VPS13A is a multitasking protein at the crossroads between organelle communication and protein homeostasis

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CHAPTER 1

Introduction and aim of the thesis

INTRODUCTION

Chorea-Acanthocytosis

Chorea-Acanthocytosis (ChAc) (MIM 200150) is a rare autosomal recessive neurodegenerative disorder and member of a family of neurological disorders broadly known as neuroacanthocytosis (NA) syndromes[1–3]. NA involves neurological abnormalities coupled with the presence of abnormally spiked red blood cells (acanthocytes) in the peripheral blood circulation[4]. NA syndromes are broadly classified into two categories; the "core" NA syndromes and NA with lipoprotein disorders[5]. The "core" NA group consists of ChAc, McLeod syndrome (MLS), Huntington's disease-like 2 (HDL-2) and pantothenate kinase associated neurodegeneration (PKAN); all of which display degeneration of basal ganglia and acanthocytosis[5].

ChAc is characterized by progressive adult onset involuntary movements, behavioral and cognitive changes, oral dystonia and occasional seizures[5–7]. Increased creatine kinase levels and a 7-50% acanthocytosis in blood circulation are common features of ChAc[8]. Causative mutations for the onset of ChAc are mapped on the *Vacuolar Protein Sorting 13A* (*VPS13A*) gene[2,8]. In most patients, these mutations lead to reduction or absence of detectable protein levels in red blood cells[10] and hence, Western blotting for VPS13A is used as a diagnostic tool in clinical setups[4,10,11].

The main cause of red blood cell abnormalities and neurodegeneration in ChAc is largely unknown. In this chapter, we will describe a general background of VPS13 family proteins with emphasis on VPS13A. Domain architecture, subcellular localizations and functions of VPS13A will be discussed in the context of various ChAc model organisms.

The human VPS13 family proteins

The human VPS13 family consists of four ubiquitously expressed proteins (VPS13A, VPS13B, VPS13C and VPS13D) that share similarity with yeast Vps13[12]. Mutations in all human *VPS13* genes are associated with the onset of neurological and developmental disorders. *VPS13A*, *VPS13B*, *VPS13C* and *VPS13D* are linked to the onsets of ChAc, Cohen syndrome, Parkinson's disease and septic shock mortality respectively[2,13–15].

The *VP13A* gene spans 73 exons and is located on chromosome 9q21. There are two splicing variants of VPS13A (variant 1a and variant 1b). Variant 1a consists of exons 1-68 and 70-73 whereas variant 1b contains only exons 1-69[12]. Mutations in ChAc patients can be found distributed randomly throughout the *VPS13A* gene and so far there are no potential hotspots identified[4].

VPS13B is mutated in patients with Cohen syndrome[13]. Cohen syndrome is a rare autosomal recessive disorder characterized by obesity, motor clumsiness, microcephaly, mental retardation, neutropenia, facial, oral and ocular abnormalities[16–18]. *VPS13B* is located on chromosome 8q22 and widely expressed in a variety of human tissues and unlike VPS13A, its expression in adult brain is marginally low[13,19]. VPS13B is required to maintain Golgi integrity and proper protein glycosylation[20,21]. Although it was initially predicted to contain 10 transmembrane domains[13], subcellular fractionation study shows that VPS13B is a peripheral

membrane protein localized at the Golgi where it interacts with Rab6 to regulate neurite outgrowth with a mechanism that remains to be determined[20,22].

VPS13C is more similar to VPS13A compared to other VPS13 family proteins[12]. *VPS13C* is located on chromosome 15q22 where truncating mutations and polymorphisms are causally linked to Parkinson's disease[14,23–25]. In addition, mutations and single polynucleotide polymorphisms (SNPs) of *VPS13C* are associated with the risk of type 2 diabetes[26–29]. VPS13C is localized at the mitochondrial membrane and its absence aggravates mitochondrial fragmentation and clearance[14].

VPS13D is a ubiquitin binding protein that regulates mitochondrial size and clearance both in *Drosophila* and human cultured cells[30]. Furthermore, a *VPS13D* gene variant is associated with increased septic shock mortality and overproduction of interleukin-6 (IL-6) in patients' plasma and cultured cells[31]. Recent molecular autopsy analysis identified *VPS13D* gene mutation as one of the genes linked to early embryonic mortality[15].

All of the human VPS13 family proteins share conserved N- and C-terminal domains[12]. Nonetheless, the diversity of diseases associated with different human VPS13 family proteins predict that each protein may function in different cellular pathways. Indeed, not all human VPS13 family proteins have similar subcellular localization patterns. VPS13B is localized to the Golgi complex while VPS13C is localized to mitochondria and lipid droplets (LDs)[14,20,22,32]. VPS13D, on the other hand, colocalizes with the lysosomal protein, LAMP1[30]. The biggest issues to be solved in VPS13A research are to define the localization of the protein in mammalian cells[33] and to identify functional domains of the VPS13A protein.

Domains of VPS13A

Sequence alignment studies identify multiple domains of VPS13A. The known domains of VPS13A include Chorein, two phenylalanines in an acidic tract (FFAT), short root transcription factor-binding domain (SHR-BD), aberrant pollen transcription 1 (APT1), ATG-C terminal domain (ATG-C) and pleckstrin homology (PH) domain (Figure 1)[12,34,35].

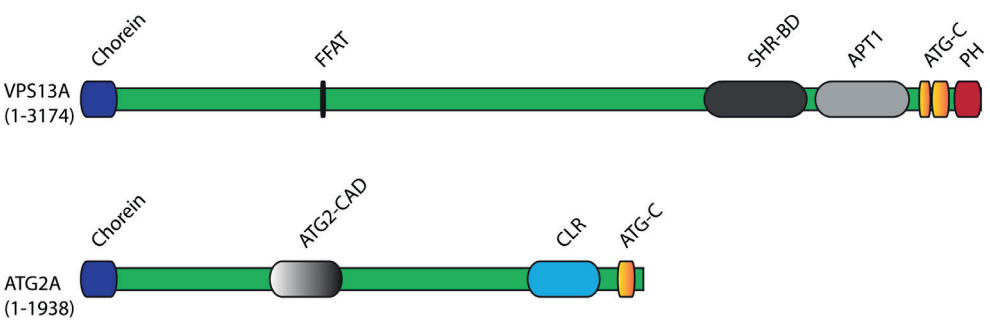


Figure 1. Schematic representations of VPS13A and ATG2. Known domains of both proteins are labelled and similar domains are color-coded. FFAT (two phenylalanines in acidic tract), SHR-BD (short root transcription factor-binding domain), APT1 (aberrant pollen transcription 1), ATG-C (ATG-C terminal domain) and PH (pleckstrin homology), ATG2-CAD (cysteine-alanine-aspartic acid triad). CLR (C-terminal localization region)[34,35,37–39].

The Chorein domain is an evolutionarily conserved domain with an unknown function[12]. The FFAT is a short stretch of amino acids commonly present in lipid transfer proteins with properties of building membrane contact sites with ER[34]. The APT1 domain was first identified in maize APT1 protein. APT1 colocalizes with a Golgi marker protein in tobacco pollen tubes and mutations in APT1 protein lead to defective pollen tube germination and transmission[36]. *In vitro*, Vps13 APT1 fragments bind specifically to PtdIns3p[35]. In the primary structure of VPS13A, the APT1 domain is located between SHR-BD and ATG-C domains[35,37].

SHR-BD is a highly conserved domain that was previously known as domain of unknown function 1162 (DUF1162)[35]. SHR-BD is present in vacuolar protein sorting (At5g24740) of *A. thaliana*. At5g24740 is also known as SHRUBBY and mutation in this gene leads to an aberrant root growth in *Arabidopsis*[40]. The SHR-BD fragment of Vps13 binds to a variety of phosphoinositides as well as to lysophosphatidic acid and phosphatidic acid[35]. Interestingly, the SHR-BD-APT1 fragment binds specifically to PtdIns3p unlike the SHR-BD alone, indicating that APT1 determines the specificity of lipid binding[35]. VPS13 also contains a PH domain and two ATG-C domains that are conserved in both yeast and human[35,37,41,42].

A PH domain is composed of approximately 100 amino acids[43] and is considered as one of the most common domains in the human proteome. PH domain containing proteins are known for their affinities to phosphoinositides; specifically to those with a pair of adjacent phosphate groups such as (PtdIns(4,5) P2 and (PtdIns(3,4,5)P3[44].

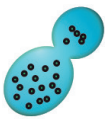
Additionally, VPS13A has two ATG-C domains that show homology with the C-terminal region of ATG2A[35]. There is a 25% identity between the C-terminal regions of VPS13A (aa 2939-3025) and ATG2A (aa 1830-1916)(Figure 1)[39]. ATG2 proteins have a membrane binding ability and are essential for autophagy and LD distribution[39,45]. Similarly, VPS13A is also required to maintain proper autophagic flux [42].

Cellular functions of VPS13A

Most of our current understanding about the cellular functions of Vps13 is derived from studies in yeast[35,46–54]. Vps13 was first identified in a genetic screen for mutants displaying impaired delivery of carboxypeptidase Y (CPY) to the vacuole[55]. Carboxypeptidase Y is a vacuolar protease synthesized in the endoplasmic reticulum (ER) as pro-CPY. Pro-CPY is transported to the Golgi complex where glycosylation occurs and subsequently delivered to the vacuole where modification to the active form takes place[56–58].

Mutants that fail to transport CPY to the vacuolar compartment secrete pro-CPY to the periplasm and ultimately to the extracellular medium[55]. By screening for secretion defects, together with morphological examinations, 41 Vps mutant strains were identified. These mutants are grouped into six classes based on their vacuolar morphology [55,59,60]. The different classes of Vps mutants and the description of their vacuolar morphology is summarized in table 1.

Table 1. Classification of Vps mutants based on their vacuolar morphology. All Vps mutants secrete CPY at various degrees[59]. Green circles (Vacuoles), small blue circles (fragmented vacuole like structures), orange circles (pre-vacuolar or class E compartment).

Class	Vps mutant	Characteristic	
A	Vps8, Vps10,Vps13,Vps29 Vps30, Vps35,Vps38, Vps44 Vps46	Normal vacuolar morphology with 1-3 large vacuoles per cell.	
B	Vps5, Vps17,Vps39,Vps41 Vps43	Large number (20-40) of small and fragmented vacuolar like compartments.	
C	Vps11, Vps16,Vps18,Vps33	Severe defect of vacuole assembly. These mutants barely show vacuoles, but instead accumulate small fragmented vesicles.	
D	Vps3, Vps6,Vps9,Vps15 Vps19, Vps21, Vps34,Vps45	One large vacuole in the parent cell, which fails to be acidified and to segregate to budding daughter cells.	
E	Vps2, Vps4,Vps20,Vps22 Vps24, Vps25, Vps27,Vps28 Vps31,Vps32, Vps36, Vps37	Possess a different population of vesicles (prevacoular endosome like compartment) that contain proteins from both late Golgi and vacuole.	
F	Vps1, Vps26	Large central vacuole surrounded by small fragments without any observable segregation defects.	

As a member of class-A Vps mutants, Vps13 mutants possess morphologically normal vacuoles[59]. Further characterization revealed that Vps13 is a peripheral membrane protein involved in the transport of membrane bound proteins between the trans-Golgi network (TGN) and pre-vacuolar compartment (PVC)[46,61] or from endosome to vacuole [62]. In vps13 mutant cells, there is an increased secretion of insulin and pro-CPY[46,63]. In control cells pro-CPY is actively sorted from late Golgi to vacuole by the sorting receptor Vps10[64]. In Vps13 mutant strains however, Vps10 is mislocalized and rapidly degraded which accounts for an apparent extracellular secretion of pro-CPY[46]. Severe impairment in the production of viable spores is also an apparent phenotype of Vps13 mutants[46].

At the earliest phase of sporulation, Vps13 is diffusely distributed throughout the cytoplasm. Whereas later in meiosis, it is localized at the prospore membrane[48]. Compared to wild type strains, Vps13 mutants have a few very small prospores that often fail to encapsulate nuclei[47].

The cellular functions of VPS13 proteins are intricately broad. Studies in several model organisms revealed that VPS13A plays an array of conserved roles to maintain protein homeostasis, phosphoinositide metabolism, actin cytoskeleton, membrane contact sites and LD homeostasis.

Knock-out of one of the six *Dictyostelium* VPS13 genes (*VPS13F*) delays intracellular destruction of phagocytic cargo attributed to failure in sensing bacterial folate without affecting phagosome maturation[65]. Similarly, *Tetrahymena* VPS13A (TtVPS13A) decorates the phagosome membrane and is required for efficient clearance of phagocytic cargo[66]. In cultured insect cells, Vps13 depletion delays endocytic processing[67]. Another *Dictyostelium* VPS13 (TipC), was identified in a screen for mutations affecting tip formation[68], similar to a phenotype that is observed in autophagy mutants[69]. *tipc*$^{-/-}$ cells accumulate ubiquitinated protein aggregates accompanied by a decreased number of GFP-LC3 and GFP-ATG18 puncta. In mammalian cells, depletion of VPS13A raises the number of GFP-LC3 puncta but decreases liberation of free GFP indicative for a slow autophagic flux[42].

Both endocytic and autophagic degradation pathways are highly regulated by phosphoinositides[70–72]. Interestingly, synthetic genetic screens revealed that Vps13 mutants show similar sets of genetic interactions with Vps30 and Vps38[73,74]. Vps30 and Vps38 are the components of yeast complex I and complex II phosphatidylinositol 3-phosphate kinase (PI3K) complexes, respectively[75,76]. A plausible importance of Vps13 in phosphoinositide metabolism is further established as Vps13 directly binds to an array of phosphoinositides[35,50]. In addition, lipids -such as phosphatidic acid (PA), phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2 and phosphatidylinositol 4-phosphate (PtdIns4p) are reduced at the prospore membrane of Vps13 mutants[48]. Phosphoinositides regulate a multiplicity of cellular processes including actin polymerization and their mis-regulation is linked to a variety of human diseases[71,77,78]. Of importance, impaired actin polymerization is apparent in ChAc patient cells, VPS13A depleted cultured cells and Vps13 mutant yeast cells. In different organisms, VPS13A forms a complex with actin[35,79].

Vps13 is localized at multiple membrane contact sites (MCS)[49,51,54]. MCSs regulate lipid distribution and maintain proper lipid gradients across membranes of different organelles[80,81]. The type and abundance of lipid species determines the subcellular localization of MCS proteins[82]. A number of proteins involved in MCSs are identified through bioinformatics, imaging, biochemical and synthetic biology screens[83–87]. ER occupies the largest intracellular space in eukaryotic cells and is essential for the biosynthesis of proteins and lipids and the ER regulates cellular calcium homeostasis. It is therefore not surprising that most organelles communicate with the ER[81,82,88–95]. Organelle communication is not limited to the ER and it is now clear that MCSs are established between LDs and mitochondria[96], peroxisomes and mitochondria[97,98], lysosomes and peroxisomes[99], LDs and endosomes[100], nucleus and vacuoles[101]and mitochondria and vacuoles[86,87].

Vps13 is recruited to ER-Mitochondrial Encounter Structure (ERMES), vacuole and mitochondria patch (vCLAMP) and NVJ (nuclear vacuole junction) depending on metabolic growth conditions[49,54]. ERMES mutants are synthetically lethal when combined with Vps13 loss of function[49,54]. When harboring a dominant point mutation (Vps13-D716H), Vps13 is able to restore growth defects of ERMES mutants suggesting that Vps13 and ERMES are functionally redundant[49,51,54]. The mammalian ERMES counterpart has yet to be identified and the role of VPS13A in organelle communication is unknown.

AIM AND OUTLINE OF THE THESIS

The overall aim of our research was to uncover the cellular functions of VPS13A in health and disease. The biggest hurdles to study the biology of VPS13A were the absence of reliable genetic model systems and limited biochemical and labelling tools. This is mainly attributed to the absence of antibodies that would detect endogenous VPS13A protein in immunolabelling experiments and partly because of the inherently big size of the protein which in turn makes cloning and overexpression difficult. Indeed, Vps13 is the fifth largest protein in the yeast proteome[49]. We initially characterized a *Drosophila* model of ChAc with an aim to investigate phenotypic consequences of VPS13A-loss of function at the organismal level. We next aimed to identify the localization and interaction partners of VPS13A in cultured human cell lines. Through combined applications molecular biology, biochemistry and cellular imaging, we uncovered previously unknown functions of VPS13A in membrane contacts and LD homeostasis.

Chapter 2: Drosophila Vps13 is required for protein homeostasis in the brain.

Reports about Vps13 function are mainly derived from studies in unicellular eukaryotes such as *Saccharomyces cerevisiae* and *Tetrahymena thermophile*. The availability of multicellular models to study ChAc is limited and there is an obvious demand to generate and validate genetic ChAc models. Although VPS13A mutant mouse models that recapitulate some of the ChAc phenotype were generated[102], it later became clear that the phenotypes were not merely caused by VPS13A loss of function but rather dependent on genetic backgrounds[103]. In this chapter, we aimed to characterize an isogenic Vps13 mutant *Drosophila* line and showed that Vps13 deficient flies have motor impairments, shorter lifespan, neurodegeneration and accumulation of ubiquitinated protein aggregates. Some of these phenotypes were reverted by ubiquitous expression of human VPS13A in Vps13 deficient lines.

Chapter 3: Drosophila Vps13 mutants show overgrowth of larval neuromuscular junctions.

Impaired synaptic communication and plasticity have been previously implicated in many neurodegenerative diseases[104–106] . The neuromuscular junction (NMJ) is a specialized type of synapse that regulates muscle movement by controlling output of neuronal signals[107]. Cytoskeletal integrity of the NMJ not only determines synaptic architecture but also the quality of neuronal impulses[107–110]. Although, VPS13A depletion leads to mis-stabilized actin cytoskeleton[111–113] and abnormal bleb formation at neurite terminals of cultured cells[114], it is unclear whether defective neuro-synaptic architecture contributes to neurodegeneration in ChAc patients or the *Drosophila* model. In this chapter, we investigated and the NMJ anatomy of Vps13 mutants that were validated in chapter 2. Body wall muscles of Vps13 mutants were equally developed as their wild type counterparts. Nonetheless, Vps13 mutant larvae were highly mobile. Our data also indicate that Vps13 loss of function is associated with a large increase in number of boutons that are smaller in size compared to wild type controls.

Chapter 4: Human VPS13A is associated with multiple organelles and required for lipid droplet homeostasis

In chapter 2, we described that *Drosophila* Vps13 co-fractionates with endosomal proteins. However, the subcellular localization of mammalian VPS13A and interaction partners were unresolved for a long time. In this chapter, we provide evidence that VPS13A is localized at the ER-mitochondria interface and directly binds to the ER resident protein, VAP-A, through a specific motif. We also show that the VPS13 C-terminal part acts as a mitochondrial localization signal. When cellular lipid is surplus, VPS13A shifts from its reticular arrangement to LDs where it halts LD mobility. Moreover, we find that, upon VPS13A loss of function, LDs accumulate in both cultured cells and *Drosophila* brain. We also discuss that improper organelle communication and LD handling could contribute to the onset and progression of ChAc.

Chapter 5: Summarizing discussion

Our research highlights a conserved function of VPS13A to control proper protein homeostasis, neuronal growth, organelle communication and LD dynamics. Initially reported as a class A Vps family in yeast, Vps13 later emerged as a protein with multiplicity of cellular functions ranging from intracellular transport, prospore formation, mitochondrial clearance and MCSs. In this chapter, we summarize and discuss the available literature in the VPS13 field and propose a model in which VPS13A is not limited to a single subcellular compartment but it is associated of with multiple organelles dependent on cellular lipid content.

REFERENCES

1. Rubio, J. P. *et al.* Chorea-Acanthocytosis: Genetic Linkage to Chromosome 9q21. *Am. J. Hum. Genet.* **61,** 899–908 (1997).
2. Rampoldi, L. *et al.* A conserved sorting-associated protein is mutant in chorea-acanthocytosis. *Nat. Genet.* **28,** 119–20 (2001).
3. Walker, R. H., Jung, H. H. & Danek, A. in *Handbook of clinical neurology* **100,** 141–151 (2011).
4. Walker, R. H. Untangling the Thorns: Advances in the Neuroacanthocytosis Syndromes. *J. Mov. Disord.* **8,** 41–54 (2015).
5. Jung, H. H., Danek, A. & Walker, R. H. Neuroacanthocytosis Syndromes. *Orphanet J. Rare Dis.* **6,** 68 (2011).
6. Prohaska, R. *et al.* Brain, blood, and iron: perspectives on the roles of erythrocytes and iron in neurodegeneration. *Neurobiol. Dis.* **46,** 607–24 (2012).
7. Velayos Baeza, A. *et al. Chorea-Acanthocytosis. GeneReviews®* (2002). at <http://www.ncbi.nlm.nih.gov/pubmed/20301561>
8. Peikert, K., Danek, A. & Hermann, A. Current state of knowledge in Chorea-Acanthocytosis as core Neuroacanthocytosis syndrome. *Eur. J. Med. Genet.* (2017). doi:10.1016/j.ejmg.2017.12.007
9. Dobson-Stone, C. *et al.* Mutational spectrum of the CHAC gene in patients with chorea-acanthocytosis. *Eur. J. Hum. Genet.* **10,** 773–81 (2002).
10. Dobson-Stone, C. *et al.* Chorein detection for the diagnosis of chorea-acanthocytosis. *Ann. Neurol.* **56,** 299–302 (2004).
11. Lupo, F. *et al.* A new molecular link between defective autophagy and erythroid abnormalities in chorea-acanthocytosis. *Blood* **128,** 2976–2987 (2016).
12. Velayos-Baeza, A., Vettori, A., Copley, R. R., Dobson-Stone, C. & Monaco, A. P. Analysis of the human VPS13 gene family. *Genomics* **84,** 536–49 (2004).
13. Kolehmainen, J. *et al.* Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. *Am. J. Hum. Genet.* **72,** 1359–69 (2003).
14. Lesage, S. *et al.* Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and Increases PINK1/Parkin-Dependent Mitophagy. *Am. J. Hum. Genet.* **98,** 500–13 (2016).
15. Shamseldin, H. E. *et al.* Molecular autopsy in maternal–fetal medicine. *Genet. Med.* (2017). doi:10.1038/gim.2017.111
16. Cohen, M. M., Hall, B. D., Smith, D. W., Graham, C. B. & Lampert, K. J. A new syndrome with hypotonia, obesity, mental deficiency, and facial, oral, ocular, and limb anomalies. *J. Pediatr.* **83,** 280–4 (1973).
17. Kivitie-Kallio, S. & Norio, R. Cohen syndrome: essential features, natural history, and heterogeneity. *Am. J. Med. Genet.* **102,** 125–35 (2001).
18. Seifert, W. *et al.* Mutational spectrum of COH1 and clinical heterogeneity in Cohen syndrome. *J. Med. Genet.* **43,** e22–e22 (2006).
19. Seifert, W. *et al.* Expanded mutational spectrum in Cohen syndrome, tissue expression, and transcript variants of COH1. *Hum. Mutat.* **30,** E404-20 (2009).
20. Seifert, W. *et al.* Cohen syndrome-associated protein, COH1, is a novel, giant Golgi matrix protein required for Golgi integrity. *J. Biol. Chem.* **286,** 37665–75 (2011).
21. Duplomb, L. *et al.* Cohen syndrome is associated with major glycosylation defects. *Hum. Mol. Genet.* **23,** 2391–2399 (2014).
22. Seifert, W. *et al.* Cohen syndrome-associated protein COH1 physically and functionally interacts with the small GTPase RAB6 at the Golgi complex and directs neurite outgrowth. *J. Biol. Chem.* **290,** 3349–58 (2015).
23. Schormair, B. *et al.* Diagnostic exome sequencing in early-onset Parkinson's disease confirms *VPS13C* as a rare cause of autosomal-recessive Parkinson's disease. *Clin. Genet.* (2017). doi:10.1111/cge.13124
24. Chen, C.-M. *et al.* Association of GCH1 and MIR4697 , but not SIPA1L2 and VPS13C polymorphisms, with Parkinson's disease in Taiwan. *Neurobiol. Aging* **39,** 221.e1-221.e5 (2016).
25. Safaralizadeh, T. *et al.* SIPA1L2 , MIR4697 , GCH1 and VPS13C loci and risk of Parkinson's diseases in Iranian population: A case-control study. *J. Neurol. Sci.* **369,** 1–4 (2016).
26. Grarup, N. *et al.* The diabetogenic VPS13C/C2CD4A/C2CD4B rs7172432 variant impairs glucose-stimulated insulin response in 5,722 non-diabetic Danish individuals. *Diabetologia* **54,** 789–794 (2011).
27. Holstein, J. *et al.* Genetic variants in GCKR, GIPR, ADCY5 and VPS13C and the risk of severe sulfonylurea-induced hypoglycaemia in patients with type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* **121,** 54–57 (2012).
28. Strawbridge, R. J. *et al.* Genome-Wide Association Identifies Nine Common Variants Associated With Fasting Proinsulin Levels and Provides New Insights Into the Pathophysiology of Type 2 Diabetes. *Diabetes* **60,** 2624–2634 (2011).
29. Windholz, J. *et al.* Effects of Genetic Variants in ADCY5, GIPR, GCKR and VPS13C on Early Impairment of Glucose and Insulin Metabolism in Children. *PLoS One* **6,** e22101 (2011).
30. Anding, A. L. *et al.* Vps13D Encodes a Ubiquitin-Binding Protein that Is Required for the Regulation of Mitochondrial Size and Clearance. *Curr. Biol.* **28,** 287–295.e6 (2018).
31. Nakada, T. *et al.* VPS13D Gene Variant Is Associated with Altered IL-6 Production and Mortality in Septic Shock. *J. Innate Immun.* **7,** 545–553 (2015).
32. Ramseyer, V. D., Kimler, V. A. & Granneman, J. G. Vacuolar protein sorting 13C is a novel lipid droplet protein that inhibits lipolysis in brown adipocytes. *Mol. Metab.* (2017). doi:10.1016/j.molmet.2017.10.014
33. Pappas, S. S. *et al.* Eighth International Chorea-Acanthocytosis Symposium: Summary of Workshop Discussion and Action Points. *Tremor Other Hyperkinet. Mov. (N. Y).* **7,** 428 (2017).
34. Mikitova, V. & Levine, T. P. Analysis of the Key Elements of FFAT-Like Motifs Identifies New Proteins That Potentially Bind VAP on the ER, Including Two AKAPs and FAPP2. *PLoS*

One **7,** e30455 (2012).

35. Rzepnikowska, W. *et al.* Amino acid substitution equivalent to human chorea-acanthocytosis I2771R in yeast Vps13 protein affects its binding to phosphatidylinositol 3-phosphate. *Hum. Mol. Genet.* **26,** 1497–1510 (2017).
36. Xu, Z. & Dooner, H. K. The maize aberrant pollen transmission 1 gene is a SABRE/KIP homolog required for pollen tube growth. *Genetics* **172,** 1251–61 (2006).
37. Rzepnikowska, W. *et al.* Yeast and other lower eukaryotic organisms for studies of Vps13 proteins in health and disease. *Traffic* **18,** 711–719 (2017).
38. Chowdhury, S. *et al.* Structural analyses reveal that the ATG2A-WIPI4 complex functions as a membrane tether for autophagosome biogenesis. *bioRxiv* 180315 (2017). doi:10.1101/180315
39. Tamura, N. *et al.* Differential requirement for ATG2A domains for localization to autophagic membranes and lipid droplets. *FEBS Lett.* (2017). doi:10.1002/1873-3468.12901
40. Koizumi, K. & Gallagher, K. L. Identification of SHRUBBY, a SHORT-ROOT and SCARECROW interacting protein that controls root growth and radial patterning. *Development* **140,** 1292–300 (2013).
41. Fidler, D. R. *et al.* Using HHsearch to tackle proteins of unknown function: A pilot study with PH domains. *Traffic* **17,** 1214–1226 (2016).
42. Muñoz-Braceras, S., Calvo, R. & Escalante, R. TipC and the chorea-acanthocytosis protein VPS13A regulate autophagy in Dictyostelium and human HeLa cells. *Autophagy* **11,** 918–27 (2015).
43. Scheffzek, K. & Welti, S. Pleckstrin homology (PH) like domains - versatile modules in protein-protein interaction platforms. *FEBS Lett.* **586,** 2662–73 (2012).
44. Lemmon, M. A. Pleckstrin homology (PH) domains and phosphoinositides. *Biochem. Soc. Symp.* **74,** 81 (2007).
45. Velikkakath, A. K. G., Nishimura, T., Oita, E., Ishihara, N. & Mizushima, N. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. *Mol. Biol. Cell* **23,** 896–909 (2012).
46. Brickner, J. H. & Fuller, R. S. SOI1 encodes a novel, conserved protein that promotes TGN-endosomal cycling of Kex2p and other membrane proteins by modulating the function of two TGN localization signals. *J. Cell Biol.* **139,** 23–36 (1997).
47. Nakanishi, H., Suda, Y. & Neiman, A. M. Erv14 family cargo receptors are necessary for ER exit during sporulation in Saccharomyces cerevisiae. *J. Cell Sci.* **120,** 908–16 (2007).
48. Park, J.-S. & Neiman, A. M. VPS13 regulates membrane morphogenesis during sporulation in Saccharomyces cerevisiae. *J. Cell Sci.* **125,** 3004–11 (2012).
49. Lang, A. B., Peter, A. T. J., Walter, P. & Kornmann, B. ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. *J. Cell Biol.* **210,** 883–90 (2015).
50. De, M. *et al.* The Vps13p–Cdc31p complex is directly required for TGN late endosome transport and TGN homotypic fusion. *J. Cell Biol.* jcb.201606078 (2017). doi:10.1083/jcb.201606078
51. John Peter, A. T. *et al.* Vps13-Mcp1 interact at vacuole–mitochondria interfaces and bypass ER–mitochondria contact sites. *J. Cell Biol.* **216,** 3219–3229 (2017).
52. Dalton, L. E., Bean, B. D. M., Davey, M. & Conibear, E. Quantitative high-content imaging identifies novel regulators of Neo1 trafficking at endosomes. *Mol. Biol. Cell* **28,** 1539–1550 (2017).
53. Xue, Y. *et al.* Endoplasmic reticulum–mitochondria junction is required for iron homeostasis. *J. Biol. Chem.* **292,** 13197–13204 (2017).
54. Park, J.-S. *et al.* Yeast Vps13 promotes mitochondrial function and is localized at membrane contact sites. *Mol. Biol. Cell* **27,** 2435–49 (2016).
55. Bankaitis, V. A., Johnson, L. M. & Emr, S. D. Isolation of yeast mutants defective in protein targeting to the vacuole. *Proc. Natl. Acad. Sci. U. S. A.* **83,** 9075–9 (1986).
56. Shiba, Yoichiro Ichikawa, Kimihisa Serizawa, Nobufusa Yoshikawa, H. No Title. *J. Ferment. Bioeng.* **86,** 545–549 (1998).
57. Klionsky, D. J. & Emr, S. D. A new class of lysosomal/vacuolar protein sorting signals. *J. Biol. Chem.* **265,** 5349–52 (1990).
58. Tabuchi, M. *et al.* Vacuolar protein sorting in fission yeast: cloning, biosynthesis, transport, and processing of carboxypeptidase Y from Schizosaccharomyces pombe. *J. Bacteriol.* **179,** 4179–89 (1997).
59. Raymond, C. K., Howald-Stevenson, I., Vater, C. A. & Stevens, T. H. Morphological classification of the yeast vacuolar protein sorting mutants: evidence for a prevacuolar compartment in class E vps mutants. *Mol. Biol. Cell* **3,** 1389–402 (1992).
60. Rothman, J. H. & Stevens, T. H. Protein sorting in yeast: mutants defective in vacuole biogenesis mislocalize vacuolar proteins into the late secretory pathway. *Cell* **47,** 1041–51 (1986).
61. Redding, K., Brickner, J. H., Marschall, L. G., Nichols, J. W. & Fuller, R. S. Allele-specific suppression of a defective trans-Golgi network (TGN) localization signal in Kex2p identifies three genes involved in localization of TGN transmembrane proteins. *Mol. Cell. Biol.* **16,** 6208–17 (1996).
62. Luo, W. j & Chang, A. Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant. *J. Cell Biol.* **138,** 731–46 (1997).
63. Zhang, B., Chang, A., Kjeldsen, T. B. & Arvan, P. Intracellular retention of newly synthesized insulin in yeast is caused by endoproteolytic processing in the Golgi complex. *J. Cell Biol.* **153,** 1187–98 (2001).
64. Marcusson, E. G., Horazdovsky, B. F., Cereghino, J. L., Gharakhanian, E. & Emr, S. D. The sorting receptor for yeast vacuolar carboxypeptidase Y is encoded by the VPS10 gene. *Cell* **77,** 579–86 (1994).
65. Leiba, J. *et al.* Vps13F links bacterial recognition and intracellular killing in Dictyostelium. *Cell. Microbiol.* **19,** e12722 (2017).
66. Samaranayake, H. S., Cowan, A. E. & Klobutcher, L. A. Vacuolar Protein Sorting Protein 13A, TtVPS13A, Localizes to the Tetrahymena thermophila Phagosome Membrane and Is Required for Efficient Phagocytosis. *Eukaryot. Cell* **10,** 1207–1218 (2011).

67. Korolchuk, V. I. *et al.* Drosophila Vps35 function is necessary for normal endocytic trafficking and actin cytoskeleton organisation. *J. Cell Sci.* **120,** 4367–76 (2007).

68. Stege, J. T., Laub, M. T. & Loomis, W. F. tip genes act in parallel pathways of earlyDictyostelium development. *Dev. Genet.* **25,** 64–77 (1999).

69. Mesquita, A. *et al.* Autophagy in *Dictyostelium* : Mechanisms, regulation and disease in a simple biomedical model. *Autophagy* **13,** 24–40 (2017).

70. Balla, T. Phosphoinositides: Tiny Lipids With Giant Impact on Cell Regulation. *Physiol. Rev.* **93,** 1019–1137 (2013).

71. Shi, Y., Azab, A. N., Thompson, M. N. & Greenberg, M. L. Inositol phosphates and phosphoinositides in health and disease. *Subcell. Biochem.* **39,** 265–92 (2006).

72. Idevall-Hagren, O. & De Camilli, P. Detection and manipulation of phosphoinositides. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **1851,** 736–745 (2015).

73. Costanzo, M. *et al.* The Genetic Landscape of a Cell. *Science (80-.).* **327,** 425–431 (2010).

74. Hoppins, S. *et al.* A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. *J. Cell Biol.* **195,** 323–340 (2011).

75. Kihara, A., Noda, T., Ishihara, N. & Ohsumi, Y. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae. *J. Cell Biol.* **152,** 519–30 (2001).

76. Vanhaesebroeck, B., Guillermet-Guibert, J., Graupera, M. & Bilanges, B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat. Rev. Mol. Cell Biol.* **11,** 329–341 (2010).

77. Vicinanza, M., D'Angelo, G., Di Campli, A. & De Matteis, M. A. Phosphoinositides as regulators of membrane trafficking in health and disease. *Cell. Mol. Life Sci.* **65,** 2833–2841 (2008).

78. Wen, P. J., Osborne, S. L. & Meunier, F. A. Dynamic control of neuroexocytosis by phosphoinositides in health and disease. *Prog. Lipid Res.* **50,** 52–61 (2011).

79. Shiokawa, N. *et al.* Chorein, the protein responsible for chorea-acanthocytosis, interacts with β-adducin and β-actin. *Biochem. Biophys. Res. Commun.* **441,** 96–101 (2013).

80. Toulmay, A. & Prinz, W. A. Lipid transfer and signaling at organelle contact sites: the tip of the iceberg. *Curr. Opin. Cell Biol.* **23,** 458–63 (2011).

81. Raiborg, C. *et al.* Repeated ER–endosome contacts promote endosome translocation and neurite outgrowth. *Nature* **520,** 234–238 (2015).

82. Mesmin, B. *et al.* A four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-Golgi tether OSBP. *Cell* **155,** 830–43 (2013).

83. Kornmann, B. *et al.* An ER-Mitochondria Tethering Complex Revealed by a Synthetic Biology Screen. *Science (80-.).* **325,** 477–481 (2009).

84. Lahiri, S. *et al.* A Conserved Endoplasmic Reticulum Membrane Protein Complex (EMC) Facilitates Phospholipid Transfer from the ER to Mitochondria. *PLoS Biol.* **12,** e1001969 (2014).

85. Gatta, A. T. *et al.* A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. *Elife* **4,** (2015).

86. Hönscher, C. *et al.* Cellular Metabolism Regulates Contact Sites between Vacuoles and Mitochondria. *Dev. Cell* **30,** 86–94 (2014).

87. Elbaz-Alon, Y. *et al.* A Dynamic Interface between Vacuoles and Mitochondria in Yeast. *Dev. Cell* **30,** 95–102 (2014).

88. Rocha, N. *et al.* Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. *J. Cell Biol.* **185,** 1209–25 (2009).

89. Hua, R. *et al.* VAPs and ACBD5 tether peroxisomes to the ER for peroxisome maintenance and lipid homeostasis. *J. Cell Biol.* jcb.201608128 (2017). doi:10.1083/jcb.201608128

90. Costello, J. L. *et al.* ACBD5 and VAPB mediate membrane associations between peroxisomes and the ER. *J. Cell Biol.* jcb.201607055 (2017). doi:10.1083/jcb.201607055

91. Giordano, F. *et al.* PI(4,5)P(2)-dependent and Ca(2+)-regulated ER-PM interactions mediated by the extended synaptotagmins. *Cell* **153,** 1494–509 (2013).

92. Galmes, R. *et al.* ORP5/ORP8 localize to endoplasmic reticulum–mitochondria contacts and are involved in mitochondrial function. *EMBO Rep.* **17,** 800–810 (2016).

93. Saheki, Y. *et al.* Control of plasma membrane lipid homeostasis by the extended synaptotagmins. *Nat. Cell Biol.* **18,** 504–515 (2016).

94. Salo, V. T. *et al.* Seipin regulates ER-lipid droplet contacts and cargo delivery. *EMBO J.* e201695170 (2016). doi:10.15252/embj.201695170

95. Stoica, R. *et al.* ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nat. Commun.* **5,** 3996 (2014).

96. Herms, A. *et al.* AMPK activation promotes lipid droplet dispersion on detyrosinated microtubules to increase mitochondrial fatty acid oxidation. *Nat. Commun.* **6,** 7176 (2015).

97. Fan, J., Li, X., Issop, L., Culty, M. & Papadopoulos, V. ACBD2/ECI2-Mediated Peroxisome-Mitochondria Interactions in Leydig Cell Steroid Biosynthesis. *Mol. Endocrinol.* **30,** 763–82 (2016).

98. Fransen, M., Lismont, C. & Walton, P. The Peroxisome-Mitochondria Connection: How and Why? *Int. J. Mol. Sci.* **18,** 1126 (2017).

99. Chu, B.-B. *et al.* Cholesterol Transport through Lysosome-Peroxisome Membrane Contacts. *Cell* **161,** 291–306 (2015).

100. Guimaraes, S. C. *et al.* Peroxisomes, lipid droplets, and endoplasmic reticulum "hitchhike" on motile early endosomes. *J. Cell Biol.* **211,** 945–54 (2015).

101. Pan, X. *et al.* Nucleus-vacuole junctions in Saccharomyces cerevisiae are formed through the direct interaction of Vac8p with Nvj1p. *Mol. Biol. Cell* **11,** 2445–57 (2000).

102. Tomemori, Y. *et al.* A gene-targeted mouse model for chorea-acanthocytosis. *J. Neurochem.* **92,** 759–766 (2005).

103. Sakimoto, H., Nakamura, M., Nagata, O., Yokoyama, I. & Sano, A. Phenotypic abnormalities in a chorea-acanthocytosis mouse model are modulated by strain background. *Biochem. Biophys. Res. Commun.* **472,** 118–124 (2016).

104. Bae, J. R. & Kim, S. H. Synapses in neurodegenerative

diseases. *BMB Rep.* **50,** 237–246 (2017).

105. Zhou, L. *et al.* Tau association with synaptic vesicles causes presynaptic dysfunction. *Nat. Commun.* **8,** 15295 (2017).

106. Milnerwood, A. J. & Raymond, L. A. Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. *Trends Neurosci.* **33,** 513–523 (2010).

107. Wu, H., Xiong, W. C. & Mei, L. To build a synapse: signaling pathways in neuromuscular junction assembly. *Development* **137,** 1017–33 (2010).

108. Dobbins, G. C., Zhang, B., Xiong, W. C. & Mei, L. The Role of the Cytoskeleton in Neuromuscular Junction Formation. *J. Mol. Neurosci.* **30,** 115–118 (2006).

109. Nelson, J. C., Stavoe, A. K. H. & Colón-Ramos, D. A. The actin cytoskeleton in presynaptic assembly. *Cell Adh. Migr.* **7,** 379–87 (2013).

110. Kapitein, L. C. & Hoogenraad, C. C. Building the Neuronal Microtubule Cytoskeleton. *Neuron* **87,** 492–506 (2015).

111. Foller, M. *et al.* Chorein-sensitive polymerization of cortical actin and suicidal cell death in chorea-acanthocytosis. *FASEB J.* **26,** 1526–1534 (2012).

112. Schmidt, E.-M. *et al.* Chorein sensitivity of cytoskeletal organization and degranulation of platelets. *FASEB J.* **27,** 2799–2806 (2013).

113. Alesutan, I. *et al.* Chorein Sensitivity of Actin Polymerization, Cell Shape and Mechanical Stiffness of Vascular Endothelial Cells. *Cell. Physiol. Biochem.* **32,** 728–742 (2013).

114. Park, J.-S., Halegoua, S., Kishida, S. & Neiman, A. M. A Conserved Function in Phosphatidylinositol Metabolism for Mammalian Vps13 Family Proteins. *PLoS One* **10,** e0124836 (2015).