

WELCOME NOTES

Dear Colleagues, Patients and Caregivers,

Welcome to Dresden, the “Florence on the Elbe”!

The 9th International Meeting on Neuroacanthocytosis Syndromes follows the tradition of the previous eight international symposia, the last of which was held in Ann Arbor, USA in May, 2016.

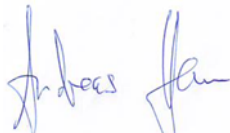
Neuroacanthocytosis Syndromes are a group of rare neurological disorders. Chorea-Acanthocytosis is the “core” disease of this family caused by mutations in the VPS13A gene encoding for chorein. As a neurodegenerative disease it leads to chorea, epilepsy, problems with mood, thinking and memory, and acanthocytosis of red blood cells. At present there are no treatments that can halt or slow down the progression of these diseases; however, our research continues to move in the direction of possible therapies.

The objectives of this meeting are to bring together basic and clinical neuroscientists and neurologists to address new discoveries in this expanding field. There will be a particular focus on important developments in basic science since 2016, in addition to the potential clinical translation of experimental treatments developed *in vitro*.

The setting of the meeting will encourage interactions, exchange of ideas and networking opportunities among all participants. Young faculty and students will have the opportunity to present their work for example during the poster session.

Patients and caregivers continue to play a critical role in this process, as we will discuss together the next steps, action points, and future studies. This is a collaborative process involving researchers, clinicians, patients, and caregivers, in our quest to advance knowledge and practice. As always, the Advocacy for Neuroacanthocytosis Patients is playing a crucial role in determining our goals.

Again, we welcome you in Dresden and hope you have an inspiring meeting.



Andreas Hermann
Chair of the Meeting



Kevin Peikert
Congress Secretary

Dear Colleagues, Patients and Families,

We are delighted to join our friends at Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden to welcome you to the 9th International Meeting on Neuroacanthocytosis Syndromes in Dresden and the second to include patients and families. Scientists and clinicians are leading contributors to the research and into the cause and treatment of neuroacanthocytosis. We are very excited to support this opportunity to continue the efforts to maintain collaboration between scientists from around the world to pursue the causes and cures of this group of neurodegenerative diseases.

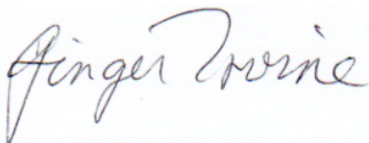
The Advocacy grew out of the spirit of collaboration in the 2002 Symposium organized by Professor Adrian Danek in the monastery at Seon in southern Bavaria. We are a movement that brings together everyone concerned with and interested in this group of debilitating diseases. Scientists see opportunities for both research and service to patients. The Advocacy helps communication between patients, families and friends, and supports the science with grants for projects.

We are grateful to Professor Andreas Hermann, Kevin Peikert and their colleagues at the University for the generous hospitality, allowing us to join in the endeavors of this cutting edge group of international experts in the field of neurodegeneration.

We all look forward to both the knowledge and the personal links that will come from this two day meeting with time for discussion and interaction.

With our deep thanks for your concern for suffering caused by neurodegeneration.

Best regards,

A handwritten signature in blue ink that reads "Ginger Irvine". The signature is written in a cursive, flowing style.

Ginger Irvine
Advocacy for Neuroacanthocytosis Patients

GENERAL INFORMATION

MEETING VENUE

The 9th International Meeting on Neuroacanthocytosis Syndromes takes place from 23 to 25 March, 2018 at

Radisson Blu Park Hotel & Conference Centre Dresden Radebeul
Nizzastrasse 55, D-01445 Dresden Radebeul, Germany

www.radissonblu.com/en/parkhotel-dresdenadebeul

The conference hotel is located just a few kilometers from Radebeul city center, it also provides proximity to Dresden.



REGISTRATION AND ACCOMODATION

We tried hard to reduce costs wherever possible. We want to encourage young scientists to attend the meeting. Registration fee includes the meeting costs and food & beverage during the day. Accommodation in the meeting's hotel is not included.

Accommodation and registration for patients and caregivers will not be charged.

Registration fee: 100 € [full meeting] / 50 € [day ticket]

Discounted registration fee*: 50 € [full meeting]

*discounts are available for students / PhD students.

CONTACT

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University Hospital
Carl Gustav Carus
THE DRESDENERS.



Meeting's website: www.ukdd.de/neuroacanthocytosis-syndromes-meeting

FINAL PROGRAM

Thursday, March 22, 2018:

Optional: Arrival of scientists, patients and caregivers

Friday, March 23, 2018:

13:00-17:00 **Registration**

Patients and Caregivers Session I

11:00-12:30 **Registration / Lunch**

12:30-12:45 **Introduction**
Ginger and Alex Irvine and Joy and Mark Williford

12:45-13:00 **Roundtable introduction of all participants**

13:00-13:30 **A patient's history**
Claudia Volger

13:30-14:00 **Short clinical overview**
Adrian Danek, Hans H. Jung and Ruth H. Walker

14:00-14:15 **New developments in translational research**
Andreas Hermann

14:15-15:00 **Medical Q&A**
Adrian Danek, Andreas Hermann and Ruth H. Walker

15:00-15:30 **Coffee break**

15:30-16:30 **Practice music-sound conversation**
Alexandra Takats (music therapist)

Scientists/Patients and Caregivers

17:00-17:20 **Conference Opening**
Andreas Hermann and Ginger Irvine

- 17:20-19:00 **Opening Session: The VPS13 gene family**
Chairs: Bernhard Landwehrmeyer and Ruth H. Walker
- 1.) **The syndrome of Chorea-Acanthocytosis**
Adrian Danek
 - 2.) **Genetics of Chorea-Acanthocytosis**
Antonio Velayos-Baeza
 - 3.) **Functional studies of the Cohen syndrome-associated protein VPS13B (COH1)**
Wenke Seifert
 - 4.) **Genetic contribution of mutated VPS13C to Lewy Body Dementia**
Christine Van Broeckhoven
 - 5.) **VPS13D, a new ataxia gene, plays an essential role in mitochondrial morphology and maintenance in Drosophila**
Catherine Collins
- 19:00 **Reception**
- 19:30 **Dinner**

Saturday, March 24, 2018:

Scientists/Patients and Caregivers

- 09:00-10:20 **Yeast and Dictyostelium as lower organism models**
Chairs: Ody C. M. Sibon and Masayuki Nakamura
- 1.) **Molecular analysis of yeast Vps13p**
Robert S. Fuller
 - 2.) **Using yeast Vps13 models of Chorea-Acanthocytosis to isolate genetic and chemical suppressors**
Teresa Zoladek
 - 3.) **The many roles of VPS13 in budding yeast**
Aaron M. Neiman
 - 4.) **The role of VPS13A in the endo-lysosomal and autophagic pathways**
Ricardo Escalante
- 10:20-10:40 **Coffee break**

10:40-11:40

Animal models

Chairs: Sami J. Barmada and Catherine Collins

1.) Drosophila oogenesis as a model system to reveal novel functions of VPS13

Ody C. M. Sibon

2.) A mouse model of Chorea-Acanthocytosis

Masayuki Nakamura

3.) Phenotypes of Vps13a, Vps13c and Vps13d knockout mice

Birgit Rathkolb

11:40-13:00

Lunch

Parallel Sessions: Scientists

13:00-13:40

Cell models I

Chairs: Lucia De Franceschi and Wenke Seifert

1.) Chorea-Acanthocytosis – characterization and functional regeneration of affected neurons

Hannes Glaß

2.) Disease-associated VPS13A mutations: consequences for neuronal function and survival

Sami J. Barmada

13:40-15:00

Disturbed pathways in Chorea-Acanthocytosis

Chairs: Ricardo Escalante and Robert S. Fuller

1.) Impaired autophagy is combined with abnormal signal transduction in Chorea-Acanthocytosis

Lucia De Franceschi

2.) Disturbed red blood cell mechanics in patients with Neuroacanthocytosis

Giel Bosman

3.) Cellular mechanisms contributing to acanthocytosis and neurodegeneration in Chorea-Acanthocytosis

Florian Lang

4.) Actin regulation in neurological diseases

Peter Claus

15:00-15:20

Coffee break

Parallel Sessions: Patients and Caregivers Session II

- 13:00-14:00 **Caregivers' issues / physical and occupational therapy**
Nico Bitter, Antonia Hanslik and Caroline Hoffmann (physical and occupational therapists)
- 14:00-15:00 **Mindfulness**
Nancy Glynn
- 15:00-15:20 **Coffee break**

Scientists/Patients and Caregivers

- 15:20-16:40 **(Para-) Clinics and standard of care in Chorea-Acanthocytosis**
Chairs: Adrian Danek and Ginger Irvine
- 1.) **Symptomatic treatment of Neuroacanthocytosis Syndromes**
Ruth H. Walker
 - 2.) **Future therapies of Neuroacanthocytosis Syndromes/Deep brain stimulation**
Bernhard Landwehrmeyer
 - 3.) **Cognition in Chorea-Acanthocytosis: Relevance of executive functions and avenues for treatment**
Christian Beste
 - 4.) **Patient centered care**
Ginger Irvine
- 16:40-17:00 **Coffee break**
- 17:00-18:00 **Poster Session**
- 19:00 **Dinner**

Sunday, March 25, 2018:

Patients and Caregivers

10:00-13:00 **Trip to the Historic Green Vault**

Scientists

9:00-11:00 **Workshop I**
Chairs: Adrian Danek and Andreas Hermann

Results and questions from two experimental treatment approaches with Dasatinib and Lithium/ Clinical trial readiness

Bonifacio group, Bosman group, Claus group, De Franceschi group, Guck group, Hermann group, Lang group, Salzer group

11:00-11:30 **Coffee break**

11:30-13:00 **Workshop II**
Chairs: Adrian Danek and Bernhard Landwehrmeyer

Future directions of scientific work

13:00-14:00 **Lunch**

SPEAKERS

Barmada	Sami J.	Department of Neurology, University of Michigan School of Medicine, Ann Arbor, Michigan, USA
Beste	Christian	Cognitive Neurophysiology, Department of Child and Adolescent Psychiatry, Technische Universität Dresden, Dresden, Germany
Bosman	Giel	Department of Biochemistry, Radboud University Medical Center, Nijmegen, The Netherlands
Claus	Peter	Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover, Germany and Center for Systems Neuroscience (ZSN) Hannover, Hannover, Germany
Collins	Catherine	Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan, USA
Danek	Adrian	Neurologische Klinik, Klinikum der Universität, Ludwig-Maximilians-Universität München, Munich, Germany
De Franceschi	Lucia	Department of Medicine, University of Verona, Verona, Italy
Escalante	Ricardo	Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, Spain
Fuller	Robert S.	Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan, USA
Glaß	Hannes	Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Dresden, Germany
Glynn	Nancy	Advocacy for Neuroacanthocytosis Patients, London, United Kingdom
Hermann	Andreas	Division for Neurodegenerative Diseases, Department of Neurology, Technische Universität Dresden, and DZNE, German Centre for Neurodegenerative Diseases, Research Site Dresden, Dresden, Germany
Irvine	Ginger	Advocacy for Neuroacanthocytosis Patients, London, United Kingdom
Jung	Hans H.	Department of Neurology, University Hospital Zurich, Zurich, Switzerland
Landwehrmeyer	Bernhard	Department of Neurology, Ulm University, Ulm, Germany

Lang	Florian	Department of Physiology I, University of Tübingen, Tübingen, Germany
Nakamura	Masayuki	Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
Neiman	Aaron M.	Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York, USA
Rathkolb	Birgit	German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, Munich, Germany, and Institute of Molecular Animal Breeding and Biotechnology, Ludwig-Maximilians-Universität München, Munich, Germany
Seifert	Wenke	Institute of Vegetative Anatomy, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
Sibon	Ody C. M.	Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
Van Broeckhoven	Christine	Neurodegenerative Brain Diseases, Center for Molecular Neurology, VIB & Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium
Velayos-Baeza	Antonio	Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom
Volger	Claudia	Patients and Caregivers, Leipzig, Germany
Walker	Ruth H.	Department of Neurology, James J. Peters Veterans Affairs Medical Center, Bronx, New York, and Mount Sinai School of Medicine, New York, New York, USA
Williford	Joy and Mark	Patients and Caregivers, Melbourne, Florida, USA
Zoladek	Teresa	Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Department of Genetics, Warsaw, Poland

ABSTRACTS – SCIENTIFIC TALKS

(In alphabetical order of presenting author's last names)

Disease-associated VPS13A mutations: consequences for neuronal function and survival

Tank E, Qiao T, Tidball A, Dauer W, Parent J, and Barmada SJ

Department of Neurology, University of Michigan School of Medicine, Ann Arbor, MI 48109, USA

Mutations in the gene encoding vacuolar protein sorting 13 homolog A (VPS13A) are responsible for Chorea-Acanthocytosis (ChAc), a neurodegenerative disorder characterized by seizures, cognitive decline, involuntary dance-like movements of the limbs (chorea) and abnormal red blood cell morphology. VPS13A participates in several essential physiologic processes, including maintenance of cytoskeletal architecture, protein turnover via autophagy, neurotransmitter release, and calcium-mediated signaling. How disease-associated VPS13A mutations affect such functions, and whether one or all of these activities are required for neuronal survival, remain unknown. To answer these questions, we pursued two complementary approaches. First, we developed a human neuron model of ChAc by generating induced pluripotent stem cells (iPSCs) from individuals with ChAc and unaffected family members, and differentiating these into excitatory forebrain-like neurons. Second, we utilized CRISPR/Cas9 genome editing to fluorescently label the VPS13A protein at its endogenous locus in human cells, thereby obviating the need for VPS13A-reactive antibodies and enabling noninvasive studies of VPS13A localization and function in living cells. In ongoing studies, we take advantage of a unique platform of automated microscopy with the capacity to link disease-related phenotypes—such as altered neuronal morphology, autophagy activity, protein clearance, and calcium homeostasis—to the probability of neuronal survival in a prospective fashion. In this way, we will highlight the relevant pathways affected by pathogenic *VPS13A* mutations, and emphasize therapeutic targets most likely to effectively prevent neuron loss in ChAc and related Neuroacanthocytosis Syndromes.

Cognition in Chorea-Acanthocytosis: Relevance of executive functions and avenues for treatment

Christian Beste

Cognitive Neurophysiology, Department of Child and Adolescent Psychiatry, TU Dresden, Germany

Cognitive deficits are a common symptom of neurodegenerative diseases like chorea acanthocytose (ChAc). Due to the progressive loss of neurons in fronto-cortical as well as subcortical areas including the basal ganglia ChAc can be summarized as “fronto-subcortical syndrome”. The effective interplay of these areas, forming fronto-subcortical functional loops, constitute the basis for many higher order cognitive functions like planning and execution of goal-directed behaviors, attention, mental flexibility and self-regulation, as well as response inhibition. These functions are essential for adapting our behavior in everyday life and are commonly subsumed under the term “Executive functions”. Using neurophysiological approaches, sub-processes of executive functions can be analyzed in detail, thereby broadening our understanding of pathological changes of functional loops associated with ChAc. We here present the current state of research on cognitive deficits in ChAc and discuss possible avenues for treatment like deep brain stimulation (DBS).

Disturbed red blood cell mechanics in patients with Neuroacanthocytosis

Giel Bosman, Dan Lazari, Joames Freitas Leal, Merel Adjobo-Hermans

Department of Biochemistry, Radboud University Medical Center, HB Nijmegen, The Netherlands

The structure of red blood cells is affected by many inborn and acquired factors, but in most cases this does not seem to affect their function or survival in physiological conditions. Often, functional deficits become apparent only when they are subjected to biochemical or mechanical stress in vitro, or to pathological conditions in vivo. Our in vitro data on the misshapen red blood cells of patients with neuroacanthocytosis suggest that abnormal red cell morphology is associated with an increase in susceptibility to osmotic and mechanical stress, and alters their rheological properties. We postulate that the underlying mutations may not only affect these red cell functions, but may also render neurons in specific brain areas more susceptible to a concomitant reduction in oxygen supply. Through this mechanism, an increased susceptibility of compromised red blood cells to physiological stress conditions may constitute an additional risk factor for vulnerable neurons.

Actin regulation in neurological diseases

Niko Hensel, Peter Claus

Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover, Germany and Center for Systems Neuroscience (ZSN) Hannover, Hannover, Germany

Coordinated regulated of the actin cytoskeleton is an essential and important process in diverse cell types. Actin dynamics comprises a polymerization of monomeric, globular (G-) actin into filamentous (F-) actin and the depolymerization of F- into G-actin. These processes are directly regulated by actin binding proteins: Profilin binds to G-actin and regulate its polymerization into F-actin and facilitates G-actin recycling. Cofilin severs actin filaments into shorter elements thereby increasing the number of actin ends for increased polymerization. Both proteins participate in this fine-tuned process called actin treadmilling. Both, Profilin and Cofilin, are downstream targets of Rho-kinase (ROCK). Therefore, ROCK is an important signaling hub and its dysregulation alters actin dynamics in multiple cell types including neurons and glia. We have analyzed the roles of Profilin, Cofilin and ROCK in the motoneuron diseases Spinal Muscular Atrophy (SMA)^{1,2} and Amyotrophic Lateral Sclerosis (ALS)³ as well as in Chorea-Acanthocytosis⁴. We propose common molecular mechanisms in SMA and for some cases of ALS based on dysregulation of these signaling cascades resulting in altered actin dynamics in these diseases.

¹Nölle A. et al (2011): The Spinal Muscular Atrophy disease protein SMN is linked to the Rho-kinase pathway via profilin. *Hum. Mol. Gen.* 20: 4865-4878.

²Rademacher S. (2017): Metalloprotease-mediated cleavage of PlexinD1 and its sequestration to actin rods in the motoneuron disease Spinal muscular atrophy (SMA). *Hum. Mol. Gen.* 26: 3946-3959.

³Hensel N. & Claus P. (2017): The actin cytoskeleton in SMA and ALS: How does it contribute to motoneuron degeneration? *Neuroscientist* 24: 54-72.

⁴Stanslowsky Net al. (2016): Neuronal dysfunction in iPSC-derived medium spiny neurons from Chorea-Acanthocytosis patients is reversed by Src-kinase inhibition and F-actin stabilization. *J. Neurosci.* 36:12027–12043.

VPS13D, a new ataxia gene, plays an essential role in mitochondrial morphology and maintenance in *Drosophila*

Ryan Insolera¹, David Lozano¹, Alec Wishnie¹, Eunju Seong², Margit Burmeister², Ody Sibon³, Catherine Collins¹

¹Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan, USA

²Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, Michigan, USA

³Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

Recent genetic studies in humans have associated mutations in *VPS13D* with adult onset ataxia. Using *Drosophila melanogaster* as a model system to study the cellular functions of *Vps13D* in the nervous system, we have identified an essential role in the maintenance of mitochondrial structure. Loss-of-function mutations in *VPS13D* lead to severe defects in mitochondrial morphology in multiple tissues, and early larval lethality. Targeted knockdown of *VPS13D* in subsets of neurons and muscle cells circumvents this early lethality to allow further analysis. In both neurons and muscle cells we observed oversized, atypical mitochondria, some of which contain mitochondrial inner membrane proteins but lack matrix proteins, and vice versa. Because autophagy machinery appears to be strongly engaged in muscle cells, we interpret these atypical mitochondria to be breakdown intermediates. Atypical mitochondria with similar appearance also accumulate in *VPS13D* depleted neurons, and strikingly, also appear in nearby glial cells. We are currently testing whether these atypical mitochondria are transferred from neurons to glia.

VPS13D knockdown also leads to accumulations of poly-ubiquitin aggregates in neurons and muscles. Because this phenotype was recently described for mutations in *VPS13* (orthologous to the A and C family members) (DOI:10.1371/journal.pone.0170106), we have begun to examine whether *Drosophila VPS13* null mutants share mitochondrial defects with *VPS13D* and note preliminary similarities in certain tissues. Altogether, these observations dovetail with findings for other *VPS13* family members suggesting an important role in the maintenance of mitochondria.

Clues to *VPS13A* function from clinical observation?

Adrian Danek

Neurologische Klinik, Ludwig-Maximilians-Universität, Munich, Germany

Chorea-Acanthocytosis (ChAc) has become increasingly well characterized clinically since *VPS13A* mutations had been discovered as its genetic basis in 2001. In spite of the wealth of *in vivo* and *in vitro* models presented at neuroacanthocytosis symposia past and present, its function has so far remained elusive (alternatively, its functions?). It may be worthwhile to review features of the disease for clues.

1, ChAc is an autosomal-recessive condition. 2, Gender distribution appears equal. 3, In the great majority of patients, the *VPS13A* product, chorein, is absent. 4, Disease manifestation typically is in the third decade. 5, ChAc affects multiple organ systems, most prominently the central and the peripheral nervous system. 6, There is progressive nerve cell loss yet relative increase of glia cells, nevertheless with brain atrophy as the final outcome. No intracellular inclusions whatsoever, the supposed hallmark of neurodegeneration these days, have yet been discovered, neither extracellular protein aggregates. 7, CK elevation occurs in probably all patients and clinical myopathy may be observed. 8, Acanthocytosis is found often and points to involvement of hemopoietic cells, but is not obligatory. 9, Connections with other so-called Neuroacanthocytosis Syndromes appear tenuous, apart from McLeod syndrome (MLS), which may be viewed as a more slowly progressing, X-linked variant of the disease process (mostly in males), with much delayed onset. 10, The involvement of *VPS13B* in hemopoiesis as well as of *VPS13C* and *VPS13D* in neurodegeneration is notable.

It appears as if *VPS13A* function is essential for neurons with their high metabolic activity and that the mere lack of chorein seems sufficient to explain ChAc. The absence of debris speaks against "toxic gain of function", too. Interestingly, about 2% of all currently known cases diagnosed by DNA analysis have a normal chorein Western blot: it may be assumed that the underlying point mutations hit a region of the protein with subsequent "loss of function".

Alternative pathways may temporarily compensate before widespread cell loss, mainly of basal ganglia neurons, becomes apparent. The many similarities of ChAc and MLS make the protein affected in the latter, Kx, a natural candidate. Additional compensatory pathways may become known from the current studies in disease models such as yeast. Nevertheless, plasticity of the brain as a whole and/or differences in the time course of specific nerve cell subpopulations, not necessarily "metabolic detours within cells" might explain delays in age of onset. Also, while in MLS heart involvement is typical, in ChAc it is a very rare exception.

With respect to the hemopoietic system, *VPS13A* mutations do not appear to be of crucial importance. Presence of acanthocytes, with wide ranges reported, seems unrelated to red cell function or survival, yet no systematic data are available, e.g. on erythrocyte life span in ChAc. Other rapidly dividing cell populations such as those of the GI tract do not seem essentially affected either. So far, no data are available on acanthocytosis or CK elevation in children with

ChAc mutations but could help to understand very early disease stages. Muscle cell affection in ChAc seems more similar to the hemopoietic than the nervous system involvement.

Another open question relates to the issue of subclinical manifestations in ChAc patients' parents. A few reports mention acanthocytosis in these carriers of a single gene copy mutation, yet all reports that initially favoured autosomal-dominant disease transmission eventually were disproven.

Concerning *VPS13A* function, currently the rare situations of point mutations in spite of apparently normal chorein expression seem of greatest clinical interest. Further, similarity/dissimilarity across the range of disease manifestations of *VPS13* gene family members needs to be better characterized.

Impaired autophagy is combined with abnormal signal transduction in Chorea-Acanthocytosis

Enrica Federti¹, Alessandro Matte¹, Annalisa Alfieri², Paola Defilippi², Mario Buffelli³, Emilia Turco², Gabriela Constatin¹, Erika Lorenzetto³, Francesca Lupo¹, Andreas Hermann⁴, Adrian Danek⁵, Ruth H Walker⁶, Seth L Alper⁷, Immacolata Andolfo⁸, Angela Siciliano¹, Paolo Fabene², Serge Cedrick M Toya¹, Elisabetta Benduce¹, Achille Iolascon⁸, Lucia De Franceschi¹

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²Dept of Molecular Biotechnologies and Life Sciences, University of Torino, Torino; Italy;

³Dept of Neurosciences, Biomedicine, and Movement Sciences, University of Verona;

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⁵Dept. of Neurology, Ludwig-Maximilians-Universität, Munich, Germany;

⁶Dept of Neurology, JJ Peters VAMC, Bronx and Mount Sinai School of Medicine, NY, USA;

⁷Division of Nephrology and Vascular Biology Research Center, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA;

⁸Dept of Molecular Medicine and Medical Biotechnologies, University Federico II, Naples; Italy

Chorea-Acanthocytosis (ChAc) is a severely invalidating, hereditary neurodegenerative disorder. Current clinical management of patients with ChAc is limited, due to lack of understanding of the VPS13a-encoded protein, chorein, that is absent or nonfunctional in ChAc. We recently identified a novel link between the absence of chorein and the impairment of autophagy, resulting in "toxic" accumulation of active Lyn kinase. Here, we generated a mouse model genetically lacking chorein (*Vps13a*^{-/-}). We confirmed the absence of chorein in red cells and in isolated basla ganglia by both RT-PCR and immunoblot analysis. Hematologic parameters were evaluated at 2, 4, 6, 8, 10 and 12 months of age. Circulating acanthocytes were present at the different time points studied and accumulation of multivesicular bodies were observed in acanthocytes from *Vps13a*^{-/-} mice similarly to human ChAc red cells (Lupo F et al Blood 2016). In addition, *Vps13a*^{-/-} mouse erythrocytes exhibited accumulation of active Lyn (P-Lyn 396) compared to wild-type cells, in agreement with human studies (Lupo F et al. Blood 2016). We then evaluated the expression of key autophagy related proteins in *Vps13a*^{-/-} mouse red cells. we found accumulation of LC3II, Ulk1, Atg4, Atg5 and Rab5. As in human ChAc erythrocytes (Lupo F et al. Blood 2016), we observed in *Vps13a*^{-/-} mouse red cells Lyn co-immunoprecipitation Ulk1, but not Atg4, suggesting a selective association between Ulk1 and Lyn. These data support an impairment of autophagy in *Vps13a*^{-/-} mouse erythrocytes, in agreement with our report on human ChAc red cells (Lupo F et al. Blood 2016). We conducted molecular analysis of isolated basal ganglia from saline buffer-perfused 12-month old *Vps13a*^{-/-} mice. In *Vps13a*^{-/-} mouse isolated basal ganglia, we found active Lyn stabilized in high molecular weight complexes, as earlier observed in human erythroid cells (Lupo F et al. Blood 2016). Fluoro-Jade C labeling revealed initial stages of neurodegeneration in basal ganglia from *Vps13a*^{-/-} mice compared to matched wild-type. we found a slight increase in LC3II in *Vps13a*^{-/-} mice compared to wild-type controls, and significant accumulation of the following autophagy related proteins: Ulk1, Atg4, Atg5-12, Atg9 and the lysosomal cargo protein p62, supporting impairment of autophagy in *Vps13a*^{-/-} mice. We then investigated beclin-1, a key initiator of

autophagy reported to be reduced in other neurodegenerative disease models (Salminen A et al. *Progress in Neurobiology* 106-107: 33, 2013; Mcknight NC et al *Plos Genetics* 10: e1004626, 2014). Beclin-1 was significantly reduced in Vps13a^{-/-} mice. Whereas, we found up-regulation of Beclin-1 mRNA levels in isolate basal ganglia from Vps13a^{-/-} mice compared to wild-type mice. We also observed an accumulation of beclin-1 complex components, Vp34 and Rab5, was increased compared to wild-type mice, suggesting a perturbation of beclin-1 related autophagy pathway in Vps13a^{-/-} mice. To further evaluate a possible link between chorein and beclin-1, we immunoprecipitated beclin-1 and immunoblotted for either chorein, Atg14L or Vps34. we found chorein co-immunoprecipitated with beclin-1 in basal ganglia from wild-type mice but not from Vps13a^{-/-} mice. In addition, we observed a reduction in Vps34 association with beclin-1 in Vps13a^{-/-} mice compared to wild-type animals. Since beclin-1 levels depend on caspase 3 activity, we evaluated caspase-3 activation by both immunoblot analysis of total cleaved caspase-3 and ELISA for caspase-3 activity. In basal ganglia of Vps13a^{-/-} mice, caspase-3 activity was increased compared to wild-type animals contributing to reduction of beclin-1 expression observed in Vps13a^{-/-} mice. It is of note that increased casapse-3 activity has been reported in brains from PD patients (Hartmann A et al. 97: 2875, 2000; Blandini F et al. 111: 1017, 2004). Collectively these preliminary data indicate that absence of chorein in 12-months-old Vps13a^{-/-} mice is associated with impairment of autophagy in basal ganglia, possibly linked to perturbation of the beclin-1 complex (reduction of beclin-1, accumulation of Vps34 and increased Caspase-3 activity).

These data are the first allowing proposal of ChAc as a novel disorder of proteostasis related to impaired autophagy due to the absence of chorein, possibly involved in formation of the beclin-1 complex.

The role of VPS13A in the endo-lysosomal and autophagic pathways

Sandra Muñoz-Braceras, Alba Tornero, Olivier Vincent, Ricardo Escalante

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VPS13 proteins are a group of conserved proteins whose mutation lead to the appearance or higher risk to suffering from different diseases. Particularly, VPS13A mutations cause Chorea-Acanthocytosis, a rare neurodegenerative disorder, for which available treatments are not able to modify the disease progression. Human VPS13A and other members of the VPS13 family have been implicated autophagy and other cellular processes, yet their molecular functions and the mechanistic details of their participation in those processes are still unknown. We propose that the VPS13 function in autophagy is part of a more general role in intracellular trafficking. This idea is supported by the altered pattern of several proteins involved at distinct steps of vesicle dependent trafficking processes in human cells lacking VPS13A and other findings in the simple model organism *Dictyostelium discoideum*. Moreover, human and *D. discoideum* VPS13 proteins interact with a key player in intracellular trafficking regulation. In addition, new avenues to explore the exact role of VPS13 proteins in autophagy come from the elucidation of VPS13A subcellular localization. Altogether, our findings provide the identification of potential targets that should be considered for the development of therapies for Chorea-Acanthocytosis.

Molecular analysis of yeast Vps13p

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Yeast Vps13p is the prototype of a family of conserved eukaryotic proteins that includes four human homologs, VPS13A-D, each the locus of an autosomal recessive neurodegenerative or neurodevelopmental disorder. Vps13p is involved in a variety of membrane transactions (homotypic fusion, vesicular transport, prospore membrane maturation) and also localizes to several membrane/organelle contact sites (nuclear-vacuolar, mitochondrial-vacuolar and ER-lipid droplet junctions). Using cell-free fusion and transport assays, we find that Vps13p is directly required for TGN homotypic fusion and TGN-to-late endosome vesicular transport. Extracts from cells with loss of function mutations in VPS13 are defective in these reactions but activity is restored by adding Vps13p purified from yeast. 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. Purified Vps13p also binds specifically to synthetic liposomes doped with phosphatidic acid (PA), and phosphorylated forms of phosphatidyl inositol including PI(3)P, PI(4)P and PI(4,5)P₂. Conserved, recombinant domains expressed in and purified from *E. coli* exhibit lipid-selective binding [N-domain, PA; Duf1162 domain, PI(3)P; C-domain, PI(4,5)P₂]. Analysis of TEM images of negatively stained Vps13p indicate that this large protein (3144 residues, 358 kDa) is folded into a flexible, curved, compact rod (28 x 6 nm) with a loop at one end that possesses a circular opening of ~6 nm in diameter. Human VPS13A in red blood cells is in a complex with β -actin and β -adducin, suggesting a role for VPS13A in actin organization [Shiokawa N, et al., (2013) BBRC 441:96-101]. A possible β -adducin-related protein in yeast, Bsp1p, known to be an actin-capping protein, is required for both TGN-late endosome transport and TGN homotypic fusion. It is hoped that molecular analysis of core functions of yeast Vps13p will help inform studies of the human disease homologs. Parts of this abstract have been published in De, M., et al. (2017) *J. Cell. Biol.* 216:425-439. Supported by the Lakritz-Weinbaum Gift Fund and the Protein Folding Disease Initiative at the University of Michigan Medical School.

Chorea-Acanthocytosis – characterization and functional regeneration of affected neurons

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Mutations in the VPS13A gene leading to depletion of Chorein protein are causative for Chorea-Acanthocytosis (ChAc), a movement disorder characterised by red blood cell acanthocytes and a degeneration of striatal neurons leading to epilepsy and hyperkinetic movement. Recently, severe cell membrane disturbances based on dysregulation of Actin cytoskeleton caused by downregulation of the PI3K pathway and hyper-activation of Lyn-kinase were identified, but to what extent these mechanisms are present and relevant in the affected neurons remains elusive. We studied the effect of Chorein absence in ChAc patients' induced pluripotent stem cells derived GABAergic medium spiny neurons. Morphology and trafficking of mitochondria and lysosomes is altered in ChAc patients. Number of both organelle types was reduced and mitochondria were shortened showing a hyperpolarization. In compartmentalized microfluidic chambers a reduction in retrograde transport was observed. Pharmacological interventions with the Src-kinase inhibitor PP2, were ineffective in treating the observed phenotypes. Electrophysiological analysis revealed a significantly elevated synaptic activity in ChAc. Treatment of cells with the actin-stabilizer phalloidin or PP2 resulted in the reduction of disinhibited synaptic activity in ChAc neurons to the level of healthy controls, suggesting an actin dependent mechanism of pathologically enhanced synaptic activity. These data suggest that the previously established treatments of Chorea Acanthocytosis related phenotypes are not effective for all pathological deficiencies. The observed changes in lysosome and mitochondria population seem to act independent of the electrophysiological phenotypes.

Cellular mechanisms contributing to acanthocytosis and neurodegeneration in Chorea-Acanthocytosis

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Chorea-Acanthocytosis (ChAc), a neurodegenerative disease, results from loss-of-function-mutations of the chorein-encoding gene VPS13A. Affected patients suffer from a progressive movement disorder including chorea, parkinsonism, dystonia, tongue protrusion, dysarthria, dysphagia, tongue and lip biting, gait impairment, progressive distal muscle wasting, weakness, epileptic seizures, cognitive impairment, and behavioral changes. Those pathologies may be paralleled by erythrocyte acanthocytosis. Chorein supports activation of phosphoinositide-3-kinase (PI3K)-p85-subunit with subsequent up-regulation of ras-related C3 botulinum toxin substrate 1 (Rac1) activity, p21 protein-activated kinase 1 (PAK1) phosphorylation, and activation of several tyrosine kinases. Chorein sensitive PI3K signaling further leads to stimulation of the serum and glucocorticoid inducible kinase SGK1, which in turn upregulates ORAI1, a Ca²⁺-channel accomplishing store operated Ca²⁺-entry (SOCE). The signaling participates in the regulation of cytoskeletal architecture on the one side and cell survival on the other. Compromised cytoskeletal architecture has been shown in chorein deficient erythrocytes, fibroblasts and endothelial cells. Impaired degranulation was observed in chorein deficient PC12 cells and in platelets from ChAc patients. Similarly, decreased ORAI1 expression and SOCE as well as compromised cell survival were seen in fibroblasts and neurons isolated from ChAc patients. ORAI1 expression, SOCE and cell survival can be restored by lithium treatment, an effect disrupted by pharmacological inhibition of SGK1 or ORAI1. Chorein, SGK1, ORAI1 and SOCE further confer survival of tumor cells. Further examination is required exploring whether the *in vitro* observations indeed reflect the *in vivo* pathology of the disease.

A mouse model of Chorea-Acanthocytosis

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We previously generated a model mouse of Chorea-Acanthocytosis (ChAc-model mouse), which carries a targeted deletion mutation in the mouse *Vps13a* gene corresponding to a human disease mutation. The mutant mice, which have a hybrid C57BL/6J and a 129/Sv genetic background, displayed variable phenotypes, strongly suggesting the existence of modifier genes. Recently, we backcrossed the model mice, and created four strains carrying the *Vps13a* mutation on C57BL/6, 129/S6, Balb/c, and FVB genetic backgrounds. We investigated the effects of the genetic background on the phenotypic variation of ChAc-model mice using a number of behavioral analyses. ChAc-model mice backcrossed to the different inbred strains exhibited differences in symptoms. We suggest that this is a result of symptom-modifying factors of ChAc in the genetic background. In this meeting, we will review the ChAc-model mouse and recent findings on phenotypes of epileptic seizure and male infertility.

The many roles of VPS13 in budding yeast

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The budding yeast, *Saccharomyces cerevisiae* has a single VPS13 gene. Mutation of VPS13 causes pleiotropic phenotypes. These phenotypes implicate VPS13 in multiple cellular processes including vesicular traffic between the Golgi and endosome, mitochondrial maintenance, sporulation, and others. Vps13 displays dynamic localization to multiple intracellular sites depending on growth conditions and alleles that alter this distribution display subsets of the null phenotype, suggesting that Vps13 activity at different subcellular sites contributes to its multiple roles. Whether the different phenotypes of the null result from loss of a single critical function or multiple, different functions of the protein is unclear. Genetic and cell biological studies suggest that Vps13 may function at membrane contact sites, which are sites of transfer of lipids and metabolites between organelles. We describe evidence that VPS13 has a role in peroxisomal maintenance that is related to membrane contact site function and discuss the model that the pleiotropic phenotypes of vps13 mutants are due to multiple defects in membrane contact sites.

Phenotypes of Vps13a, Vps13c and Vps13d knockout mice

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The International Mouse Phenotyping Consortium (IMPC) phenotypically characterizes mouse knockout lines to identify genes involved in disease development and progression and to establish and offer mouse models for human diseases to the scientific community. In humans, mutations in genes of the VPS13 gene family could be associated with several pathological phenotypes: VPS13A and VPS13C mutations have been shown to cause neurological disorders and hematological alterations, belonging to the so-called Neuroacanthocytosis syndromes. Vps13B mutations were associated with the rare Cohen-Syndrome, and genetic alterations of VPS13D were found in cases of fetal death. GWAS studies also suggested an association of VPS13C variants with certain types of cancer and type II diabetes. Three of the four known members of this mammalian gene family were analyzed by the IMPC: Vps13a, Vps13c and Vps13d.

For the Vps13a gene, homozygous mutant young adult mice were investigated at the age of two to five months. Besides lower body weight but higher relative body fat content, effects on bone metabolism and reduced variability of red blood cell size were detected in these mice. We characterized Vps13c mutant mice at an age of two to five months and, in addition, after aging at an age of 12 to 15 months in the German Mouse Clinic. Irrespective of age homozygous mutant animals of this line showed reduced body mass with reduced relative body fat content, altered eye morphology including thinner retinas, chronic progressive pancreatitis and increased anisocytosis of erythrocytes. Aged mice also showed further signs of altered energy metabolism, and mild differences in behavioral and neurological tests. Vps13d homozygous mutations were lethal during early stages of embryonic development, while heterozygous mutant mice showed no clear phenotype.

Our mouse models suggest novel pleiotropic effects of Vps13a and Vps13c mutations that have not yet been described but could be relevant for translation to human patients. A more detailed characterization of pathogenic effects in the Vps13c mutant mouse line, especially regarding eye morphology and vision, neurological consequences and energy metabolism is currently under way at our institute. Further studies will be required to get a full picture of VPS13 gene mutations associated pathologies in humans and mice.

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. We identified the encoded VPS13B (3997aa), as a peripheral scaffold protein that localizes to the Golgi complex and contributes to its structural maintenance and function. Another study showed that disturbed Golgi complex homeostasis affects glycan maturation and that VPS13B-deficient cells display a reduced quantity of early endosomes and abnormally enlarged lysosomes, pointing to a role of VPS13B in endosomal-lysosomal trafficking. We show that RNAi-mediated knockdown of the small GTPase RAB6A/A', which tethers vesicles to the Golgi membrane and controls several trafficking steps, prevents Golgi localization of VPS13B. Co-immunoprecipitation experiments and mass spectrum analyzes confirmed the physical interaction of VPS13B with RAB6, which is in line with studies on yeast Vps13p. Our ongoing work focusses on Vps13b expression analyses, identification of other VPS13B interactors similar to the known yeast Vps13p network, and cortical development studies using RNAi. Depletion of VPS13B in primary neurons from the cortex negatively interferes with neurite outgrowth, indicating a causal link between the integrity of the Golgi complex and abnormal intracellular trafficking. Using in utero electroporation of shRNA we induced a selective neuronal Vps13b knock down in mice at different developmental stages. Initial data demonstrate that Golgi orientation and neuronal migration is affected.

Together, we conclude that VPS13B is a RAB6 effector protein and that reduced brain size in patients with Cohen syndrome likely results from impaired VPS13B function at the Golgi complex causing decreased neuritogenesis and subsequently altered neuronal migration.

Drosophila oogenesis as a model system to reveal novel functions of VPS13

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Chorea-Acanthocytosis (ChAc) is a rare neurodegenerative disorder characterized by progressive movement disorders and spiky formed red blood cells called acanthocytes. The disease is caused by loss of function mutations in the *vps13A* gene that lead to the absence of the Vps13A protein. Knowledge about the underlying pathophysiology of the disease and mechanisms involved is limited and mainly based on studies in unicellular organisms or cultured cells. The *Drosophila* ovary system is widely used to study biological and cellular processes because of its easy accessibility and the availability of many genetic tools. Using CRISPR/Cas9, we created mutant fly lines lacking Vps13 (*Vps13^{null}*), as well as lines containing GFP-tagged endogenous Vps13, to further investigate the function and localization of Vps13. Vps13 protein is highly abundant in the *Drosophila* ovary and analysis of mutant ovaries showed the absence of Vps13 protein. In addition mutant females have a deficit in egg lay and produce lower numbers of offspring. Immunofluorescent labeling with DAPI revealed higher numbers of dying egg chambers in mutant ovaries. Furthermore, an accumulation of persistent nurse cell nuclei (PNCN) in late stage egg chambers was observed. Presence of Vps13-GFP signal in close proximity to the accumulating PNCN implicate a novel role for Vps13 in proper egg development and removal of cells that undergo programmed cell death.

Genetic contribution of mutated *VPS13C* to Lewy Body Dementia

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VPS13C was first identified as a novel risk gene for Parkinson's disease (PD) in a genome-wide association meta-analysis for Parkinson's disease (Nalls et al. *Nature Genetics*, 2014). Homozygous or compound heterozygous truncating-mutations in *VPS13C* were associated with a distinctive phenotype of rapid and severe progression suggesting that loss-of-function of *VPS13C* is a cause of autosomal recessive early-onset PD (Lesage et al. *Am J Hum Genet.* 2016). Diagnostic exome sequencing in 80 individuals with PD symptoms at early age and a negative family history, identified in one patient compound heterozygous variants affecting canonical splice sites in *VPS13C*, confirming the causal role of protein-truncating variants to autosomal-recessive early-onset PD (Schormair et al. *Clin Genet.* 2017).

We identified compound heterozygous non-synonymous variants reducing *VPS13C* expression that were associated with Lewy Bodies disease (LBD). Lewy bodies disease (DLB) is the second most prevalent cause of neurodegenerative dementia with a frequency of 10 to 25%. The heterozygous missense mutations were identified by whole genome sequencing on an affected sib pair with early-onset dementia of which the index patient was pathological diagnosed with Lewy body disease (LBD) and the parents were not affected. Screening of *VPS13C* in a Belgian DLB cohort resulted in the identification of two additional carriers of compound heterozygous *VPS13C* mutations, a pathologically confirmed LBD patient and a clinically diagnosed DLB patient. In patient-derived lymphoblast cells, the presence of the two mutant alleles decreased endogenous *VPS13C* protein expression by almost 90%. Expression of *VPS13C* in autopsy brains of the two DLB carriers of compound heterozygous *VPS13C* variants showed reduced expression in the prefrontal and temporal cortex as well as in the hippocampus and cerebellum. Our genetic and expression data suggested that the *VPS13C* non-synonymous variants contributed by a loss-of-function mechanism comparable to the effect of protein-truncating variants. In addition, they underscore that the *VPS13C* variants affect residues that are essential for the functioning of the protein. The cellular expression studies indicated a co-localization with late endosomes and lysosomes in accordance with the subcellular distribution of *VPS13C* in the study of Lesage et al. 2016. Additional functional studies will be needed to understand the contribution of *VPS13C* to DLB and PD.

Genetics of Chorea-Acanthocytosis

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Research on Chorea-Acanthocytosis (ChAc), an adult-onset rare neurodegenerative disorder, has reached now 50 years. It started in 1968 when two families from New England and Kentucky (USA) with several members affected with a neurological disease with acanthocytosis were independently reported, respectively, by Irvine Levine and Edmund Critchley, and led to the denomination of this new condition as the Levine-Critchley syndrome. Different similar cases were reported later on, many of them under the generic denomination of neuroacanthocytosis. This, however, is an umbrella term including several other similar conditions, all of which contributed to create a certain degree of confusion in the field that was only solved by the identification of the genetic causes at play. For ChAc this was achieved about 30 years after the original case reports, when the CHAC gene, in chromosome 9q21, was described, allowing confirmation of the recessive nature of the disorder. This gene was later renamed VPS13A due to its similarity to the yeast VPS13 gene, and identified as a member of a family containing four genes (VPS13A, B, C, D), all of which have now been associated with recessive disorders.

The identification of the genetic basis of ChAc has allowed us to undoubtedly demonstrate that the disorder described by Critchley in 1968 was indeed ChAc, after a fortunate turn of events involving a member of the original reported family getting in contact with a member of the rather small ChAc-scientific community. The same, however, cannot be said about the condition described by Levine since genetic analysis of his original family has not yet been possible.

Extensive screening for pathogenic mutations in affected patients revealed ChAc results from a loss-of-function of chorein, the protein encoded by the CHAC/VPS13A gene. These mutations have a gene-wide distribution, with no hot-spots, and include an extensive range of mutations: large deletions, splicing-site mutations, small insertion/deletions, nonsense mutations, or missense mutations. These analyses provide important insight about the function of chorein. First, the very C-terminal region of the protein is essential for, at least, its ChAc-related function(s) since patients with mutations in the last exons of VPS13A gene, resulting not in absence but in the production of mutant chorein altered only at the C-terminus, present the typical symptomatology of the disorder. Second, only a reduced number of missense changes have been described as pathogenic mutations, suggesting that the specific individual residues modified in these cases might be particularly relevant for some of the functions of chorein.

In any case, the overwhelming majority of described pathogenic ChAc mutations lead to absence of the protein, either by a mechanism of degradation of the mutated RNA or by instability and degradation of the mutant protein. This fact allowed us to develop a semi-diagnostic test based on the analysis by western blotting of protein extracts from blood, an approach much faster and cheaper than DNA sequencing. Although not all ChAc cases would be detected at this way, most of them are, implying that the positive cases detected by this test do not require further analysis unless a particular interest in knowing the causative mutations exists.

Finally, there has been in these years a certain debate over the existence of "dominant ChAc", that is, the description of patients presenting with ChAc syndrome but having only one VPS13A allele mutated while the second one would be normal. This would imply the single mutation present in these cases has a dominant nature and its mere presence in an individual would lead to development of ChAc. This was not only a question of scientific debate but also a cause of deep worry for patients and families for the possibility that carriers of VPS13A mutations (such as children of ChAc patients) could indeed develop the disease. This debate, however, seems to have been settled by the demonstration that some of the cases initially reported as "dominant" had indeed mutations in both VPS13A alleles, but one had been missed in the initial report. While theoretically it could be possible that a mutation in the VPS13A gene could have a dominant effect leading to ChAc, it is important to convey the message that not such a mutation has been described so far and that ChAc is only developed when both VPS13A alleles present pathogenic mutations.

Symptomatic treatment of Neuroacanthocytosis Syndromes

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The two core Neuroacanthocytosis Syndromes, Chorea-Acanthocytosis and McLeod syndrome, are progressive neurodegenerative disorders which affect primarily the basal ganglia. The characteristic phenotype comprises a variety of movement disorders, including chorea, dystonia, and parkinsonism, and also psychiatric and cognitive symptoms attributable to basal ganglia dysfunction. These disorders are managed symptomatically and on a case-by-case basis, with very few practitioners seeing more than a single case in their careers. There have been no blinded, controlled trials, and only one retrospective case series of patients undergoing deep brain stimulation for Chorea-Acanthocytosis. The various therapies which have been used in the Neuroacanthocytosis Syndromes will be summarized. Management remains at present purely symptomatic, and thus is similar in principle to other more common basal ganglia neurodegenerative disorders, such as Huntington's disease and Parkinson's disease, in terms of treatment both the movement disorders and the psychiatric issues. There are in addition specific issues particular to these conditions which merit attention, such as the early and prominent speech and swallowing issues in Chorea-Acanthocytosis, and the cardiac and hematologic issues in McLeod syndrome. An integrated multidisciplinary approach is the ideal management strategy for these complex and multifaceted neurodegenerative disorders.

Using yeast Vps13 models of Chorea-Acanthocytosis to isolate genetic and chemical suppressors

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Defects in the expression and structure of all human VPS13 (hVPS13A-D) genes are linked to multiple disorders, such as neurodegeneration, cancers and diabetes. In particular, mutations in the hVPS13A gene lead to a complex rare neurodegenerative disease known as Chorea-Acanthocytosis (ChAc). The roles of Vps13 proteins in specific molecular processes are still unclear. Vps13 proteins are conserved in eukaryotes and in recent years a number of studies have taken advantage of simple experimental models, such as yeast *Saccharomyces cerevisiae*, to investigate the function of Vps13 proteins. There is one Vps13 protein in yeast which is involved in vacuolar protein transport and, like hVps13A, participates in phospholipid metabolism. One of the mutation found in ChAc patients causes I2771R amino acid substitution in hVps13A protein which affects its localization in skeletal muscle cells. To dissect the mechanism of pathogenesis of I2771R, we created and analyzed a yeast strain carrying the equivalent mutation. We show that in yeast, substitution I2749R causes dysfunction of Vps13 protein in the actin cytoskeleton organization, endocytosis and vacuolar transport, and the defect of growth on media supplemented with various chemicals. We also show that Vps13, like hVps13A, binds actin and this ability is not disrupted in mutant vps13-I2749R. Moreover, we show that Vps13 binds phospholipids, especially phosphatidylinositol 3-phosphate (PI3P), via its SHR_{BD} and APT1 domains and substitution I2749R in APT1 domain attenuates this ability. To find out if vps13 Δ or vps13-I2749R defects could be overcome we screen yeast genomic library for multicopy suppressor genes which restore growth of mutant cells on tester plates. Five multicopy suppressor genes were isolated, including RCN2 which encodes negative regulator of calcineurin phosphatase and fragment of MYO3 encoding actin cytoskeleton protein which binds calmodulin. This indicates the connection between Vps13 functioning and calcium signaling and shows that the effects of deletion mutation could be overcome by changing other cellular pathways. With this knowledge, we screen the library of FDA-accepted drugs for those which restore growth of vps13 Δ strain on tester plates. We have identified six hits which are analyzed further for specificity. Modelling of ChAc in yeast can shed light on the pathological mechanisms underlying the disease and may also serve as experimental platform for drug testing.

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ABSTRACTS – POSTERS

(In alphabetical order of first author's last names)

The phenotypic variation in Chorea-Acanthocytosis model mice and the search for modifier genes

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We previously produced a ChAc-model mice encoding a human disease mutation with deletion of exons 60-61 in the VPS13A gene. The behavioral and pathological phenotypes of the model mice showed strain differences. To establish the effect of the genetic background on phenotype, we produced ChAc-model mice on two different inbred strains: 129S6 and FVB. In ChAc-model mice on the FVB, hyperactivity in open field test and neurodegeneration in striatum was observed. In ChAc-model mice on the 129S6, memory dysfunction in the novel objects recognition test and neurodegeneration in hippocampus was observed. They also showed decrease of prepulse inhibition of startle response. Both strains showed motor dysfunction in the balance beam test. These findings indicate that strain background genes affect phenotype variation.

Furthermore, we found that the incidence of seizures in ChAc-model mice on 129S6 strain vary according to pedigrees. We are now trying to identify the genes that modify seizure susceptibility.

“Levine syndrome”: neither Chorea-Acanthocytosis nor McLeod syndrome?

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Although the extended New England family reported in the 1960s by Irving M. Levine and collaborators was seminal for the development of the neuroacanthocytosis concept, a final diagnosis had not been arrived at. We collated data on its individual members from the various, sometimes contradictory reports. We identified individual names and biographic dates in publicly available archival material to re-draw the pedigree in a modern fashion and to properly place novel data in context that previously were only reported in a meeting abstract or were contained in patient charts of family members seen at the National Institutes of Health.

The novel information presented here describes the long-term disease course of six family members: all showed definite acanthocytosis in their blood and five of them (four siblings and the son of one sister) developed findings suggestive of a Neuroacanthocytosis Syndrome, such as chorea, seizures, cognitive impairment, hyporeflexia or CK-elevation. We regard their mother's neurological findings, however, as unrelated. The reconstructed pedigree is incompatible with the autosomal-recessive transmission seen in Chorea-Acanthocytosis (OMIM #200150). In addition, the respective ages of symptom manifestation, the absence of feeding dystonia as well as the patients' life duration would be atypical. Even if there is some resemblance to rare pedigrees of X-linked McLeod syndrome (OMIM #300842) where female mutation carriers had manifested disease symptoms, this diagnosis also appears unlikely as no relevant heart disease was reported as well as one instance of male-to-male transmission.

In summary, we suggest that “Levine syndrome” might represent another, as yet genetically unidentified type of neuroacanthocytosis that is transmitted as an autosomal-dominant trait. Unfortunately, none of the tissue samples that had been collected could be recovered for further, including molecular, analyses, and so far, to our knowledge, no descendants of the cases reported have been reassessed. Final clarification of the status of the syndrome thus seems to depend on serendipitous discoveries of members of the family that had first been studied by Levine and collaborators more than half a century ago.

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Functional link between Vps13 and Myo3, type I myosin

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Mutations in human VPS13 family genes cause several diseases. In particular mutations in hVPS13A gene lead to Chorea-Acanthocytosis. A number of studies have taken advantage of simple model organisms, like yeast, to elucidate the mechanisms underlying diseases. One of the powerful yeast methods is genetic screen for multicopy suppressors. Such dosage suppression is a genetic interaction in which overexpression of one gene rescues a mutant phenotype of another. Increasing gene dosage provides a means of probing gene function as it tends to cause an increase in respective gene product activity. We used power of this type of screen to study Vps13 function. We identified, among others, a fragment of MYO3 gene which, when overexpressed, restored growth of vps13 mutant on tester plates. MYO3 gene encodes myosin implicated in organization of actin cytoskeleton and endocytosis. The identified fragment of Myo3 protein contains N-terminal motor head domain and a linker with IQ motifs responsible for binding of a negative regulator of Myo3 - calmodulin (Cmd1). The substitutions of amino acids which disturb Myo3 fragment interaction with Cmd1, as assayed in two hybrid system, resulted in loss of Myo3 ability to suppress vps13 growth defect. Moreover, we found that overproduction of Cmd1 variant, which is unable to bind Myo3, also restores growth of vps13. These results show that defect of vps13 could be overcome and point to functional connection between Vps13, Myo3 and calmodulin.

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Bivalent cations-dependent phosphoinositide binding by chorein APT1 domain

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VPS13A is human gene encoding chorein, which physiological function at the molecular level is poorly understood. Studies show chorein involvement in actin cytoskeleton organization and phospholipid metabolism. Defects in chorein cause neurodegenerative disease Chorea-Acanthocytosis. Large size of the chorein makes the functional characterization a difficult task. To overcome this issue single or double domains of chorein need to be studied. Chorein as well as Vps13 protein from yeast

(Vps13 hereafter) have SHR binding domain (SHR_BD) and adjacent APT1 domain. Function of these domains is still unknown, however previous studies of our team revealed that double SHR_BD-APT1 domain from Vps13 binds phosphatidylinositol-3-phosphate (PI3P). To find evolutionary conservation of chorein domains, mentioned above, chimeric proteins were tested for their ability to complement defects of *vps13Δ* mutant. Chimeric proteins consisted of Vps13 protein in which single APT1 or double SHR_BD-APT1 domains were substituted for domains originated from chorein. Western blot analysis demonstrated that chimeric proteins are expressed at sufficient level in yeast cells. Results of phenotypic analysis showed lack of complementation for all chimeric proteins tested, hence features comparison between APT1 domains from chorein and Vps13 was conducted. Since APT1 from Vps13 binds PI3P, protein-lipid overlay assay was performed for APT1 from chorein. This analysis revealed that chorein APT1 binds PI3P, however it binds phosphatidylinositol-5-phosphate (PI5P) with higher affinity. Phospholipid binding on flat surface differs from interaction within membrane. Broader analysis of PI3P binding using liposomes revealed that chorein APT1 domain binds PI3P in bivalent cation dependent manner. Altered specificity in phospholipid binding of yeast and human APT1 domains may explain lack of complementation by chimeric Vps13 of *vps13* phenotypes. It supports the view that phosphoinositide binding via APT1 domain is essential for most Vps13 functions and is regulated by bivalent cations. Further studies are required to find out if PI5P binding by APT1 of chorein is also regulated by bivalent cations.

Clinical, genetic, and biophysical characterization of Chorea-Acanthocytosis – the Portuguese experience

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Chorea-acanthocytosis (ChAc) is inherited in an autosomal recessive manner. In 2012, we diagnosed clinically and genetically the only known patient with ChAc in Portugal. Subsequently, with the help of the Advocacy for Neuroacanthocytosis Patients, we genetically analysed cases from Brazil, Bulgaria, and the UK. Whenever available, the diagnosis of ChAc was confirmed by chorein Western blot on red blood cell membranes.

Genetic analyses of the VPS13A gene supported the clinical diagnoses. We found one splice site mutation (Brazilian case) one non-sense (Bulgarian case) and 3 different frameshift mutations (Bulgarian and UK cases) in total. Three of the mutations were novel. Interestingly, the mutation detected in the Brazilian patient was different of those described in another Brazilian case from the same city (Florianópolis).

We also analysed the blood sample of our Portuguese patient for changes of morphology and membrane elasticity of the red blood cells, compared with samples of seven healthy blood donors. Samples were analyzed by atomic force microscopy (AFM), haemorheological parameters, zeta-potential and fluorescence generalized polarization.

The initial results demonstrate that the red blood cells of the ChAc patient are softer than those of controls (255 ± 19 Pa vs. 553.8 ± 35.6 Pa for the control; $p < 0.0001$) and have higher penetration depth (1349 ± 17.4 nm vs. 811.3 ± 7.3 nm; $p < 0.0001$). Therefore, red blood cells from ChAc patient seem to be more capable of deforming than those from the control group.

AFM scanning images of red blood cells from both groups revealed that those from the ChAc patient are thicker than those of the controls (0.714 ± 0.006 μ m vs. 0.514 ± 0.004 μ m; $p < 0.0001$) and the ChAc patient presented lower red blood cell membrane roughness than the controls ($p < 0.0001$). Zeta-potential analysis did not show significant changes on the electrical charge of the membranes.

Fluorescence spectroscopy revealed more fluid RBC membranes of the ChAc patient.

Conclusions: The genetic results can help in the (i) confirmation of the clinical diagnosis, (ii) obtaining data about mutation frequency and disease prevalence, and (iii) analysis of genotype-phenotype associations in ChAc. AFM and other biophysical techniques can add further results on the physical characteristics of acanthocytes. We offer our genetic and biophysical expertise for further collaborations.

Characterization of human *VPS13A*, *VPS13C* and *VPS13A VPS13C* knockout cells

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The human vacuolar protein sorting (VPS) 13 family consists of four paralogs; *VPS13A*, *VPS13B*, *VPS13C*, and *VPS13D*. Mutations in different VPS13 family genes are linked to multiple disorders including neurodegeneration, neuronal diseases, diabetes, and autism. In particular, genetic alterations in *VPS13A* and *VPS13C* lead to a complex neuronal disorder Chorea-Acanthocytosis and an early-onset parkinsonism, respectively. The physiological roles of the different VPS13 proteins, and whether they have distinct or overlapping cellular functions remains unknown. Eukaryotic VPS13 genes are evolutionarily highly conserved even in unicellular budding yeast. Since yeast possesses a single VPS13 gene, studying cellular functions of yeast VPS13 could help to understand the basics of human VPS13 family better. Several different biological roles of yeast VPS13 have been described; 1) differentiation (sporulation), 2) endosomal vesicle traffic and 3) mitochondrial homeostasis. When disease-causing missense mutations in *VPS13A* or *VPS13C* were characterized in yeast VPS13 mitochondrial dysfunction was a common phenotype, suggesting that this may be the basis for the disease state in humans. To examine whether *VPS13A* or *VPS13C* are important for mitochondrial maintenance in human cells and to test for possible redundancy between the human genes, we obtained human HAP1 cell lines in which *VPS13A*, *VP13C*, or both *VPS13A* and *VPS13C* have been inactivated using CRISPR. Our progress in characterizing these cell lines will be discussed.

Chorea, psychosis, acanthocytosis, and prolonged survival associated with ELAC2 mutations

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Introduction: ElaC ribonuclease 2 (ELAC2) has been identified as a subunit of the RNaseZ complex, associated with the processing of mtDNA-encoded transcripts. Mutations in the ELAC2 gene are associated with rare autosomal recessive mitochondrial disease, leading to hypertrophic cardiomyopathy and usually death during childhood. Movement disorders have not been described as part of the ELAC2-associated disease spectrum.

Methods and results: Here we described an Assyrian 69 year old female affected by Huntingtonism, waddling gait, olfactory hallucinations, type 2 diabetes and hearing loss requiring aids. Her motor features have been slowly progressive. EMG revealed myopathy, a muscle biopsy revealed COX negative and red ragged fibers. Repeated blood smears have revealed acanthocytosis. Cognitive decline was also evident. MRI and FDG-PET scan of the brain revealed widened perisylvian sulci and reduced metabolism in these regions. Huntington's disease (HD) and other HD phenocopies were ruled out (pathological expansions of c9orf72, SCA17, and HDL2, and mutations in the PRNP gene). Further investigations with whole exome sequencing revealed the new trans variants c.394G>A and c.1040C>T in the ELAC2 gene. Repeated echocardiography demonstrated mild ventricular septum hypertrophy. Although we did not find any evidence of respiratory chain defect in the muscle biopsy, molecular characterization of patient fibroblasts revealed the accumulation of unprocessed mitochondrial transcripts but normal steady-state levels of mitochondrial mRNAs and tRNAs. Furthermore, patient fibroblasts showed severe growth impairment, when using galactose as energy source.

Discussion and conclusion: This is the first time ELAC2 mutations are associated with Huntingtonism and long survival. Other mitochondrial diseases associated with chorea include Leigh syndrome and MELAS. One MELAS case has been reported to be associated with acanthocytosis. Myopathy, hearing loss, diabetes and polyneuropathy are signs of mitochondrial disease but not HD. Myopathy and polyneuropathy are manifestations of Neuroacanthocytosis Syndromes (specifically Chorea-Acanthocytosis and McLeod syndrome (MLS)). Dilated, but not hypertrophic, cardiomyopathy is typical of MLS. This case illustrated that

mutations in ELAC2 are not exclusively confined to a lethal pediatric cardiomyopathy but may have a presentation that could be included as one of the Neuroacanthocytosis Syndrome.

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Key words: ELAC2, mitochondrial disease, Huntingtonism, acanthocytosis.

Male infertility associated with Chorea-Acanthocytosis

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Chorea-Acanthocytosis (ChAc) is a rare hereditary neurodegenerative disease due to VPS13A deficiency leading to a spectrum of neurological symptoms and acanthocytosis of red blood cells. Furthermore, male infertility has been reported in ChAc-model mice in the past. Herein, we present insights into the involvement of the male reproductive system in two ChAc patients and in a Vps13a knockout mouse model. It is therefore tempting to hypothesize that male infertility has to be considered as a new symptom complex associated with ChAc. However, studies with a higher case number investigating fertility in male ChAc patients are urgently needed to further address this question. Additionally, at the pathophysiological level, the so far unknown function of VPS13A in spermatozoa and spermatogenesis remains to be revealed.

Mechanical characterization of blood cells in Chorea-Acanthocytosis during experimental treatment with Dasatinib or Lithium

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The ability to deform and change shape is a vital feature of erythrocytes to withstand stresses, as they pass vessels just a fraction of their own size. It is hypothesized that the mechanical parameters of RBCs change when they adopt the typical acanthocytic phenotype. We demonstrate changes in deformability of healthy RBCs and Acanthocytes, as well as changes of the cells during experimental Chorea-Acanthocytosis patient treatment with Dasatinib or Lithium by using three different microfluidic techniques. Our data provides additional information to patient monitoring during treatment.

RCN2 encoding the calcineurin regulator is a suppressor of vps13 mutations in yeast Chorea-Acanthocytosis model

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Chorea-Acanthocytosis (ChAc) is a fatal rare genetic neurodegenerative disease linked with mutations in hVPS13A gene, one of four VPS13 genes in human. Mutations in hVPS13B and hVPS13C are also implicated in human neurodegenerative disorders and effective cure for any of these diseases is lacking. VPS13 genes are conserved from yeast to humans. Thus, yeast is a good model system to study function of Vps13 proteins, the effect of human mutations on cell physiology and to screen for suppressors of mutations in VPS13 gene. In yeast there is one VPS13 gene and it is most homologous to hVPS13A. The deletion of VPS13 gene in yeast impairs many functions such as intracellular trafficking, actin cytoskeleton organization and sporulation. A point mutation vps13-I2749R, which mimics the point mutation found in ChAc patient, also exhibits loss of function phenotypes. We identified RCN2 gene as a multicopy suppressor of vps13 Δ , as well as vps13-I2749R mutation. RCN2, next to RCN1, is the regulator of calcineurin, a calcium and calmodulin dependent protein phosphatase. While RCN1, depending on the expression level and phosphorylation state, can stimulate and inhibit calcineurin, RCN2 shows only inhibitory activity when overexpressed. Here we show that overexpression of RCN2 diminishes sensitivity to canavanine and improves actin cytoskeleton organization of vps13 mutant cells. Our results suggest possible link between calcium signaling and function of Vps13.

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Huntington's disease-like disorders in Latin America and the Caribbean

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Objective: To review the genetic causes of chorea which have been documented to date in Latin America and the Caribbean. Documentation of the presence of these conditions will contribute to providing appropriate diagnostic and clinical care.

Background: As testing for Huntington's disease (HD) is becoming increasingly available in previously resource-limited regions, it is becoming apparent that there are a number of patients with other rare conditions which result in an HD-like phenotype.

Methods: The literature was surveyed for publications reporting a variety of genetic choreic disorders, and in particular HD-like 2 (HDL2), Chorea-Acanthocytosis (ChAc), and McLeod syndrome (MLS), in addition to the inherited ataxias. Movement disorders specialists from countries in Latin America and the Caribbean were contacted regarding their experiences with these disorders; they in turn recommended other colleagues who might have informative experience in the area. Additional publications were identified from the local literature. Contributions in Spanish and Portuguese were encouraged, if appropriate.

Results: For HDL2, 27 cases were identified from 19 families from Latin America. The majority were from Brazil, with others being from Venezuela and Mexico. There were 9 patients from 3 families originating from the Caribbean (Fig. 1). For ChAc, 32 patients from 18 families were identified from Latin America; there were 5 patients from 3 families from Puerto Rico (Fig. 2). For MLS, there were 25 patients from 5 families, the majority from 2 families from Chile (Fig. 3). The incidence of some of these disorders is likely determined by factors such as variations in ethnic background and settlement patterns; for example, HDL2 is particularly prevalent in regions or countries where the population has African ancestry. Patients have also been documented with chorea due to other conditions, such as the inherited ataxias.

Conclusions: While rare, a significant number of patients affected by the non-HD choreas are evidently present in Latin America and the Caribbean. When not available locally, international collaborations can facilitate diagnosis of rare genetic disorders. As genetic resources and awareness of these disorders improve, more patients are likely to be identified, and have the potential to benefit from education, support, and ultimately molecular therapies.

Life expectancy and mortality in Neuroacanthocytosis Syndromes

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Objective: To document life expectancy and causes of mortality in patients with Chorea-Acanthocytosis (ChAc) and McLeod syndrome (MLS).

Background: ChAc and MLS are rare progressive neurodegenerative conditions which cause a spectrum of neurological syndromes. There are no data regarding life expectancy and causes of death. This information will be valuable for disease management.

Methods: We reviewed our personal databases and the published literature to identify cases of ChAc and McLeod syndrome for whom adequate information was available regarding age of disease onset, age at death, cause of death, and clinical information such as presence of seizures or cardiac disease.

Results: Adequate information was obtained on 44 patients with ChAc and 28 with McLeod syndrome. Causes of death included pneumonia, cardiac disease, seizure, suicide, and sepsis. Mean disease duration for ChAc was 11 years, while for McLeod syndrome it was 20 years.

Conclusions: Causes of death in ChAc and McLeod syndrome are similar to those for the phenotypically-similar Huntington's disease, with additional risks due to the presence of seizures and cardiac disease. Suicidality was seen in 10% of patients with ChAc. In the absence of disease-modifying agents, disease management should focus upon treating symptoms which may contribute to morbidity

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