Yeast and other lower eukaryotic organisms for studies of Vps13 proteins in health and disease

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Synopsis

Vps13 proteins have a prominent contribution to human health and disease, although their function is obscured. Studies using simple eukaryotes greatly extended the knowledge about Vps13 proteins, indicated their role in actin cytoskeleton, vesicular trafficking, regulation of membrane contact sites, mitochondrial functioning and autophagy, and allow to classify the yeast Vps13 as a lipid-binding protein. Moreover, these studies revealed the pathogenic mechanism of the mutations found in *VPS13*-associated neurodegenerative disorder Chorea-acanthocytosis. Recent findings in the field are presented and discussed.

Abstract

Human Vps13 proteins are associated with several diseases, including the neurodegenerative disorder Chorea-acanthocytosis (ChAc). However, the biology of these proteins is relatively poorly understood. Studies in lower eukaryotic models, such as *Saccharomyces cerevisiae* and *Dictyostelium discoideum,* point to the involvement of Vps13 in many key cellular processes, the actin cytoskeleton organization, vesicular trafficking, regulation of membrane contact sites, mitochondrial functioning and autophagy. Recent findings revealed that yeast Vps13 binds phosphatidylinositol lipids via at least four different lipid binding regions. Modelling of ChAc-associated mutations in yeast has revealed that particular amino acid substitutions give rise to specific phenotypes. Noteworthy, a mutation in the APT1 domain of Vps13 results in diminished lipid binding and disturbances of all processes studied, including the actin cytoskeleton organization, vacuolar transport, endocytosis and sporulation. This review describes the great potential of simple eukaryotes to decipher disease mechanisms and highlights novel insights into the pathological role of Vps13 towards ChAc.

Introduction

There are growing evidences that members of the human Vps13 protein family have a prominent contribution in health and disease [1]. Defects in the expression and structure of all human *hVPS13* genes are linked to multiple disorders, such as neurodegeneration and neurological diseases, cancers and diabetes. In particular, mutations in *hVPS13A* gene lead to a complex and fatal disease known as Chorea-acanthocytosis [2]. Despite their relevance, the functioning of Vps13 proteins in cells and their molecular role in particular cellular processes are still unclear. In the last years, an increasing number of studies have taken advantage of simple experimental models to deeply investigate the function of Vps13 proteins. In this review we summarize the recent reports regarding the domain structure and functions of Vps13 proteins, and modelling of mutations found in ChAc disease, especially focusing on the simple eukaryote *Saccharomyces cerevisiae*. Other experimental models such as *Tetrahymena thermophila*, *Dictyostelium discoideum* and *Drosophila melanogaster* are also contributing to understand the complexity of Vps13 proteins. These studies have revealed potential pathways affected in the diseases, which include actin cytoskeleton organization, vesicle trafficking, regulation of membrane contact sites between organelles, mitochondrial function, phagocytosis and autophagy. Modelling of diseases in simple eukaryotes can shed more light into the pathological mechanisms and may serve as experimental platforms for drug testing.

Human diseases associated with *VPS13* genes

In the human genome, there are four widely expressed genes: *hVPS13A*, *hVPS13B*, *hVPS13C* and *hVPS13D* encoding hVPS13 proteins (here referred to as hVps13, for clarity) [1]. Mutations or alternations in the expression of these genes are associated with various human disorders (Table 1). Mutations in *hVPS13A* lead to the rare hereditary disease – Chorea-acanthocytosis (ChAc; OMIM 200150) [2–4], an autosomal recessive disorder characterized by adult-onset chorea, progressive neurodegeneration and abnormal erythrocyte morphology – acanthocytosis [5]. Mutations in *hVPS13B* cause Cohen syndrome (OMIM 216550) [6], a rare autosomal recessive disorder characterized by non-progressive psychomotor retardation, microcephaly, characteristic facial features, retinal dystrophy, and intermittent neutropenia in children [7], but there are also reports of correlation with autism and other neurological disorders (Table 1). In turn, *hVPS13C* mutations are implicated in a distinct form of early-onset Parkinsonism (OMIM 616840) characterized by rapid and severe disease progression and early cognitive decline [8]. Genetic studies have also revealed that variants of *hVPS13C* are associated with increased risk of diabetes [9–12]. On the other hand, it was identified a genetic variation in the *hVPS13D* allele, which contributes to septic shock mortality [13]. There are also reports of putative associations of *hVPS13A*, *hVPS13C* and *hVPD13D* variations with schizophrenia [14,15]. Finally, alterations in the expression level and/or mutations in all human *hVPS13* are found in some cancers [16–21].

The complex roles of Vps13 proteins in mammalian cells

Despite the obvious fact that human *hVPS13* genes are involved in several disorders, little is still known about the role of hVps13 proteins in cells and their exact molecular function(s). The most studied member of hVps13 protein family, hVps13A, is apparently involved in cytoskeletal structure organization and dynamics, such as actin filaments [22–26], microtubule and intermediate filament (desmin and cytokeratin) networks [27]. It also regulates vesicular transport [22,79], autophagy [28] and is involved in the metabolism of phosphoinositides (PIPs) [29]. The hVps13B is a peripheral membrane protein localized to the Golgi complex, required for maintenance of the Golgi complex morphology [30] and essential for normal glycosylation of proteins [31]. The hVps13C protein is enriched in the outer membrane of mitochondria [8], lipid droplets (LD) [32] and, based on cell fractionation studies, in early endosomes (EE) [8]. The hVps13C seems to be involved in maintenance of mitochondrial morphology and vulnerability to stress [8,33]. Moreover, it participates in adipogenesis by binding galectin-12, a protein required for adipogenic signalling and adipocyte differentiation *in vitro* [32]. In turn, hVps13D regulates interleukin-6 (IL-6) production and/or transport, since silencing of a gene encoding hVps13D in HeLa cells significantly increased the IL-6 levels in the medium in both non-stimulated and stimulated conditions [13]. To sum up, the hVps13 proteins are apparently involved in many key cellular processes. Hence, disturbances in their functioning lead to severe disorders affecting various tissues.

Recent insights in Vps13 function from studies on *Dictyostelium*, *Tetrahymena* and *Drosophila* species

The Vps13 proteins are not unique for humans but are evolutionary conserved in eukaryotic organisms. The members of Vps13 family are found in yeast *Saccharomyces cerevisiae* [34], a ciliated protozoan *Tetrahymena thermophila* [35], a social amoeba *Dictyostelium discoideum* [28], insects [36], mouse [37] and others [1]. The high degree of conservation suggests that the functions of Vps13 family members are preserved during evolution and concern basic cellular processes. This fact encourages researchers to use model organisms to study Vps13 proteins as well as the mechanisms of their related diseases.

In the amoeba *D. discoideum*, the absence of one of its six Vps13 homologues, called TipC (or DdVps13C), leads to an aberrant multicellular structure in development and defective sporulation, phenotypes observed in many autophagy mutants. Autophagic flux was impaired in the mutant cells, which consistently accumulated ubiquitin positive structures, probably representing protein aggregates [28]. Ubiquitinated proteins were also accumulated in a *Drosophila melanogaster* DmVps13 mutant [38] and α-synuclein-mediated neurodegeneration was increased in *D. melanogaster* retina lacking the same Vps13 homologue [33]. The *D. melanogaster* protein has been co-fractioned with endosomal proteins and its absence resulted in reduced endocytosis [38,39]. The morphology and acidification of endosomal compartments were shown to be unaltered in a *D. discoideum* mutant lacking another Vps13 homologue (DdVps13F), characterized by a slight delayed growth on *Klebsiella pneumoniae* lawns. This defect was mainly attributed to a defective intracellular killing of *K. pneumoniae*, as phagocytic internalisation was not significantly altered in that mutant after a short incubation with heat-inactivated *K. pneumoniae* or fluorescent latex beads [40]. However, in the *tipC* mutant of *D. discoideum*, the reduced phagocytosis was observed upon longer incubation times [28]. Another *D. discoideum* mutant, with *vps13A* knock-out, showed defective growth dependent on phagocytosis of *Micrococcus luteus* [40]. Moreover, phagocytosis was reduced and phagocytic digestion was delayed in *T. thermophila* lacking a Vps13 homologue [35], which was identified as a component of phagosomes in a mass spectrometry analysis [41] and shown to localize at the membrane of phagosomes [35]. Altogether, the study of functions of the Vps13 proteins in these model organisms points to a role of Vps13 proteins in processes highly dependent on membranes, vesicle trafficking and lysosomal degradation pathways. It is well-known that a deficient degradative capacity of cells is involved in multiple human diseases, including the major neurodegenerative diseases, and thus it should be taken into account as a possible therapeutic target of *hVPS13*-related diseases.

Yeast as a model to study Vps13 proteins and associated diseases

The budding yeast *S. cerevisiae* is one of the most studied and best-characterized eukaryotic models. Its extensive use as a model organism to understand the intricacies of human diseases is motivated by the high evolutionary conservation of fundamental cellular processes with higher eukaryotes. Indeed, yeast encodes orthologues of many human disease genes and, conversely, some human genes can complement the defects caused by the lack of native yeast genes [42–45]. In addition, yeast is genetically tractable, has a short generation time, and is cheap and easy to culture. All these features place *S. cerevisiae* as a one of the most potent eukaryotic model organisms to study the molecular mechanisms underlying human diseases [46] and the effect of particular mutations on protein function. Yeast has proven its utility in the study of mitochondrial diseases [47], neurodegenerative disorders [48–50], inherited peripheral neuropathies [51] and other diseases [52]. Moreover, yeast is suitable for high-throughput screening approaches to identify drugs that may turn out to be effective in the treatment of human disorders [53,54].

Yeast possesses a single intron-less *VPS13* (*YLL040C*) gene encoding a Vps13 protein, which may execute many of the activities performed by different human hVps13 proteins. Consequently, functional complementation by other protein isoforms is not possible. This feature simplifies the study of the underlying mechanisms of Vps13 associated disorders and the impact of disease causing mutations and, together with all the advantages described above, makes yeast a powerful and simple model to study the function of hVps13 proteins and associated diseases.

Vps13 in yeast

The Vps13 of *S. cerevisiae* is a large protein (358 kDa) peripherally associated with membranes [34]. Vps13 is most similar to human hVps13A [1] presenting a comparable domain structure: Chorein_N, VPS13 domain, VPS13_mid_rpt, SHR_BD (previously called DUF1162, domain of unknown function No. 1162) and VPS13_C (Fig.1A); according to the Pfam database (http://pfam.xfam.org) [1,35,55,56]. The ATG_C (Autophagy-related protein C-terminal domain) region was recognized in yeast Vps13 and human hVps13A [28], and using the HHpred program other authors revealed the presence of two ATG_C domains in yeast Vps13 – a full-length domain (amino acids (aa) 2921-3005) and a truncated one (aa 2845-2909), which overlap with the VPS13_C domain [57]. Moreover, the APT1 domain between SHR-BD and ATG_C was identified in both human hVps13A and yeast Vps13 (Fig.1A) [57]. An independent HHsearch at the C-terminal region of Vps13 revealed the presence of homology to the pleckstrin homology domains (PH), which are typical docking sites for phosphatidylinositol lipids [58], and homology to this domain was also found in hVps13A in our recent analysis using Phyre2 (K. Flis, unpublished result). Recently, using single-particle electron microscopic analysis of negatively stained

protein, an approximate 3D architecture of yeast Vps13 was visualized (Fig.1B) [59]. Vps13 is folded into a compact rod-shaped density (20×4 nm) with a flexible portion in its middle, a characteristic loop structure with internal diameter of ~6 nm at one end and a hook-like density on the opposite end that can be found on the same or opposite site (Fig.1B) [59]; however, the position of N- and C-terminal regions in this structure is still unknown. Vps13 was very recently found to be a lipid-binding protein as it binds to phosphatidic acid (PA), mono- and diphosphorylated phosphatidylinositol derivatives (PIP and PIP_2, respectively), lysophosphatidic acid (LPA) and, with very low affinity, to phosphatidylserine (PS) [59]. Interestingly, Vps13 possesses at least four lipid-binding regions, which may independently bind to different phospholipids: the N terminus (aa 1-437) interacts with PA, $PI(4,5)P_2$, and PI4P; the C terminus (aa 2905-3144) binds to $PI(4,5)P_2$ [59]; the central SHR_BD domain containing region (aa 2158-2575) binds all phosphorylated phosphatidylinositol derivatives (PIPs), PA and lysophosphatidic acid (LPA); and the APT1 domain region (aa 2492-2844) interacts almost exclusively with PI3P (Fig.1A) [57].

Yeast Vps13 is involved in cellular transport and was originally identified by its role in vesicular trafficking between the Golgi apparatus and vacuole. The null mutant of *VPS13* (*vps13*Δ) exhibits mislocalization of the native vacuolar enzyme carboxypeptidase Y (CPY) and the proteases Pep4 and Prb1 to the cell surface [34,60,61]. Moreover, Vps13 is also required for Golgi-to-vacuole transport of an adaptor membrane protein Sna3 [57]. The *vps13*Δ mutant also shows disturbed retrograde transport from the late endosome (LE) to the Golgi apparatus and accelerated vacuolar degradation of the sorting receptor Vps10 [34]. Recently, it was reported that Vps13 is directly required for transport from the Golgi network to the LE compartment and for *trans*-Golgi homotypic fusion [59]. Vps13 is also involved in the endocytic transport of lipids and proteins from the plasma membrane to vacuole, as determined by internalization of the lipophilic dye FM4-64 [57,62] and Can1 transporter [57]. Defects of *vps13*Δ in endosomal recycling of proteins were also reported [63].

Similar to hVps13A, Vps13 is involved in the regulation of the actin cytoskeleton network. Vps13 was found among proteins captured in *in vitro* actin assembly assay using microbeads coated with nucleation promoting factor Las17 (yeast WASP) [64] and it binds actin in immunoprecipitation assays [57]. Moreover, the *vps13*Δ strain displays defects in actin cytoskeleton organization [57]. Additionally, it is worth mentioning that the internal diameter (~6 nm) of the loop in Vps13 structure is similar to the diameter of an F-actin filament [59], further suggesting that Vps13 may directly interact and regulate the actin cytoskeleton network.

The actin cytoskeleton is important for many processes based on membrane dynamics, such as endocytosis, retrograde transport [65,66] and that list also includes mitochondrial inheritance and integrity [67,68]. Therefore, it was not surprising that, similarly to hVps13C, yeast Vps13 was found to be involved in mitochondrial homeostasis. The *vps13*Δ cells show increased rate of nuclear transfer of mitochondrial DNA (mtDNA) indicating defects in mtDNA stability and mitochondrial integrity [69]. This could be also the reason for the increased rate of mitophagy that was observed in the *vps13*Δ strain [69].

Vps13 is also involved in sporulation, which is the yeast equivalent of gametogenesis [70]. During this process, four haploid nuclei are produced by meiosis and enveloped within a novel intracellular membrane called the prospore membrane. Sporulation efficiency is reduced ~2,500-fold in *vps13*Δ [34], as a result of defects in the formation of prospore membranes, which are smaller and only about a quarter of them capture nuclei [71]. Upon sporulation, Vps13 translocates to the prospore membrane where it promotes expansion of the membrane and regulates its bending, being also required for cytokinesis [72]. The observed defects in *vps13*Δ during sporulation are probably associated with the reduced levels of $PI(4,5)P_2$ and its precursor, PI4P, in the prospore membrane [72].

Initially, it was claimed that Vps13 localized to the cytoplasm and endosomes [73] and later to the prospore membrane [72]. Recently, it was demonstrated that Vps13 is also localized to the membrane contact sites (MCSs), where it may hypothetically regulate their formation or function (Fig.2) [69,74]. The MCSs are structures where two membranes are tethered in close apposition, but not fuse. These junctions allow the exchange of ions, metabolites and lipids between membranes and organelles [75]. Vps13 was found in mitochondria and vacuole contacts called vacuole and mitochondria patch (vCLAMP), between vacuole and the ER-derived nuclear envelope–vacuole junction (NVJ) [69,74], and at the interface between the endosomes and mitochondria (IEM) [69], depending on the carbon source. Deletion of *VPS13* is synthetically lethal in combination with mutations of *MMM1*, encoding an integral ER membrane protein that is a subunit of the ER-mitochondria encounter structure (ERMES) complex, tethering the ER and mitochondria [69,74]. Also, dominant mutations in *VPS13* suppress all known ERMES complex-deficient phenotypes, probably by increasing the formation or function of vCLAMP [74]. This role in MCS is well-matched with the current reports presenting that purified Vps13 exhibits ATP-stimulated binding to yeast membranes and interacts with various membrane lipids, as described above [59]. Moreover, association of Vps13 with lipids is important for its localization, as microscopy studies showed that Vps13-GFP localization is altered in strains with decreased PI3P level due to the lack of Vps30 and Vps38 subunits of the phosphatidylinositol 3-kinase (PI3K) complex; Vps13-GFP is accumulated in large perivacuolar membrane compartments of endocytic origin [57]. As the MCSs between particular membranes regulate the dynamics and functions of organelles through the trafficking of various molecules, it is possible that the observed disturbances in vesicular transport, sporulation and mtDNA stability in the *vps13*Δ cells are a result of changes in MCSs

formation or function. Thus, it is not surprising that the depletion of Vps13 results in such pleiotropic effects.

Summarizing, the single yeast Vps13 protein seems to participate in multiple cellular processes including membrane and protein trafficking, actin cytoskeleton organization and mitochondrial stability. It binds to several membranes and is able to interact with multiple phospholipids through at least four regions dispersed from N to C terminus. It also participates in the formation or function of multiple MCSs. However, the molecular mechanism by which Vps13 regulates such a variety of processes is still unknown. It is possible that a single property, such as the role in MCSs, may impact and explain the defects observed by the loss of Vps13, or alternatively, Vps13 may display different molecular functions associated perhaps with the different conserved domains.

Exploring the yeast Vps13 to decipher ChAc mechanisms

As mentioned above, mutations in human *VPS13* genes are involved in several human diseases and yeast Vps13 may provide a valuable research model to study the molecular underpinnings on the pathological mechanisms. With reference to *hVPS13A* mutations leading to ChAc, most of the described mutations cause a premature STOP codon (nonsense mutations) or a reading frame shift (frameshift mutations, i.e. insertions or deletions) and are relatively easy to interpret, since they result in a truncated protein. However, missense mutations resulting in amino acid substitutions in hVps13A have also been described and they require in-depth studies. So far, five yeast *VPS13* mutations corresponding to ChAc-associated *hVPS13A* missense mutations were investigated: L66P, C89K, L1107P, Y2702C and I2749R (see Table 2 for a list of human missense mutations). Intriguingly, the effect of each of those mutations on Vps13 functioning in yeast cells was different suggesting that a universal mechanism leading to ChAc disease may not exist. No phenotypes were observed for *vps13-Y2702C* variant, indicating that this mutation does not alter the function of the yeast protein in the processes tested [69]. In contrast, the *vps13-L1107P* mutant strain shows a defect in CPY sorting and *mmm1*Δ complementation. Moreover, *vps13-L66P* and *vps13-C89K* were also synthetically lethal with *mmm1*Δ, demonstrating the inability of these variants to compensate for the loss of ERMES [69]. Finally, the *vps13-I2749R* yeast variant was inactive in all processes tested, suggesting a loss of function [57] (see Table 2 for a description of the associated phenotypes to these mutations). Importantly, the I2749R substitution, which is located in the APT1 domain, diminished binding of Vps13 fragment to PI3P, suggesting that this binding is essential to all Vps13 functions [57]. Based on the homology of Vps13 to hVps13A and these recent findings, a model suggesting a possible mechanism of ChAc pathogenesis was proposed where I2771R substitution may diminish the binding of APT1 domain of hVps13A to PI3P-enriched membranes affecting the hVps13A localization and/or function [57].

Conclusions and future perspectives

The huge contribution of simple model organisms for the state of art knowledge on the molecular and cellular mechanisms underlying many human diseases is undeniable. Because *S. cerevisiae* is the simplest eukaryotic organism surrounded by a plethora of available genetic and molecular tools, the yeast-based models have a prominent role as living test tubes to address several fundamental aspects associated with pathological processes of many diseases. Although yeast has greatly contributed to the study of neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases [50,76], its exploitation for the study of rare genetic diseases is still limited. The studies summarized in this review are a testimony of the potential of yeast models to unveil the intricacies of human Vps13-related diseases, particularly ChAc. Furthermore, ChAc molecular targets amenable for therapeutic intervention could also be identified using yeast as a powerful toolbox for high-throughput genetic screenings. In addition, high-throughput assay formats could also be explored as primary drug-screening platforms to filter for natural/chemical small molecules with cytoprotective action towards ChAc pathological processes, addressing the lack of effective drugs as an important knowledge-gap on ChAc research.

Other models, such as the social amoeba *D. discoideum* can complement the studies in yeast. In several aspects, this organism is more similar to animal cells than to fungi for processes such as phagocytosis, cell motility, development, and the lysosomal and autophagic pathways [77]. The presence of 6 different *vps13* genes and the apparently non-similar phenotypes associated with their mutations in *D. discoideum* suggest that in multicellular organisms, such as humans, there may have been a specialization of functions that could facilitate the study of specific processes.

In conclusion, *S. cerevisiae* and other lower eukaryotic organisms such as *D. discoideum* represent an invaluable tool for the study of the molecular function of Vps13 proteins and a better understanding of the diverse set of processes potentially affected in the associated human diseases and they hopefully will open up new avenues for future treatments.

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Bibliography

1. Velayos-Baeza A, Vettori A, Copley RR, Dobson-Stone C, Monaco AP. Analysis of the human *VPS13* gene family. Genomics 2004;84:536–549.

2. Rubio JP, Danek A, Stone C, Chalmers R, Wood N, Verellen C, Ferrer X, Malandrini A, Fabrizi GM, Manfredi M, Vance J, Pericak-Vance M, Brown R, Rudolf G, Picard F, Alonso E, Brin M, Németh AH, Farrall M, Monaco AP. Chorea-acanthocytosis: genetic linkage to chromosome 9q21. Am J Hum Genet 1997;61:899–908.

3. Rampoldi L, Dobson-Stone C, Rubio JP, Danek A, Chalmers RM, Wood NW, Verellen C, Ferrer X, Malandrini A, Fabrizi GM, Brown R, Vance J, Pericak-Vance M, Rudolf G, Carrè S, Alonso E, Manfredi M, Németh AH, Monaco AP. A conserved sorting-associated protein is mutant in chorea-acanthocytosis. Nat Genet 2001;28:119–120.

4. Ueno S, Maruki Y, Nakamura M, Tomemori Y, Kamae K, Tanabe H, Yamashita Y, Matsuda S, Kaneko S, Sano A. The gene encoding a newly discovered protein, chorein, is mutated in chorea-acanthocytosis. Nat Genet 2001;28:121–122.

5. Walker RH. Untangling the thorns: advances in the neuroacanthocytosis syndromes. J Mov Disord 2015;8:41–54.

6. Kolehmainen J, Black GCM, Saarinen A, Chandler K, Clayton-Smith J, Träskelin A-L, Perveen R, Kivitie-Kallio S, Norio R, Warburg M, Fryns J-P, de la Chapelle A, Lehesjoki A-E. 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 2003;72:1359–1369.

7. Douzgou S, Petersen M. Clinical variability of genetic isolates of Cohen syndrome. Clin Genet 2011;79:501–506.

8. Lesage S, Drouet V, Majounie E, Deramecourt V, Jacoupy M, Nicolas A, Cormier-Dequaire F, Hassoun SM, Pujol C, Ciura S, Erpapazoglou Z, Usenko T, Maurage C-A, Sahbatou M, Liebau S, Ding J, Bilgic B, Emre M, Erginel-Unaltuna N, Guven G, Tison F, Tranchant C, Vidailhet M, Corvol J-C, Krack P, Leutenegger A-L, Nalls MA, Hernandez DG, Heutink P, Gibbs JR, Hardy J, Wood NW, Gasser T, Durr A, Deleuze J-F, Tazir M, Destée A, Lohmann E, Kabashi E, Singleton A, Corti O, Brice A, French Parkinson's Disease Genetics Study (PDG), International Parkinson's Disease Genomics Consortium (IPDGC), International Parkinson's Disease Genomics Consortium IPDGC. Loss of VPS13C function in autosomal-recessive Parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. Am J Hum Genet 2016;98:500–513.

9. Grarup N, Overvad M, Sparsø T, Witte DR, Pisinger C, Jørgensen T, Yamauchi T, Hara K, Maeda S, Kadowaki T, Hansen T, Pedersen O. The diabetogenic *VPS13C/C2CD4A/C2CD4B* rs7172432 variant impairs glucose-stimulated insulin response in 5,722 non-diabetic Danish individuals. Diabetologia 2011;54:789–794.

10. Saxena R, Hivert M, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, Kao WHL, Li M, Glazer NL, Manning AK, Luan J, Stringham HM, Prokopenko I, Johnson T, Grarup N, Boesgaard TW, Lecoeur C, Shrader P, O'Connell J, Ingelsson E, Couper DJ, Rice K, Song K, Andreasen CH, Dina C, Köttgen A, Le Bacquer O, Pattou F, Taneera J, Steinthorsdottir V, Rybin D, Ardlie K, Sampson M, Qi L, van Hoek M, Weedon MN, Aulchenko YS, Voight BF, Grallert H, Balkau B, Bergman RN, Bielinski SJ, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Böttcher Y, Brunner E, Buchanan TA, Bumpstead SJ, Cavalcanti-Proença C, Charpentier G, Chen Y-DI, Chines PS, Collins FS, Cornelis M, J Crawford G, Delplanque J, Doney A, Egan JM, Erdos MR, Firmann M, Forouhi NG, Fox CS, Goodarzi MO, Graessler J, Hingorani A, Isomaa B, Jørgensen T, Kivimaki M, Kovacs P, Krohn K, Kumari M, Lauritzen T, Lévy-Marchal C, Mayor V, McAteer JB, Meyre D, Mitchell BD, Mohlke KL, Morken MA, Narisu N, Palmer CNA, Pakyz R, Pascoe L, Payne F, Pearson D, Rathmann W, Sandbaek A, Sayer AA, Scott LJ, Sharp SJ, Sijbrands E, Singleton A, Siscovick DS, et al. Genetic variation in *GIPR* influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 2010;42:142–148.

11. Strawbridge RJ, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, Rybin D, Petrie JR, Travers ME, Bouatia-Naji N, Dimas AS, Nica A, Wheeler E, Chen H, Voight BF, Taneera J, Kanoni S, Peden JF, Turrini F, Gustafsson S, Zabena C, Almgren P, Barker DJP, Barnes D, Dennison EM, Eriksson JG, Eriksson P, Eury E, Folkersen L, Fox CS, Frayling TM, Goel A, Gu HF, Horikoshi M, Isomaa B, Jackson AU, Jameson KA, Kajantie E, Kerr-Conte J, Kuulasmaa T, Kuusisto J, Loos RJF, Luan J, Makrilakis K, Manning AK, Martínez-Larrad MT, Narisu N, Mannila MN, Öhrvik J, Osmond C, Pascoe L, Payne F, Sayer AA, Sennblad B, Silveira A, Stančcáková A, Stirrups K, Swift AJ,

Syvänen AC, Tuomi T, Van't Hooft FM, Walker M, Weedon MN, Xie W, Zethelius B, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, Zeggini E, Huth C, Aulchenko YS, Thorleifsson G, Mcculloch LJ, Ferreira T, Grallert H, Amin N, Wu G, Willer CJ, Raychaudhuri S, Mccarroll SA, Hofmann OM, Qi L, Segre A V., Van Hoek M, Navarro P, Ardlie K, Balkau B, Benediktsson R, Bennett AJ, Blagieva R, Boerwinkle E, Bonnycastle LL, Bostrom KB, Bravenboer B, Bumpstead S, Burtt NP, Charpentier G, 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 2011;60:2624–2634.

12. Windholz J, Kovacs P, Tönjes A, Dittrich K, Blüher S, Kiess W, Stumvoll M, Körner A. efects of genetic variants in *ADCY5, GIPR, GCKR* and *VPS13C* on early impairment of glucose and insulin metabolism in children. PLoS One 2011;6:e22101.

13. Nakada T, Boyd JH, Russell JA, Aguirre-Hernández R, Wilkinson MD, Thair SA, Nakada E, McConechy MK, Fjell CD, Walley KR. *VPS13D* gene variant is associated with altered IL-6 production and mortality in septic shock. J Innate Immun 2015;7:545–553.

14. McCarthy SE, Gillis J, Kramer M, Lihm J, Yoon S, Berstein Y, Mistry M, Pavlidis P, Solomon R, Ghiban E, Antoniou E, Kelleher E, O'Brien C, Donohoe G, Gill M, Morris DW, McCombie WR, Corvin A. *De novo* mutations in schizophrenia implicate chromatin remodeling and support a genetic overlap with autism and intellectual disability. Mol Psychiatry 2014;19:652–658.

15. Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, Georgieva L, Rees E, Palta P, Ruderfer DM, Carrera N, Humphreys I, Johnson JS, Roussos P, Barker DD, Banks E, Milanova V, Grant SG, Hannon E, Rose SA, Chambert K, Mahajan M, Scolnick EM, Moran JL, Kirov G, Palotie A, McCarroll SA, Holmans P, Sklar P, Owen MJ, Purcell SM, O'Donovan MC. *De novo* mutations in schizophrenia implicate synaptic networks. Nature 2014;506:179–184.

16. An CH, Kim YR, Kim HS, Kim SS, Yoo NJ, Lee SH. Frameshift mutations of vacuolar protein sorting genes in gastric and colorectal cancers with microsatellite instability. Hum Pathol 2012;43:40–47.

17. Andersen JN, Sathyanarayanan S, Di Bacco A, Chi A, Zhang T, Chen AH, Dolinski B, Kraus M, Roberts B, Arthur W, Klinghoffer RA, Gargano D, Li L, Feldman I, Lynch B, Rush J, Hendrickson RC, Blume-Jensen P, Paweletz CP. Pathway-based identification of biomarkers fortargeted therapeutics: personalized oncology with PI3K pathway inhibitors. Sci Transl Med 2010;2:43ra55.

18. Furukawa T, Kuboki Y, Tanji E, Yoshida S, Hatori T, Yamamoto M, Shibata N, Shimizu K, Kamatani N, Shiratori K. Whole-exome sequencing uncovers frequent *GNAS* mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep 2011;1:161.

19. Morisaki T, Yashiro M, Kakehashi A, Inagaki A, Kinoshita H, Fukuoka T, Kasashima H, Masuda G, Sakurai K, Kubo N, Muguruma K, Ohira M, Wanibuchi H, Hirakawa K. Comparative proteomics analysis of gastric cancer stem cells. PLoS One 2014;9:e110736.

20. Park HY, Lee S-B, Yoo HY, Kim SJ, Kim WS, Kim JI, Ko YH. Whole-exome and transcriptome sequencing of refractory diffuse large B-cell lymphoma. Oncotarget 2016;7:86433–86445.

21. Yang Y, Zhang Y, Qu X, Xia J, Li D, Li X, Wang Y, He Z, Li S, Zhou Y, Xie L, Yang Z. Identification of differentially expressed genes in the development of osteosarcoma using RNA-seq. Oncotarget 2016;7:87194–87205.

22. Schmidt EM, Schmid E, Münzer P, Hermann A, Eyrich AK, Russo A, Walker B, Gu S, Vom Hagen JM, Faggio C, Schaller M, Föller M, Schöls L, Gawaz M, Borst O, Storch A, Stournaras C, Lang F. Chorein sensitivity of cytoskeletal organization and degranulation of platelets. FASEB J 2013;27:2799–2806.

23. Foller M, Hermann A, Gu S, Alesutan I, Qadri SM, Borst O, Schmidt E-M, Schiele F, Muller vom Hagen J, Saft C, Schols L, Lerche H, Stournaras C, Storch A, Lang F. Chorein-sensitive polymerization of cortical actin and suicidal cell death in chorea-acanthocytosis. FASEB J 2012;26:1526–1534.

24. Shiokawa N, Nakamura M, Sameshima M, Deguchi A, Hayashi T, Sasaki N, Sano A. Chorein, the protein responsible for chorea-acanthocytosis, interacts with β-adducin and β-actin. Biochem Biophys Res Commun 2013;441:96–101.

25. De Franceschi L, Tomelleri C, Matte A, Brunati AM, Bovee-Geurts PH, Bertoldi M, Lasonder E, Tibaldi E, Danek A, Walker RH, Jung HH, Bader B, Siciliano A, Ferru E, Mohandas N, Bosman GJCGM. Erythrocyte membrane changes of chorea-acanthocytosis are the result of altered Lyn kinase activity. Blood 2011;118:5652–5663.

26. Alesutan I, Seifert J, Pakladok T, Rheinlaender J, Lebedeva A, Towhid ST, Stournaras C, Voelkl J, Schäffer TE, Lang F. Chorein sensitivity of actin polymerization, cell shape and mechanical stiffness of vascular endothelial cells. Cell Physiol Biochem 2013;32:728–742.

27. Honisch S, Gu S, Vom Hagen JM, Alkahtani S, Al Kahtane AA, Tsapara A, Hermann A, Storch A, Schöls L, Lang F, Stournaras C. Chorein sensitive arrangement of cytoskeletal architecture. Cell Physiol Biochem 2015;37:399–408.

28. Muñoz-Braceras S, Calvo R, Escalante R. TipC and the chorea-acanthocytosis protein VPS13A regulate autophagy in *Dictyostelium* and human HeLa cells. Autophagy 2015;11:918–927.

29. Park J-S, Halegoua S, Kishida S, Neiman AM. A conserved function in phosphatidylinositol metabolism for mammalian Vps13 family proteins. PLoS One 2015;10:e0124836.

30. Seifert W, Kühnisch J, Maritzen T, Horn D, Haucke V, Hennies HC. Cohen syndrome-associated protein, COH1, is a novel, giant Golgi matrix protein required for Golgi integrity. J Biol Chem 2011;286:37665–37675.

31. Duplomb L, Duvet S, Picot D, Aral B, Chehadeh-djebbar S El, Marle N, Carmignac V, Thevenon J, Lopez E, Rivie J, Philippe C, Droin N, Blair E, Delva L, Michalski J, Solary E, Bellanne C, Faivre L. Cohen syndrome is associated with major glycosylation defects. 2014;23:2391–2399.

32. Yang R-Y, Xue H, Yu L, Velayos-Baeza A, Monaco AP, Liu F-T. Identification of VPS13C as a galectin-12-binding protein that regulates galectin-12 protein stability and adipogenesis. PLoS One 2016;11:e0153534.

33. Jansen IE, Ye H, Heetveld S, Lechler MC, Michels H, Seinstra RI, Lubbe SJ, Drouet V, Lesage S, Majounie E, Gibbs JR, Nalls MA, Ryten M, Botia JA, Vandrovcova J, Simon-Sanchez J, Castillo-Lizardo M, Rizzu P, Blauwendraat C, Chouhan AK, Li Y, Yogi P, Amin N, van Duijn CM, International Parkinson's Disease Genetics Consortium (IPGDC), Morris HR, Brice A, Singleton AB, David DC, Nollen EA, Jain S, Shulman JM, Heutink P. Discovery and functional prioritization of Parkinson's disease candidate genes from large-scale whole exome sequencing. Genome Biol 2017;18:22.

34. Brickner JH, Fuller RS. *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 1997;139:23–36.

35. Samaranayake HS, Cowan AE, Klobutcher LA. Vacuolar protein sorting protein 13A, TtVPS13A, localizes to the *Tetrahymena thermophila* phagosome membrane and is required for efficient phagocytosis. Eukaryot Cell 2011;10:1207–1218.

36. Li Z, Blissard G. The vacuolar protein sorting genes in insects: A comparative genome view. Insect Biochem Mol Biol 2015;62:211–225.

37. Tomemori Y, Ichiba M, Kusumoto A, Mizuno E, Sato D, Muroya S, Nakamura M, Kawaguchi H, Yoshida H, Ueno SI, Nakao K, Nakamura K, Aiba A, Katsuki M, Sano A. A gene-targeted mouse model for chorea-acanthocytosis. J Neurochem 2005;92:759–766.

38. Vonk JJ, Yeshaw WM, Pinto F, Faber AIE, Lahaye LL, Kanon B, van der Zwaag M, Velayos-Baeza A, Freire R, van IJzendoorn SC, Grzeschik NA, Sibon OCM. *Drosophila* Vps13 is required for protein homeostasis in the brain. PLoS One 2017;12:e0170106.

39. Korolchuk VI, Schutz MM, Gomez-Llorente C, Rocha J, Lansu NR, Collins SM, Wairkar YP, Robinson IM, O'Kane CJ. *Drosophila* Vps35 function is necessary for normal endocytic trafficking and actin cytoskeleton organisation. J Cell Sci 2007;120:4367–4376.

40. Leiba J, Sabra A, Bodinier R, Marchetti A, Lima WC, Melotti A, Perrin J, Burdet F, Pagni M, Soldati T, Lelong E, Cosson P. Vps13F links bacterial recognition and intracellular killing in *Dictyostelium*. Cell Microbiol 2017;e12722.

41. Jacobs ME, DeSouza L V, Samaranayake H, Pearlman RE, Siu KWM, Klobutcher LA. The *Tetrahymena thermophila* phagosome proteome. Eukaryot Cell 2006;5:1990–2000.

42. Bilsland E, Bean DM, Devaney E, Oliver SG. Yeast-based high-throughput screens to identify novel compounds active against *Brugia malayi.* PLoS Negl Trop Dis 2016;10:e0004401.

43. Zhang N, Osborn M, Gitsham P, Yen K, Miller JR, Oliver SG. Using yeast to place human genes in functional categories. Gene 2003;303:121–129.

44. Estela A, Pla-Martín D, Sánchez-Piris M, Sesaki H, Palau F. Charcot-Marie-Tooth-related gene *GDAP1* complements cell

cycle delay at G2/M phase in *Saccharomyces cerevisiae fis1* gene-defective cells. J Biol Chem 2011;286:36777–36786.

45. Menezes R, Tenreiro S, Macedo D, Santos C, Outeiro T. From the baker to the bedside: yeast models of Parkinson's disease. Microb Cell 2015;2:262–279.

46. Oliveira A V, Vilaça R, Santos CN, Costa V, Menezes R. Exploring the power of yeast to model aging and age-related neurodegenerative disorders. Biogerontology 2017;18:3–34.

47. Lasserre J-P, Dautant A, Aiyar RS, Kucharczyk R, Glatigny A, Tribouillard-Tanvier D, Rytka J, Blondel M, Skoczen N, Reynier P, Pitayu L, Rotig A, Delahodde A, Steinmetz LM, Dujardin G, Procaccio V, di Rago J-P. Yeast as a system for modeling mitochondrial disease mechanisms and discovering therapies. Dis Model Mech 2015;8:509–526.

48. Franssens V, Bynens T, Van den Brande J, Vandermeeren K, Verduyckt M, Winderickx J. The benefits of humanized yeast models to study Parkinson's disease. Oxid Med Cell Longev 2013;2013:760629.

49. Sere YY, Regnacq M, Colas J, Berges T. A *Saccharomyces cerevisiae* strain unable to store neutral lipids is tolerant to oxidative stress induced by α-synuclein. Free Radic Biol Med 2010;49:1755–1764.

50. Tenreiro S, Rosado-Ramos R, Gerhardt E, Favretto F, Magalhães F, Popova B, Becker S, Zweckstetter M, Braus GH, Outeiro TF. Yeast reveals similar molecular mechanisms underlying alpha- and beta-synuclein toxicity. Hum Mol Genet 2016;25:275–290.

51. Vester A, Velez-Ruiz G, McLaughlin HM, NISC Comparative Sequencing Program, Lupski JR, Talbot K, Vance JM, Züchner S, Roda RH, Fischbeck KH, Biesecker LG, Nicholson G, Beg AA, Antonellis A. A loss-of-function variant in the human histidyl-tRNA synthetase (*HARS*) gene is neurotoxic *in vivo*. Hum Mutat 2013;34:191–199.

52. Dunham MJ, Fowler DM. Contemporary, yeast-based approaches to understanding human genetic variation. Curr Opin Genet Dev 2013;23:658–664.

53. Couplan E, Aiyar RS, Kucharczyk R, Kabala A, Ezkurdia N, Gagneur J, St. Onge RP, Salin B, Soubigou F, Le Cann M, Steinmetz LM, di Rago J-P, Blondel M. A yeast-based assay identifies drugs active against human mitochondrial disorders. Proc Natl Acad Sci 2011;108:11989–11994.

54. Tardiff DF, Jui NT, Khurana V, Tambe MA, Thompson ML, Chung CY, Kamadurai HB, Kim HT, Lancaster AK, Caldwell KA, Caldwell GA, Rochet J-C, Buchwald SL, Lindquist S. Yeast reveal a "druggable" Rsp5/Nedd4 network that ameliorates α-synuclein toxicity in neurons. Science 2013;342:979–983.

55. Mizuno E, Nakamura M, Agemura A, Kusumoto A, Ichiba M, Kurano Y, Muroya S, Sano A. Brain-specific transcript variants of 5′ and 3′ ends of mouse *VPS13A* and *VPS13C*. Biochem Biophys Res Commun 2007;353:902–907.

56. Koizumi K, Gallagher KL. Identification of SHRUBBY, a SHORT-ROOT and SCARECROW interacting protein that controls root growth and radial patterning. Development 2013;140:1292–1300.

57. Rzepnikowska W, Flis K, Kaminska J, Grynberg M, Urbanek A, Ayscough KR, Zoladek T. Amino acid substitution equivalent to human chorea-acanthocytosis I2771R in yeast Vps13 protein affects its binding to phosphatidylinositol 3-phosphate. Hum Mol Genet 2017;26:1497–1510.

58. Fidler DR, Murphy SE, Courtis K, Antonoudiou P, El-Tohamy R, Ient J, Levine TP. Using HHsearch to tackle proteins of unknown function: A pilot study with PH domains. Traffic 2016;17:1214–1226.

59. De M, Oleskie AN, Ayyash M, Dutta S, Mancour L, Abazeed ME, Brace EJ, Skiniotis G, Fuller RS. The Vps13p-Cdc31p complex is directly required for TGN late endosome transport and TGN homotypic fusion. J Cell Biol 2017;216:425–439.

60. Bankaitis V a, Johnson LM, Emr SD. Isolation of yeast mutants defective in protein targeting to the vacuole. Proc Natl Acad Sci U S A 1986;83:9075–9079.

61. Robinson JS, Klionsky DJ, Banta LM, Emr SD. Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. Mol Cell Biol 1988;8:4936–4948.

62. Luo WJ, Chang A. Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant. J Cell Biol 1997;138:731–746.

63. Dalton LE, Bean BDM, Davey M, Conibear E. Quantitative high-content imaging identifies novel regulators of Neo1 trafficking at endosomes. Mol Biol Cell 2017;mbc.E16-11-0772.

64. Michelot A, Costanzo M, Sarkeshik A, Boone C, Yates JR, Drubin DG. Reconstitution and protein composition analysis of endocytic actin patches. Curr Biol 2010;20:1890–1899.

65. Aghamohammadzadeh S, Ayscough KR. Differential requirements for actin during yeast and mammalian endocytosis. Nat Cell Biol 2009;11:1039–1042.

66. Seaman MNJ, Gautreau A, Billadeau DD. Retromer-mediated endosomal protein sorting: All WASHed up! Trends Cell Biol 2013;23:522–528.

67. Anesti V, Scorrano L. The relationship between mitochondrial shape and function and the cytoskeleton. Biochim Biophys Acta - Bioenerg 2006;1757:692–699.

68. Vevea JD, Swayne TC, Boldogh IR, Pon LA. Inheritance of the fittest mitochondria in yeast. Trends Cell Biol 2014;24:53–60.

69. Park JS, Thorsness MK, Policastro R, McGoldrick LL, Hollingsworth NM, Thorsness PE, Neiman AM. Yeast Vps13 promotes mitochondrial function and is localized at membrane contact sites. Mol Biol Cell 2016;27:2435–2449.

70. Neiman AM. Sporulation in the budding yeast *Saccharomyces cerevisiae*. Genetics 2011;189:737–765.

71. Nakanishi H, Suda Y, Neiman AM. Erv14 family cargo receptors are necessary for ER exit during sporulation in *Saccharomyces cerevisiae*. J Cell Sci 2007;120:908–916.

72. Park J-S, Neiman AM. VPS13 regulates membrane morphogenesis during sporulation in *Saccharomyces cerevisiae*. J Cell Sci 2012;125:3004–3011.

73. Huh W-K, Falvo J V, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK. Global analysis of protein localization in budding yeast. Nature 2003;425:686–691.

74. Lang AB, Peter ATJ, Walter P, Kornmann B. ER–mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. J Cell Biol 2015;210:883–890.

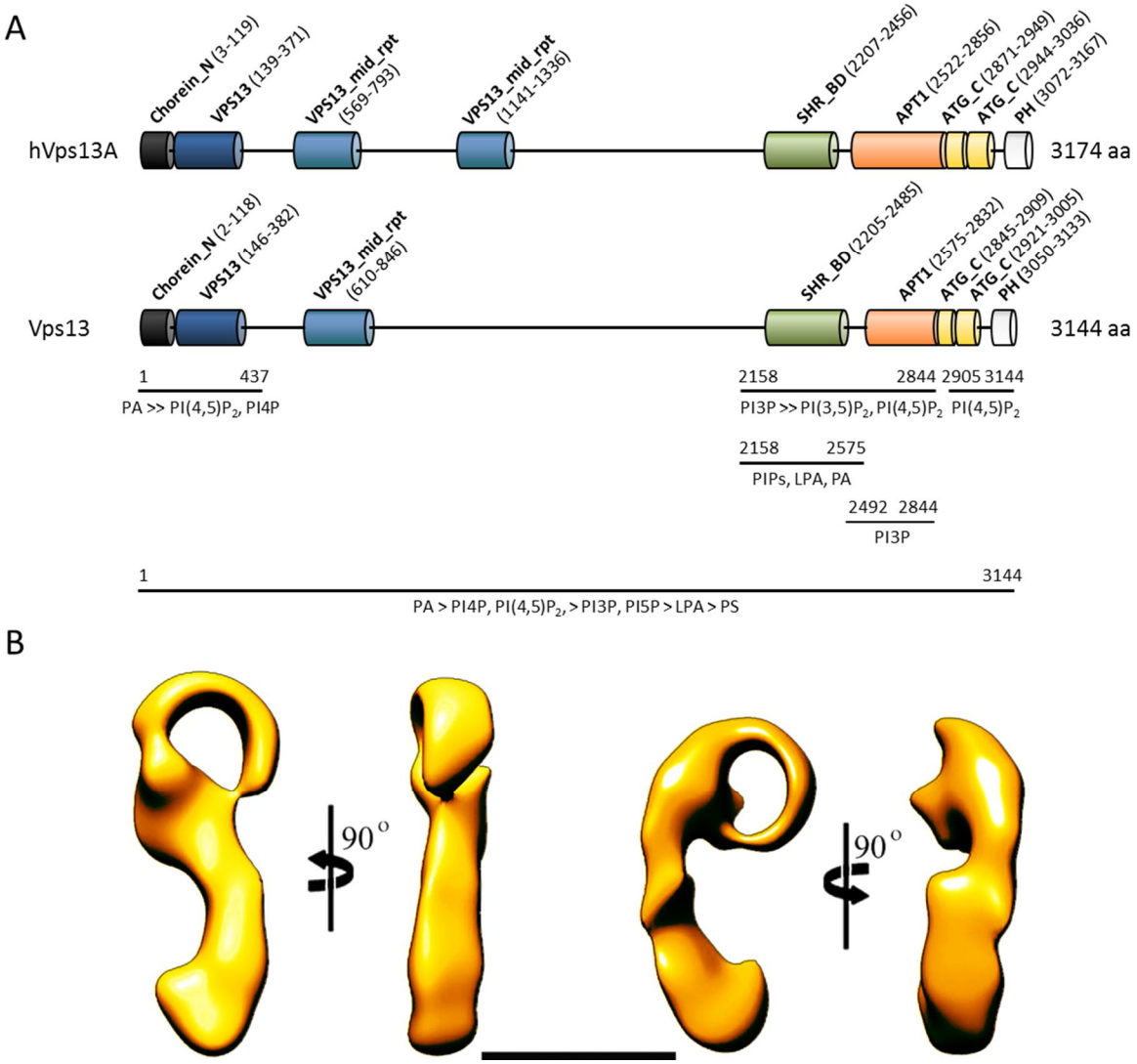
75. Schrader M, Godinho LF, Costello JL, Islinger M. The different facets of organelle interplay—an overview of organelle interactions. Front Cell Dev Biol 2015;3:56.

76. Bharadwaj PR, Verdile G, Barr RK, Gupta V, Steele JW, Lachenmayer ML, Yue Z, Ehrlich ME, Petsko G, Ju S, Ringe D, Sankovich SE, Caine JM, Macreadie IG, Gandy S, Martins RN. Latrepirdine (dimebon) enhances autophagy and reduces intracellular GFP-Aβ42 levels in yeast. J Alzheimers Dis 2012;32:949–967.

77. Mesquita A, Cardenal-Muñoz E, Dominguez E, Muñoz-Braceras S, Nuñez-Corcuera B, Phillips BA, Tábara LC, Xiong Q, Coria R, Eichinger L, Golstein P, King JS, Soldati T, Vincent O, Escalante R. Autophagy in *Dictyostelium* : Mechanisms, regulation and disease in a simple biomedical model. Autophagy2017;13:24–40.

78. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 2015;10:845–858.

79. Honisch S, Fehrenbacher B, Lebedeva A, Alesutan I, Castor T, Alkahtani S, Alarifi S, Schaller M, Stournaras C, Lang F. Chorein sensitive dopamine release from pheochromocytoma (PC12) cells. Neurosignals. 2015;23:1-10.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SHR_BD (2207-2456)
APT1 (2522-2856)
ATG_C (2871-2949)
ATG_C (2944-3036)
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ATG_C (2921-3005)
PH (3050-3133)
3144 aa
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PA >> PI(4,5)P2, PI4P
2158 2844 2905 3144
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2158 2575
PIPs, LPA, PA
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Figure 1

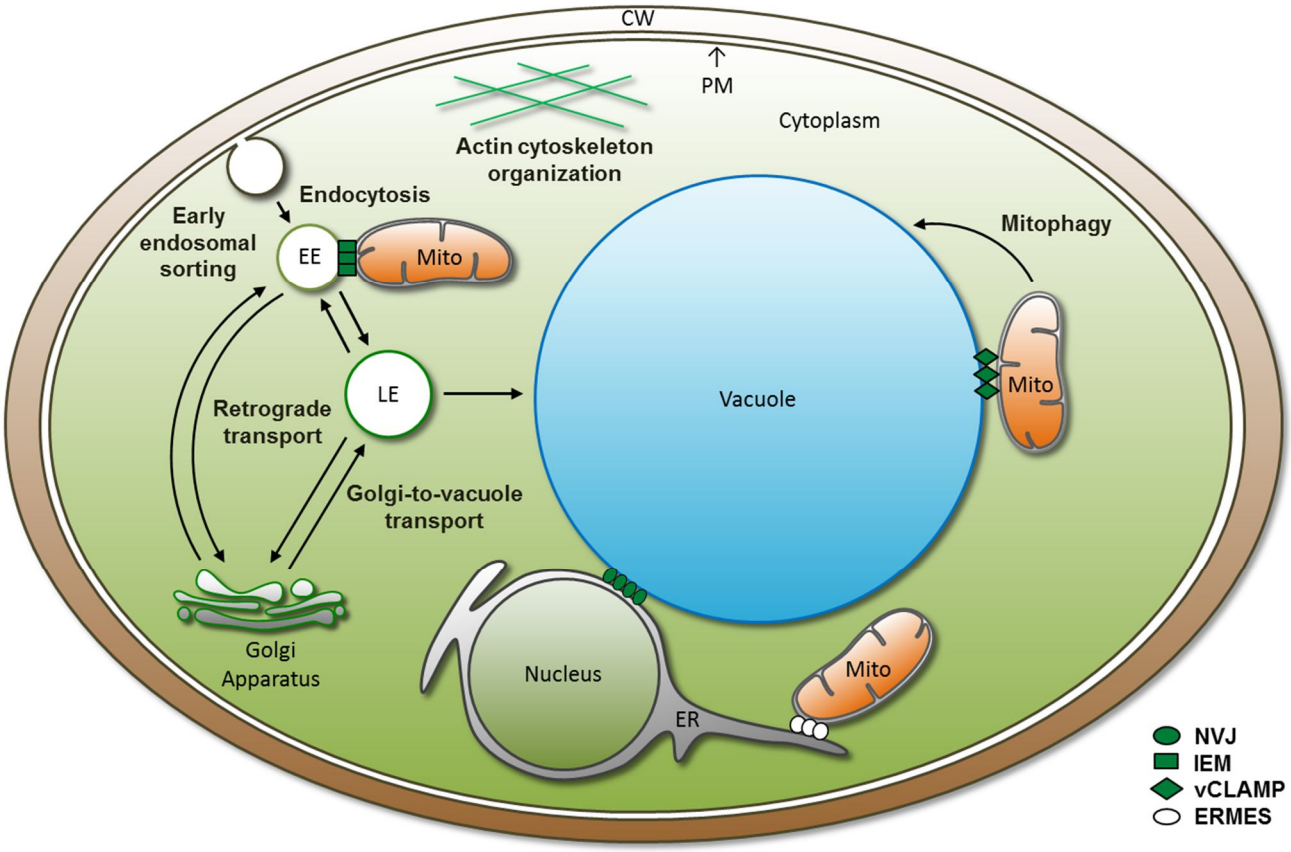
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Figure 2

Figure Legends and Tables

FIGURE 1. Structure of both yeast Vps13 and human Vps13A proteins.
A. The schematic representation of domain architecture of Vps13 proteins based on Pfam database records, recent results and unpublished analysis done using the Phyre2 software (http://www.sbg.bio.ic.ac.uk/phyre2) [78]. The regions of yeast Vps13 with *in vitro* recognized lipid-binding ability are marked as horizontal bars with lipids listed: LPA, lysophosphatidic acid; PA, phosphatidic acid; PI, phosphatidylinositol; PIPs, phosphorylated PI derivatives; PI3P, PI3-phosphate; PI4P, PI4-phosphate; PI5P, PI5-phosphate; $PI(3,5)P_2$, PI(3,5)-bisphosphate; $PI(4,5)P_2$, PI(4,5)-bisphosphate; PS, phosphatidylserine.
B. Two examples of 3D reconstructions at ~30Å level of TAP-tag purified Vps13 obtained from particle projections based on electron microscopy images of negatively stained protein. Scale bar, 20 nm. Figure reprinted with small modifications, with publisher (Rockefeller University Press) permission, from original paper [59] (© 2017 De M. et al. *Journal of Cell Biology*; 216:425–439. doi: 10.1083/jcb.201606078).

FIGURE 2. Localization of Vps13-GFP and involvement of Vps13 in various processes in yeast cells. The intracellular sites of Vps13-GFP localization are schematically shown in tones of green and Vps13 relevance for particular processes is indicated. Abbreviations for intracellular junctions: NVJ, ER-derived nuclear envelope-vacuole junctions; vCLAMP, vacuole and mitochondria patch; IEM, interface between the endosomes and mitochondria; ERMES, endoplasmic reticulum-mitochondria encounter structure. Components of a cell: CW, cell wall; EE, early endosome; ER, endoplasmic reticulum; LE, late endosome; Mito, mitochondrion; PM, plasma membrane.

TABLE 1. Mutation types found in the *hVPS13* genes[1]

Point mutations in coding regions				Splicing	Small rearrangements			Gross rearrangements			Total[2]
Missense	Nonsense	Total[2]	SNP[3]		Deletions	Insertions/Duplications	Indels	Deletions	Insertions/Duplications	Complex	
VPS13A (Aliases: *CHAC*, *CHOREIN*; Chromosomal location: 9q21.2)											
Chorea-acanthocytosis (108) Schizophrenia (1)											
9	26	35	31	21	37	6	1	6	1	2	109
VPS13B (Aliases: *CHS1*, *COH1*; Chromosomal location: 8q22.2)											
Cohen syndrome (165) Cohen syndrome, cutis verticis gyrata and sensorineural deafness (1) Cohen syndrome with autistic-like features (1) Autism (7) Intellectual disability (3) Intellectual disability and microcephaly (1) Microcephaly, mild cortical atrophy (1) Malformations of cortical development (2) Neutropaenia with retinopathy (2) Retinal dystrophy (1)											
17 (16)[4]	37	54	72	20	42 (43)[4]	22	6	33	6	1	184
VPS13C (Alias: *PARK23*; Chromosomal location: 15q22.2)											
Parkinsonism (5) Schizophrenia (1)											
	1	1		2	2	1					6
VPS13D (No aliases found; Chromosomal location: 1p36.22)											
Schizophrenia (1)											
1		1									1

[1] Data compilation according to the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php) ver. Professional 2016.4; below the gene names, the diseases are listed with the numbers of associated mutations indicated in the parentheses.

[2] Total numbers of known point mutations without SNPs.

[3] Nucleotide sequence variations according to the Single Nucleotide Polymorphism Database (dsSNP; https://www.ncbi.nlm.nih.gov/SNP/) causing amino acid substitutions, which are not connected yet to any disease/phenotype.

[4] One of mutations, i.e. M1I, is a missense mutation, but from the physiological point of view should be considered as a small deletion, since it causes start of translation from the second in-frame ATG codon resulting in 10-aa shortening of the protein at the N terminus.

TABLE 2. Conversion table of human Vps13 amino acid substitutions to yeast equivalents

hVps13A	Yeast Vps13	Domain	Resulting yeast phenotype[1]	Reference
L67P	L66P	Chorein_N	– Synthetically lethal with *mmm1*Δ	[69]
I90K	C89K	Chorein_N	– Synthetically lethal with *mmm1*Δ	[69]
A1095P	L1107P	Region between Vps13_mid_rpt	– CPY secretion (→ defect of transport to the vacuole) – Synthetically lethal with *mmm1*Δ	[69]
Y2721C	Y2702C	APT1	– None found	[69]
I2771R	I2749R	APT1	– Diminished binding ability of Vps13 to PI3P – Actin cytoskeleton disturbances – CPY secretion (→ defect of transport to the vacuole) – FM4-64 internalization (→ endocytosis defect) – Canavanine hypersensitivity (Can^S; → endocytosis defect) – Sna3 Golgi-to-vacuole transport delay (→ inefficient MVB sorting) – Synthetically lethal with *mmm1*Δ – Sporulation defect	[57]

[1] MVB, multivesicular body