Eighth International Chorea–Acanthocytosis Symposium: Summary of Workshop Discussion and Action Points

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Abstract

Chorea-Acanthocytosis (ChAc) is a rare hereditary neurological disorder characterized by abnormal movements, red blood cell pathology, and progressive neurodegeneration. Little is understood of the pathogenesis of ChAc and related disorders (collectively Neuroacanthocytosis). The Eighth International Chorea-Acanthocytosis Symposium was held in May 2016 in Ann Arbor, MI, USA, and focused on molecular mechanisms driving ChAc pathophysiology. Accompanying the meeting, members of the neuroacanthocytosis research community and other invited scientists met in a workshop to discuss the current understanding and next steps needed to better understand ChAc pathogenesis. These discussions identified several broad and critical needs for advancing ChAc research and patient care, and led to the definition of 18 specific action points related to functional and molecular studies, animal models, and clinical research. These action points, described below, represent tractable research goals to pursue for the next several years.

Keywords: Chorea Acanthocytosis, Neuroacanthocytosis, VPS13, VPS13A, Chorein

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Introduction

The Eighth International Chorea–Acanthocytosis Symposium was held on May 14 and 15, 2016, in Ann Arbor, MI, USA. Previous conferences were held in Seeon, Germany; Montreal, Québec, Canada; London and Oxford, UK; Kyoto, Japan; Bethesda, MD, USA; Ede, The Netherlands; and Stresa, Italy. Two comprehensive summary volumes were among the outcomes of these meetings.1,2 The most
The recent neuroacanthocytosis symposium in Ann Arbor was accompanied by an organized parallel patient meeting. It was attended by 16 families with patients affected by chorea–acanthocytosis (ChAc) or McLeod syndrome, and was organized by the Advocacy for Neuroacanthocytosis Patients (www.naadvocacy.org).

The focus of the current scientific symposium was ChAc, functions of the causative protein chorein (encoded by \textit{VPS13A}; Table 1), and related intracellular transport mechanisms (a full list of speakers and topics is available at https://sites.google.com/a/umich.edu/chacsymposium/home).

Accompanying the meeting, members of the neuroacanthocytosis research community and other invited scientists met in a workshop format to discuss current progress and critical next steps and experiments needed to better understand ChAc pathogenesis. Topics related to three broad themes were discussed: 1) the burgeoning knowledge of yeast Vps13p function as a foundation for understanding \textit{VPS13A} biology and ChAc pathogenesis; 2) the status of animal models of ChAc; 3) critical needs in ChAc clinical research and patient care.

This document summarizes the workshop discussion and sets forth a list of tractable action points for the ChAc research community. Readers are directed to previous review articles and volumes for comprehensive overviews of the field.\textsuperscript{1–3}

### Current understanding of \textit{VPS13} function: insights from yeast models

Dr. Robert Fuller (University of Michigan) led a discussion outlining current knowledge regarding the known functions of the Vps13 protein (Vps13p) in yeast. Extensive work in yeast has provided a large number of identifiable Vps13p cellular localizations, functions, and interactions (Table 2, Figure 1). The yeast work is a rich source of hypotheses about the functions of mammalian \textit{VPS13} homologues.\textsuperscript{4} Although there is

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**Table 1. \textit{VPS13} Nomenclature**

| Gene     | Protein                                                                 |
|----------|-------------------------------------------------------------------------|
| \textit{S. cerevisiae} |                                                                 |
| \textit{VPS13} | Vacuolar Protein Sorting-associated protein 13 (Vps13p)                 |
| \textit{D. melanogaster} |                                                                 |
| \textit{Vps13} | Vacuolar Protein Sorting-associated protein 13 (VPS13)                   |
| \textit{H. sapiens} |                                                                 |
| \textit{VPS13A} | Chorein                                                                  |
| \textit{VPS13B} | Vacuolar Protein Sorting-associated protein 13A (VPS13A)                |
| \textit{VPS13C} | Vacuolar Protein Sorting-associated protein 13B (VPS13B)                |
| \textit{VPS13D} | Vacuolar Protein Sorting-associated protein 13D (VPS13D)                |

**Table 2. Known Vps13p (yeast) Localizations, Functions, and Interactions**

| Localizations                      | Functions                              | Interactions      |
|-----------------------------------|----------------------------------------|-------------------|
| Late endosome/PVC                 | TGN to PVC transport                   | Cdc31p–yeast centrin |
| TGN                               | TGN homotypic fusion                   |                   |
| Nuclear–vacuolar junction         | Unknown                                |                   |
| Prospore membrane                 | Prospore membrane maturation and fusion | Spo71p            |
| Vacuolar–mitochondrial junction   | Mitochondrial integrity                | Mcp1p             |
| Lipid droplet aggregates          | Unknown                                |                   |

Abbreviations: PVC, Prevacuolar Compartment; TGN, Trans-Golgi network.
significant understanding of Vps13p in yeast, the extent to which these reflect the biological consequences of chorein function (and loss of function) in mammalian cells and human tissue is unknown, and represents a major gap in the field (Box 1).
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Vps13p may stimulate the synthesis of several lipids, including PI(4)P, through positive feedback loops. For example, recent data suggest that phosphatidylinositol/phosphatidyl choline transport protein, Sec14p, both function during this lag. The role of Sec14p in stimulating synthesis of PI(4)P at the Golgi suggests that the lag may represent the rebuilding of PI(4)P pools on TGN membranes. Because there is evidence that Vps13p stimulates PI(4)P synthesis on prospore membranes, it may similarly stimulate PI(4)P synthesis in the TGN to PVC reaction. A number of possible mechanisms could explain this putative role (e.g., interaction with PIK1 or blockade of phosphatase function), which begins to invoke potentially druggable enzymes. However, the relevance of these pathways to human cells is unknown.

As another example, introduction of three different point mutations found in ChAc patients into the yeast Vps13 results in separation-of-function alleles. These mutations show specific defects in the mitochondrial integrity aspect of Vps13 function. The common loss of PI(4)P synthesis in the TGN to PVC reaction, which begins to invoke potentially druggable enzymes. However, the relevance of these pathways to human cells is unknown.

Further studies using this type of pathway-oriented approach in yeast, red blood cells (RBCs), and other model systems (as described below) may implicate additional potentially druggable targets. Beyond in vitro studies, these putative pathways could be manipulated in cell and animal models as a next step toward viable therapies.

**Action points: VPS13 functional studies**

1. Characterize the rare human missense mutations that result in disease, but do not cause loss of protein product.
2. Characterize chorein localization in mammalian/human cells.
3. Define associated proteins/complexes.
4. Establish functional readouts/assays for mammalian cells.
5. Define the structure of human chorein isoforms.
6. Perform unbiased lipid profiling studies in mutant and control cells.
7. Perform yeast synthetic tether experiments to model roles at junctions.
8. Assess mitochondrial (and other organelle) function/dysfunction in human cells.
9. Characterize autophagosome–lysosome fusion.
10. Consider antisense transcript in knockout models.
Animal models

Given the rarity of ChAc and related disorders and the relative difficulty of clinical research, valid animal models are a high priority for the field. Prior work has included mouse, tetrahymena, and dictyostelium models. Drs. Ody Sibon (Groningen) and Andreas Hermann (Dresden) led a discussion of the initial findings from newly developed drosophila and mouse knockout models.

Dr. Sibon described a new Drosophila VPS13 knockout model that is characterized by age-associated neurodegeneration, reduced locomotor function in climbing assays, and premature death, which are partially rescued with human VPS13A overexpression. This represents the first ChAc model to exhibit overt neurodegeneration associated with motor behavioral abnormalities. One notable feature of the drosophila model is abrupt early mortality, which should be considered further as a way to examine underlying pathophysiological mechanisms and as a screening tool (Box 2). Further studies aiming to identify rescue interventions of the fly model could provide a link toward human therapies.

Dr. Hermann (Dresden) and Dr. Lucia De Franceschi (Verona) discussed early work from FPS13 knockout mouse models (in addition to a published, but not generally available prior mouse model), including conditional knockout lines. Some similarities emerge through this work, including infertility (particularly in male mice) and impaired autophagy. To date, no overt neurological phenotypes have been observed in these mouse lines, and neuropathological examinations have not been completed systematically.

Pathway-based approaches have been useful to generate hypotheses in animal modeling research. For example, studies of RBCs from ChAc patients provided first insights into possible functions of human chorenin. While RBC membrane lipid composition appears essentially normal, patient erythrocytes demonstrate increased Lyn kinase activity. Unpublished data presented at the workshop demonstrated phenotypic rescue in RBC and patient-derived neuronal cells with Lyn kinase inhibition, suggesting Lyn kinase inhibition as a potential druggable target. It is important to confirm if these abnormalities are present in neurons and if they warrant future rescue studies with Lyn kinase inhibitors. Compensation by other VPS family members, genetic background effects, and age should be considered in the evaluation of mouse models.

Action points: ChAc animal model studies

11. Definitively characterize behavioral and neuropathological phenotypes in new animal models.
12. Perform genetic and drug screening studies in Drosophila model.
13. Complete neuropathology and lipid profiling studies in FIPS13 knockout mice.

Clinical research and patient care

While the first discussion was led by Drs. Bernhard Landwehrmeyer (Ulm) and Adrian Daneck (Munich) and addressed our understanding of the clinical features of ChAc, the ultimate goal of clinical research in ChAc is to produce useful interventions and disease-modifying therapies. A detailed understanding of phenotypic variation and natural history is needed for planning good intervention studies (Box 3). Because ChAc is a very rare disease, intervention studies will be handicapped by very small numbers of participants. Studies using clinical endpoints must be based on well-characterized clinical instruments and will probably require very large predicted intervention effect sizes. Development of efficient biomarkers reflecting disease activity will be crucial to circumvent these problems.

Another key objective of a clinical research network is the further collection of patient and control biosamples for biomarker research. Ideally, this would include a wide range of fluids (blood, serum, cerebrospinal fluid), tissues, and post-mortem specimens. Facilitation of such efforts is crucial for advancing our understanding of ChAc.
of biomarker studies should be a high priority. An accessible repository, building upon a collection based at the neuropathology institute of the University of Munich (blood and DNA samples, ChAc muscle biopsies and brain tissue) will facilitate hypothesis-based, goal-directed studies with appropriate materials. These materials are crucial for validation of pathogenesis and biomarker studies from animal models. Other related disorders, including McLeod syndrome, diseases based on mutations of VPS13C and VPS13D (poster at this symposium), and other HD-like phenotypes could be included, particularly because these patients are being cared for by many of the same physicians as ChAc patients.

It is possible that some of these activities could be done in parallel with efforts in HD research, given the similar clinical context. Working within the structures already in place for the study of HD will increase geographical coverage, providing medical professionals who can assist and perform quality monitoring, thereby creating more and higher quality data for collaborative studies. Importantly, this along with the longstanding support and extensive network activity by the Advocacy for Neuroacanthocytosis Patients would also facilitate recruitment of patients for imaging studies and for donation of biomaterials.

Bench researchers working on VPS13 biology or with animal models should think actively about biological features in their studies that could translate to markers of underlying pathologies and potential biomarkers in patients. Plausible, well-defined functional readouts are needed. Therapeutics should be based on the observed pathogenesis and follow the identification of targets, rather than focusing on untargeted broad high throughput approaches. Development of good biomarkers is challenging and this will require many levels of collaboration between caregivers, clinical research, basic research, and translational studies.

In parallel with this research network, another important project would be to develop an internet platform for clinicians to easily discuss experiences with management of ChAc patients. Because performing multiple clinical trials is not feasible, sharing of clinical experience and discussions among clinicians, patients, and caregivers offers the most plausible route for near term improvement of the care of ChAc patients.

**Action points: clinical care and research**

14. Define natural history and progression of ChAc.
15. Develop standard rating instrument(s).
16. Develop a well-curated biorepository.
17. Conduct prospective, focused biomarker studies.
18. Further develop an internet-based resource and exchange for physicians and medical professionals who assist patients.

**Summary**

The workshop discussions identified three broad critical needs for advancing ChAc research:

i. Defining VPS13A functions that are critical to neuronal function and survival.
ii. Definitive evaluation and exploitation of emerging animal and cell models, including human cell models.
iii. Development of a comprehensive clinical research database, including a well curated biorepository, particularly with a view to defining suitable biomarkers.

ChAc research in the next several years should focus on these needs and attempt to address the action points described above.

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