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Tremor and Other Hyperkinetic Movements

Conference Proceedings

Proceedings of the Ninth International Meeting on Neuroacanthocytosis Syndromes

Editors: Kevin Peikert[1] & Andreas Hermann[1,2,3]

[1] Department of Neurology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [2] Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Dresden, Germany, [3] German Center for Neurodegenerative Diseases (DZNE) Dresden, Dresden, Germany

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Introduction

The 9th International Meeting on Neuroacanthocytosis Syndromes was held on March 23th–25th, 2018 in Dresden, Germany. The conference followed the tradition of the previous eight international symposia, the last of which was held in Ann Arbor, USA in May, 2016. Following the positive response to the previous meeting, a major component of this year's symposium was the participation of patients, their families, and caregivers. The conference focused primarily on chorea-acanthocytosis as one of the "core" diseases of neuroacanthocytosis syndromes, with some discussion also of McLeod syndrome. These neurodegenerative diseases lead to chorea, epilepsy, cognitive and behavioral problems, and acanthocytosis of red blood cells. While chorea-acanthocytosis is caused by mutations in the VPS13A gene, other "VPS13opathies" (VPS13B-D) were also topic of the meeting.

At present there are no treatments that can halt or slow down the progression of these diseases. However, two pathways seem to be prominently involved in chorea-acanthocytosis – namely the PI3K (phosphoinositide 3-kinase) pathway and the Lyn kinase pathway – both of which are potential "druggable" targets. Model organisms including *Dictyostelium*, *Drosophila*, yeast, and mice, and human cell models were presented. Finally, clinical translation and clinical trial readiness in such a rare disease were extensively discussed.

The VPS13 Gene Family

1

Clues to VPS13A Function from Clinical Observation?

A. Danek

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

Chorea-acanthocytosis (ChAc) has become increasingly well characterized clinically since VPS13A mutations were 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 (or functions) has so far remained elusive. 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 occurs in the third decade. 5) ChAc affects multiple organ systems, most importantly the central and the peripheral nervous system. 6) There is progressive nerve cell loss yet a relative increase in glia cells, with brain atrophy as the final outcome. No intracellular inclusions whatsoever, the supposed hallmark of neurodegeneration, have yet been discovered, or 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.

VPS13A function appears to be essential for neurons with their high metabolic activity, and the mere lack of chorein seems sufficient to explain ChAc. The absence of debris also speaks against "toxic gain of function". Interestingly, about 2% of all currently known cases diagnosed via 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 between ChAc and MLS make the protein affected in the latter, Kx, a natural candidate to

provide such compensation. Additional compensatory pathways may become known from 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. The 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 that may help to understand very early disease stages. Muscle cell affection in ChAc seems more similar to hemopoietic than to nervous system involvement.

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

Concerning VPS13A function, currently the rare situations of point mutations despite 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.

2

Genetics of Chorea-acanthocytosis

A. Velayos-Baeza

Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK

Research on chorea-acanthocytosis (ChAc), an adult-onset rare neurodegenerative disorder, has been ongoing for 50 years. It started in 1968, when two families from New England and Kentucky (USA), with several members affected by a neurological disease with acanthocytosis, were independently reported by Irvine Levine and Edmunt Critchley, respectively, after whom this new condition was named the Levine–Critchley syndrome. Other similar cases were reported later, many of them under the generic denomination of neuroacanthocytosis. This, however, is an umbrella term that includes several other similar conditions, which contributed to create confusion in the field that was only resolved by the identification of the genetic causes. For ChAc, this was achieved about 30 years after the original case reports, when the ChAc gene on chromosome 9q21 was described, allowing confirmation of the recessive nature of the disorder. This gene was later renamed VPS13A because of its similarity to the yeast VPS13 gene, and was 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 that 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 hotspots, and include an extensive range of mutations: large deletions, splicing-site mutations, small insertion/deletions, nonsense mutations, or missense mutations. These analyses provide important insights into the function of chorein. First, the 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 the VPS13A gene, resulting not in the 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 led to absence of the protein, either by a mechanism involving the 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 analysis of protein extracts from blood by western blotting, which is a much faster and cheaper approach than DNA sequencing. Most, although not all, ChAc cases would be detected in this way, 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 debate over the existence of ''dominant ChAc'', that is, the description of patients presenting with ChAc syndrome but having only one mutated VPS13A allele while the second one is normal. This would imply the single mutation present in these cases has a dominant nature, and that 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 that 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 is possible that a mutation in the VPS13A gene has a dominant effect leading to ChAc, it is important to note that such a mutation has not been described so far, and that ChAc is only developed when both VPS13A alleles present pathogenic mutations.

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3

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

S. Lommatzsch[1], M. Zorn[1], J. Kühnisch[2], S. Bachmann[1], D. Horn[3], V. Haucke[4], W. Seifert[1]

[1] Institute of Vegetative Anatomy, Charité – Universitätsmedizin Berlin, Berlin, Germany, [2] Experimental and Clinical Research Center (ECRC), Berlin, Germany, [3] Institute for Medical and Human Genetics, Charité – Universitätsmedizin Berlin, Berlin, Germany, [4] Department of Molecular Pharmacology and Cell Biology, Leibniz-Institute for Molecular Pharmacology, Berlin, Germany

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 (3,997 aa), 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 focuses 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 knockdown in mice at different developmental stages. Initial data demonstrate that Golgi orientation and neuronal migration are 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.

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Genetic Contribution of Mutated VPS13C to Lewy Body Dementia

C. Van Broeckhoven, S. Smolders, S. Van Mossevelde, S. Philtjens

Neurodegenerative Brain Diseases, Center for Molecular Neurology, VIB & Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

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.[1] 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.[2] Diagnostic exome sequencing in 80 patients with PD symptoms at early age and a negative family history identified compound heterozygous variants in one patient affecting canonical splice sites in VPS13C, confirming the causal role of protein-truncating variants to autosomal-recessive early-onset PD.[3]

In our study, we identified compound heterozygous, non-synonymous variants that reduced VPS13C expression and were associated with Lewy body disease (LBD). LBD is the second most prevalent cause of neurodegenerative dementia, with a frequency of 10–25%. Compound heterozygous missense mutations were identified by whole-genome sequencing in an affected sib pair with early-onset dementia. The index patient was pathologically diagnosed with LBD and the parents were not affected. Screening of VPS13C in a Belgian LBD cohort resulted in the identification of two additional carriers of compound heterozygous VPS13C missense mutations, a pathologically confirmed LBD patient and a clinically diagnosed LBD 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 brains at autopsy of the two LBD 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 contribute by a loss-of-function mechanism similar to the effect of protein-truncating variants. In addition, they underscored that the VPS13C variants affect residues that are essential for protein function. Cellular expression studies indicated 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 are needed to understand the contribution of VPS13C to LBD and PD.

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5

VPS13D, a New Ataxia Gene, Plays an Essential Role in Mitochondrial Morphology and Maintenance in *Drosophila*

R. Insolera[1], D. Lozano[1], A. Wishnie[1], E. Seong[2], M. Burmeister[2], O. Sibon[3], C. Collins[1]

[1] Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, USA, [2] Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI, USA, [3] Department 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 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. The autophagy machinery appears to be strongly engaged in muscle cells, suggesting that these atypical mitochondria represent breakdown intermediates. Atypical mitochondria with a similar appearance also accumulate in VPS13D-depleted neurons, and, strikingly, 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),[1] we have begun to examine whether *Drosophila* VPS13-null mutants share mitochondrial defects with VPS13D, and have noted 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.

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Yeast and *Dictyostelium* as Lower Organism Models

6

Molecular Analysis of Yeast Vps13p

R. S. Fuller[1], M. De[1], M. Abazeed[1], E. J. Brace[1], R. E. Diaz[1,2], M. Ayyash[1] A. N. Oleskie[1,3], L. V. Mancour[1,3], G. Skiniotis[1,3]

[1] Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA, [2] Summer Research Opportunities Program, University of Michigan, Ann Arbor, MI, USA, [3] Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA

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 found 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; however, activity is restored by adding Vps13p purified from yeast. Soluble, purified Vps13p is monomeric and occurs 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 *Escherichia 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 indicates that this large protein (3,144 residues, 358 kDa) is folded into a flexible, curved, compact rod (28 × 6 nm), with a loop at one end, that possesses a circular opening ~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.[1] 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.

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Using Yeast Vps13 Models of Chorea-acanthocytosis to Isolate Genetic and Chemical Suppressors

P. Soczewka, D. Kolakowski, W. Rzepnikowska, M. Grabiec, K. Flis, J. Kaminska, T. Zoladek

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

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; 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. One Vps13 protein in yeast is involved in vacuolar protein transport and, like hVps13A, participates in phospholipid metabolism. One of the mutations found in ChAc patients causes a I2771R amino acid substitution in hVps13A protein that 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, which results in impaired actin cytoskeleton organization, endocytosis and vacuolar transport, as well as growth defect on media supplemented with various chemicals. We also show that Vps13, like hVps13A, binds actin, and that 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 determine whether vps13Δ or vps13-I2749R defects could be overcome, we screened the yeast genomic library for multicopy suppressor genes that restore growth of mutant cells on tester plates. Five multicopy suppressor genes were isolated, including *RCN2*, which encodes the negative regulator of calcineurin phosphatase, and a 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 screened the library of FDA-accepted drugs for those that restore growth of the vps13Δ strain on tester plates. We identified six hits, which have been analyzed further for specificity. Modeling of ChAc in yeast may shed light on the pathological mechanisms underlying the disease and may also serve as experimental platform for drug testing.

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Bivalent Cation-dependent Phosphoinositide Binding by the Chorein APT1 Domain

D. Kolakowski, T. Zoladek, J. Kaminska

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

VPS13A is human gene encoding chorein, whose physiological function at the molecular level is poorly understood. Studies show chorein involvement in actin cytoskeleton organization and phospholipid metabolism. Defects in chorein cause the neurodegenerative disease chorea-acanthocytosis. The large size of chorein makes functional characterization a difficult task. To overcome this issue, single or double domains of chorein need to be studied. Chorein and Vps13 protein from yeast (Vps13 hereafter) have an SHR-binding domain (SHR_BD) and an adjacent APT1 domain. The function of these domains is still unknown; however, previous studies by our team have revealed that a double SHR_BD-APT1 domain from Vps13 binds phosphatidylinositol-3-phosphate (PI3P). In order to analyze the evolutionary conservation of the chorein domains mentioned above, chimeric proteins were tested for their ability to complement defects of the vps13Δ mutant. Chimeric proteins consisted of Vps13 protein in which single APT1 or double SHR_BD-APT1 domains were substituted for domains originating from chorein. Western blot analysis demonstrated that chimeric proteins are expressed at sufficient levels in yeast cells. The results of phenotypic analysis showed lack of complementation for all chimeric proteins tested; hence, comparison between APT1 domains from chorein and Vps13 was conducted. Since APT1 from Vps13 binds PI3P, a 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 a flat surface differs from interaction within a membrane. Broader analysis of PI3P binding using liposomes revealed that the chorein APT1 domain binds PI3P in a bivalent cation-dependent manner. Altered specificity in phospholipid binding of yeast and human APT1 domains may explain a lack of complementation by chimeric Vps13 of vps13Δ phenotypes. This supports the view that phosphoinositide binding via the APT1 domain is essential for most Vps13 functions and is regulated by bivalent cations. Further studies are required to find out whether PI5P binding by APT1 of chorein is also regulated by bivalent cations.

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Functional Link between Vps13 and Myo3, Type I Myosin

J. Kaminska, P. Soczewka, T. Zoladek

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

Mutations in the human VPS13 family of genes cause several diseases. In particular, mutations in the 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 most powerful methods using yeast is genetic screening for multicopy suppressors. Such dosage suppression is a genetic interaction in which the overexpression of one gene rescues a mutant phenotype of another. Increasing the gene dosage provides a means of probing gene function, as it tends to cause an increase in the corresponding gene product activity. We used this type of screen to study Vps13 function. We identified, among others, a fragment of the *MYO3* gene that, when overexpressed, restored growth of the vps13Δ mutant on tester plates. The *MYO3* gene encodes myosin implicated in the organization of actin cytoskeleton and endocytosis. The identified fragment of Myo3 protein contains an N-terminal motor head domain and a linker with IQ motifs responsible for binding a negative regulator of Myo3: calmodulin (Cmd1). Substitution of amino acids that disturb Myo3 fragment interaction with Cmd1, as assayed in a two-hybrid system, resulted in the loss of ability of Myo3 to suppress the vps13Δ growth defect. Moreover, we found that overproduction of the Cmd1 variant, which is unable to bind Myo3, also restores growth of vps13Δ. These results show that a defect of vps13Δ could be overcome and point to a functional connection between Vps13, Myo3, and calmodulin.

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RCN2 Encoding the Calcineurin Regulator Is a Suppressor of vps13 Mutations in a Yeast Chorea-acanthocytosis Model

P. Soczewka, M. Grabiec, J. Kaminska, T. Zoladek

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

Chorea-acanthocytosis (ChAc) is a fatal, rare, genetic, neurodegenerative disease linked with mutations in the hVPS13A gene, one of four VPS13 genes in humans. Mutations in hVPS13B and hVPS13C are also implicated in human neurodegenerative disorders and an 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 the function of Vps13 proteins, the effect of human mutations on cell physiology, and to screen for suppressors of mutations in the VPS13 gene. In yeast, there is one VPS13 gene and it is most homologous to hVPS13A. The deletion of the 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 the *RCN2* gene as a multicopy suppressor of vps13Δ, as well as the vps13-I2749R mutation. *RCN2*, next to *RCN1*, encodes the regulator of calcineurin, a calcium- and calmodulin-dependent protein phosphatase. *Rcn1*, depending on the expression level and phosphorylation state, can stimulate and inhibit calcineurin; however, *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 a possible link between calcium signaling and the function of Vps13.

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The Many Roles of VPS13 in Budding Yeast

J.-S. Park, A.M. Neiman

Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY, USA

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 many 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 showing that the pleiotropic phenotypes of vps13 mutants are due to multiple defects in membrane contact sites.

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Characterization of Human VPS13A-, VPS13C-, and VPS13A and VPS13C double knockout cells

J.-S. Park, A.M. Neiman

Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY, USA

The human vacuolar protein sorting (VPS) 13 family consists of four paralogs: VPS13A, VPS13B, VPS13C, and VPS13D. Mutations in various 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 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 the 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 found to be a common phenotype, suggesting that this may be the basis for the disease state in humans. To examine whether VPS13A and 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 had been inactivated using CRISPR. Our progress in characterizing these cell lines will be discussed.

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The Role of VPS13A in the Endolysosomal and Autophagic Pathways

S. Muñoz-Braceras, A. Tornero-écija, O. Vincent, R. Escalante

Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, Spain

VPS13 proteins are a group of conserved proteins whose mutations lead to the appearance or elevated risk of various diseases. Particularly, VPS13A mutations cause chorea-acanthocytosis, a rare neurodegenerative disorder, for which available treatments are not able to modify disease progression. Human VPS13A and other members of the VPS13 family have been implicated in autophagy and other cellular processes; however, their molecular functions and the mechanistic details of their participation in these processes are still unknown. We propose that the function of VPS13 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 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.

Animal Models

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Drosophila Oogenesis as a Model System to Reveal Novel Functions of Vps13

A.E. Faber, O.C.M. Sibon

Department of Cell Biology, University Medical Center Groningen, University of Groningen, The Netherlands

Chorea-acanthocytosis (ChAc) is a rare neurodegenerative disease characterized by progressive movement abnormalities and a spiked form of red blood cell called acanthocytes. The disease is caused by loss-of-function mutations in the VPS13A gene and in most cases leads to the absence of the Vps13A protein. Knowledge of the underlying pathophysiology of the disease and mechanisms involved is limited and mainly based on studies in unicellular organisms or cultured cells. The *Drosophila melanogaster* ovary system is widely used to study biological and cellular processes because of its accessibility and the availability of numerous genetic tools. Using CRISPR/Cas9, we created fly lines harboring strong loss-of-function Vps13 ($Vps13^{null}$) mutations to further investigate the function and localization of Vps13. Vps13 protein is abundant in the *Drosophila* ovary, and analysis of mutant ovaries showed the absence of Vps13 protein. In addition, mutant females exhibit a striking deficit in egg laying and produce lower numbers of offspring. Preliminary results revealed a higher number of dying egg chambers in mutant ovaries. By further investigating the ovary phenotype of Vps13 mutant females, we are attempting to gain insights into the cellular processes in which Vps13 is involved.

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A Mouse Model of Chorea-acanthocytosis

M. Nakamura, H. Sakimoto, O. Nagata, K. Arai, A. Sano

Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Previously, we generated a mouse model of chorea-acanthocytosis (ChAc-model mouse) that 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; this strongly suggested 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 various 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.

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The Phenotypic Variation in Chorea-acanthocytosis Model Mice and the Search for Modifier Genes

K. Arai, H. Sakimoto, O. Nagata, M. Nakamura, A. Sano

Department of Psychiatry, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Previously, we constructed a ChAc mouse model 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 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 the hippocampus was observed. They also showed a decrease in 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 attempting to identify the genes that modify seizure susceptibility.

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Phenotypes of Vps13a-, Vps13c-, and Vps13d-Knockout Mice

B. Rathkolb[1,3], O. Amarie[2], L. Garrett[2], L. Becker[1], J. Rozman[1], J. Calzada-Wack[1], P. da Silva-Buttkus[1], N.F. Chhabra, The IMPC consortium, J. Graw[2], S. Hölter[2], V. Gailus-Durner[1], H. Fuchs[1], M. Hrabe de Angelis[1]

[1] German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum München, Munich, Germany, [2] German Mouse Clinic, Institute of Developmental Genetics, Helmholtz Zentrum München, Munich, Germany, [3] Institute of Molecular Animal Breeding and Biotechnology, Ludwig-Maximilians-Universität München, Munich, Germany

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 may 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. Genome-wide association studies (GWAS) 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 2–5 months. In addition to 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 2–5 months and, in addition, after aging at an age of 12–15 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 may 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 are required to obtain a full picture of the pathologies associated with VPS13 gene mutations in humans and mice.

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Cell Models

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Chorea-acanthocytosis – Characterization and Functional Regeneration of Affected Neurons

H. Glaß[1,2], A. Pal[1,2] N. Stanslowsky[3*] P. Reinhardt[2†], L. de Franceschi[4], P. Claus[5], J. Sterneckert[2], A. Storch[6,7], F. Wegner[3], A. Hermann[1,2,8]

[1] Department of Neurology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [2] DFG-Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Dresden, Germany, [3] Department of Neurology, Hannover Medical School, Hanover, Germany, [4] Department of Medicine, Section of Internal Medicine, University of Verona, Policlinico GB Rossi, AOUI, Verona, Italy, [5] Institute of Neuroanatomy, Hannover Medical School, Hanover, Germany, [6] Department of Neurology, University of Rostock, Rostock, Germany, [7] German Center for Neurodegenerative Diseases (DZNE) Rostock, Rostock, Germany, [8] German Center for Neurodegenerative Diseases (DZNE) Dresden, Dresden, Germany

Current address: *Zellkraftwerk GmbH, Leipzig, Germany, †AbbVie Deutschland GmbH & Co KG, Neuroscience discovery, Ludwigshafen, Germany

Mutations in the VPS13A gene leading to depletion of chorein protein are causative for chorea-acanthocytosis (ChAc), a movement disorder characterized by red blood cell acanthocytes and 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 hyperactivation of Lyn kinase were identified; however, to what extent these mechanisms are present and relevant in the affected neurons remains elusive. We studied the effect of chorein absence in GABAergic medium spiny neurons derived from induced pluripotent stem cells of ChAc patients. Morphology and trafficking of mitochondria and lysosomes is altered in ChAc patients. The number of both organelle types is reduced and mitochondria are 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 phallacidin or PP2 resulted in the reduction of disinhibited synaptic activity in ChAc neurons to the level in 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 mitochondrial populations seem to act independent of the electrophysiological phenotypes.

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Dopaminergic Neurons Derived from Chorea-acanthocytosis Patients Are More Susceptible to Mitochondrial Stress

P. Neumann[1], H. Glaß[1,2], A. Pal[1], A. Storch[1,3,4], A. Hermann[1,2,3]

[1] Division for Neurodegenerative Diseases, Department of Neurology, Technische Universität, Dresden, Germany, [2] CRTD, Center for Regenerative Therapies Dresden, Technische Universität, Dresden, Germany, [3] DZNE, German Center for Neurodegenerative Diseases, Rostock, Germany, [4] Department of Neurology, University of Rostock, Rostock, Germany

Abstract: Mutations in the VPS13A gene encoding the protein chorein cause chorea-acanthocytosis (ChAc), an autosomal recessive inherited rare neurodegenerative disease. Patients suffer from a variety of symptoms: primarily a movement disorder with involuntary movements. Although impairment in the cytoskeletal architecture in erythrocytes and neurons of ChAc patients has been shown, the cause of neuronal degeneration, however, remains not fully understood. As patients also show parkinsonism, we used induced pluripotent stem cell (iPSC)-derived human midbrain dopaminergic neurons (mDAN) from ChAc patients and healthy donors and investigated them for in vitro phenotypic abnormalities.

Methods: Neural progenitor cells (NPCs) were derived from iPSCs obtained by reprogramming skin fibroblasts of ChAc patients and controls.[1,2] NPCs were differentiated over the course of 30 days into mDAN (2). Neuronal growth speed was evaluated using fluorescence labeling of actin and measuring the cumulative length of all neurites, number of branching points, and length of longest neurite at 24, 48, and 72 hours after seeding. Survival of mDAN cultures upon stress with rotenone and arsenite was quantified with the resazurin-based PrestoBlue® assay and measurement of LDH levels in stressed cultures. PrestoBlue®-measured viability activity was normalized to that of the untreated control, and LDH levels were normalized to those of the permeabilized untreated control.

Results: Neuronal growth of wild-type (WT) and ChAc mDAN at defined time points after seeding was similar. The number of branching points, longest single neurite, and the number of neurites per neuron did not differ either. Mature mDAN were stressed with rotenone and arsenite at different concentrations. To assess their viability, the PrestoBlue® assay was performed. Rotenone-treated mDANs from ChAc patients showed significant lower cell viability at rotenone concentrations of 100 and 200 nM. Arsenite-treated ChAc and WT neurons displayed similar viability at arsenite concentrations of 5, 1, and 0.2 µM. LDH levels were measured at 3 and 6 days. There was no difference in the levels released by WT and ChAc neurons. When the released LDH amount was normalized to the remaining cell viability after stressing, there was no difference observed as well.

Conclusions: Midbrain dopaminergic neurons derived from healthy donors and ChAc patients display similar neuronal growth in vitro. mDAN derived from ChAc patients were significantly more vulnerable than wild-type control neurons when complex 1 (NADH dehydrogenase)

of the mitochondrial respiratory chain was inhibited using rotenone. This may indicate alterations of the respiratory chain, and thus of ATP production, in midbrain dopaminergic neurons with VPS13A mutations.

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Disease-associated VPS13A Mutations: Consequences for Neuronal Function and Survival

E. Tank, T. Qiao, A. Tidball, W. Dauer, J. Parent, S.J. Barmada

Department of Neurology, University of Michigan School of Medicine, Ann Arbor, MI, 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, remains 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 non-invasive 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.

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Disturbed Red Blood Cell Mechanics in Patients with Neuroacanthocytosis

G. Bosman, D. Lazari, J. Freitas Leal, M. Adjobo-Hermans

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

The structure of red blood cells is affected by numerous inborn and acquired factors; however, 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.

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Mechanical Characterization of Blood Cells in Chorea-acanthocytosis During Experimental Treatment with Dasatinib or Lithium

F. Reichel, M. Kräter, P. Rosendahl, M. Herbig, A. Rivera Prieto, J. Guck

CMCB – TU, Dresden, Germany

The ability to deform and change shape is a vital feature of erythrocytes to withstand stresses as they pass through vessels just a fraction of their own size. It is hypothesized that the mechanical parameters of red blood cells (RBCs) change when they adopt the typical acanthocytic phenotype. We demonstrate changes in deformability of healthy RBCs and acanthocytes, as well as changes to these cells during experimental chorea-acanthocytosis patient treatment with dasatinib or lithium, by using three different microfluidic techniques. Our data provide additional information to guide patient monitoring during treatment.

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Cellular Mechanisms Contributing to Acanthocytosis and Neurodegeneration in Chorea-acanthocytosis

F. Lang[1], L. Pelzl[1], L. Schöls[2,3], S. Hauser[2,3], A. Hermann[4], M. Föller[5], C. Stournaras[1,6]

[1] Department of Physiology I, University of Tübingen, Tübingen, Germany, [2] German Center for Neurodegenerative Diseases, Tübingen, Germany, [3] Department of Neurology and Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany, [4] Department of Neurology and Center for Regenerative Therapies Dresden (CRTD), Technische Universität, Dresden, Germany, & DZNE, German Center for Neurodegenerative Diseases, Dresden, Germany, [5] Institute of Agricultural and Nutritional Sciences, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany, [6] Department of Biochemistry, University of Crete Medical School, Heraklion, Greece

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. These pathologies may be paralleled by erythrocyte acanthocytosis. Chorein supports activation of the phosphoinositide-3-kinase (PI3K) p85 subunit with subsequent upregulation of Ras-related C3 botulinum toxin substrate 1 (Rac1) activity, and p21 protein-activated kinase 1 (PAK1) phosphorylation. Chorein-sensitive PI3K signaling further leads to stimulation of the serum and glucocorticoid inducible kinase SGK1, which in turn upregulates ORAI1, a Ca^{2+} channel accomplishing store-operated Ca^{2+} 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. Additional examination is required exploring whether the in vitro observations indeed reflect the in vivo pathology of the disease.

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Actin Regulation in Neurological Diseases

N. Hensel[1], P. Claus[1,2]

[1] Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hanover, Germany, [2] Center for Systems Neuroscience (ZSN) Hannover, Hanover, 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: profilins bind 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 the 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-acanthocytosis.[4] 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.

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Clinical Aspects and Standard of Care in Chorea-acanthocytosis

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Symptomatic Treatment of Neuroacanthocytosis Syndromes

R.H. Walker[1,2]

[1] Department of Neurology, James J. Peters Veterans Affairs Medical Center, New York, NY, USA, [2] Department of Neurology, Mount Sinai School of Medicine, New York, NY, USA

The two core neuroacanthocytosis syndromes, chorea-acanthocytosis and McLeod syndrome, are progressive neurodegenerative disorders that primarily affect 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 that 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 that 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.

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Clinical, Genetic, and Biophysical Characterization of Chorea-acanthocytosis – The Portuguese Experience

C.S. Lopes[1], **F.A. Carvalho**[1], **P. Bernardi**[2], **K. Lin**[3], **S. Mehrabian**[4], **A. Radunovic**[5], **M. Joao Azevedo**[6], **Ginger. Irvine**[7], **Glenn Irvine**[7], **B. Bader**[8], **R.H. Walker**[9,10], **A. Danek**[8], **N. C. Santos**[1], **M. de Carvalho**[1], **G. Miltenberger-Miltenyi**[1]

[1] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, [2] Núcleo de Genética Clínica, Departamento de Clínica Médica, Hospital Universitário, UFSC, Florianópolis, Brazil, [3] Serviço de Neurologia, Departamento de Clínica Médica, Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, Brazil, [4] Department of Neurology, University Hospital Alexandrovska, Sofia, Bulgaria, [5] Great Ormond Street Hospital for Sick Children, NHS Trust, London, UK, [6] Serviço de Medicina Física e de Reabilitação, Hospital Da Senhora Da Oliveira, Guimaraes, Portugal, [7] Advocacy for Neuroacanthocytosis Patients, London, UK, [8] Neurologische Klinik, Ludwig-Maximilians-Universität, Munich, Germany, [9] Department of Neurology, James J. Peters VAMC, New York, NY, USA, [10] Department of Neurology, Mount Sinai School of Medicine, New York, NY, USA

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 analyzed 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 three 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 from those described in another Brazilian case from the same city (Florianópolis).

We also analyzed 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), hemorheological parameters, zeta-potential, and fluorescence generalized polarization.

The initial results demonstrated that the red blood cells of the ChAc patient were 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 the 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 were thicker than those of the controls (0.714 ± 0.006 μm vs. 0.514 ± 0.004 μm; $p < 0.0001$); further, 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.

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

Conclusions: The results of genetic analysis are useful for 1) confirmation of the clinical diagnosis, 2) obtaining data about mutation frequency and disease prevalence, and 3) analysis of genotype-phenotype associations in ChAc. AFM and other biophysical techniques may be used for further elucidation of the physical characteristics of acanthocytes. We offer our genetic and biophysical expertise for further collaborations.

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Huntington's Disease-like Disorders in Latin America and the Caribbean

R.H. Walker[1], E.M. Gatto[3], M.L. Bustamente[4], O. Bernal-Pacheco[5], F. Cardoso[6], R.M. Castilhos[7], P. Chana[8], M. Cornejo-Olivas[9], I. Estrada Bellmann[10], L.B. Jardim[11,12], R. López-Castellanos[13], R. López-Contreras[14], D.P. Maia[6], P. Mazzetti[9] M. Miranda[15], M. Rodríguez-Violante[16], H. Teive[17], V. Tumas[18]

[1] Department of Neurology, James J. Peters Veterans Affairs Medical Center, New York, NY, USA, [2] Mount Sinai School of Medicine, New York, NY, USA, [3] Sanatorio Trinidad Mitre, INEBA, Buenos Aires, Argentina, [4] Biomedical Sciences Institute, Faculty of Medicine, Universidad de Chile, Santiago, Chile, [5] Neurologo Clinico-Universidad Militar Nueva Granada, Colombia, [6] The Federal University of Minas Gerais, Belo Horizonte, Brazil, [7] Serviço de Neurologia, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil, [8] Universidad de Santiago de Chile, Santiago, Chile, [9] Neurogenetics Research Center, Instituto Nacional de Ciencias Neurologicas, Lima, Peru, [10] Hospital Universitario Monterrey, Nuevo León, México, [11] Departamento de Medicina Interna, Universidade Federal do Rio Grande do, Sul, Brazil, [12] Medical Genetics Service, Hospital de Clínicas de, Porto Alegre, Brazil, [13] Department of Neurology and Rehabilitation Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA, [14] Clínica de Especialidades Neurológicas, San Salvador, El Salvador, [15] Department of Neurology, Clinica Las Condes, Santiago, Chile, [16] Instituto Nacional De Neurologia Y Neurocirugia, Mexico City, Mexico, [17] Movement Disorders Unit, Neurology Service, Internal Medicine Department, Hospital de Clínicas, Federal University of Parana, Curitiba, Brazil, [18] Department of Neuroscience and Behavior Sciences, Ribeirao Preto School of Medicine, University of Sao Paulo, Ribeirao Preto, Brazil

Objective: To review the genetic causes of chorea 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: The increased availability of testing for Huntington's disease (HD) in previously resource-limited regions has revealed that there are a number of patients with other rare conditions that 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 nine patients from three families originating from the Caribbean. For ChAc, 32 patients from 18 families were identified from Latin America; there were five patients from three families from Puerto Rico. For MLS, there were 25 patients from five families, the majority from two families from Chile. 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 because of 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 may facilitate diagnosis of rare genetic disorders. As genetic resources and awareness of these disorders improve, more patients are likely to be identified, with the potential to benefit from education, support, and, ultimately, molecular therapies.

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Life Expectancy and Mortality in Neuroacanthocytosis Syndromes

R.H. Walker[1], M. Miranda[3], A. Danek[4]

[1] Department of Neurology, James J. Peters Veterans Affairs Medical Center, New York, NY, USA, [2] Mount Sinai School of Medicine, New York, NY, USA, [3] Department of Neurology, Clinica Las Condes, Santiago, Chile, [4] Department of Neurology, Ludwig-Maximilians-Universität München, Munich, Germany

Objective: The aim was 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 that cause a spectrum of neurological syndromes. There are no data regarding life expectancy and causes of death. This information would 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 for 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 that may contribute to morbidity.

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Male Infertility Associated with Chorea-acanthocytosis

K. Peikert[1], R. Proschmann[2], H. Glaß[1,3], R. Jung[4], R. Naumann[5], A. Hermann[1,3,6]

[1] Department of Neurology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [2] Department of Urology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [3] Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Dresden, Germany, [4] Experimentelles Zentrum, Medical Faculty, Technische Universität Dresden, Dresden, Germany, [5] Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany, [6] German Center for Neurodegenerative Diseases (DZNE) Dresden, Dresden, Germany

Chorea-acanthocytosis (ChAc) is a rare hereditary neurodegenerative disease caused by 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 impairment of the male reproductive system in two ChAc patients showing asthenoteratozoospermia and oligoasthenozoospermia, respectively, and in Vps13a-knockout mice with similar abnormalities as evidenced by semen analysis. While it is tempting to hypothesize that male infertility should be considered as a new symptom complex associated with ChAc, 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.

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"Levine Syndrome": Neither Chorea-acanthocytosis nor McLeod Syndrome?

A. Danek[1], K. Mente[2], B. Karp[2], A. Ramamurthy[3], D. Scharre[3]

[1] Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität München, Munich, Germany, [2] Human Motor Control Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA, [3] Division of Cognitive Neurology, Department of Neurology, Ohio State University, Columbus, OH, USA

Although the report of the extended New England family 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 the individual members of this family from the various, sometimes contradictory, reports. We identified individual names and biographic dates in publicly available archival material to redraw the pedigree in a modern fashion and to properly place novel data in context that previously were only reported in a meeting abstract or 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 mothers 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, and life duration of the patients would be atypical. Despite some resemblance to rare pedigrees of X-linked McLeod syndrome (OMIM #300842) where female mutation carriers had manifested disease symptoms, this diagnosis appears unlikely as no relevant heart disease was reported; in addition, one instance of male-to-male transmission was observed.

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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Chorea, Psychosis, Acanthocytosis, and Prolonged Survival Associated with ELAC2 Mutations

M. Paucar[1,2], A. Pajak[3,4], C. Freyer[3,4], Å. Bergendal[5], M. Döry[6] J. Laffita[1], H. Stranneheim[4], I. Savitcheva[7], R.H. Walker[8,9], A. Wedell[3,4] A. Wredenberg[3,4*], P. Svenningsson[1,2*]

[1] Department of Neurology, Karolinska University Hospital, Stockholm, Sweden, [2] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, [3] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [4] Center for Inherited Metabolic Diseases, Karolinska University Hospital, Stockholm, Sweden, [5] Department of Neuroimaging, Karolinska Institutet, Stockholm, Sweden, [6] Department of Laboratory Medicine, Karolinska University Hospital, Stockholm, Sweden, [7] Department of Nuclear Medicine, Karolinska University Hospital, Stockholm, Sweden, [8] Department of Neurology, James J. Peters Veterans Medical Affair Center, New York, NY, USA, [9] Department of Neurology, Mount Sinai School of Medicine, New York, NY, USA

*These authors contributed equally.

Introduction: ElaC ribonuclease 2 (ELAC2) has been identified as a subunit of the RNaseZ complex that is 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 a 69-year-old Assyrian female affected by huntingtonism, waddling gait, olfactory hallucinations, type 2 diabetes, and hearing loss requiring aids. Her motor features had been slowly progressive. EMG revealed myopathy and a muscle biopsy revealed COX-negative and red ragged fibers. Repeated blood smears 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 via 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. We did not find any evidence of respiratory chain defect in the muscle biopsy; however, 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 report of the association of *ELAC2* mutations with huntingtonism and long-term 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 illustrates that mutations in *ELAC2* are not exclusively confined to a lethal pediatric cardiomyopathy, but may have a presentation that could be included under the neuroacanthocytosis syndromes.

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