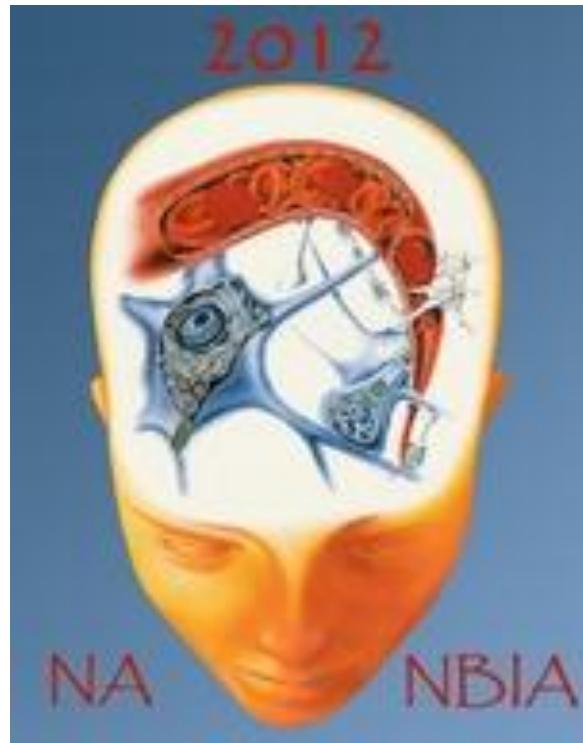


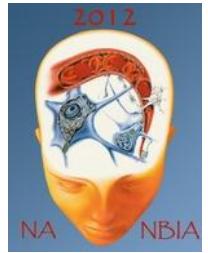
# **New Breakthroughs in NA/NBIA**

## **The 2nd Joint International Symposium on Neuroacanthocystosis and Neurodegeneration with Brain Iron Accumulation**

**October 26 – 27, 2012**

**Hotel & Congrescentrum De Reehorst, Ede, The Netherlands**





## Introduction

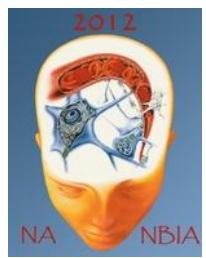
We are very happy to welcome you to the 2nd joint symposium on neuroacanthocytosis (NA) and neurodegeneration with brain iron accumulation (NBIA).

This symposium is part of a series of meetings that, throughout the years and all over the world, aim to bring together not only the recently acquired knowledge on NA and NBIA, but especially all the people involved in this endeavour in the past, in the present and, perhaps most importantly, in the future. The very nature of NA and NBIA requires a multidisciplinary approach, and this is only possible if all those involved make an effort to understand each other's way of thinking. In the design of the programme of this meeting, our primary goal has been to create a surrounding that facilitates a free exchange of results and ideas between patients, patient organisations, neurologists, hematologists and cell biologists.

Since the previous symposium in Bethesda in 2010, breakthroughs have been made in the understanding of the mechanisms underlying NA and NBIA at the molecular and cellular level, and new NBIA genes have been discovered. We hope that our getting together in Ede will create an atmosphere that inspires you all to a further unraveling, and especially to open the ways for treatment and therapy.

Giel Bosman and Ody Sibon

Ede, October 26, 2012



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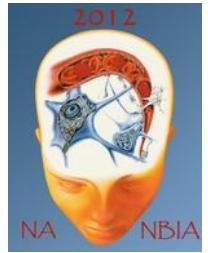
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## Speakers

Giel Bosman, The Netherlands

Dario Brunetti, Italy

Adrian Danek, Germany

Lucia de Franceschi, Italy

Tobias Haack, Germany

Susan Hayflick, United States of America

Andreas Hermann, Germany

Penelope Hogarth, United States of America

Alex and Ginger Irvine, England

Suzy Jackowski, United States of America

Thomas Klopstock, Germany

Angelika Klucken, Germany

Paul Kotzbauer, United States of America

Sonia Levi, Italy

Marieke von Lindern, The Netherlands

Thomas Meitinger, Germany

Aaron Neiman, United States of America

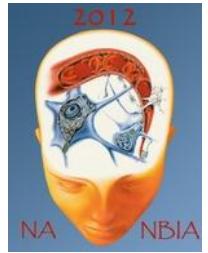
Rainer Prohaska, Austria

Fulvio Reggiori, The Netherlands

Ody Sibon, The Netherlands

Jan Vonk, The Netherlands

Ruth Walker, United States of America

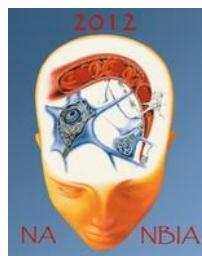


## Organizing committee

Giel Bosman, The Netherlands

Judith Cluitmans, The Netherlands

Ody Sibon, The Netherlands



## Program NA-NBIA meeting: October 26-27 2012

### Thursday 25<sup>th</sup> October

19.00 Welcome buffet

### Friday 26<sup>th</sup> October

9.00 Welcome

09.15-10.45 Topic 1: Clinical studies: Chair: Benedikt Bader

09.15-09.45 **Ruth Walker:** Clinical perspectives on neuroacanthocytosis

09.45-10.15 **Susan Hayflick:** Genetic and Clinical Delineation of NBIA

10.15-10.45 **Thomas Klopstock:** Treat Iron-Related Childhood-Onset Neurodegeneration (TIRCON) - an integrated strategy under FP7 to improve research, treatment and care in NBIA

10.45-11.15 **Coffee/tea**

11.15-12.15 Topic 2: New genes: Chair Susan Hayflick

11.15-11.45 **Thomas Meitinger:** Absence of a newly discovered orphan mitochondrial protein causes a distinct clinical subtype of neurodegeneration with brain iron accumulation.

11.45-12.15 **Tobias Haack:** BPAN: identification of a new NBIA disease gene by whole exome sequencing of a clinically preselected group of 14 patients.

12.15-13.30 **Poster Lunch I**

13.30-14.30 Topic 3: Autophagy Chair: Rainer Prohaska

13.30-14.00 **Fulvio Reggiori:** Autophagy in health and disease.

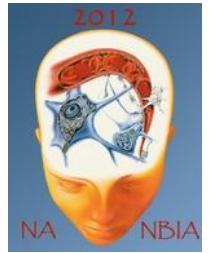
14.00-14.30 **Penelope Hogarth:** title to be announced TITEL?

15.00-17.00 Social program: Visit Kröller-Müller Museum\*

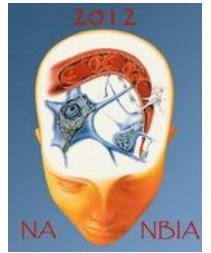
*\*The Kröller-Müller Museum has a world-renowned collection of mainly 19<sup>th</sup> and 20<sup>th</sup> century visual art, including a large collection of the work by Vincent van Gogh, and a sculpture garden. ([www.kmm.nl](http://www.kmm.nl)).*

19.00 **Dinner**

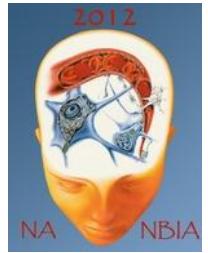
**Saturday 27<sup>th</sup> October**



- 08.30-12.00 Topic 4: Pathophysiology and basic research in NA: Chair: Giel Bosman
- 08.30-09.00 **Rainer Prohaska:** Fundamental insights in neuroacanthocytosis
- 09.00-09.30 **Lucia de Franceschi :** Erythrocyte membrane changes of chorea acanthocytosis revealed by kinomics.
- 09.30-10.00 **Judith Cluitmans:** Acanthocytosis in NA and NBIA: a molecular investigation into erythrocyte morphology and function.
- 10.00-10.20 **Aaron Neiman:** VPS13 regulates prospore membrane morphogenesis through control of phosphatidylinositol phosphates.
- 10.20-10.40 **Jan Vonk:** A Drosophila melanogaster model for Chorea-Acanthocytosis shows impaired autophagic substrate degradation and a decreased life span.
- 10.40-11.00 **Coffee/tea break**  
Chair: Lucia de Franceschi
- 11.00-11.30 **Andreas Herman:** Chorein-sensitive polymerization of cortical actin and suicidal cell death in chorea-acanthocytosis.
- 11.30-12.00 **Marieke von Lindern:** In Vitro Erythropoiesis as a Model System to Study Erythroid Defects
- 12.00-13.00 **Poster Lunch II**
- 13.00-15.00: Topic 5: Pathophysiology and basic research in NBIA: Chair: Ody Sibon
- 13.00-13.35 **Paul Kotzbauer:** PLA2G6
- 13.35-14.10 **Suzy Jackowski:** Physiological roles of the pantothenate kinases
- 14.10-14.30 **Dario Brunetti:** Pantothenate kinase-associated neurodegeneration: altered mitochondrial membrane potential and defective respiration in Pank2 knock out mouse model.
- 14.30-15.00 **Sonia Levi:** The involvement of iron in pantothenate kinase-associated neurodegeneration.
- 15.00-15.30 **coffee/tea break**
- 15.30-17.00 Topic 6: Patient/family/scientists discussion: Chair: Penelope Hogarth



- 15.30-15.50 **Alex and Ginger Irvine:** perspective of an NA patient's day to day life
- 15.50-16.10 **Angelika Klucken:** patient organizations and their role in EU funded projects
- 16.10-16.40 **Ody Sibon:** Development of pantethine-based therapies, FP7 funded research
- 16.40-17.00 Discussions
- 17.00-17.30 **Adrian Danek** Round-up discussion (Introduction and Chair)
- 17.30-18.30 Reception: Meet and connect to all groups
- 19.00 **Dinner**



## General information

### Venue

The 2nd Joined International Symposium on Neurocanthocytosis and Neurodegeneration with Brain Iron Accumulation takes place at the Hotel and Congrescentrum De Reehorst, Ede, The Netherlands.

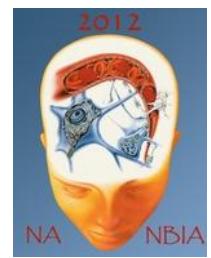
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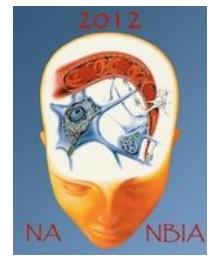
### Travel info

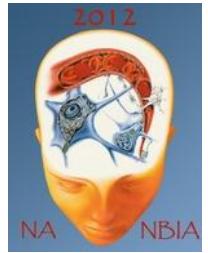
De Reehorst is situated in Ede, at the very centre of the Netherlands. From Schiphol Airport, it is easily accessible by train and car. Please visit [www.reehorst.nl/en/route](http://www.reehorst.nl/en/route) for more details.

## NOTES



## ORAL PRESENTATIONS

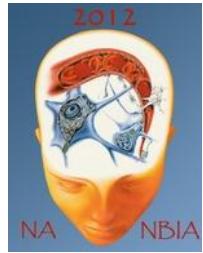




## OP 1 Clinical perspectives on neuroacanthocytosis

Walker, Ruth (James J. Peters VAMC, USA)

The core neuroacanthocytosis syndromes are chorea-acanthocytosis (ChAc) and McLeod syndrome (MLS). Acanthocytosis and hyperkinetic movements are also features of PKAN and Huntington's disease-like 2. These four disorders are genetically distinct, and it is not yet known why acanthocytes are generated, nor why the basal ganglia are preferentially affected. There are other intriguing similarities between ChAc and MLS, which resulted in diagnostic confusion until identification of their respective causative genes, such as the involvement of peripheral nerve and muscle, liver and spleen, and seizures. In both disorders the onset is in adulthood, albeit typically at a younger age for ChAc. Psychiatric features are often an early feature, resulting in attribution of motor symptoms to medication side-effects. In neither of these disorders is specific neuropathology found, rather there is non-specific neuronal loss and gliosis of the basal ganglia. Treatment remains symptomatic, with a focus upon maintaining communication, function and nutritional status.

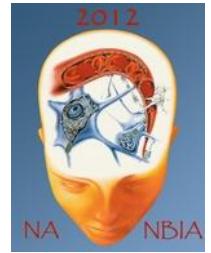


## OP2 Genetic and Clinical Delineation of NBIA

Hayflick, Susan (OHSU, USA)

Neurodegeneration with brain iron accumulation (NBIA) comprises four major clinically distinctive single gene disorders. Mutations in *PANK2*, *PLA2G6*, *C19orf12* and *XLBIA*\* lead to recognizable phenotypes. By combining clinical, radiographic and genetic data, physicians can narrow their differential diagnosis to one or two specific forms of NBIA and target diagnostic testing to those underlying genes. As new genes have been discovered, our understanding of the pathophysiology of individual forms of NBIA has evolved. At the intersection of the specific pathophysiologies is a common pathway leading to damage of the selectively vulnerable basal ganglia, for reasons that remain elusive. Within their limitations, animal models have provided important insights into the various forms of NBIA and offer a platform in which to develop rational therapeutics.

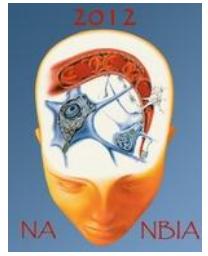
\*to be identified at the conference



## **OP 3 Treat Iron-Related Childhood-Onset Neurodegeneration (TIRCON) - an integrated strategy under FP7 to improve research, treatment and care in NBIA**

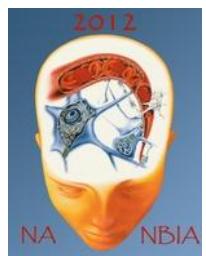
*Thomas Klopstock*

Neurodegeneration with brain iron accumulation (NBIA) is a clinically and genetically heterogeneous group of rare hereditary neurodegenerative disorders characterized by high levels of brain iron. Many NBIA cases are characterized by early childhood onset and rapid progression to disability and death. The most frequent form of NBIA 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. Thus, the absence of adequately powered randomized clinical trials is not due to a lack of therapeutic options but to the rarity of the disease, the lack of patient registries and the fragmentation of therapeutic research worldwide. In TIRCON (Treat Iron-Related Childhood-Onset Neurodegeneration), for the first time, an international group of scientists and clinicians have elaborated a collaborative project with patient representatives and innovative companies committed to orphan products. TIRCON's goals are 1) the setup of an international NBIA registry and 2) biobank, 3) the development of biomarkers for the disease, 4) the conduction of a randomized clinical trial of the iron-chelating drug deferiprone in PKAN, and 5) the preclinical development of pantethine derivatives for the treatment of PKAN.



## OP 4 Absence of a newly discovered orphan mitochondrial protein, causes a distinct clinical subtype of neurodegeneration with brain iron accumulation

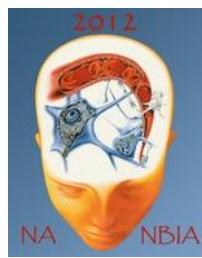
*Thomas Meitinger*



## OP 5 BPAN: identification of a new NBIA disease gene by whole exome sequencing of a clinically preselected group of 14 patients

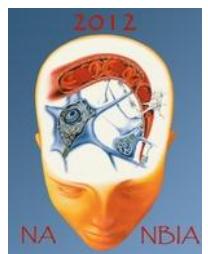
Haack, Tobias (Institute of Human Genetics, Technische Universität München and Helmholtz Zentrum München, Munich, Germany, MUNICH, DEU); Hogarth, Penelope (Department of Molecular & Medical Genetics and Department of Neurology, Oregon Health & Science University, Portland, 97239 USA, USA); Kruer, Michael (Sanford Children's Health Research Center, Sioux Falls, SD 57104 USA, USA); Gregory, Allison (Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, 97239 USA, NLD); Wieland, Thomas (Institute of Human Genetics, Helmholtz Zentrum München, Munich, Germany, DEU); Schwarzmayr, Thomas (Institute of Human Genetics, Helmholtz Zentrum München, Munich, Germany, DEU); Graf, Elisabeth (Institute of Human Genetics, Helmholtz Zentrum München, Munich, Germany, DEU); Meyer, Esther (Neurosciences Unit - Institute of Child Health, University College London, UK, GBR); Kara, Eleanna (Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK, GBR); Harik, Sami (Department of Neurology, University of Arkansas for Medical Sciences, Little Rock, AR, 72205 USA, ITA); Dandu, Vasuki (Department of Neurology, University of Arkansas for Medical Sciences, Little Rock, AR, 72205 USA, ITA); Nardocci, Nardo (Foundation IRCCS Neurological Institute C. Besta, ITA); Zorzi, Giovanni (Unit of Child Neurology, Department of Pediatric Neuroscience IRCCS Foundation Neurological 27 Institute "Carlo Besta", 20133 Milan Italy, ITA); Dunaway, Todd (Private practice, Tulsa OK 74104 USA, USA); Tarnopolsky, Mark (Departments of Pediatrics and Medicine, McMaster Children's Hospital and Hamilton Health Sciences Foundation, Hamilton, Canada, CAN); Skinner, Steven (Greenwood Genetic Center, Greenwood, SC 29646 USA, USA); Frucht, Steven (Department of Neurology, Mount Sinai School of Medicine, New York, NY 10029 USA, USA); Hanspal, Era (Parkinson's Disease and Movement Disorders Center, Albany Medical Center, and Department of Neurology, Albany Medical College, NY 12208 USA, USA); Schranden-Strumpel, Connie (Department of Clinical Genetics, Academic Hospital Maastricht, University of Limburg, 6229 GT Maastricht, Netherlands, NLD); Héron, Delphine (Clinical Genetics Unit, Centre de Référence des Déficiences Intellectuelles de Causes Rares, Groupe Hospitalier Pitié-Salpêtrière, 75651 Paris, France, FRA); Mignot, Cyril (Clinical Genetics Unit, Centre de Référence des Déficiences Intellectuelles de Causes Rares, Groupe Hospitalier Pitié-Salpêtrière, 75651 Paris, France, FRA); Garavaglia, Barbara (Unit of Molecular Neurogenetics, IRCCS, Foundation Neurological Institute "Carlo Besta", 20133 Milan, Italy, ITA); Bhatia, Kailash (Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK, GBR); Hardy, John (Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK, GBR); Strom, Tim (Institute of Human Genetics, Technische Universität München and Helmholtz Zentrum München, Munich, Germany, DEU); Boddaert, Nathalie (Department of Paediatric Radiology, Hôpital Necker Enfants Malades, 75743 Paris, France, FRA); Houlden, Henry (Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK, GBR); Kurian, Manju (Neurosciences Unit - Institute of Child Health, University College London, UK, GBR); Meitinger, Thomas (Institute of Human Genetics, Technische Universität München and Helmholtz Zentrum München, Munich, Germany, DEU); Prokisch, Holger (Institute of Human Genetics, Technische Universität München and Helmholtz Zentrum München, Munich, Germany, DEU); Hayflick, Susan (Departments of Molecular & Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University, Portland, USA, USA)

The three main forms of NBIA caused by mutations in PANK2, C19orf12, and PLA2G6 account for about 70% of cases. The application of next generation sequencing technologies is an option to identify the molecular genetic correlate in the remaining undiagnosed individuals. However, clinical



and locus heterogeneity are major hurdles hindering the prioritization of few candidate genes. This is especially true for NBIA subtypes associated with a dominant mode of inheritance where several hundred genes carrying rare predicted deleterious variants are left in a single exome.

We here report a dual strategy based on (i) clinical stratification and subsequent (ii) targeted next generation sequencing. We accordingly analyzed a clinically preselected group of 14 NBIA individuals lacking a previous molecular diagnosis using whole exome sequencing. Based on the clinical stratification we postulated a fraction of patients to share mutations in the same gene. This assumption allowed us to search for mutated genes shared by an increasing number of patients. Filters for rare compound heterozygous or homozygous variants failed to identify obvious candidates. Contrariwise, a filter for rare heterozygous variants lead to the identification of a gene predicted defective in 13 out of 14 cases. Screening of additional patients with suggestive phenotypes identified mutations in six of them, for a total of 18 different disease alleles in 19 cases. Investigation of parental DNAs suggested that the mutations are de novo. The new disease gene codes for a protein with a putative role in autophagy, a pathway regarded increasingly important also in the pathogenesis of frequent neurodegenerative disorders.

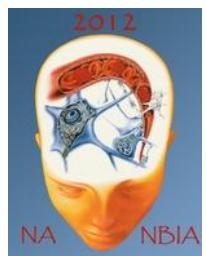


## OP 6 Autophagy in Health and Disease

*Fulvio Reggiori, Department of Cell Biology, University Medical Center Utrecht, The Netherlands*

Autophagy is a conserved degradative pathway essential in a growing number of physiological processes and associated to the physiopathology of numerous diseases including cancer and neurodegeneration. Despite the medical relevance of autophagy, the mechanism and regulation of this pathway remain largely unclear. Those are studied in my laboratory using yeast *Saccharomyces cerevisiae* as a model organism, and biochemical, cell biological and electron microscopy methods in combination. My long-term objective is to understand the role of autophagy in specific medically-relevant pathological situations. The first step in this direction has been to study how coronaviruses subvert the autophagy machinery to invade host cells. This type of research has now been extended to other viruses and represents an alternative approach to investigate the molecular mechanism of autophagy.

In my presentation, I will give an extensive introduction about the regulatory and mechanistic principles of autophagy in order to provide researchers in the field of the NA/NBIA diseases with concepts and notions that could be important for their investigations. Subsequently, I will go into some of the recent results obtained in my laboratory the mechanism of autophagy.



## OP 7 Phenotypic analysis of PBAN

Penelope Hogarth (MD1,2)

Tobias B. Haack, MD, PhD3,4; Michael C. Kruer, MD5; Allison Gregory, MS2; Manju A. Kurian, MA, MRCPCH, PhD6,7; Henry H. Houlden, MBMS, PhD8; James Anderson, MD9; Nathalie Boddaert, MD10; Lynn Sanford, BS2; Nardo Nardocci, MD11; Giovanna Zorzi, MD11; Sami Harik, MD12; Todd Dunaway, MD13; Mark Tarnopolsky, MD, PhD14; Steven Skinner, MD15; Steven Frucht, MD16; Era Hanspal, MD17; Connie Schrander-Stumpel, MD18; Cyril Mignot, MD19; Delphine Héron, MD19; Barbara Garavaglia, PhD20; Kailash Bhatia, MD, DM21; John Hardy, PhD8,22; Thomas Meitinger, MD, PhD3,4; Holger Prokisch, PhD3,4; Susan J. Hayflick, MD1,2,23.

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3Institute of Human Genetics, Technische Universität München, 85748 Munich, Germany

4Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany

5Sanford Children's Health Research Center, Sioux Falls, SD 57104 USA

6Neurosciences Unit - Institute of Child Health, University College London, UK

7Department of Paediatric Neurology, Great Ormond Street Hospital, London, UK

8Reta Lilla Weston Laboratories, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

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17Parkinson's Disease and Movement Disorders Center, Albany Medical Center, and Department of Neurology, Albany Medical College, NY 12208 USA

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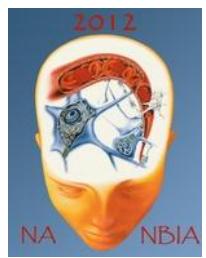
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21Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

22Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

23Department of Pediatrics, Oregon Health & Science University, Portland, 97239 USA

Introduction: The various single-gene disorders associated with high basal ganglia iron can largely be distinguished from one another by their associated clinical and neuroimaging features. Careful phenotypic characterization can guide diagnostic workup, prognostication and treatment.

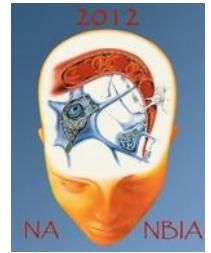


Objective: The aim of the present study was to characterize the phenotype associated with a newly identified X-linked dominant form of neurodegeneration with brain iron accumulation (NBIA).

Methods: We identified mutation-positive subjects from a large international cohort of idiopathic NBIA patients. We reviewed medical records on all and personally examined selected subjects. Longitudinal clinical, laboratory, and radiologic data were available in several cases.

Results: Nineteen mutation-positive subjects were identified (16 females). The natural history of their disease was remarkably uniform, with global developmental delay in childhood, followed by regression in early adulthood with progressive parkinsonism, dystonia, and dementia. The phenotypes of male and female subjects were indistinguishable from one another. Common early comorbidities included spasticity, seizures, disordered sleep, and stereotypies reminiscent of Rett syndrome. The symptoms of parkinsonism improved with dopaminergic drugs, in some cases, dramatically so; however, all patients experienced early motor fluctuations that quickly progressed to disabling dyskinesias, warranting discontinuation of medications in some cases. Brain MRI showed iron in the substantia nigra and globus pallidus, as well as a halo of T1 hyperintense signal in the substantia nigra. All patients harbored de novo mutations on the X chromosome encoding a protein postulated to play a role in autophagy.

Conclusions: This first X-linked form of NBIA to be described is recognizable by a unique combination of clinical, natural history and neuroimaging features.

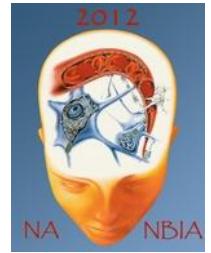


## OP 8 Fundamental insights in neuroacanthocytosis

Rainer Prohaska

Neuroacanthocytosis (NA) syndromes are basically characterized by neurodegeneration of basal ganglia and the appearance of acanthocytes in the patients' blood. It is hypothesized that a defective pathway common to erythroid and neuronal cells leads to both phenotypes. The core NA syndromes are known to be caused by defects in either the VPS13A gene resulting in Chorea-Acanthocytosis (ChAc), or in the XK gene resulting in McLeod Syndrome (MLS). Defects in JPH3 (junctophilin-3) lead to Huntington's disease-like 2 (HDL-2), and in PANK2 causes pantothenate kinase-associated neurodegeneration (PKAN). Acanthocytosis is typical for the core NA syndromes but less common in HDL-2 and PKAN.

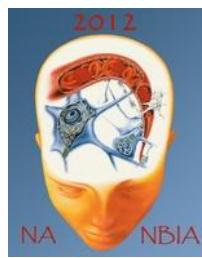
Recently there has been considerable progress elucidating ChAc-relevant signaling pathways and functional aspects of VPS13A/chorein. In a large collaborative effort, De Franceschi et al. (2011) showed that ChAc acanthocyte membrane proteins band 3, beta-spectrin, and adducin are highly phosphorylated on tyrosine-residues by Lyn kinase. The reason for Lyn-activation is currently unclear; however, band3-phosphorylation leads to altered linkage with cytoskeleton and may thus explain the acanthocytic shape. A bioinformatics approach also revealed the involvement of Lyn and a kinase network connecting ChAc and MLS (De Franceschi et al., 2012). This wealth of data proves again the relevance of studying erythrocyte membranes as a model system even for a neurological disease. In a collaboration of German researchers (Föller et al., 2012) it was shown that ChAc red cells have decreased phosphoinositide-3-kinase (PI3K) phosphorylation, reduced Rac1 activity, and decreased PAK1 kinase phosphorylation. Therefore, cortical actin is largely depolymerized, thus explaining the morphological deviation. Chorein down-regulation in K562 cells induced apoptosis. Japanese researchers have studied chorein localization in differentiated PC12 cells (Hayashi et al., 2012) and found it colocalizing with synaptotagmin I in dense core vesicles (DCV), which contain dopamine. It is speculated that chorein might be involved in the regulation of DCV exocytosis. These new insights will be integrated in common cellular pathways.



## OP 9 Erythrocyte membrane changes of chorea acanthocytosis revealed by kinomics.

De Franceschi, Lucia (University of Verona, ITA), Matte, Alessandro (University of Verona, Dept of Medicina, NLD); Lupo, Francesca (University of Verona, Dept of Medicina, ITA); Danek, Adrian (University of Munich, Germany); Bader, Benedikt (University of Munich, Germany); Walker, Ruth H (Department of Neurology, Mount Sinai School of Medicine, New York, USA); Santos Franco, Sara (University of Verona, Dept of Medicina, ITA); Mazzucco, Sara (University of Verona Dept of Neurology, ITA); Dotti, Maria Teresa (University of Siena, Dept of Neurology, ITA); De Franceschi, Lucia (University of Verona, ITA)

Neuroacanthocytosis syndromes (NA) are a group of hereditary neurodegenerative disorders that include chorea-acanthocytosis (ChAc). Although progresses have been made in identification of molecular defects in NA, the diseases pathophysiology and the related red blood cell (RBC) abnormalities are still under investigation. In ChAc-RBCs we have recently shown independent and strong activation of Lyn, a Tyr kinase of the Src family associated to the membrane. Since in RBCs Lyn is partitioned between the cytoplasm and the membrane, we evaluated the functional state of Lyn in the cytoplasm of ChAc-RBCs. We immunoprecipitated Lyn from cytoplasm of ChAc-RBCs and we found that in ChAc-RBCs Lyn was present as activated form differently from normal-RBCs, suggesting a complex scenario in which Lyn may be already abnormally activated when cells leave the bone marrow. We then studied the in vitro erythropoiesis of 2 ChAc patients. We observed a reduction of cell growth in ChAc compared to controls. We characterized erythroid cell maturation by FACS analysis using the CD36-GPA-CD71 strategy (Merryweather-Clarke AT, Blood 2011). This allows the detection of four different populations: CFU-E, Pro-E, Int-E and late-Erythroblasts. In ChAc we observed reduced CFU-E and increased Int-E at day 7, and 11 of culture compared to normal controls. When we analysed the appearance of GPA, we found that in ChAc the GPA positive cells appeared earlier than in normal cells, indicating that the reduction in cell growth in ChAc may be possibly related to early cell maturation. In ChAc the cytopsin analysis revealed signs of dyserythropoiesis such as cellular bridges and binuclear-cells, suggesting that abnormalities of chorein affect maturation of erythroid cells. Further studies should be carried out to better characterize the signaling pathway in both circulating red cells and erythropoiesis in ChAc.



## OP 10 Acanthocytosis in NA and NBIA: a molecular investigation into erythrocyte morphology and function

Giel J.C.G.M. Bosman

Judith C.A. Cluitmans<sup>1</sup>\*, Carlo Tomelleri<sup>2</sup>\*, Sip Dinkla<sup>1</sup>, Zuhal Yapici<sup>3</sup>, Giel J.C.G.M. Bosman<sup>1</sup>

\* Both authors contributed equally to this work.

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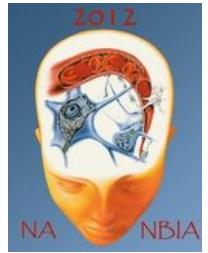
Deformed red blood cells with thorny protrusions (acanthocytes) are characteristic for patients with neuroacanthocytosis (NA). The association of the - still largely undefined - alterations in the red blood cell (RBC) membrane with selective neurodegeneration of the basal ganglia suggests a common pathogenic pathway. Acanthocytes are also found in the blood of patients with neurodegeneration with brain iron accumulation (NBIA).

In order to understand the mechanisms underlying acanthocyte formation we studied the RBCs of several patients with choreoacanthocytosis, McLeod disease, and pathotenate kinase-associated neurodegeneration (PKAN) and their relatives, using qualitative and semi-quantitative morphological, biochemical and functional analyses.

Our data indicate that, in addition to acanthocytes, other RBC morphologies are detectable in patients with NA and PKAN and, in some PKAN families, also in relatives without neurological symptoms. Analysis of the membrane/cytoskeleton structure of these cells with immunoblot and confocal microscopy did not reveal any consistent differences. Measurement of the HbA1c and HbF concentrations indicated cellular aging-related and erythropoiesis-related variations within patients' and relatives' samples.

The association of abnormal RBC morphology with functional changes in deformability and vesiculation was studied using newly developed technologies. A microfluidic device was used to study the deformability of RBCs within the microcapillaries, and a spleen-mimicking device to study the passage of RBCs through the spleen.

Our data suggest that, in NBIA and NA patients, perturbation of RBC membrane organization is not limited to the homozygous state and to the presence of neurological symptoms, but may be present in various degrees in all carriers.

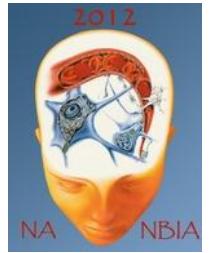


## OP 11 VPS13 regulates prospore membrane morphogenesis through control of phosphatidylinositol phosphates

Neiman, Aaron (STONY BROOK, USA)

In vegetatively growing yeast, Vps13 localizes to endosomes where it is involved in the delivery of proteins to the vacuole. During sporulation, VPS13 is important for formation of the prospore membrane that encapsulates the daughter nuclei to give rise to spores. We have found that VPS13 is required for multiple aspects of prospore membrane morphogenesis: 1) VPS13 promotes membrane expansion via activation the phospholipase D, Spo14; 2) VPS13 is required for a late step in closure of the prospore membrane at the cell division that gives rise to spores; and 3) VPS13 regulates a membrane bending activity that can generate intraluminal vesicles.

In sporulating cells, Vps13p localizes to the prospore membrane. This localization requires the sporulation-specific gene SPO71, and loss of SPO71 produces similar effects on prospore membrane formation as deletion of VPS13. We provide evidence that all of these effects are caused by the reduction of levels of phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate in the vps13 mutant. These results suggest a model in which induction of SPO71 leads to Vps13 recruitment to the prospore membrane where it influences the activity of a phosphatidylinositol-4-kinase, probably Stt4. If regulation of phosphatidylinositol phosphates is a conserved function of VPS13 family proteins this has important implications for understanding the basis of chorea acanthocytosis.



## OP 12 A *Drosophila melanogaster* model for Chorea-Acanthocytosis shows impaired autophagic substrate degradation and a decreased life span.

Vonk, Jan (GRONINGEN, NLD); Lahaye, Liza (UMC Groningen, NLD); Sibon, Ody (University of Groningen, NLD)

### Introduction:

The neurodegenerative disease Chorea-Acanthocytosis (ChAc) is part of a group of disorders called the Neuroacanthocytosis syndromes, which are characterized by neurodegeneration in the brain and the presence of acanthocytes. ChAc is a genetically recessive inherited disorder caused by mutations in the VPS13A gene. The patients develop movement disorders, like chorea and parkinsonism, and psychiatric problems. The pathogenesis of ChAc is largely unknown.

### Objective:

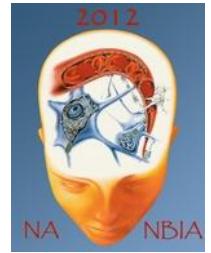
To use *Drosophila melanogaster* as a model organism to study the underlying molecular mechanisms that play a role in ChAc.

### Methods and results:

Mutations in the VPS13A gene have been suggested to have an effect on autophagy. Autophagy is a cellular degradation mechanism involved in the degradation of protein aggregates and organelles. We used an RNAi based approach to knock down the *Drosophila* ortholog of VPS13A, called dVPS13, in *Drosophila* S2 cells and we studied the process of autophagy. In the dVPS13 depleted cells we found an impairment of cells to degrade the autophagosomal targets Ref(2)p and Htt128Q. In order to establish a fly model for Chorea-Acanthocytosis we acquired a fly line which has a transposable element inserted in the dVPS13 gene, thereby causing disruption of this gene. We confirmed that this fly line has very little dVPS13 protein expression and subsequent life span studies showed that these flies have a decreased life span. We also studied the locomotor behavior of these flies by climbing assays. They showed that mutant flies are not impaired in their locomotor function when they are young, however develop locomotor problems when they are at an older age.

### Conclusion:

All the current data support the fact that we can use this *Drosophila* model to study the function of the VPS13 protein and the possible underlying pathological mechanisms that may play a role in ChAc.



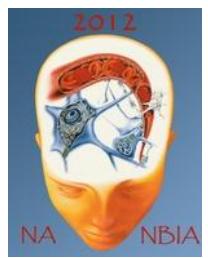
## OP 13 Chorein-sensitive polymerization of cortical actin and suicidal cell death in chorea-acanthocytosis

Hermann, Andreas (Division of Neurodegenerative Diseases, Department of Neurology, University of Technology, Dresden, Germany and DZNE, German Center for Neurodegenerat, DEU); Föller, Michael (Department of Physiology, University of Tübingen, DEU); Gu, Suchen (Department of Physiology, University of Tübingen, DEU); Alesutan, Ioana (Department of Physiology, University of Tübingen, DEU); Qadri, Syed (Department of Physiology, University of Tübingen, DEU); Borst, Oliver (Department of Physiology, University of Tübingen, DEU); Schmidt, Eva-Maria (Department of Physiology, University of Tübingen, DEU); Reinhardt, Peter (Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, DEU); Sterneckert, Jared (Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, DEU); Schöler, Hans (Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, DEU); Schiele, Franziska (Department of Neurology, University of Tübingen, Germany, DEU); Müller vom Hagen, Jennifer (Department of Neurology, University of Tübingen, DEU); Saft, Carsten (Department of Neurology, Ruhr University, Bochum, DEU); Schöls, Ludger (Department of Neurology, University of Tübingen, DEU); Lerche, Holger (Department of Neurology, University of Tübingen, DEU); Christos Stournaras, Christos (Department of Biochemistry, University of Crete Medical School, Heraklion, GRC); Lang, Florian (Department of Physiology, University of Tübingen, DEU); Storch, Alexander (Division of Neurodegenerative Diseases, Department of Neurology, University of Technology, Dresden, Germany and DZNE, German Center for Neurodegenerat, DEU)

Chorea-acanthocytosis (ChAc) is an inevitably lethal genetic disease characterized by a progressive hyperkinetic movement disorder, cognitive and behavioural abnormalities as well as acanthocytosis. The disease is caused by loss-of-function mutations of *VPS13A* encoding chorein, a protein with unknown function expressed in various cell types. How chorein deficiency leads to the pathophysiology of chorea-acanthocytosis remained enigmatic. Here, we show decreased PI3K-p85-subunit phosphorylation, Rac1 activity and PAK1 phosphorylation as well as depolymerised cortical actin in erythrocytes from chorea-acanthocytosis patients and in K562-erythrocytic cells following chorein silencing. Pharmacological inhibition of PI3K, Rac1 or PAK1 similarly triggered actin depolymerization. Moreover, in K562 cells both chorein silencing and PAK1 inhibition with IPA-3 decreased phosphorylation of Bad, a Bcl2-associated protein promoting apoptosis by forming mitochondrial pores, followed by mitochondrial depolarization, DNA fragmentation and phosphatidylserine exposure at the cell surface, all hallmarks of apoptosis. Our observations reveal chorein as a novel powerful regulator of cytoskeletal architecture and cell survival thus explaining erythrocyte misshape and possibly neurodegeneration in chorea-acanthocytosis. Current research is focused on above mentioned pathway in neurons from ChAc patients.

### Acknowledgements

We thank all patients and their families as well as the healthy control subjects for participation. This study was supported by the Carl-Zeiss-Stiftung, and the Deutsche Forschungsgemeinschaft, GRK 1302, SFB 773, La 315/13-3, and the Neuroacanthocytosis Advocacy, London, UK.



## OP 14 In Vitro Erythropoiesis as a Model System to Study Erythroid Defects

*Marieke von Lindern,*

*Emile van den Akker, Elina Ovchynnikova, Nurcan Yagci, Klaske Thiadens, Marieke von Lindern  
Sanquin Research and Landsteiner Laboratory, AMC/UvA, Amsterdam, The Netherlands*

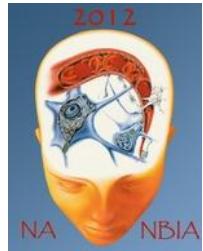
Erythroblasts can be expanded from hematopoietic tissues, including peripheral blood, in serum-free medium supplemented with erythropoietin (Epo), stem cell factor (SCF) and glucocorticoids (dexamethasone). Differentiation of erythroblasts to haemoglobinised, enucleated reticulocytes can be induced in presence of Epo, thyroid hormone and human serum. In vitro erythropoiesis can expose cellular and molecular processes because it allows for separating expansion and differentiation of the erythroid compartment, and enables the expansion of large numbers of erythroblasts. To study erythroid differentiation defects of patients with congenital anaemia we aim to immortalise our cultures by generating induced pluripotent stem cells (iPS cells), to start erythroblast cultures from iPS, and to differentiate these cells to erythrocytes.

To generate iPS we transduce erythroblasts cultured from PBMC with a lentiviral vector expressing Sox2, Klf4, Oct4, and cMyc. The iPS that arise from these cultures stably express pluripotency markers Sox2, Oct2/4, Tra1-60 and SSEA4. Upon induction of embryoid bodies 80% of the cells express Brachyury, and all embryoid bodies develop blood islands after 20 days of differentiation. The erythroid cells in these islands express high level CD71, a marker for erythroblasts. Conditions that further improve erythroid commitment, expansion of iPS-derived erythroblasts and their differentiation to erythrocytes are being optimised.

We are currently able to expand erythroblasts to such extent that we could generate an additional unit of packed red cells from the PBMC in the buffy coat of a single blood donation. Whereas commitment of stem cells to erythroblasts and differentiation to erythrocytes is orchestrated by transcription factors at the level of gene transcription, the regulation of expansion versus differentiation of erythroblasts is controlled at the level of mRNA translation, which is dictated by the availability of translation factors and the structure of the untranslated regions of the mRNA. One of the translationally controlled mRNAs is Use1 (Unusual SNARE in the ER 1), whose translation (i) requires the PI3kinase/mTOR pathways and (ii) is inhibited by unfolded proteins in the ER or lack of haem. By controlling the retrograde transport of cargo-receptors and chaperons from the Golgi back to the ER, Use1 controls the rate of anterograde transport of lipids and proteins from the ER to the Golgi, and thus the production of glycosylated lipids and proteins in the plasma membrane. We study control of translation of Use1 mRNA and identified both positive and negative regulatory elements in the 5'UTR.

Currently we are able to differentiate 90% of cultured erythroblasts to enucleated, haemoglobinised reticulocytes. However, the maturation of cultured reticulocytes to stable biconcave erythrocytes that can be stored and have a proper half-life of 120 days *in vivo*, remained an unresolved challenge in the field of generating erythrocytes for transfusion. Band3 is a crucial anchor point for the connection of the spectrin cytoskeleton to the reticulocyte/erythrocyte membrane. The function of the Band3 macrocomplex has been investigated extensively in freshly isolated erythrocytes. We investigated how the Band3 complex associates during maturation in cultured cells, and which posttranslational modifications control its function during the maturation from reticulocytes to erythrocytes.

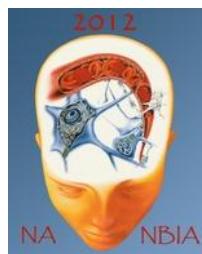
Understanding the processes that govern expansion and differentiation of erythroid cells can benefit from studying mutations found in congenital anaemia's. Therefore we aim to set up a bank of patient material that can be used to generate iPS and study the specific defect.



## OP 15 PLA2G6

Paul Kotzbauer, Washington University School of Medicine, St. Louis, Missouri, USA

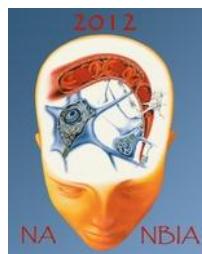
Mutations in the PLA2G6 gene cause infantile neuroaxonal dystrophy (INAD), a subgroup of neurodegeneration with brain iron accumulation (NBIA). INAD affects the central and peripheral nervous system and is defined pathologically by the presence of neuroaxonal spheroids containing accumulated membranes in distal axons. The PLA2G6 gene encodes the protein known as group VIA calcium-independent phospholipase A2 (PLA2G6). Our studies demonstrate that human PLA2G6 hydrolyzes both phospholipids and lysophospholipids to produce free fatty acids, and that disease-associated mutations dramatically impair the catalytic function of the protein. These results indicate that PLA2G6 plays an important role in the production of fatty acids from phospholipids, and predict two pathological pathways in INAD: accumulation of PLA2G6 substrates (phospholipids) and deficiency of products (free fatty acids). Accumulation of PLA2G6 substrates explains membrane accumulation within neuroaxonal spheroids in INAD. The corresponding defect in free fatty acid production caused by loss of PLA2G6 function in INAD likely restricts rates of new lipid synthesis and remodeling, but could also restrict the availability of fatty acids for other processes such as beta oxidation. Neuroaxonal spheroids are recapitulated in a Pla2g6-KO mouse model and accompany the progressive neurological impairment observed in these mice. The defect in free fatty acid and subsequent acyl CoA production caused by PLA2G6 mutations could be addressed by approaches that increase acyl CoA production through increased activity of fatty acid synthase and acyl CoA synthetase enzymes. Studies in the Pla2g6-KO mouse line support this hypothesis and ongoing studies are evaluating this therapeutic approach in INAD by characterizing the ability of drug-like compounds to increase acyl CoA production and improve neurological impairment in Pla2g6-KO mice.



## OP 16 Physiological Roles of the Pantothenate Kinases

Jackowski, Suzanne (St. Jude Children's Research Hospital, USA)

Pantothenate kinase-associated neurodegeneration, called PKAN, is a rare, inborn error of metabolism characterized by iron accumulation in the basal ganglia of the brain. Mutations in pantothenate kinase 2 (PanK2) cause this disorder. PanK phosphorylates pantothenic acid (vitamin B<sub>5</sub>) and controls the overall rate of coenzyme A (CoA) biosynthesis. Our laboratory investigates the metabolic consequences of modification of PanK isoform expression in mice. Both humans and mice express 4 active PanK isoforms that are encoded by 3 genes. The PanK isoforms differ from each other in biochemical regulation and subcellular localization. The PanK1 isoforms are more important to CoA levels in liver hepatocytes whereas PanK2 contributes more to CoA synthesis in the neurons of the brain. Deletion of the PanK2 isoform does not elicit a PKAN-like neuromuscular control deficit, indicating that the activities of the PanK1 and PanK3 isoforms can compensate for loss of PanK2 in mice. Deletion of both PanK2 and PanK3 is embryonic lethal. Deletion of both PanK2 and PanK1 results in viable mice that survive for 2 weeks after birth. The PanK1/PanK2 double knockout mice fail to thrive due to their inability to maintain serum glucose levels and to utilize ketone bodies as fuel. A line of transgenic mice expressing the human PanK2 (hPanK2) gene was established. Characterization of the hPanK2 transgenic mice shows that hPanK2 drives whole-body oxidative metabolism. These data suggest that the normal function of hPanK2 is to support mitochondrial energy production. (Research supported by NIH GM062896 and ALSAC.)



## OP 17 Pantothenate kinase-associated neurodegeneration: altered mitochondria membrane potential and defective respiration in Pank2 knock-out mouse model

Brunetti D1, Dusi S1, Morbin M2, Uggetti A2, Moda F2, D'Amato I1, Giordano C4, d'Amati G., Anna Cozzi 4, Sonia Levi 4,5, Hayflick S3, Tiranti V1.

1Division of Molecular Neurogenetics and 2Division of Neurology and Neuropathology, IRCCS Foundation Neurological Institute C. Besta, Milan, Italy, 4San Raffaele Scientific Institute and 5Division of Neuroscience, Vita-Salute San Raffaele University, Milan, Italy; 3Departments of Molecular & Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University, Portland, USA.

### INTRODUCTION

Mutations in the PANK2 gene, underlie an autosomal recessive inborn error of coenzyme A metabolism, called pantothenate kinase-associated neurodegeneration (PKAN). PKAN is characterized by dystonia, dysarthria, rigidity, pigmentary retinal degeneration and brain iron accumulation. The pathogenesis of this disorder is poorly understood and, although PANK2 is a mitochondrial protein, perturbations in mitochondrial bioenergetics have not been reported. A knock-out mouse model of PKAN exhibits retinal degeneration and azoospermia but lacks any neurological phenotype.

### OBJECTIVE

Objective of this work was the study of Pank2<sup>-/-</sup> mouse under a mitochondrial prospective in order to understand if a mitochondrial phenotype can be detected.

### METHODS

Sub-mitochondrial localization of mouse Pank2 protein was performed by western-blot analysis using a commercially available antibody on total homogenate, cytosol and mitochondria isolated from brain and fibroblasts of Pank2<sup>+/+</sup> and Pank2<sup>-/-</sup> mice.

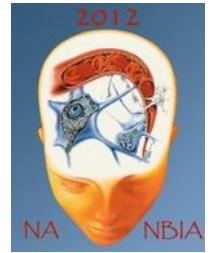
Mitochondrial bioenergetics status was evaluated with a Seahorse Bioscience XF96 analyzer on mitochondria isolated from mouse brain. Mitochondrial morphology and integrity was studied in cultured neurons using mitochondrion specific dyes and by TEM in CNS and PNS of Pank2<sup>+/+</sup> and Pank2<sup>-/-</sup> mice.

### RESULTS

We demonstrated that murine Pank2 protein is located in the mitochondrial inter-membrane space. Microscale oxygraphy detected an alteration in the respiratory profile of mitochondria derived from Pank2<sup>-/-</sup> as compared to Pank2<sup>+/+</sup> brains. Moreover we detected an alteration of mitochondrial membrane potential, which was confirmed by electron microscopy in Pank2<sup>-/-</sup> neurons showing the presence of swollen mitochondria with aberrant cristae and a complete modification of the matrix.

### CONCLUSION

These findings clearly demonstrated the presence of mitochondrial dysfunction in Pank2<sup>-/-</sup> mice, suggesting the investigation of mitochondrial and bioenergetics alterations in PKAN patients.



## OP 18 The involvement of iron in Pantothenate Kinase Associated Neurodegeneration

Levi, Sonia (Vita-Salute San Raffaele University, MILANO, ITALY)

Privitera, Daniela (Vita-Salute San Raffaele University, ITA); Cozzi, Anna (San Raffaele Scientific Institute, ITA); Santambrogio, Paolo (San Raffaele Scientific Institute, ITA); Broccoli, Vania (San Raffaele Scientific Institute, ITA); Rotundo, Ida Luisa (San Raffaele Scientific Institute, ITA); Garavaglia, Barbara (IRCCS Foundation Neurological Institute C. Besta, ITA); Brunetti, Dario (IRCCS Foundation Neurological Institute C. Besta, ITA); Tiranti, Valeria (IRCCS Foundation Neurological Institute C. Besta, ITA);

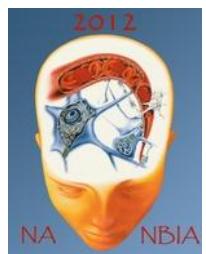
**Introduction.** Pantothenate Kinase2 Associated Neurodegeneration (PKAN) is a genetic movement disorder characterized by abnormal iron accumulation and degeneration in the brain basal ganglia. Despite the massive iron overload occurring in patients' brains the relationships between defects of Pank2 activity and brain iron deposition is not yet clarified.

**Objective** We are characterizing cellular and animal models of PKAN in order to define the molecular mechanism leading to iron homeostasis dysfunction.

**Material and Methods.** Primary skin fibroblasts from three PKAN patients and three unaffected subjects, dopaminergic neurons (iDAN) obtained by patients fibroblast's direct reprogramming and PANK2-/ mice testis were analyzed for iron homeostasis parameters and oxidative damage.

**Results.** Analysis of iron homeostasis parameters on patient's fibroblasts suggested that Pank2 deficiency promotes an increased oxidative status that is further enhanced by the addition of iron. This effect is expected to be more severe in neurons and thus we developed a new human neuronal model of disease. The iDANs were obtained by infection with lentivirus carrying the three-transcription factors-Mash1, Nurr1 and Lmx1a. The efficiency of fibroblasts reprogramming is ~5%, as identified by the expression of TuJ1, TH and N-CAM neuronal markers. We are collecting data on oxidative status and mitochondria functionality at single cell level. The preliminary data suggest an increment of ROS and a mild decrease of mitochondrial membrane potential in iDAN patients. Ferritins evaluation in testis tissues from 4 Pank2-/ and 4 controls (2 wt and 2 Pank 2+/-) mice indicate that FtL is increased (~1,6 fold) in KO mice. On the contrary the expression of antioxidant proteins, like FtMt and SOD1 appears strongly decreased (~20 fold and ~2,5 fold respectively) in KO mice respect to the control ones.

**Conclusion.** The data obtained are suggestive of oxidative damage of tissues possibly due to iron parameters deregulation.



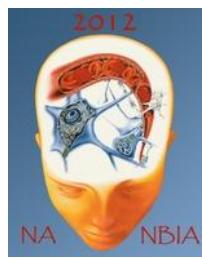
## OP 19 Perspective of an NA patient's day to day life

*Alex and Ginger Irvine (with contributions by Glenn Irvine)*

We all know that each NA patient has individual needs and symptoms, caring delivery and coping mechanisms to live with this disease. Alex and Ginger will illustrate some of Alex's daily activities and tell you about some of her thinking regarding what she wishes to accomplish and how she operates with NA.

From the days before NA manifested itself through the diagnosis and to changes in abilities over the last sixteen years, Alex has led the reaction and approaches to the challenges presented by the reality of this disease. Her determination to be the best she can in terms of living with such a rare condition has been the overriding response during these long years. She models the motto described first by our German patient Pamela Korb, "I have the disease, but it doesn't have me."

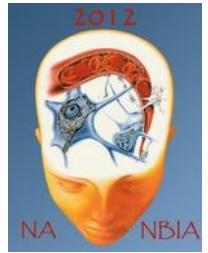
Included in Alex's daily life are the things many of us take for granted: dressing and speaking, eating and drinking and mobility and exercise. Activities high on the list of Alex's day are hobbies and work, her pet and visits from friends and of course, family get-togethers and celebrations. As a result of her parents involvement with the Advocacy, Alex is called upon to fulfill fundraising duties but she also has time for fun: the spa at the pool and the consumption of home-made chocolate chip cookies.



## OP 20 Patient organizations and their role in EU funded projects

*Klucken, Angelika (Hoffnungsbaum e.V. Verein zur Förderung der Erforschung und Behandlung von NBIA (vormals: Hallervorden-Spatz-Syndrom), VELBERT, DEU)*

Hoffnungsbaum e.V. (HoBa) is the German NBIA patient organization, dedicated to foster research and treatment in NBIA. Since 2002 HoBa has become an interface between many stakeholders in the field of NBIA and with the upcoming Rare Diseases Movement in Germany and Europe. Given the extraordinary rareness of NBIA the goal of HoBa's networking activities has always been to initiate and support international collaborative efforts in NBIA research as the probably best way to success. As TIRCON-partner HoBa is mainly responsible for Dissemination-related tasks of the consortium. Like the US patient group NBIA Disorders Association HoBa is also involved in the TIRCON work packages for the NBIA Patient Registry, the Deferiprone trial and Ethics - thus trying to represent the patients' interests within the EU funded Research Consortium.

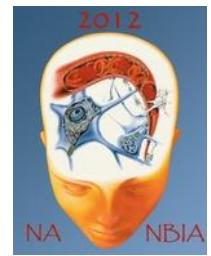


## OP 21 Development of pantethine based therapies, FP7 funded research

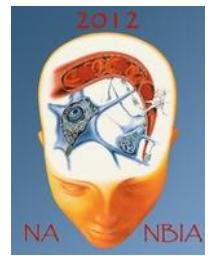
Ody C.M. Sibon, Department of Cell Biology, University Medical Center Groningen, University of Groningen, The Netherlands

Patients suffering from Pantothenate Kinase-Associated Neurodegeneration (PKAN) carry mutations in the human gene coding for pantothenate kinase 2. Pantothenate kinase is an essential enzyme required for the de novo biosynthesis of Coenzyme A. Coenzyme A is a metabolic cofactor involved in numerous biochemical reactions inside cells. There is now mounting evidence that at least part of the disease characteristics in PKAN patients are due to decreased amounts of cellular Coenzyme A. We have recently demonstrated in a fruitfly and in cell models for PKAN that impairment of pantothenate kinase leads to a strong decrease in Coenzyme A levels and this in turn leads to increased oxidative stress, neurodegeneration and a reduced life span. We also demonstrated using fruitfly and cell models that in the presence of impaired function of pantothenate kinase, levels of Coenzyme A can be restored by the compound pantethine. Pantethine also rescues apparent phenotypes of these PKAN models. Our results suggest that pantethine can be used as a possible starting point to develop a therapy for PKAN. Currently in the EU funded project TIRCON the potential of pantethine as a possible therapy for PKAN is further investigated. In addition, several pantethine-derivatives are being synthesized and their potential will be investigated as well. Strategies and current developments will be presented.

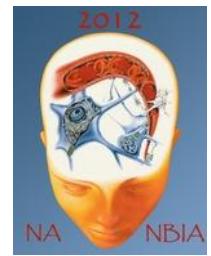
## NOTES

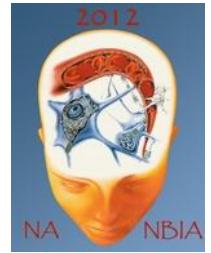


## NOTES



## POSTER PRESENTATIONS





# PP 1 Manipulation of kinases and phosphatases affects the morphology and deformability of erythrocytes

Nils Rother<sup>1</sup>, Judith C.A. Cluitmans<sup>1</sup>, Venkat Chokkalingam<sup>2</sup>, Giel J.C.G.M. Bosman<sup>1</sup> and Merel J.W. Adjobo-Hermans<sup>1</sup>

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## Introduction

Recently, abnormal activation of Lyn kinase has been described in erythrocytes of choreo-acanthocytosis patients. A percentage of the erythrocytes derived from these patients are malformed, i.e. non-discoid. Here, we hypothesize that the morphology and the deformability of erythrocytes in general is governed by the phosphorylation status of proteins that are involved in connecting the membrane with the cytoskeleton.

## Objective

In order to test our hypothesis, we selected a set of compounds that are known to modulate kinases and phosphatases and thereby the phosphorylation status of proteins. We tested these compounds in fresh, stored and patient-derived erythrocytes.

## Methods

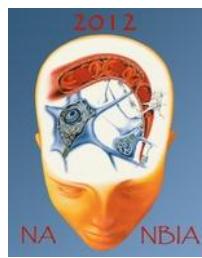
The morphology of the treated erythrocytes was studied by means of light microscopy. Their deformability was investigated by means of a microfluidic device in order to simulate the passage of the erythrocytes through capillaries.

## Results

To quantify the effect of the tested compounds on erythrocyte morphology, we made a classification that designates 5 different erythrocyte morphologies. Importantly, we observed that orthovanadate treatment, which inhibits phosphatase activity, induced an echinocytic morphology comparable to that found in patient-derived and blood bank-derived erythrocytes. In contrast, PP2, which inhibits Src-family kinase activity, induced a decrease in the percentage of echinocytes. Interestingly, certain treatments also caused changes in the deformability and relaxation of the erythrocytes.

## Conclusion

Effects of the treatments are observed, however the proteins that are responsible for the observed changes still have to be described. We will start out with the study of band 3 and its phosphorylation status upon treatment of erythrocytes with (combinations of) the various treatments. Moreover, we will implement a novel strategy to study the effect of kinases and phosphatases on erythrocyte morphology and deformability. For this, we will apply light-inducible protein dimerizers that enable the activation of specific kinases and phosphatases on-demand in transduced erythroblasts.



## PP2 A novel splice site mutation in a Brazilian chorea-acanthocytosis case

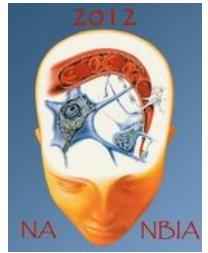
Miltenberger-Miltenyi, Gabriel (Instituto de Medicina Molecular, Faculdade de Medicina-Universidade de Lisboa, Portugal, PRT)

Bernardi, Priscila (Núcleo de Genética Clínica, Departamento de Clínica Médica Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil, BRA); Silva, Ines (Instituto de Medicina Molecular, Faculdade de Medicina-Universidade de Lisboa, Portugal, PRT); Enguita, Francisco J (Instituto de Medicina Molecular, Faculdade de Medicina-Universidade de Lisboa, Portugal, PRT); Danek, Adrian (Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität München, DEU); Bader, Benedikt (Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität München, DEU); Lin, Katia (Serviço de Neurologia, Departamento de Clínica Médica Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil, BRA);

**Background:** Chorea-acanthocytosis (ChAc) follows an autosomal recessive inheritance. An autosomal dominant pattern has been claimed for a few cases but never substantiated. To date, three patients have been reported from Brazil, where the diagnosis of ChAc was confirmed by chorein Western blot on red cell membranes. Genetic analyses of the VPS13A gene found compound heterozygous mutations in two cases, of which one harboured a gross deletion. Only a single heterozygous nonsense mutation was detected in the third case.

**Case report:** We report the fourth case from Brazil, a 42 year-old Caucasian male from non-consanguineous, asymptomatic parents, who reported walking difficulties starting 5 years previously. Approximately 1.5 years ago the patient showed dysphagia, weight loss, involuntary movements of the head and limbs, as well as depression. CPK was elevated and erythrocytes showed acanthocytosis (50%; Giemsa staining). Brain MRI showed atrophy of the caudate nuclei, slight dilatation of the anterior horns of both lateral ventricles and slight generalized cerebral cortical atrophy. Electromyography revealed sensory axonopathy. The most recent neurologic evaluation disclosed chorea, coprolalia, oro-facial dyskinesia, generalized hypotonia, absence of tendon reflexes with preserved muscle strength. The patient's sister (39 years) displays a similar movement disorder. We carried out mutation search on the VPS13A gene with PCR and direct sequencing. This analysis revealed one novel genetic variant in heterozygous state, which we presume as pathogenic. Investigation for a gross deletion/duplication using quantitative PCR is currently performed.

**Conclusions:** We report on the fourth Brazilian ChAc patient and discuss the difficulties of genetic analysis in this condition without obvious mutation hotspots and a variety of underlying alterations of the VPS13A gene. As long as not all cases with only a single detected mutation have been properly analysed the claim of autosomal dominant transmission should not be propagated.



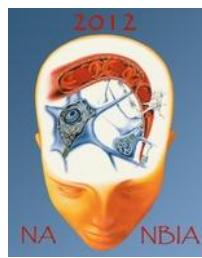
## PP3 Chorea-acanthocytosis: the first case from Bulgaria

Mehrabian, Shima (Department of Neurology, UH "Alexandrovska", Sofia, BGR); Bader, Benedikt (Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität München, DEU); Danek, Adrian (Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität München, DEU); Raycheva, Margarita (Department of Neurology, UH "Alexandrovska", Sofia, BGR); Skelina, Sylvia (Department of Neurology, UH "Alexandrovska", Sofia, BGR); Pavlova, Radka (Department of Neurology, UH "Alexandrovska", Sofia, BGR); Velizarova, Reana (Department of Neurology, UH "Alexandrovska", Sofia, BGR); Traykov, Latchezar (Department of Neurology, UH "Alexandrovska", Sofia, BGR)

Chorea-acanthocytosis (ChAc) is a rare progressive neurodegenerative disorder characterized by movement disorders, neuropsychiatric and cognitive disturbances, seizures, neuromuscular disorders and acanthocytosis, caused by mutations of *VPS13A*. This gene produces the protein chorein which is reduced or absent in patients with ChAc.

We report a case of a 37-year-old woman of Turkish origin, born from non-consanguineous marriage, with no family history of neurological disorders, a sewer by profession. She presented with hyperkinetic movements and behavior abnormalities for seven years. Neurological examination revealed a wide range of movement disorders with choreatic hyperkinesia and dystonia, head drops and myoclonus. These findings were associated with dysarthria, areflexia, seizures, myopathy and raised serum levels of CK and LDH. Acanthocytes were also observed. Molecular testing for Huntington's disease was negative. Neuropsychological assessment showed mild cognitive impairment (MMSE=25) with pronounced dysexecutive type cognitive profile. Dopamine transporter imaging (DAT-SPECT) revealed striatal DAT binding reduction. This is the first Bulgarian patient with ChAc confirmed by red cell membrane chorein Western blot. Results of *VPS13A* gene mutation analyses are pending.

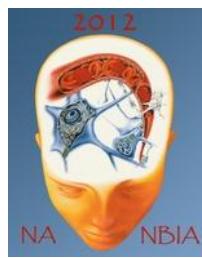
In **conclusion**, chorea-acanthocytosis should be considered in any early onset movement disorder with behavior disturbances and elevated CK levels.



## PP4 CoA biosynthesis in mechanisms of pharmacological activities of pantothenate derivatives

Moiseenok, Andrey (Institute of Bioorganic Chemistry, NAS of Belarus, BLR); Omelyanchik, Sofia (Institute of Bioorganic Chemistry, NAS of Belarus, BLR); Gurinovich, Valery (Institute of Bioorganic Chemistry, NAS, BLR); Sheibak, Vladimir (Grodno State Medical University, BLR); Slyshenkov, Vyacheslav (Yanka Kupala State University of Grodno, BLR); Khomich, Tamara (Institute of Bioorganic Chemistry, NAS of Belarus, BLR); Kanunnikova, Nina (Grodno State University, BLR); Katkovskaya, Inna (Institute of Bioorganic Chemistry, NAS of Belarus, BLR); Shevalye, Anna (Institute of Bioorganic Chemistry, NAS of Belarus, BLR); Bashun, Natalya (Yanka Kupala State University of Grodno, BLR)

We studied biotransformation and metabolic activities of pantothenic acid derivatives (PaA-Ca, 4'-phospho-PaA, pantethine, S-benzoyl-pantethine, S-sulfopantetheine, panthenol and 4'-phosphopantetheine) and CoA substance. We found a phenomenon of pantothenate kinase deinhibition due to CoA-SH by formation of symmetric or mixed coenzyme disulfides with cystine, pantethine and oxidized glutathione. During biotransformation of [<sup>14</sup>C] PaA derivatives, production of cytosolic CoA-S-S protein, 56 kDa, was detected which bound up to 46% of the vitamin radionuclide in PaA deficiency. A limiting role and a share of amino acid precursors (cysteine, methionine) in CoA biosynthesis were determined. In experiments with subchronic administration of [<sup>14</sup>C]PaA, HPLC isolated dephospho-CoA as the dominating intracellular form. Features of [<sup>3</sup>H]PaA derivatives transport and biotransformation in the CNS were established and a high level of 4'-phospho-PaA accumulation in neurostructures, especially in the hippocamp, was revealed. In terms of the possibility of intramolecular cycle formation the role of P-PaA was considered as chelating (e.g., in Fe<sup>+2</sup> excess) or antidote in relation to cysteine excitotoxicity, whereas pantethine effects were realized via the pantetheine kinase reaction, thiol disulfide mechanisms and S-containing metabolic products. We showed a universal capacity of the CoA biosynthetic precursors to prevent lipid peroxidation activation in different membrane structures and models. The main physiologic function of CoA system is suggested to be participation in formation of glutathione redox potential, redox signaling and maintenance of biological membrane stability, including that in neurostructures.



## PP5 Establishing a sensitive method to measure total Coenzyme A and other related thiols, with relevance to PKAN

Srinivasan, Balaji (University of Groningen, GRONINGEN, NLD); Grzeschik, Nicola (University of Groningen, NLD); Reijngoud, Dirk-Jan (University of Groningen, NLD); Sibon, Ody (University of Groningen, NLD)

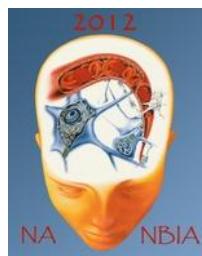
Coenzyme A (CoA) is an essential metabolic cofactor and a major thiol metabolite involved in various biochemical pathways. CoA and other thiol metabolites like glutathione, cysteamine, cysteine and pantetheine are considered to have inter-regulating mechanisms to maintain thiol redox-homeostasis at the cellular level. Understanding the dynamics between CoA and thiol regulation is of interest in various diseases linked to mitochondrial dysfunction, especially in Pantothenate-Kinase-Associated-Neurodegeneration (PKAN), a major form of NBIA. Our previous research showed that the neurodegenerative phenotype in *Drosophila dPANK/fbl* mutants is due to reduced CoA levels. We are currently focusing to investigate metabolic thiol regulation with respect to PKAN.

**Objective:** 1) To establish a robust, simple and sensitive method for total CoA estimation. 2) To determine how levels of various thiol metabolites change in response to reduced CoA levels.

**Methods:** HPLC-fluorescence detection of compounds after pre-column thiol derivatization with SBD-F. Chemical knock-out with hopantenate and RNAi approach were adopted to inactivate or downregulate the CoA synthesizing enzyme (PANK) in tissue culture cells and in a PKAN fruitfly model system.

**Results:** Optimized chromatographic separation has been established to estimate CoA and other thiol metabolites with higher sensitivity than HPLC-UV detection. We were able to reliably detect the reduction in CoA levels under hopantenate and RNAi downregulation of CoA synthesizing enzyme. Our method is also applicable to estimate various thiol metabolites in both drosophila and mammalian (HEK293) cells. Moreover, preliminary results also showed that glutathione levels were affected in PKAN models .

**Conclusion:** Our method is highly sensitive to determine total CoA levels and other thiol metabolites in *Drosophila* and mammalian cells. Impairment of CoA biosynthesis might lead to thiol homeostatic imbalance followed by increased mitochondrial/cellular stress. Understanding CoA and thiol homeostasis could be of primary importance in revealing insights of neurodegenerative diseases linked to metabolic disorders.



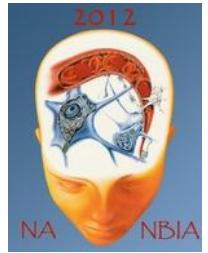
## PP6 HOMOPANTOTHENIC ACID (HOPANTENATE) - TRANSPORT IN THE BRAIN, BIOTRANSFORMATION, AND MECHANISMS OF ACTION

Kanunnikova, Nina (Grodno State University, BLR); Gurinovich, Valery (Institute of Bioorganic Chemistry, NAS, BLR); Moiseenok, Andrey (Institute of Bioorganic Chemistry, NAS, BLR)

Hopantenate contains GABA instead of the  $\beta$ -alanine in the molecule in contrast with D-pantothenic acid. It is unable to metabolize to CoA and does not catabolize to GABA practically. As a medicine, hopantenate has pronounced nootropic, anticonvulsant and neuroprotector effects but its mechanisms of action in the brain are rather uncertain. It is known that hopantenate influences the GABA-receptors but range of its effects in the brain is wider than effects on the GABA system only. Hopantenate is a concurrent inhibitor of a pantothenate kinase, a key enzyme in the CoA biosynthesis. Nevertheless, our experiments showed that the levels and CoA fraction structures did not change after injections of the hopantenate in the rat brain but not in the liver. It may be owing to acyl-CoA hydrolase activations that leads to alternative (aside from the CoA biosynthesis) increase of the CoA level, in spite of the inhibition of pantothenate kinase activity.

We studied hopantenate biotransformation following intraperitoneal or intragastral ingestions to rats of 3H-hopantenate (1 mg/kg, 0,55 mCu/mg) which was administered using thermic tritium activation technique (Dr Badun G.A., Moscow State University). Maximum accumulation of the radioactive substance was observed in brain hippocampus and hemispheres. We showed 4'-phosphohomopantothenate formation in the above structures. Addition of a CoA biosynthesis precursor D-panthenol (10 mg/kg) increased accumulation of the hopantenate metabolite in the structures.

We suppose the neurotropic mechanisms of the hopantenate may include its interaction with GABA receptors, activation of the alternative ways of CoA-SH stabilization, and formation of the hopantenate biotransformation product - 4'-phosphohomopantothenic acid.



## PP7 Cofilin/Twinstar phosphorylation levels increase in response to impaired Coenzyme A metabolism

Grzeschik, Nicola

Coenzyme A (CoA) is a metabolite essential for many fundamental cellular processes including energy, lipid and amino acid metabolism. Pantothenate kinase (PANK), catalysing the first step in the conversion of pantothenic acid to CoA, is associated with the neurodegenerative disorder Pantothenate-Kinase-Associated-Neurodegeneration (PKAN), a sub-type of NBIA; the consequences of impaired PANK activity are, however, poorly understood.

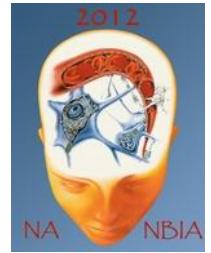
The *Drosophila* dPANK/fbl gene was initially identified in a screen for male sterility and mutants show cell division errors and cytokinesis defects with abnormal F-actin dynamics. These defects observed in dPANK/fbl mutant flies suggest a link between CoA levels and actin-regulation, but so far the underlying mechanism remains elusive and relatively little is known about whether/how the metabolic state of a cell influences cytoskeletal dynamics.

Objective: To determine how PANK/CoA levels are linked to the regulation of actin dynamics.

Methods: RNAi treatment and chemical inhibition with hopantenate was employed to reduce PANK activity and CoA synthesis in fly and mammalian cell cultures. Actin morphology and regulation was analysed by immunofluorescence, live-cell-imaging and Western analysis.

Results: Decrease of dPANK/fbl in *Drosophila* cells causes actin abnormalities. Cells deficient for dCofilin/Twinstar display comparable phenotypes. Twinstar is hyperphosphorylated and thereby inhibited in CoA depleted cells. Cdi kinase and Slingshot phosphatase are involved in Twinstar hyperphosphorylation associated with decreased CoA levels, although the status of their activity is currently unknown. In mammalian neuronal cells PANK inhibition inactivates Cofilin and adversely affects neurite outgrowth, a process dependent on actin-remodelling.

Conclusions: Disruption of PANK activity and decreased CoA levels can lead to alterations in actin dynamics and neuronal dysfunction by causing hyperphosphorylation and inactivation of cofilin/Twinstar. This mechanism is conserved in fly and human cell lines. However, whether cofilin inactivation and/or actin abnormalities indeed underlie the pathology of PKAN, remains to be determined.



## PP8 Revealing differences between acanthocytes and normal erythrocytes.

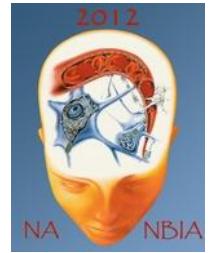
Claudia Siegle-Roos

Acanthocytes, spiky erythrocytes, are a constant companion of the Neuroacanthocytosis Syndromes Chorea Acanthocytosis (ChAc) and McLeod Syndrome (MLS), but occur in only 8 % of Pantothenate Kinase-Associated Neurodegeneration (PKAN) cases. Although these misshapen red cells are not known to contribute to the disease, they are used for diagnosis of Neuroacanthocytosis.

For functional analysis of acanthocytes, we used drugs such as primaquine, chlorpromazine, and imipramine that are known to induce endovesiculation. This is not a physiological function of erythrocytes but essential for neurons. By using lysophosphatidic acid (LPA), we stimulated phosphatidylserine exposure on the erythrocyte surface and Ca-influx into the cell, which are physiological processes for all cell types including neurons. The results show that acanthocytes are less inducible than normal discocytes regarding endovesiculation and phosphatidylserine exposure. Ca-influx was significantly reduced only in ChAc acanthocytes while PKAN samples with and without acanthocytes did not show this effect. LPA is secreted from platelets and acts mainly through G-protein-coupled receptors. Their activation results in Ca-influx leading to activation of scramblase and exposure of phosphatidylserine but also to PI3K-dependent activation of protein kinase PKC $\zeta$ . Consequently, we studied the membrane association of PKC $\zeta$  along with Lyn kinase, which was recently shown to be highly active in ChAc acanthocytes (De Franceschi et al., 2011).

To study the role of VPS13A/chorein in erythrocytes, we analyzed red cell membranes biochemically. Several solubilization assays and proteinase treatments were developed to analyze the membrane association of VPS13A using erythrocytes from healthy donors and NBIA patients.

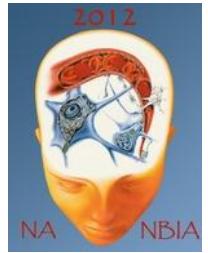
The differences in endovesiculation, phosphatidylserine exposure, and Ca-influx may be linked to the pathophysiology of Neuroacanthocytosis syndromes. Our findings implicate that there are different mechanisms leading to the characteristic acanthocyte morphology in ChAc and PKAN. Those mechanisms may also be involved in neurodegeneration.



## PP9 Metabolic consequences of mitochondrial coenzyme A deficiency in patients with PANK2 mutations

*Tiranti, Valeria*

Pantothenate kinase-associated neurodegeneration (PKAN) is a rare, inborn error of metabolism characterized by iron accumulation in the basal ganglia and by the presence of dystonia, dysarthria, and retinal degeneration. Mutations in pantothenate kinase 2 (PANK2), the rate-limiting enzyme in mitochondrial coenzyme A biosynthesis, represent the most common genetic cause of this disorder. How mutations in this core metabolic enzyme give rise to such a broad clinical spectrum of pathology remains a mystery. To systematically explore its pathogenesis, we performed global metabolic profiling on plasma from a cohort of 14 genetically defined patients and 18 controls. Notably, lactate is elevated in PKAN patients, suggesting dysfunctional mitochondrial metabolism. As predicted, but never previously reported, pantothenate levels are higher in patients with premature stop mutations in PANK2. Global metabolic profiling and follow-up studies in patient-derived fibroblasts also reveal alterations in bile acid conjugation and lipid metabolism, pathways that require Coenzyme A. These findings raise a novel therapeutic hypothesis, namely, that dietary fats and bile acid supplements may hold potential as disease-modifying interventions. Our study illustrates the value of metabolic profiling as a tool for systematically exploring the biochemical basis of inherited metabolic diseases.



## PP 10 Distribution of chorein in human beings

Liu, Jia

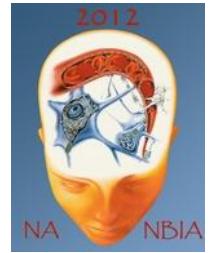
**Introduction:** Chorea-acanthocytosis (ChAc) is a rare autosomal recessive hereditary neurodegenerative disease. Chorein is a 360 kDa protein encoded by VPS13A, which is reduced on ChAc patients, e.g. in red cell membranes.

**Objectives:** We systematically investigated the chorein distribution in human tissues.

**Methods:** Frozen tissues were acquired from 10 normal controls and 3 ChAc patients at autopsy. Quantitative Western immunoblot was used to examine presence and levels of chorein.

**Results:** Chorein was found in all brain regions of normal controls including cortex, caudate nucleus, putamen, globus pallidus, thalamus, hippocampus, cerebellum and white matter, but was absent in the ChAc brains. In peripheral organs of normal controls, chorein was highly expressed in the testis, heart, bone marrow and muscle.

**Conclusion:** Chorein is widely distributed in normal brains. Absence of chorein in ChAc brains might contribute to the neurological changes. In normal controls, the high chorein expression was found in the peripheral organs derived from mesoderm, which might be a result of VPS13A upregulating in consequence of mesoderm differentiation.

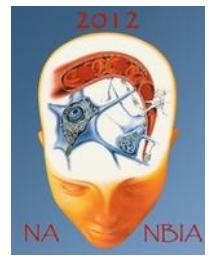


## PP11 CoA biosynthesis in mechanisms of pharmacological activities of pantothenate derivatives

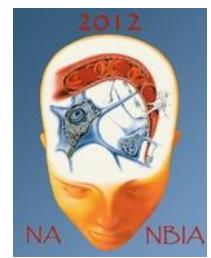
*Moiseenok, Audrey*

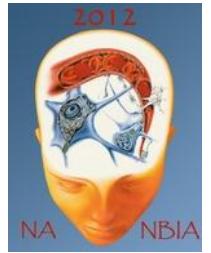
We studied biotransformation and metabolic activities of pantothenic acid derivatives (PaA-Ca, 4'-phospho-PaA, pantethine, S-benzoyl-pantethine, S-sulfopantetheine, panthenol and 4'-phosphopantetheine) and CoA substance. We found a phenomenon of pantothenate kinase deinhibition due to CoA-SH by formation of symmetric or mixed coenzyme disulfides with cystine, pantethine and oxidized glutathione. During biotransformation of [<sup>14</sup>C] PaA derivatives, production of cytosolic CoA-S-S protein, 56 kDa, was detected which bound up to 46% of the vitamin radionuclide in PaA deficiency. A limiting role and a share of amino acid precursors (cysteine, methionine) in CoA biosynthesis were determined. In experiments with subchronic administration of [<sup>14</sup>C]PaA, HPLC isolated dephospho-CoA as the dominating intracellular form. Features of [<sup>3</sup>H]PaA derivatives transport and biotransformation in the CNS were established and a high level of 4'-phospho-PaA accumulation in neurostructures, especially in the hippocampus, was revealed. In terms of the possibility of intramolecular cycle formation the role of P-PaA was considered as chelating (e.g., in Fe<sup>+2</sup> excess) or antidote in relation to cysteine excitotoxicity, whereas pantethine effects were realized via the pantetheine kinase reaction, thiol disulfide mechanisms and S-containing metabolic products. We showed a universal capacity of the CoA biosynthetic precursors to prevent lipid peroxidation activation in different membrane structures and models. The main physiologic function of CoA system is suggested to be participation in formation of glutathione redox potential, redox signaling and maintenance of biological membrane stability, including that in neurostructures.

## NOTES



## NOTES





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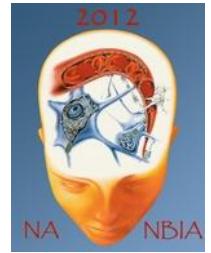
The *Movement* Disorder Society  
**Movement Disorders**



**NBIA**

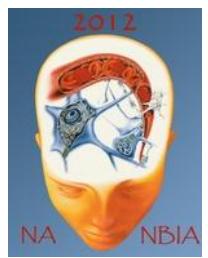


**NWO**



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