RBC of ChAc patients. In ChAc RBCs we found higher levels of phospho-Lyn-396, corresponding to active Lyn, compared to controls. We then evaluated whether classical Lyn inhibitors such as PP2 or Dasatinib, a pharmacological Lyn inhibitor, might block Lyn in ChAc RBCs. We found that both PP2 (0.1µM) or Dasatinib (0.1 µM) were able to efficiently inhibit Lyn in both ChAc and healthy RBCs. These data suggest

that in ChAc (i) the abnormal activation of Lyn affects RBC membrane mechanical stability weakening both multiprotein complexes bridging the membrane to the cytoskeleton; (ii) Lyn activity is inhibited by either PP2 or Dasatinib, suggesting Lyn as possible new therapeutic target in ChAc.

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* These two authors contributed equally.

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Analysis of the Effects of the Knock-Down of VPS13A in Cell Lines, IPS Cells and Primary Cultures

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Chorea-acanthocytosis is caused by loss-of-function mutations in the VPS13A gene. Functional annotation of VPS13A as a protein involved in vesicle transport and lysosomal degradation largely relies on data collected in yeast, and the mammalian VPS13A protein remains largely uncharacterized. We chose an ecotropic lentiviral knock-down approach to study VPS13A function in human cell lines and primary cells. Knock-down efficacies of different shRNA constructs were analysed by Q-PCR and Western blotting, and a reduction below 20% of control VPS13A protein levels could be obtained. The most efficient constructs were used for knock-down of VPS13A in HeLa cells and iPS cells. In HeLa cells, a distinct change in cell morphology was observed in response to VPS13A shRNA treatment. Preliminary analysis points towards problems with cellular adhesion or migration, which are observed in response to the VPS13A knock-down. An in-depth analysis of the phenotype, which fits well with previous reports on cytoskeletal abnormalities in VPS13A-deficient cells, is currently on-going.

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Multiple Functions of Vps13 in Yeast

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In yeast, *VPS13* plays roles in at least three distinct processes. First, *VPS13* is required for the trafficking of certain proteins to the vacuole, the yeast equivalent of the lysosome. Second, during the developmental process of sporulation, the *VPS13* protein translocates to the prospore membrane where it is required for proper morphogenesis of this compartment. Third, though usually dispensable for growth, *VPS13* becomes essential for cell viability in genetic backgrounds that lack en-

doplasmic reticulum/mitochondrial contact sites, suggesting a role for *VPS13* in mitochondrial homeostasis.

These three phenotypes may reflect distinct functions of *VPS13* or they may indicate a requirement for the same molecular function in different processes at different locations within the cell. In either case, these activities are separable genetically. For example, we have identified alleles of *VPS13* that are defective only in the mitochondrial function as well as yeast strains in which the *VPS13* mutant displays the mitochondrial and sporulation phenotypes, but no trafficking defect.

To better understand the molecular basis for the different roles of *VPS13*, we have begun a structure-function analysis of the protein to generate mutants defective in only one aspect of *VPS13* function and to correlate these phenotypes with changes in protein-protein interactions as determined by co-immunoprecipitation and mass spectrometry. In addition, to seek insight into what function(s) of *VPS13* is relevant to chorea-acanthocytosis, cognate mutations corresponding missense alleles found in ChAc patients have been introduced into the yeast gene. Examination of the effect of these mutations on different *VPS13* functions will be described.

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Wide Clinical Phenotype Variability of Chorea-acanthocytosis

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Chorea-acanthocytosis (ChAc) is a rare neurodegenerative disease characterized by progressive movement disorder, cognitive and behaviour changes, seizures and myopathy. The typical clinical features are limb chorea and oral dystonia. Here we report the clinical features of two brothers affected with ChAc. Their parents were healthy; however, non-specific movement disorders were reported in relatives. The 47 year-old man was affected by a mild mental retardation and a drug-resistant temporal lobe epilepsy diagnosed since he was 27 years-old. At age 45 years-old he started to develop choreic movements and dystonia affecting upper limbs and oral region. Dysarthria, dysphagia, asymmetric plastic rigidity and areflexia at upper and lower limbs were also present. Brain MRI showed mild enlargement of ventricular cavities associated with focal malacia of the right temporal lobe. A pacemaker was implanted after a cardiac arrest when he was 45 years-old. In contrast his 53 year-old sister started to complain progressive impaired gait with frequent falls and slurred speech in her 50s. In few years she developed dysphagia and progressive cognitive impairment. A neurological examination disclosed the presence of extrapyramidal signs characterised by bradykinesia and mask-like expressionless face, without involuntary movements. Brain MRI showed bilateral hyperintensities of striatum on T2-weighted images. Both patients presented acanthocytes in peripheral blood and

Western blot analysis showed reduced chorein expression. This report highlights the wide intra-familial phenotypic variability of ChAc and suggests the consideration of ChAc in the differential diagnosis of juvenile parkinsonism and temporal epilepsy with mental retardation.

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Clinical Spectrum and Differential Diagnosis of Chorea-acanthocytosis

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Chorea-acanthocytosis (ChAc) is an autosomal recessive neurodegenerative disorder due to mutation of the *VPS13A* (*CHAC*) gene on chromosome 9q21 encoding chorein. Clinical features include mixed movement disorders (chorea, dystonia with prominent orofacial involvement and self-mutilation, tics, parkinsonism, head thrusts), eye movement abnormalities suggestive of brain stem involvement, seizures, subcortical dementia and psychiatric features with impairment of frontal lobe function. Myopathy and neuropathy are often present. Neuroimaging strongly resembles that in Huntington disease. Blood tests reveal the presence of acanthocytosis in the blood smear but this may not always be detectable. Elevated creatine kinase is also typical and may be detected before the appearance of neurological symptoms or signs. Protein assays or genetic testing can confirm the diagnosis.

ChAc belongs to the group of neuroacanthocytosis syndromes alongside with X-linked McLeod syndrome, pantothenate kinase-associated neurodegeneration and Huntington disease-like 2. Furthermore, the differential diagnosis includes other forms of inherited chorea (Huntington disease, other forms of the Huntington-like disorders, other syndromes of neurodegeneration with brain iron accumulation [NBIA], Wilson disease and others), but acquired causes should also be considered.

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Involvement of Vps13 Protein in Protein Trafficking and Actin Cytoskeleton in Yeast

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Yeast *Vps13* was first discovered as a protein involved in the delivery of proteins from Golgi apparatus to the vacuole. Null mutant *vps13D* displayed defects in localization of several proteins, such as vacuolar carboxypeptidase Y and Golgi apparatus proteins *Kex2*, *Vps10* and *Ste13*. *Vps13* is also required for sporulation. However, the molecular function of *Vps13* is unknown. In humans there are four homologs of yeast *Vps13* and mutations in *VPS13A* and *VPS13B* cause the hereditary disorders chorea-acanthocytosis and Cohen syndrome, respectively.

We showed that *Vps13D* mutant has impairment of endocytosis. The

null mutant shows delay in internalization of FM4-64 lipophilic dye from the plasma membrane to vacuole, and slower growth on canavanine-containing medium, indicating defective endocytosis of Can permease. This mutant also has defects in actin cytoskeleton organization: actin patches are not well polarized and abnormal actin clumps are present in cells. Dynamic actin cytoskeleton is important for endocytosis but also in selective autophagy pathways. Selective cytoplasm-to-vacuole (Cvt) pathway was only weakly delayed, as documented by Western blot analysis of preAper maturation. Nonselective autophagy, measured by GFP-Atg8 degradation, was not defective when compared with control strain. A Vps13D strain also showed defects in vacuole staining by CMAC, a pH-sensitive dye. This mutant was impaired in vacuolar localization of Vph1 protein, the subunit of vacuolar ATP-ase, and showed slower growth on rich medium at low pH. This may indicate disturbances in pH homeostasis of cells which in turn may affect various trafficking steps.

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Neuroacanthocytosis – McLeod Syndrome

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The Hematology of McLeod Syndrome – Diagnostic and Therapeutic Challenges

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McLeod Syndrome (MLS) is one of the neuroacanthocytosis syndromes, characterized by hematological, immunohematological, neuro-psychiatric and other cardinal symptoms. The genetic defect is located on the short arm of X chromosome at position Xp21.1 (XK). Currently 27 aberrations such as single nucleotide polymorphism, stop codons, insertion/deletion mutation and splice site mutations are recognized. There is no well-defined genotype-phenotype correlation of XK mutation. Clinical manifestations are heterogeneous and variable, from asymptomatic carrier status to debilitating neuro-psychiatric and cardiodegenerative sequelae. Lead diagnostic findings are the pathognomonic aberrations of the Kell and Kx blood group antigen systems as well as red cell acanthocytosis. In addition, X-chromosomal inheritance with carrier status of the mothers of involved males is pivotal for MLS diagnosis. Immunohematological phenotyping of Kell antigens and flow-cytometric assessment of expression of Kell glycoprotein, as well as structured molecular characterization of the X-chromosomal defect, constitute the key findings for the diagnosis of MLS. When there are extended deletions of Xp21.1, involving centromeric or telomeric genes such as the *CYBB*, *RPGR*, *OTC* or *DMD* loci, the prototypic hematological findings of MLS may be dominated by the phenotypic manifestations of affected neighbouring genes of XK (contiguous gene syndrome).

Cellular and subcellular manifestation of XK mutations in hematological as well as extra-erythropoietic tissues will be reviewed. *KEL*/*XK*-KO mouse models, as well as assessment of transmembrane cation transport regulation of MLS red blood cells, may give insights into the

pathomechanism of MLS manifestation in extra-hematological organs. Clinical aspects of MLS patients, in addition to patients with X-linked CGD syndrome as examples of contiguous gene syndrome, will be presented.

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News on McLeod Syndrome

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The X-linked McLeod neuroacanthocytosis syndrome (MLS) is characterized by the association of erythrocyte acanthocytosis and progressive striatal neurodegeneration. As the autosomal recessive chorea-acanthocytosis (ChAc), MLS has a Huntington disease-like phenotype consisting of a hyperkinetic, mostly choreatic, movement disorder, psychiatric manifestations and cognitive decline, with a relentlessly progressive course over several decades. In addition, MLS patients may have a multi-system involvement including motor-dominant axonal neuropathy, myopathy and cardiomyopathy. McLeod syndrome is exceptionally rare with an estimated prevalence of less than 1 to 5 per 1,000,000 inhabitants. In the last years, it has been recognized that MLS has a worldwide distribution. MLS is caused by mutations in the XK gene. Although the association of the acanthocytic membrane abnormality with selective striatal degeneration suggests a common pathogenetic pathway, the possible mechanisms by which these mutations cause striatal neurodegeneration are still a matter of debate and research. The diagnosis of MLS involves (often negative) blood smears to detect acanthocytosis, but also the determination of serum creatine kinase (CK), which is elevated in virtually all MLS patients. Cerebral MRI may demonstrate striatal atrophy. Kell and Kx blood group antigens are reduced or absent, thus delivering an accurate diagnosis of MLS. Identification of a distinct mutation in the XK gene is confirmatory. The course of McLeod syndrome is relentlessly progressive, and there is no curative therapy yet known. However, regular cardiologic studies and avoidance of transfusion complications are mandatory. The hyperkinetic movement disorder may be treated as in Huntington disease. Other symptoms including psychiatric manifestations should be managed in a symptom-oriented manner.

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Alterations in Magnesium and Potassium but not Sodium Transporters Characterize McLeod Syndrome Erythrocytes

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search Institute of the New York Blood Center, New York, NY, USA

We have recently reported a novel alteration in erythrocyte Mg^{2+} and K^+ homeostasis in cells from Xk knockout mice, a model of McLeod Syndrome (MLS). We now show that in an untreated, asymptomatic MLS subject alterations in cellular ion content were associated with changes in the activity of 4 major cation transporters in erythrocytes. There was an 18% increase in erythrocyte K^+ (245.3-298.5 mmol/Kg Hb), Mg^{2+} (5.6-7.5 mmol/Kg Hb) and total Ca^{2+} levels (0.29-0.52 mmol/Kg Hb) when compared to control cells, suggesting altered ion homeostasis. Consistent with this hypothesis, Na^+ -independent Mg^{2+} permeability (2.0 to 0.6 mmol/ 10^{13} cell x h) was significantly reduced, implying an abnormality in Mg handling. We also observed that Na^+ /Mg exchanger (NME) activity was significantly decreased. It has been shown that Kell/XK complex are phosphorylated by Casein kinase II (CKII). We observed that TBB (4,5,6,7-tetrabromobenzotriazole), a specific CKII blocker, did not block Big ET-3-stimulated NME activity in Xk-mutated erythrocytes, unlike in control cells. These results suggest that alterations in CKII signaling mediate disordered Mg levels in MLS. K^+ transport mediated by the Gardos channel was increased when compared to controls (18 to 26.8 mmol/ 10^{13} cell x h). In addition, the volume-stimulated and Cl independent K/Cl co-transporter was almost two-fold lower when compared to controls. These results are in agreement with a higher K^+ content in these cells. In contrast, no differences in Na^+ transporter activities were observed. Thus, our results provide novel insights into potential mechanisms by which alterations in Kx protein lead to abnormalities in erythrocytes transporters, which may play critical roles in acanthocytosis development.

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NBIA – Pantothenate Kinase-Associated Neurodegeneration

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Best Practices in the Care and Management of People with NBIA: Development of a Guideline for PKAN

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Introduction: Pantothenate kinase-associated neurodegeneration (PKAN) is the major genetic subtype of Neurodegeneration with Brain Iron Accumulation (NBIA), accounting for 35-50% of cases. The progressive nature and intractable dystonia in this ultra-rare disorder make it a challenge to manage. To date, there is no comprehensive guideline for the diagnosis and management of these patients.

Aims and Methods: We aimed to develop a best practice guideline for PKAN, leveraging the wisdom of a small pool of experts, to facilitate holistic management. Our team comprised multidisciplinary professionals (neurologists, geneticists, surgeons, genetic counsellors, therapists and others) and family members with practical PKAN expertise. These experts drew on their direct experiences and trials and errors with PKAN to address key issues. A detailed PubMed search was also utilised to identify and review all relevant published data. The team developed, drafted, and prepared a consensus guideline for publication.

Results: Management of PKAN patients is complex and needs to be tailored from diagnosis to end-stage disease. Our guideline addressed these relevant issues:

- Diagnostic evaluation
- Initial management (medical, genetic implications, psychosocial)
- Pharmacologic and surgical management
- Monitoring for disease complications
- Emergency management
- Educational management
- Nutrition
- Psychosocial support

Conclusion: A comprehensive document outlining the care and management of patients with PKAN benefits the entire NBIA community. This guideline not only serves to support new families as they are diagnosed, but also provides clear guidance to physicians and allied health-care professionals. The experience we gain will inform the development of similar guidelines for other forms of NBIA.

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Overview of the NBIA Disorders - Neurodegeneration with Brain Iron Accumulation

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Neurodegeneration with brain iron accumulation comprises a clinically and genetically heterogeneous group of disorders. The NBIA disorders are recognizable by their clinical and radiographic features, which include changes arising from the accumulation of iron in the basal ganglia. As the genetic bases of these disorders have been identified, a unifying view of disease pathophysiology is becoming clear. While therapeutics for the NBIA disorders still focus on symptom management, the era of rational therapeutics to target the primary disease processes is within our view. This session will focus on the group of NBIA disorders, their clinical and MRI features, the genes and proteins that underlie them, and the diagnostic approach, which will highlight distinguishing features of each of these disorders.

NBIA Disorder	Key Clinical Features	Key MRI Features
PKAN	dystonia, pigmentary retinopathy, parkinsonism, acanthocytosis	T2 GP central hyperintensity with surrounding hypointensity – 'eye of tiger' sign
PLAN	psychomotor regression, dystonia, parkinsonism, ataxia, optic atrophy	Cerebellar atrophy, GP and SN T2 hypointensity later childhood or adulthood
MPAN	spasticity, dystonia, peripheral neuropathy, seizures	GP and SNT2 hypointensity
BPAN	developmental disability, seizures, sleep problems, young-onset parkinsonism	GP and SNT2 hypointensity; SNT1 hyperintense 'halo'

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News on TIRCON

T. Klopstock

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TIRCON (Treat Iron-Related Childhood-Onset Neurodegeneration) is an international collaborative project funded by the European Commission's Seventh Framework Programme and coordinated at the Ludwig-Maximilians-Universität of Munich, Germany. TIRCON aims at improving medical care, research infrastructure and therapeutic options for neurodegeneration with brain iron accumulation (NBIA), a group of rare hereditary neurodegenerative disorders characterized by high levels of brain iron. The most common form, accounting for approximately 50% of NBIA cases, is pantothenate kinase-associated neurodegeneration (PKAN). Currently, there is no proven therapy to halt or reverse PKAN or any other form of NBIA. This is especially unfortunate as both the iron accumulation in NBIA and the biochemical defect in PKAN are predicted to be amenable to drug-based treatment.

TIRCON, for the first time, addresses this urgent and unmet need for a therapy for NBIA/PKAN by conducting a randomized placebo-controlled clinical trial of the iron chelator deferiprone in PKAN (duration per patient 18 months). The four study sites in Munich (Germany), Oakland (USA), Milan (Italy) and Newcastle (UK) have (as of November 2014) already randomized more than 60 PKAN patients to this pivotal trial, approaching the planned number of 90 patients. Recruitment will be completed until February 2015, and the last patient visit is therefore scheduled for August 2016. An extension trial, sponsored by the manufacturing company ApoPharma, has already started for the patients who completed the randomized trial. Regular meetings of the Data Safety Monitoring Board identified no major safety issues so far and unequivocally recommended continuation of the trial.

Another TIRCON work-package pursues essential preclinical work on pantethine and pantethine derivatives to treat PKAN. It has been shown in *Drosophila* that pantethine acts as an alternative precursor for CoA synthesis bypassing PANK2, the defective enzyme in PKAN, and leads to rescue of neurodegeneration and increase in life span. TIRCON is working on enhanced compounds and investigates their efficacy in mammalian models. This approach will lead to a firm conclusion of

whether it is worthwhile to develop pantethine or pantethine-derivatives into an established drug that could be used alone or in combination with deferiprone to treat PKAN.

The current lack of controlled clinical trials in NBIA is due to the rarity and heterogeneity of these disorders and to previous fragmentation of research efforts. These limitations are most effectively overcome by an international patient registry. To this end, TIRCON has set up a harmonized international patient registry and biomaterial bank for all forms of NBIA to allow for natural history studies and biomarker development, two critical needs in the NBIA research community. As of November 2014, more than 100 NBIA patients have been enrolled prospectively into the registry, and one-year follow-up data are currently available in 25 of them. In addition, retrospective data have been entered from more than 50 NBIA patients.

In conclusion, TIRCON has already succeeded in bringing together into one cohesive group the existing outstanding but scattered expertise in NBIA research and care at the European and international level. Medical care and research infrastructure have already noticeably improved, and the NBIA community is eagerly awaiting the outcome of the therapy research outlined above.

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Synthesis and Pharmacokinetic Properties of 4-Thiobutyltriphenylphosphonium Pantetheine, a Novel Pantetheine Derivative

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It has been shown that pantethine, the disulphide of pantetheine, rescues a *Drosophila* model of pantothenate kinase-associated neurodegeneration (PKAN). However, the pharmacology of pantethine is known to be incompatible with its use as a possible replacement therapeutic for this neurodegenerative disease: the compound is known to be unstable in serum and is unlikely to permeate the blood-brain barrier. In an attempt to overcome these pharmacological difficulties, we successfully synthesized 4-thiobutyltriphenylphosphonium pantetheine (TBTP-pantetheine), a pantetheine derivative, and compared its serum stability and pharmacological properties to those of pantethine.

It is shown that TBTP-pantetheine is stable under aqueous conditions; however, the compound is enzymatically degraded in fetal calf serum at a higher rate than its parent compound pantethine. In a PAMPA system (Parallel Artificial Membrane Permeability Assay, an *in vitro* setup to determine passive membrane transport), TBTP-pantetheine shows increased lipophilicity by a significantly higher tendency to associate with the membrane; however, permeability coefficients are not significantly different. We conclude that TBTP-pantetheine is unable to overcome the pharmacological hurdles emerging from a pantethine-based thera-

py for PKAN. Nevertheless, this research shows that pharmacological properties can be altered by derivatization of the parent molecule pantothenine thus strengthening the rationale to synthesize and test novel derivatives of pantothenine and related molecules within TIRCON, in order to facilitate a possible replacement strategy in the future.

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Pantothenate Kinase-Associated Neurodegeneration: Clinical Assessment and Genetic Characterization by Means of a Spanish Multi-Centre Research Network

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Background: Pantothenate kinase associated neurodegeneration (PKAN) is the most common defect causing NBIA in children. Despite advances in the research of novel therapies for PKAN, validated international clinical rating scales to be used in clinical trials are lacking, and significant gaps concerning phenotype-genotype correlations and disease progression persist.

Main Objective: We aimed to identify and genetically characterize the Spanish population with NBIA and to design and validate a quantitative method for clinical assessment of PKAN patients.

Methodology: We performed a cross-sectional multi-centre study, with NBIA patient recruitment through professional associations. Design of a Disease Rating Scale for PKAN (PKAN-DRS) including four subscales: cognitive, behavioral, physical, and functional assessment. Items were scored from 0 (normal) to 3 or 4 (maximum severity), (total scores 0 (no disease) to 140 (maximum severity)). For validity and reliability assessment, four independent examiners rated PKAN patients using recorded videotapes. Sanger sequencing of NBIA genes was performed in undiagnosed NBIA families.

Results: To date, 37 NBIA patients (mean age 20 years, range 3-52; 13 males) have been identified at 15 centres in Spain: PKAN (N=22); PLAN (N=8); undiagnosed NBIA (N=7). The PKAN-DRS was applied to 10 PKAN patients (mean age 29, 5 years, range 14-53), total scores ranging from 35 to 67. Physical scores positively correlated with functional scores, and age at disease onset inversely correlated with total scores. The c.1583C>T mutation in the PANK2 gene was found in homozygosis in 4 gypsy patients from two consanguineous families from Northern Spain, suggesting a common origin for this nucleotide change.

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Targeting Brain Iron in Neurodegenerative Disease

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Perturbation of iron homeostasis and accumulation of iron in specific regions of the brain play an important role in neurodegenerative processes in all forms of NBIA, Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and others. Iron deposition, whether a primary or secondary pathological event, may underlie or contribute to progression of neurodegeneration by promoting generation of reactive oxygen species (ROS), leading to oxidative cell damage and brain inflammation.

ApoPharma's marketed iron chelator, deferiprone, is a brain-permeable 3,4-hydroxypyridinone (HP) with favourable activity in multiple animal models of neurodegenerative disease. Deferiprone has recently been shown to be effective in clinical trials in PD, and is being studied in pantothenate kinase-associated neurodegeneration (PKAN) and ALS patients. We have now developed a series of new HP iron chelators with improved properties. Model HPs penetrate the blood-brain barrier, cells and subcellular organelles, and selectively chelate Fe(III) with an affinity that facilitates transfer of iron to endogenous acceptors for reuse, avoiding significant systemic iron depletion.

Using cellular and animal models of neurological cell protection, PD and AD, and an ataxic ceruloplasmin-hephestin double knockout (DKO) mouse, we have demonstrated the efficacy of selected HPs in alleviating brain iron deposition, neuroinflammation, and ROS-related cellular damage. Significant effects on disease-related endpoints included improvement of cognitive function in mouse AD and motor function in rat PD models, and prevention of ataxia in the DKO mouse. These findings may be generalized to other neurodegenerative disorders in which brain iron accumulation leads or contributes to disease pathology.

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Mitochondrial Dysfunction in Neurodegeneration with Brain Iron Accumulation (NBIA)

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Pantothenate kinase 2 catalyzes the key, rate-limiting step in coenzyme A biosynthesis, therefore mutations in the gene encoding this enzyme have the potential to disrupt a number of metabolic processes by disrupting cellular CoA pools. Elevations in lactate suggest that mutations in this mitochondrial enzyme could cause general mitochondrial dysfunction.

Particularly relevant for neurodegenerative processes is the relationship between mitochondria and iron. The mitochondrion upholds the synthesis of iron–sulfur clusters and heme, therefore a fraction of incoming iron must go through this organelle before reaching its final destination. In turn, the mitochondrial respiratory chain is the source of reactive oxygen species (ROS) derived from leaks in the electron transport chain. The co-existence of both iron and ROS in the secluded space of the mitochondrion makes this organelle particularly prone to hydroxyl radical-mediated damage. Additionally, mitochondrial dysfunction and ROS production promotes the selective degradation of damaged mitochondria by autophagy which must be associated to increased mitochondrial biogenesis for compensating the loss of mitochondrial mass.

Our project aims to understand the molecular mechanism and modulation of mitophagy and mitochondrial biogenesis in cellular models of NBIA.

1) Characterization of iron metabolism alterations, mitochondrial dysfunction and the molecular mechanisms involved in mitophagy activation in fibroblasts harbouring PANK2 mutations.

2) Molecular analysis of pathways involved in mitochondrial biogenesis as a compensatory mechanism in response to mitophagy activation in fibroblasts harbouring PANK2 mutations.

3) Screening of modulators of autophagy and mitochondrial biogenesis from commercial libraries capable of restoring the physiopathological alterations in PANK2 mutant fibroblasts.

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Expression and Functional Analysis of the Zebrafish *Pank2* Gene: A Possible Approach for the Study of PKAN, a Human NBIA

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PKAN is the most common form of Neurodegeneration with Brain Iron Accumulation (NBIA); it is associated with mutations in the *Pank2* gene, coding for the rate limiting enzyme in CoA biosynthesis. We started to explore *Pank2* biological function in *Danio rerio*, an excellent vertebrate system to study developmental biology, gene function and to model human diseases. A bioinformatic analysis revealed the presence of a single *pank2* gene on chromosome 13, with three different transcripts. The longest one encodes for a putative protein of 437 aminoacids with a good homology (65%) to the human counterpart. A reciprocal BLAST approach supports the hypothesis that the gene represents the *Danio rerio* ortholog of *hPank2*. This is further confirmed by the presence of synteny between the chromosomal regions in the two species. Contrary to the human ortholog that resides in the mitochondria, the zebrafish protein shows prevalent localization in the cytosol when expressed in mammalian cells. qRT-PCR analysis revealed that the gene is expressed in all developmental stages, including the earliest

ones, while in the adult organs it is highly represented in brain. The in situ hybridization technique confirmed the qRT-PCR results, showing high expression in different brain structures and also in the vascular system. Preliminary results, obtained by the microinjection of a specific splicing-morpholino, revealed the presence of severe perturbation in the development of the nervous and vascular system at 24 and 48 hpf. The animal model could provide relevant information about the function of *Pank2* and the mechanisms underpinning PKAN.

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NBIA – Other

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Neurodegeneration with Brain Iron Accumulation (NBIA): Insights from Gene Co-Expression Networks

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Neurodegeneration with brain iron accumulation (NBIA) constitutes a group of neurodegenerative diseases characterized by a prominent extrapyramidal movement disorder, intellectual deterioration and characteristic iron deposition in the basal ganglia. Ten genes have been identified so far, but limited information exists regarding expression and function of these genes within the human brain.

To address possible relationships between known NBIA genes, predict their functions, and identify overlapping pathways, we used a systems-biology approach based on whole transcriptome gene expression analysis. As part of the UK Human Brain Expression Consortium (UKBEC), we analysed the expression profiles of 101 neuropathologically normal individuals (10 brain regions each). Weighted gene co-expression network analysis (WGCNA) was used to cluster genes into co-expression modules. The overrepresentation of NBIA transcripts in basal ganglia modules (substantia nigra and putamen studied) was assessed.

Six modules containing NBIA genes were found for the substantia nigra, but there was no evidence of significant overrepresentation. Two putamen modules were significantly enriched for NBIA transcripts, namely the brown (PANK2, ATP13A2, C19orf12, COASY; P= 0.003) and green (FTL, DCAF17, FA2H; P= 0.021) modules. Enrichment analysis of these two putamen modules revealed an overrepresentation of gene ontology terms and KEGG pathways, including: brown - synaptic vesicle endocytosis and axon cargo transport (biological processes), synaptic membrane (cellular component), and synaptic vesicle cycle KEGG pathway; green - ensheathment of neurons, neuronal action potential and oligodendrocyte development (biological processes), myelin sheath (cellular component), and cadherin binding (molecular function). Our data suggests shared processes and pathways in NBIA gene networks.

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NBIA Alliance

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The NBIA Alliance is the informal international umbrella organization for NBIA lay advocacy groups. Launched in 2012 under the patronage of the European Union Seventh Framework Programme (FP7 EU) project TIRCON (Treat Iron-Related Childhood-Onset Neurodegeneration), the NBIA Alliance's main objectives are to increase awareness of NBIA and to support cooperative research into NBIA Disorders. As an interface and contact point for all people in public and health policy with a special interest in NBIA, the NBIA Alliance and its members are bringing together patients, clinicians, researchers, and further stakeholders. The NBIA Alliance comprises 6 legal entities in France, Germany, Italy, the Netherlands, Spain, and the United States, and one patient group still under development in the United Kingdom. New NBIA advocacy groups are just emerging in Argentina and Canada.

The long-standing groups, Hoffnungsbaum e.V. in Germany and the NBIA Disorders Association in the U.S., are partners in TIRCON and have been fostering the establishment of further national NBIA lay advocacy groups and of the international NBIA Alliance. Herein these advocacy groups that are lesser-known to the international NBIA community introduce themselves and describe their impact on NBIA research. AISNAF (Associazione Italiana Sindromi Neurodegenerative da Accumulo di Ferro) was established in 2006 as the third NBIA patient organization. It is a contact point for NBIA families in Italy, has raised awareness on NBIA and co-funded scientific symposia as well as NBIA research projects. It aims to help harmonize and spread clinical best practices in Italy.

The French AIDNAI (Association Internationale de Dystrophie Neuro Axonale Infantile), launched in 2000 as a patient organization for NBIA subtype PLAN, supported research in PLAN, and extended its scope to all NBIA Disorders when becoming member of the NBIA Alliance in 2012.

Inspired by an NBIA Alliance meeting at the 2nd joint NA/NBIA symposium in Ede (the Netherlands) in October 2012, the Dutch Stichting IJzersterk was established in 2013. Using social media as an additional platform resulted in contact with 10 patient families within a year and fostered successful fundraisers for NBIA research.

The establishment of the Spanish ENACH Asociación (Asociación de Enfermedades Neurodegenerativas por Acumulación Cerebral de Hierro) in 2013, following the set-up of the NBIA Alliance, is focused on gaining visibility and extensive networking among NBIA families, researchers and physicians. A Scientific and Medical Advisory Board is already in place. Identifying a large patient cohort in Spain, the involvement of 15 hospitals, setting up a research infrastructure and 4 ongoing

research projects have been achieved within less than 2 years.

Additionally, NBIA UK, NBIA Canada and an Argentinian patient representative have taken their first steps to setup organizations or to join the NBIA community.

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The Launch of the International Umbrella Organization NBIA Alliance in Relation to the EU-Funded FP7 Project TIRCON

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Neurodegeneration with Brain Iron Accumulation (NBIA) is a group of ultra-rare neurological movement disorders characterized by high iron levels in the basal ganglia and a progressive degeneration of the nervous system. Currently, there is no cure or proven therapy for any of the NBIA forms.

Due to the lack of treatment options, the international European Union Seventh Framework Programme (FP7 EU) consortium TIRCON (Treat-Iron-Related-Childhood-Onset-Neurodegeneration) is dedicated to the investigation of NBIA. 13 partners from eight countries are conducting a clinical therapy study and set up a patient registry/ biobank in order to improve the infrastructure for NBIA research and care.

The lay advocacy groups, NBIA Disorders Association (NBIADA, USA) and Hoffnungsbaum e.V. (HoBa, Germany), are full partners in TIRCON. Based upon their experience in such collaborative projects, NBIADA and HoBa have been promoting the creation of further patient groups worldwide. The NBIA Alliance, launched during the TIRCON kickoff meeting in 2012, is the first informal international umbrella association for NBIA lay advocacy groups. Currently, seven NBIA patient organizations are involved in the Alliance. Its main objectives are to increase awareness for NBIA among the health care community by translating scientific knowledge into patient-friendly information and to support cooperative research. The NBIA Alliance is an information platform and a contact point for patients and clinicians where dedicated structures are not yet in place. The poster presents the launch, development, goals, activities, constraints and future options of the NBIA Alliance. It will underline the mutual empowerment of the NBIA patients' movement and the NBIA research and care community in TIRCON.

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Exploring Novel Therapeutic Strategies for PLA2G6-Associated Neurodegeneration (PLAN)

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Neurodegeneration with brain iron accumulation (NBIA) comprises a heterogeneous group of disorders with radiologically discernible brain iron. Phospholipase-associated neurodegeneration (PLAN) is the second most common subtype, accounting overall for 20% patients with NBIA. Three clinical subtypes are recognised, including classical infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (NAD) and PLA2G6-related dystonia-parkinsonism.

It is well recognized that PLAN is a devastating condition with an aggressive and relentless disease course. Children with INAD have progressive cognitive and motor difficulties, culminating in significant morbidity and reduced life expectancy. Currently, there are no disease-modifying agents to halt the progression of this disorder, let alone a cure, and therefore all available treatments for PLAN currently focus on symptom palliation to maximize quality of life.

At Great Ormond Street Hospital, we provide a tertiary service for many children with PLAN. I will discuss our current rationale for treatment strategies in PLAN, namely the management of spasticity, dystonia and secondary complications. I will also present data regarding the first reported use of deep brain stimulation in atypical NAD.

The paucity of disease-ameliorating treatments in PLAN has driven us to seek novel therapeutic strategies, and to that aim, we are currently undertaking a proof-of-concept gene therapy study in the INAD mouse model. This murine model recapitulates many of the clinical and histopathological features of the human phenotype, thereby rendering the model ideal for our study project. We will perform systemic intravenous delivery of an AAV9 viral vector to deliver therapeutic PLA2G6 gene to the INAD mouse model with the aim of rescuing and/or preventing neurodegenerative symptoms. Our ultimate long-term aim is to translate this work to clinical trials for our patients with INAD and other forms of PLAN.

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The UK Genotypic and Phenotypic Spectrum of Childhood Neurodegeneration with Brain Iron Accumulation

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Background: Neurodegeneration with brain iron accumulation (NBIA)

comprises a clinically and genetically heterogeneous group of progressive disorders characterised by high brain iron. Different genetic forms of NBIA encountered in childhood and early adolescence have been reported including pantothenate kinase-associated neurodegeneration (PKAN), PLA2G6-associated neurodegeneration (PLAN), mitochondrial membrane protein-associated neurodegeneration (MPAN), fatty acid hydroxylase-associated neurodegeneration (FAHN), beta-propeller protein-associated neurodegeneration (BPAN) and COASY protein-associated neurodegeneration (CoPAN). To further define this group of disorders, we describe the clinical phenotypes and associated genotypes in our cohort of UK childhood-onset NBIA patients.

Methods: We undertook (i) clinical assessment and/or detailed analysis of the clinical notes and (ii) mutational analysis for relevant candidate genes.

Results: We studied 37 childhood-onset cases (5 familial and 26 sporadic) and confirmed a genetic diagnosis of NBIA in 19 (51.4%) patients (3 PKAN, 6 PLAN, 1 MPAN, 6 BPAN, 3 patients with a novel unpublished gene defect). Beside the classical progressive motor symptoms and cognitive regression, many patients had additional extrapyramidal features, ocular findings and neuropsychiatric features. We also identified unusual/rare clinical features in classical disease (e.g. absent eye-of-the-tiger sign in PKAN) and a number of atypical disease presentations (e.g. atypical PLAN, early recognition of BPAN in infancy).

Conclusion: This review highlights that NBIA is both clinically and genetically heterogeneous, and for many cases in the UK the genetic aetiology is yet to be discovered. Further gene identification will significantly improve the understanding of disease mechanisms as well as identifying novel therapeutic targets for these neurodegenerative disorders.

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Decreased Brain Transcription of a Subset of NBIA-Related Genes with Possible Roles in Myelin Insights from an Hfe^{-/-}-Xtfr2^{mut} Mouse Model of Brain Iron Loading

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We have studied brain iron loading and related changes in a mouse model with mutations in the iron-regulatory genes Hfe and transferrin receptor 2 (Tfr2) and without known mutations in NBIA-related genes. Brain iron and ferritin levels were increased (>1.7-fold, p<0.025, n≥5 mice/group) and iron distribution altered in male Hfe^{-/-}-xTfr2^{mut} AKR mice at 12 weeks of age after 3 weeks on a high iron diet (2% carbonyl

iron) compared to age- and gender-matched controls. Methods used included inductively coupled-atomic emission spectroscopy, non-heme iron assay, ferritin immunoblotting, micro X-ray fluorescence mapping of metal distribution and micro X-ray absorption near edge spectroscopy analysis of iron species.

Brain RNA arrays revealed transcript decreases ($p < 0.05$) for five NBIA-related genes: phospholipase A2, group VI (PLA2G6, 1.7-fold), fatty acid 2-hydroxylase (Fa2h, 1.4-fold), chromosome 19 open reading frame 12 (C19orf12, 1.3-fold), ATPase13A2 (1.2-fold) and ceruloplasmin (Cp, 1.2-fold). Four other NBIA-related transcripts for pantothenate kinase 2 (PANK2) and CoA synthase (COASY), both involved in CoA metabolism, WD-repeat domain 45 (implicated in autophagy) and the nuclear transmembrane protein DCAF17 were unchanged. Aside from the ferroxidase Cp, transcripts showing changes are distinguished by having myelin-related roles. At least 6 other myelin-related transcripts also decreased ($p < 0.05$; ≥ 1.2 -fold).

These findings provide evidence that excess iron loading can cause decreases in the expression of a subset of the genes implicated in NBIA, notably those genes which may also have roles pertaining to myelin. Iron excess may therefore cause changes which resemble, although to a lesser degree, the effects of pathogenic, loss-of-function, NBIA-causing mutations in these genes.

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Resolution of an Undefined Case of NBIA with Miseq Technology

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Neurodegeneration with brain iron accumulation (NBIA) includes a clinically and genetically heterogeneous group of disorders with progressive extrapyramidal signs and neurological deterioration, characterized by iron accumulation in the basal ganglia. In our biobank, which is part of the Telethon Biobank Network, a considerable number of clinically associated NBIA cases without a genetic diagnosis are stored. In order to characterize this group we are studying our cohort of patients by the TruSeq Custom Amplicon panel (MiSeq Illumina platform) formed by 16 NBIA-associated genes. We screened, using Sanger sequencing, one of three affected sisters for the most commonly NBIA-associated genes, identifying a heterozygous mutation in PANK2 (p.Gly521Arg), that was shared by the other two sisters. MLPA analysis ruled out exon rearrangements on the other allele. In order to extend the genetic screening, we sequenced the NBIA panel for one of these two sisters, discovering an additional mutation in PANK2 (p.Val517Met), that was absent in the first sister analyzed but present in the third sister.

Reviewing the sisters' clinical history we observed differences between one sister and the other two with respect to age of onset and type of symptoms, differences that segregated appropriately with the different

genetic findings in PANK2.

The sister carrying only the p. Gly521Arg is likely to be affected by a different disease and show a different phenotype due to the combined effect of the mutation and unknown susceptibility factors. The sequencing of the NBIA panel for this sister could be a powerful approach to complete the molecular diagnosis.

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Iron Metabolism in the Central Nervous System and Implications for Human Disease

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Abnormal accumulation of brain iron has been detected in various neurodegenerative diseases, but the contribution of iron overload to pathology remains unclear. In a group of distinctive brain iron overload diseases known as Neurodegeneration with Brain Iron Accumulation (NBIA), nine disease genes have been identified. In the NBIA, brain iron accumulation was observed with MRI and at autopsy in the globus pallidus and other brain regions in patients and was associated with severe dystonia and gait abnormalities. Only two of these diseases, aceruloplasminemia and neuroferritinopathy, are directly caused by abnormalities in iron metabolism, which affect mainly astrocytes in the former and neurons in the latter. Understanding the early molecular pathophysiology of these diseases should provide insights into the role of iron and to the design of specific therapeutic approaches.

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Drosophila as a Tool to Identify Treatment Options for Pantothenate Kinase-Associated Neurodegeneration

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Pantothenate kinase-associated neurodegeneration (PKAN) is a rare inherited disease characterized by progressive movement impairment and iron accumulation in specific areas of the brain. Currently there are no treatment options for this devastating autosomal recessive disorder. Affected individuals carry mutations in pantothenate kinase, the first enzyme required to synthesize Coenzyme A, suggesting that impaired metabolism of Coenzyme A is one of the causes of the disease. This is further underscored by the recent finding that patients with mutations in Coenzyme A synthase, the last enzyme of the Coenzyme A biosynthesis pathway suffer from comparable symptoms as PKAN patients. Coenzyme A is an essential metabolic cofactor required for over 100 metabolic reactions. Why mutations in genes coding for Coenzyme A biosynthesis enzymes induce neurodegeneration is currently not clear.

We and others have investigated *Drosophila* neurodegenerative dPANK/fumble mutants and demonstrated that these mutants can be used to understand basic mechanisms behind PKAN and to identify rescuing compounds which can be further explored for their use as possible treatment options. We have identified various promising rescuing molecules able to rescue the observed abnormalities in dPANK/fumble mutants. Embedded within an international multidisciplinary consortium (TIRCON) we are aiming to understand the rescue potential of the compounds. The valuable contribution of *Drosophila* to develop possible therapies for PKAN will be discussed.

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Other

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VPS13C Mutations Associate with Frontotemporal Lobar Degeneration and Decreased Protein Expression

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Frontotemporal lobar degeneration (FTLD) represents a group of neurodegenerative dementias mainly affecting people younger than 65 years of age. Up to 50% of patients have a family history of dementia and in most cases familial segregation is compatible with autosomal dominant inheritance. Although mutations in seven genes have been identified, the genetic cause remains unknown in about 60% of familial FTLD. We aimed to find novel FTLD genes in a whole genome sequencing approach in 13 unrelated familial patients and three relatives affected with FTLD, selected from a Belgian cohort of 590 FTLD patients. The genome sequences were annotated using GenomeComb and variants were filtered and prioritized using multiple genetic and functional criteria. Analysis of selected variants in all FTLD patients and 1314 matched controls revealed the presence of two novel coding missense variants (p.W395C and p.A444P) in the vacuolar protein sorting 13 homolog C gene (*VPS13C*). Further screening of the 86 coding exons of *VPS13C* in the Belgian FTLD cohort resulted in the identification of 18 additional mutations that were absent in the control group. The overall mutation frequency was 4.1% (24 of 590 patients). Immunocytochemistry of lymphoblast cells of five patients with a *VPS13C* mutation suggested a 44-80% decreased expression ($p < 0.001$) of *VPS13C* protein compared to mutation-negative controls ($n=4$), while mRNA expression was not altered. Extended genetic, neuropathological and cell biological studies

are needed to support the role of *VPS13C* in FTLD.

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Approaching Our Goals Over Two Decades: A Summary of the 7th Neuroacanthocytosis Symposium

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From their start in 2002, and until very recently, the international neuroacanthocytosis (NA) symposia reported little tangible progress with respect to understanding the specific disease mechanisms that we anticipate will eventually form the basis of causal treatment. Our association with colleagues from the neurodegeneration with brain iron accumulation (NBIA) field was not only a strategic alliance within the rare disease field but also a stimulus to speed the pace of discovery, 20 and 13 years, respectively, after the discovery of the genes for McLeod syndrome (MLS: XK, encoding for Xk, identified in 1994) and chorea-acanthocytosis (ChAc *VPS13A*, encoding for chorein, identified in 2001).

The small, close NA community, organized around the Advocacy for Neuroacanthocytosis Patients (www.naadvocacy.org), had been successful in teaching our clinical colleagues about the NA syndromes, offering the free Western blot testing service to facilitate diagnosis of ChAc (instructions: www.euro-hd.net/html/na/diseases/chac), and providing information to fellow scientists. However, only with the European Multidisciplinary Initiative on Neuroacanthocytosis (EMINA) which terminated in early 2014, and its currently active successor, EMINA-2, have new basic science discoveries come at a quicker pace. The most recent development is the very welcome interest by the European collaboration program in science and technology (COST) and its “proteostasis action” (proteostasis-2014.cipf.es).

The current NA symposium presented a number of potential ChAc models, in yeast, slime mould, *drosophila*, and human induced pluripotent stem cells. New insights were presented into the roles of *VPS13A* and chorein, in particular a possible association with mitochondrial function. Important progress was also reported on *VPS13B* and *VPS13C* function, the first causing Cohen syndrome (a progressive disease only with respect to retinopathy), the latter linked to a frontotemporal dementia syndrome. *VPS13D* still seems enigmatic. Little progress has been reported in MLS, which much rarer than ChAc. Huntington disease-like type 2 (HDL-2) was not covered at the current meeting.

There has been great progress in the NBIA syndromes, especially in pantothenate kinase-associated neurodegeneration (PKAN), with the identification of new syndromes caused by novel genes, with significant progress in disease models, and with treatment being offered in the TIRCON project. Given the new genetic data and the well-organized patient cohorts with NBIA syndromes, the relationship of PKAN and the other syndromes to acanthocytosis may be further elucidated once blood smears and acanthocyte counts are systematically performed in

these subjects. These findings will also inform us about the future utility of NA and NBIA cooperation.

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Functional Studies of the Cohen Syndrome-Associated Protein VPS13B (COH1)

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Cohen syndrome is an autosomal recessive disorder caused by mutations in the gene VPS13B (COH1). Prominent clinical features are intellectual disability, postnatal microcephaly, pigmentary retinopathy, and intermittent neutropenia. However, the biochemical characteristics, cellular localization, or functional role of the encoded protein COH1 remain largely unidentified. Our cell biological analysis showed localization of COH1 to the Golgi complex. Further biochemical analysis demonstrated that COH1 is a peripheral membrane protein similar to its remote yeast homologue Vps13p. Vps13p regulates anterograde and retrograde vesicular transport of transmembrane proteins between the prevacuolar compartment and the trans-Golgi network. We found that loss of COH1 impairs the ability of the Golgi complex to (re)assemble and thus induces fragmentation into mini-stacks. Furthermore, COH1 regulates the formation of Golgi-derived membrane tubules consistent with its likely function in intracellular trafficking. In summary, our study identifies COH1 as a Golgi matrix protein required for maintaining Golgi integrity and function. Another study showed that disturbed Golgi complex homeostasis upon loss of COH1 causes major defects of glycan maturation. Experiments blocking secretion using Brefeldin A identified no major intracellular retrograde trafficking defects. However, COH1-deficient cells display a reduced amount of early endosomes and abnormally enlarged lysosomes, pointing to a role of COH1 in endosomal-lysosomal trafficking. Together, these results provide an improved insight into the molecular function of COH1 in Golgi-associated intracellular trafficking, which in the future may help to unravel its role in brain development, neuronal function, and general pathology in patients with Cohen syndrome.

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Diagnosis and Treatment of Chorea and Dystonia

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Chorea and dystonia are hyperkinetic movements which can be seen in a number of basal ganglia neurodegenerative disorders. These movements can often coexist - it is important to properly identify the phenomenology as the underlying mechanisms are likely to be distinct, and different therapies may be indicated. Both types of involuntary movements can be caused by a large number of disorders, including pharmacologic, structural, metabolic, genetic and degenerative etiologies. Features of the history, including family history, medical history, disease onset, and progression, inform the diagnosis. These features guide diagnostic testing which often includes brain imaging, and laboratory testing for potentially reversible etiologies and informative biomarkers. New technologies are permitting us to identify causative genes in rare disorders and expanding the phenotype of known disorders. Therapy for genetic diseases is in its infancy, and has not yet been successfully employed for diseases affecting the central nervous system. Despite this, it is essential to pursue the diagnosis in order to provide genetic counseling, guidance regarding future disease progression, and to expand our knowledge of rare neurodegenerative disorders. Current treatments are focused upon reducing symptoms and maintaining and optimizing function. This can be optimally accomplished using a multidisciplinary team approach. Pharmacologic and focal neurotoxin injections can help reduce involuntary movements, but are likely to be more effective in combination with other modalities, such as speech, occupational, and physical, therapies. Deep brain stimulation likely has some utility, but therapeutic goals and potential benefits should be clearly identified before performing an invasive procedure in progressive, neurodegenerative disorders.

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