REVIEW

Physiological and Pathogenesis Significance of Chorein in Health and Disease

Saad ALKAHTANI[1], Abdullah A. ALKAHTANE[1], Saud ALARIFI[1]

[1]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

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Summary

This comprehensive review explores the physiological and pathophysiological significance of VPS13A, a protein encoded by the VPS13A gene. The VPS13A gene is associated with Chorea-acanthocytosis (ChAc), a rare hereditary neurodegenerative disorder. The review covers essential aspects, beginning with the genetics of VPS13A, highlighting its role in the pathogenesis of ChAc, and addressing the spectrum of genetic variants involved. It delves into the structure and function of the VPS13A protein, emphasizing its presence in various tissues and its potential involvement in protein trafficking and lipid homeostasis. Molecular functions of VPS13A in the brain tissue and other cell types or tissues with respect to their role in cytoskeletal regulation and autophagy are explored. Finally, it explores the intriguing link between VPS13A mutations, lipid imbalances, and neurodegeneration, shedding light on future research directions. Overall, this review serves as a comprehensive resource for understanding the pivotal role of VPS13A in health and disease, particularly in the context of ChAc.

Key words

Chorein • Tumor • Actin • Microfilament • Gene expression • Chorea-acanthocytosis

Corresponding author

Saad Alkahtani, Department of Zoology, College of Science, King Saud University, P. O. Box 2455, Riyadh, 11451, Saudi Arabia. Email: salkahtani@ksu.edu.sa

Introduction

Chorea-acanthocytosis (ChAc) is a rare, hereditary neurodegenerative disorder characterized by adult-onset chorea, acanthocytosis in erythrocytes, psychiatric symptoms, epilepsy, peripheral neuropathy, and myopathy [1]. Neurodegeneration of the striatum is a hallmark of ChAc, and causative mutations have been identified in the vacuolar protein sorting 13 homolog A (VPS13A) gene, which encodes chorein, a 360-kDa protein. In gene-targeted mice without chorein, apoptosis of striatal neurons is evident, recapitulating this key feature of the disease [2]. Chorein's involvement in intracellular transport and vesicle-mediated sorting has been suggested as the key influence in the disease pathomechanism based on studies in model organisms like *Saccharomyces cerevisiae* [3].

Recent research has demonstrated chorein's (interchangeably referred to as VPS13A) biochemical properties, uncovering its unique structural characteristics and molecular interactions. Advanced techniques such as X-ray crystallography and cryo-electron microscopy have offered insights into chorein's three-dimensional structure and potential binding partners [4]. Moreover, chorein's role in intracellular trafficking, including membrane-bound organelle transport, and its interactions with cytoskeletal proteins have highlighted its role in regulating key cellular processes [5]. Post-translational modifications, such as phosphorylation and ubiquitination, have also garnered attention, hinting at their potential roles in chorein's regulation.

While chorein is ubiquitously expressed in various tissues, its precise molecular function remains to be fully understood. Functional studies have implicated chorein in cytoskeletal regulation, cell survival, and modulation of Phosphoinositide 3-kinase (PI3K)-p85-subunit activity, influencing pathways related to actin

polymerization, phosphorylation of Bad, and mitochondrial depolarization [5]. For example, research has shown that chorein plays a role in regulating the cytoskeleton, a dynamic framework of protein filaments essential for maintaining cell shape and enabling cell motility [6], [7]. Studies have also highlighted chorein's influence on actin polymerization, which is crucial for processes like cell division and intracellular transport [7]. Regarding cell survival, chorein's role appears to be associated with the modulation of the PI3K-p85-subunit, a component of a key cell signalling pathway involved in cell growth and survival [8]. By sensitizing this subunit to tyrosine phosphorylation, chorein may activate downstream effectors that promote cell survival.

Beyond the brain and erythrocytes, chorein expression has been observed in diverse tissues, contributing to blood platelet regulation, endothelial cell stiffness, and likely more yet-to-be-discovered functions. However, there is gap in knowledge on the role of chorein on physiological and pathophysiological processes. This review aims to comprehensively explore chorein's physiological and pathophysiological properties, delving into its multifaceted roles and implications in both health and disease and shedding light on the functional significance of this intriguing protein.

Chorein: molecular structure and cellular expression

Molecular structure

The VPS13A gene encodes a large, multi-domain protein that is primarily located in the endoplasmic reticulum (ER) and is involved in various cellular processes. It has been identified as a lipid transfer protein, facilitating the movement of lipids between organelles [9]. The protein is highly conserved across species, highlighting its fundamental role in cellular homeostasis.

VPS13A consists of multiple domains, including an N-terminal chorein domain, a central domain with a series of HEAT repeats, a lipid-binding domain, and a C-terminal pleckstrin homology (PH) domain. The N-terminal chorein domain is thought to play a crucial role in the regulation of actin cytoskeleton dynamics [10]. The lipid-binding domain is essential for its lipid transfer functions, enabling the transport of lipids between organelles.

AlphaFold predictions showed that VPS13A exhibits a continuous hydrophobic groove along the entire length (Fig. 1A). This groove, running uninterrupted from one end to the other, mirrors the

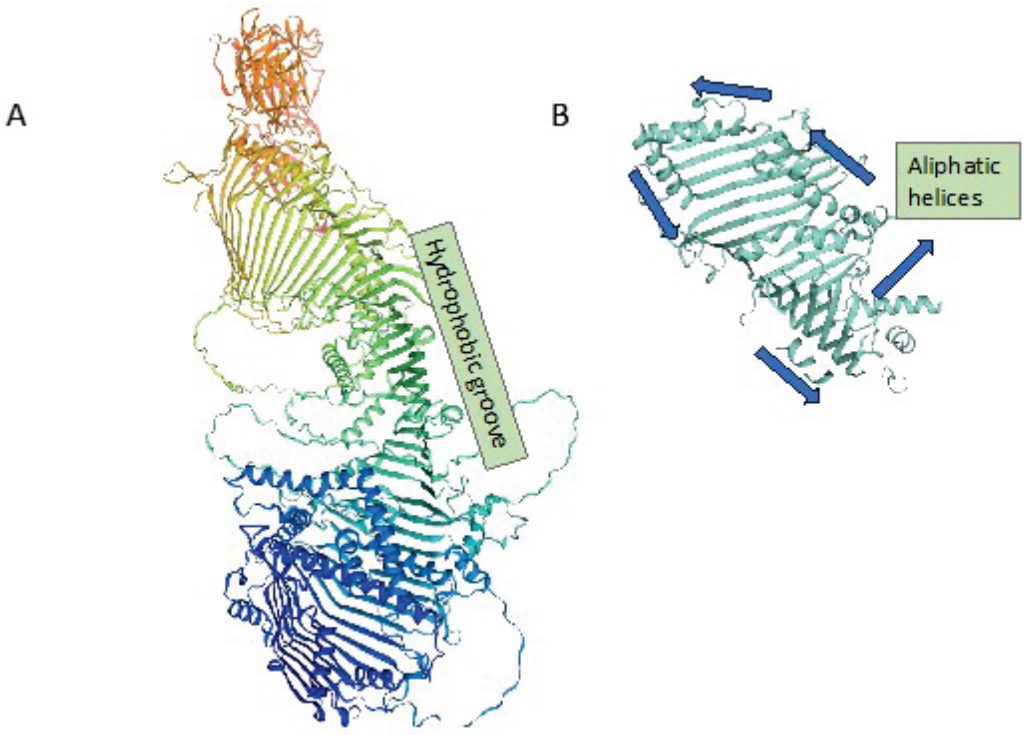

Fig. 1. Alpha-fold prediction of VPS13A tertiary structure (**A**) hydrophobic groove of VSP13A running through the length of the protein and (**B**) aliphatic helices of the N-terminal which likely aids the insertion of the protein in the cell membrane.

structural features found in other members of the VSP13 protein superfamily. This structural insight hints at VPS13A's ability to directly interact with lipid bilayers. VPS13A protein is part of the repeating beta groove (RBG) superfamily, which is composed of just one domain called the RBG domain [11]. This domain builds into rod-shaped multimers with a hydrophobic-lined groove and hydrophilic exterior. At the N-termini of the RBG multimers, an amphipathic helix is a common feature between VSP13A and SHIP164, which begins with an unstructured hydrophobic loop, enhancing membrane insertion [11]. VPS13A's N-terminus starts with an amphipathic helix, well-conserved and suited for insertion into organelle membranes with many packing defects and low anionic headgroups, such as the endoplasmic reticulum (ER) (Fig. 1B).

The N-terminal of VPS13A features an amphipathic helix, which exhibits a tubular structure containing a hydrophobic cavity capable of transporting glycerolipids across membranes [12]. This amphipathic helix serves as a structural element employed by certain proteins for interactions with lipid droplets. In the case of VPS13A, the amphipathic helix shares a nearly identical sequence with VPS13C, specifically in VPS13A (amino acids 2,993–3,027) and VPS13C (amino acids 3,550–3,584) [12]. The hydrophobic side of this helix embeds itself within the bilayer, while the hydrophilic side interacts with lipid headgroups. Consequently, VPS13A can establish connections with the endoplasmic reticulum (ER), anchoring it to mitochondria and lipid droplets. This interaction assumes a critical role in facilitating lipid transfer between the ER and mitochondria, given the absence of direct membrane connections between these organelles. Thus, it is likely that any disruptions in membrane lipid homeostasis, arising from mutations in VPS13A, may lead to the development of neurological disorders.

Chorein and the VPS13 Protein Family

The *VPS13A* gene in humans, previously known as *CHAC*, was identified in 2001 as the gene altered in patients with ChAc. The protein it encodes, consisting of 3,174 amino acids, is referred to as chorein. Functional information about similar proteins to human VPS13A is scanty but does exist within the literature. Two such proteins pertain to the yeast Vps13p and the TipC proteins in *Dictyostelium discoideum*. In this section, we aim to summarize the data from these lower organisms and from the analysis of other similar proteins.

- *The Yeast Vps13p Protein*

Vps13p, a yeast protein homologous to chorein, consists of 3,144 aa and weighs 358kDa. It is encoded by the VPS13 gene, also known as SOI1, which was cloned by complementing a sporulation defect. TipC is another yeast Vsp-like protein that is involved in cell type differentiation, autophagic flux and tip formation during the early development of *Dictyostelium* [13]. This protein is peripherally associated with membranes, forming a high molecular weight hetero- or homo-oligomeric complex. Similarly, Vps13p is involved in the efficient trans-Golgi network (TGN) localization of three transmembrane proteins Ste13p, Kex2p, and Vps10p [14]. The initial discovery of budding yeast VPS13 occurred when it was designated as VPT2 during a screening process aimed at identifying mutants with impairments in the sorting of carboxypeptidase Y (CPY) to the vacuole [15]. Additionally, in a separate investigation focused on identifying suppressors of a mutation affecting the signal TLS1, responsible for late endosome/prevacuolar compartment (PVC) to Golgi retrieval, loss-of-function alleles of VPS13, referred to as soi1 mutations, were also uncovered [15]. These mutations were linked to the cytosolic tail of Kex2p. Vps13p is not required for the transport of proteins between the TGN and the prevacuolar compartment (PVC; equivalent to the mammalian late endosome) *per se* but it seems to regulate this transport. The authors hypothesize a double mechanism for this regulation, through interaction with localization signals present in the cytoplasmic domain of the transmembrane proteins subject to inter-compartmental transport.

These localization signals called TLS1 and TLS2 for Kex2p, would promote the retrieval of the protein from the PVC to the TGN (TLS1) and retain the protein at the TGN (TLS2). Vps13p would antagonize the TGN-retention function of TLS2, facilitating transport from TGN to PVC, and would be required for the full function of TLS1, which would promote trafficking in the opposite direction. Thus, Vps13p is thought to be involved in recruiting TGN membrane proteins into transport vesicles leaving both the TGN and PVC. However, there is no evidence about the nature of the interaction between Vps13p and TLS1/TLS2 signals. It could be either a direct physical interaction or an indirect effect through other cytosolic factors.

- *Human homologues of VSP13A*

In the human genome, the VPS13 gene family

consists of four homologous genes; VPS13A, VPS13B, VPS13C, and VPS13D, each with its unique characteristics (Table 1). These proteins when initially discovered were found to be similar to the Vps13p protein in yeast. There is evidence that VPS13A plays a crucial role in intracellular vesicle trafficking and lipid transport, contributing to the maintenance of organelle structure and overall cellular function. different transcripts of this protein have been reported in the literature [12]. For example, two variants of *VPS13A* have been reported in neuroacanthocytosis patients, including the nonsense variant c.4411C>T (p.Arg1471Ter), the splicing variant c.145-2A>T and a truncated mutation with c.4326 T>A (p.Tyr1442*) [16], [17].

VPS13B generates a substantial protein product, approximately 4,022 amino acids in size, often referred to as COH1 or CHS1. Its primary function involves the organization and maintenance of cellular structures, particularly the Golgi apparatus, and active participation in membrane trafficking and vesicle transport [18]. Mutations in *VPS13B* underlie Cohen syndrome, a genetic condition characterized by developmental delay, intellectual disability, distinctive facial features, and systemic symptoms [18]. VPS13C produces a protein of varying sizes depending on the isoform, typically it's a 3700 amino acids protein. Its exact role is still under investigation, but it a previous study has linked mutation in the *VPS13C* gene with mitochondrial dysfunction in Parkinson's disease [19].

The protein product of *VPS13D* is substantial, comprising approximately 4,197 amino acids. VPS13D plays a critical role in lipid transport and the maintenance of membrane integrity within cells [20]. It is also responsible for regulating lipid droplet dynamics and membrane contact sites. Mutations in *VPS13D* are associated with the development of spastic ataxia, a neurological condition characterized by the combination of cerebellar ataxia with spasticity and other pyramidal feature [10].

All human VPS13 genes are broadly expressed and several alternative splicing variants have been identified over the years [21]. The expression patterns of three specific genes, variant 1 of *VPS13B*, variant 2 of *VPS13C*, and variant 1 of *VPS13D*, exhibit notable differences in the brain when compared to other tissues [22–24]. These variants are predominantly expressed in the brain, which contrasts with their expressions in other bodily tissues. These distinct patterns have been reported to imply that certain modifications due to changes in exons 28, 6/7, and 40 in the respective proteins VPS13B, C, and D are especially critical for their functions within the brain [22]. Furthermore, human VPS13 genes exhibit a range of alternative splicing variants. Many of these variants result in shorter protein versions due to the inclusion of alternative 3' end exons or the appearance of stop codons, either from frameshifts or alternative exons containing such codons [25]. In most cases, the

Table 1. VPS13 human homologues, their cellular localization, functions and associated diseases

Protein Name	Amino Acid Length	Cellular Localization	Function	Associated Diseases, (References)	
VPS13A	3.174	Endoplasmic reticulum membrane	Lipid transport and metabolism, autophagy	Chorea-acanthocytosis (ChAc), a rare neurodegenerative disorder	[26]
VPS13B	4.022	Endoplasmic reticulum membrane	Lipid transport and metabolism	Cohen syndrome, a rare genetic disorder	[25]
VPS13C	3.756	Endoplasmic reticulum membrane	regulation of neuronal mitochondrial function via endolysosomal pathway	Parkinson's disease	[27]
VPS13D	4.388	Endoplasmic reticulum membrane	Unknown[1]	Spinocerebellar Ataxia, spastic paraplegia, dystonia	[28]

introduction of stop codons in these mRNA variants would activate the nonsense-mediated mRNA decay response, leading to their degradation. These complex splicing events contribute to the diversity of VPS13 gene products in different tissues and cellular contexts.

VPS13 Proteins in different species

In the yeast *Saccharomyces cerevisiae*, *Vps13p* is the sole protein of the *VPS13* family [29]. However, this is not the case in most organisms. Genes orthologous to the human *VPS13* proteins, which encode equivalent proteins, are found in animal model organisms such as *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly), and *Takifugu rubripes* (pufferfish), as well as in mammals such as elephant, mouse, and dogs) [30]. Similarly, *vsp13a* of *Danio Rerio* (zebrafish) is a homolog of the human *VSP13A* and the only homologue found in this species. However, not all human *VPS13* genes have a counterpart in these organisms. For instance, *C. elegans* only has orthologous genes for *VPS13A* and *VPS13B*, while *D. melanogaster* lacks an orthologue for *VPS13C* [31]. Two of the most phylogenetically distant species to humans with four *VPS13* genes are the *Callorhinchus milii* and *Takifugu rubripes*, indicating that this gene family dates back at least to the group of bony vertebrates. It has been inferred from phylogenetic data that the *VPS13* gene family evolved from a single gene (yeast) to a group of four, implying a process of specialisation in the function performed by the *VPS13* proteins in higher organisms [32]. The fact that alteration of *VPS13A* or *VPS13B* proteins leads to ChAc or Cohen syndrome indicates that the other, non-altered, *VPS13* proteins cannot compensate for such defects, suggesting that it is not an example of functional redundancy.

Cellular expression of VPS13A and associated molecular functions

Although chorein's tissue expression profile spans across different organs such as the brain, kidney, spleen, and testis [33,34]; it is study investigating molecular function and expression of chorein has mainly been on brain/neuronal cells, underscoring its potential significance in various physiological processes within the brain. However, despite its prevalence in all of these tissues, the precise molecular function of chorein remained elusive.

Expression in brain cells

VPS13A exhibits significant expression in the brain, with a particular emphasis on neurons [35]. This neuronal expression is of paramount importance as it signifies potential roles in neurological health and function. Within neurons, VPS13A localizes to specific subcellular compartments, notably the endoplasmic reticulum (ER) and mitochondria-associated membranes (MAMs), thus implying involvement in processes related to these organelles, such as lipid transport and homeostasis [36,37]. Moreover, VPS13A's presence at MAMs and ER-mitochondria contact sites suggests its participation in the coordination of essential cellular processes, including calcium signaling and the regulation of mitochondrial function.

The connection between VPS13A and neurological health is further underscored by its association with neurological disorders. Mutations in the *VPS13A* gene, responsible for encoding chorein, have been linked to chorea-acanthocytosis, a rare neurodegenerative disorder [35]. While ongoing research aims to elucidate the precise mechanisms by which VPS13A mutations lead to neurological symptoms, it is evident that chorein/VPS13A plays a pivotal role in maintaining neuronal well-being.

A significant aspect of VPS13A's potential function in the brain relates to lipid transport. Given its presence at ER-mitochondria contact sites, chorein is believed to contribute to the transport of lipids between these organelles [9,38]. This role is crucial for ensuring the availability of essential lipids required for various neuronal activities, including membrane integrity, neurotransmitter release, and myelin formation. Furthermore, chorein/VPS13A might serve a neuro-protective function by safeguarding neurons from oxidative stress and other detrimental factors, thereby maintaining mitochondrial function and promoting overall neuronal health [36,39]. Consequently, the multifaceted roles of chorein in the brain encompass lipid transport, neuroprotection, and the regulation of ER-mitochondria contacts.

The conservation of VPS13A function in neuronal cells is not confined to only human species. In a previous study focused on elucidating the spatiotemporal distribution of VPS13A within the murine brain, notable findings emerged, shedding light on the expression patterns and potential roles of VPS13A in neural tissues [40]. Notably, this investigation revealed that VPS13A expression commences during the

embryonic developmental stage, persisting at relatively stable levels throughout adulthood. Both mRNA and protein distributions of VPS13A exhibited a harmonious pattern within the mature murine brain. A ubiquitous distribution of VPS13A across various brain regions was also observed, with particularly robust expression profiles detected in the pons, hippocampus, and cerebellum [40]. Intriguingly, the authors found that the basal ganglia exhibited comparatively subdued levels of VPS13A expression. Furthermore, immunostaining for VPS13A unveiled its presence in diverse neuronal subtypes, encompassing glutamatergic, GABAergic, and cholinergic neurons, while showing limited occurrence in glial cells. In support of this, a case study reported a sensorimotor axonal polyneuropathy and an elevation of glutamine in cerebrospinal fluid of a male CHAc patient exhibiting a homozygous novel truncating mutation (c.4326 T>A; p.Tyr1442*) in the VPS13A [17]. The patient had initially presented with unprovoked bilateral tonic-clonic seizures and also displayed persistent elevation of creatine kinase and GABA, suggesting mitochondrial disorder.

Expression in other cell types

As alluded to earlier, the specific expression patterns and functions of chorein in other cells other than the neurons remain poorly elucidated. However, there are pockets of evidence in the literature implicating chorein in the regulation of physiological processes in other cell types other than the brain.

In HeLa cells, VPS13A was found to associate with lipid droplets (LD), suggesting a potential role in cellular lipid metabolism. LDs are crucial for lipid storage and metabolism, and their dysregulation is linked to various diseases, including obesity and metabolic disorders [41]. VPS13A's presence at the LD periphery may imply involvement in lipid transport or interactions with LD-associated proteins.

A previous study conducted using human embryonic kidney 293 (HEK293) cells elucidated VPS13A role in kidney cells [42]. It was demonstrated that chorein overexpression has a protective effect on HEK293 cell viability under conditions of nutrient deprivation. Additionally, it was revealed that chorein's interaction with α-tubulin and histone deacetylase 6, a known α-tubulin deacetylase and a central component of basal autophagy. These findings suggest that chorein may have a significant role in kidney cell stress responses, particularly in the context of nutrient scarcity. In HEK293 cell lines, VPS13A localizes at the ER-mitochondrion junction, in a similar way to the localisation on yeast Vps13p at the interfaces connecting mitochondria to the vacuole and also the nuclear-vacuole junction (NVJ) linking the vacuole to the endoplasmic reticulum [10,41]. VPS13A depleted cells exhibited elongated mitochondria causing reduced contact site between the ER and mitochondria, subsequent tly impairing lipid transport. An intriguing conjecture here is that VPS13A facilitated the transfer of lipids between the ER and mitochondria as well as various other cellular membranes. Furthermore, VPS13A's role here may serve as an alternative, indirect route for the exchange of specific lipids between the ER and mitochondria by way of the vacuole.

Another study found that chorein is expressed in muscle cells and exerts an influence on cell survival. The highest levels of chorein mRNA and chorein protein were detected in drug-resistant, poorly differentiated human rhabdomyosarcoma cells [8]. Silencing chorein expression led to a significant reduction in the ratio of phosphorylated (and consequently activated) to total phosphoinositide 3 kinase (PI3K), indicating the inactivation of this critical pro-survival signalling molecule in this cell type. it appears from these studies that chorein function may be cell-type or tissue specific. however, unravelling the basic molecular mechanism of chorein still requires further research.

VPS13A and Pathophysiology of Chorea-acanthocytosis

ChAc is a rare neurodegenerative disease characterized by neurological syndromes and a condition where red blood cells exhibit spike-like membrane protrusions called acanthocytosis [35]. The disease, which mainly affects the basal ganglia, is often mistaken for Huntington’s disease. However, the pathophysiology of ChAc especially regarding the role of VPS13A has been classified based on the molecular mechanism of VPS13A in cell membrane structure and function, autophagy, and the regulation of cytoskeletal framework in different cells with red blood cells and neurons being the most implicated of all cell types.

VSP13A in cell membrane structure and function

Initial insights into the potential pathophysiological impacts of VPS13A in patient samples were obtained from the red blood cells (RBC) of

ChAc patients. The structure and function of the RBC membrane are altered in ChAc, with up to 50 % of RBC exhibiting acanthocytic phenotype [43]. Recent reports have stated that the clinical implications of acanthocytic RBCs are in their susceptibility to being trapped and destroyed within the spleen due to their shape, ultimately resulting in hemolytic anemia [44,45]. The membrane skeletal network in ChAc RBC was reported to be compact in some areas but less compact in others, suggesting disturbances in membrane fluidity. The alterations of the cytoskeletal structure, especially of the cortical actin network have been described in RBC. Interestingly, expression of cytoskeletal proteins such as β-Adducin and β-actin are significantly reduced in membranes of erythrocytes harbouring VPS13A mutations [46]. This suggests that VPS13A may be involved in the regulation of the expression of cytoskeletal proteins in the maintenance of the cell's membrane structure and fluidity.

Chorein is also expressed in blood platelets where the ratio of globular to filamentous actin is higher for ChAc patients than in those from healthy volunteers [47]. This adds to the evidence that as seen in erythrocytes and fibroblasts, the deficiency of chorein in ChAc platelets results in actin depolymerization. This reorganization of the cytoskeleton is again paralleled by altered phosphorylation of the PI3K subunit p85, and p21 protein-activated kinase (PAK1).

Chorein is also expressed in endothelial cells. Knockout of *VPS13A* in endothelial cells causes weakening of actin filaments followed by an increase in the ratio of soluble G-actin to filamentous F-actin, cell membrane softening, and altered cell shape [5]. These effects are paralleled by and at least partially due to a decrease in FAK phosphorylation. Silencing *VPS13A* also triggers immune cell death through disruption of VSP13A phospholipid scrambling activities. This results in compromised cell membrane and subsequently cytolysis [48]. Therefore, chorein is a potent regulator of cytoskeletal architecture, cell shape, mechanical stiffness, and apoptosis in vascular endothelial cells and immune cells. It's worth noting that the stiffness of endothelial cells affects the release of NO, and thus peripheral vascular resistance and blood pressure.

Increased ion efflux coupled with reduced levels of intracellular potassium has been previously reported in ChAc RBCs [49]. Recent studies demonstrated that ChAc RBCs show a reduced response in drug-induced endo-vesiculation, a lysophosphatidic acid-induced phosphate-dylserine exposure, and calcium uptake [50], [51]. It is believed that these changes in functional membrane properties arise from the acanthocytic cell shape itself, as this was only seen in RBC samples from PKAN patients with acanthocytosis, contrasting with RBC samples from PKAN patients without acanthocytosis.

VPS13A's role in regulation of cytoskeletal organization and cell morphology

The role for VPS13A in actin cytoskeleton organization and polymerization dynamics has been demonstrated in erythrocytes from ChAc patients. In the erythrocytes, lack of chorein leads to the disappearance of the cortical actin filament network, resulting in the characteristic shape change of acanthocytosis [7,52]. Chorein deficiency also influences the organization of actin microfilaments in fibroblasts from ChAc patients. Studies have shown a significant decrease in F-actin content and an increase in the G-to-F-actin ratio in both erythrocytes and fibroblasts from ChAc patients compared to healthy individuals [52,53]. Furthermore, chorein deficiency in fibroblasts from ChAc patients also affects the organization of microtubules [6,52]. Microtubular network staining revealed diminished microtubules and tubulin depolymerization in ChAc patient fibroblasts compared to healthy controls. These observations are indicative of chorein involvement in the organization and stability of microtubule structures.

The role of VPS13A in cytoskeletal protein regulation has been demonstrated beyond erythrocytes and fibroblasts. Chorein has also been found to be expressed in vascular endothelial cells. The knockdown of VPS13A in human umbilical vein endothelial cells (HUVECs) resulted in decreased actin polymerization, as evidenced by a higher G/F-actin ratio and reduced filamentous actin staining [7]. This suggests that chorein is involved in the regulation of actin cytoskeleton organization in endothelial cells. Furthermore, these cytoskeletal proteins are directly or indirectly involved in the maintenance of membrane fluidity and subsequently, cell morphology. In HUVECs, chorein deficiency was found to significantly alter cell morphology, as a result of decreased filamentous actin levels, more prominent lamellipodia formation, decrease in cell stiffness and changes in cell shape [7]. This suggests that chorein is involved in the regulation of endothelial cell mechanical properties. The changes in cell stiffness may have implications for endothelial cell function, including endothelial nitric oxide (NO) formation and vasodilation.

The impact of chorein on intermediate filament structures, such as desmin and cytokeratins, has also been investigated in fibroblasts from ChAc patients. PCR analysis and morphological evaluation revealed reduced gene expression of desmin and cytokeratins as well as disarranged intermediate filament networks, with reduced staining indicating a diminished presence of these critical intermediate filament proteins in ChAc patient fibroblasts [6,52,54]. These findings suggest that chorein deficiency may disrupt the assembly and organization of intermediate filaments, possibly compromising the cell structure integrity which results in the cytolysis often observed in cells deficient in `chorein.

Chorein's impact on actin cytoskeleton organization and cell stiffness has also been demonstrated to correlate with changes in focal adhesion kinase (FAK) phosphorylation levels in HUVECs [7]. FAK plays a crucial role in the formation of focal adhesions (FAs), which serve as a convergence point for mechanical signaling mediated by integrins and growth factors [55]. FAK is instrumental in maintaining cell rigidity by promoting a stable and highly aligned contractile cytoskeleton.

The VPS13a activities seem to be crucial to cytoskeletal organization and cellular function since chorein deficiency in ChAc patients leads to disarranged cytoskeletal structures, including actin microfilaments, microtubules, and intermediate filaments. Inadvertently, the disruption of cytoskeletal organization may contribute to the pathophysiology of ChAc, which affects multiple organs and presents with a range of clinical features. Additionally, the role of chorein in endothelial cell function suggests its potential involvement in vascular diseases and blood pressure regulation. Further research is needed to fully understand the molecular mechanisms underlying the effects of chorein on cytoskeletal organization and cellular function and to explore potential therapeutic strategies for ChAc and related disorders.

VPS13A and autophagy

Autophagy is a vital cellular process that involves the degradation and recycling of unnecessary or dysfunctional cellular components [56]. In RBCs, autophagy plays a crucial role in maintaining cellular homeostasis and integrity. During the maturation of RBCs, autophagy aids in the removal of organelles like mitochondria and ribosomes, a process essential for the formation of mature, enucleated erythrocytes [57]. This process is also involved in the response to cellular stress and the prevention of premature erythrocyte death, which could lead to conditions such as anaemia [45,58].

VPS13A has been found to play significant roles in autophagy within red blood cells. A study demonstrated reduced autophagy in the absence of VPS13A expression which may have stemmed from a broader impairment in endocytic trafficking and lysosomal degradation processes [59]. Similarly in HeLa cells, reduced levels of VPS13A have been shown to induce the expression of markers associated with autophagy such as LC3 and WIP2, and disrupt the normal flow of the autophagic process [60]. Dysfunctions in these proteins can lead to impaired autophagy and contribute to the development of various associated disorders. One of the key abnormalities observed in ChAc is the accumulation of an active form of active Src family tyrosine kinase, Lyn kinase [2]. Lyn kinase is a protein that plays a crucial role in various cellular processes such as cell proliferation, metabolism, and apoptosis [61].

Furthermore, Lyn also holds a significant position in controlling cell adhesion and mobility by phosphorylating proteins within the cytoskeleton [62]. The accumulation of active Lyn kinase in ChAc is due to impaired autophagy, which may be linked to the reduced VPS13A in ChAc cells [63]. Lyn's inhibition of autophagy has been demonstrated to also be linked to the activities of the PI3K/Akt pathway [64]. PIP3 in the PI3K signalling is known to form a complex with VPS34 and VPS15 to facilitate autophagosome formation [65,66], providing a possible mechanism of action for VPS13A in the PI3K axis in autophagy induction (Fig. 2). Interestingly, an increased abundance of Lyn, associated with the red cell membrane has been shown in a larger number of chorea-acanthocytosis patients [67], further suggesting a link between Lyn activities and lack of functional VPS13A.

In individuals with ChAc, a recent study showed that active Lyn kinase in red blood cells forms high-molecular-weight complexes with heat shock proteins HSP90-70, sequestering and safeguarding Lyn from proteasomal degradation, contributing to Lyn kinase accumulation [63]. Findings from this study suggest an association between active Lyn kinase accumulation and autophagy in ChAc, indicated by coimmunoprecipitation of Lyn with autophagy-related proteins Ulk1 and Atg7 and the presence of Ulk1 in Lyn-containing high-molecular-weight complexes.

Moreover, research has revealed that the absence of VPS13A results in defective autophagy flux, indicating

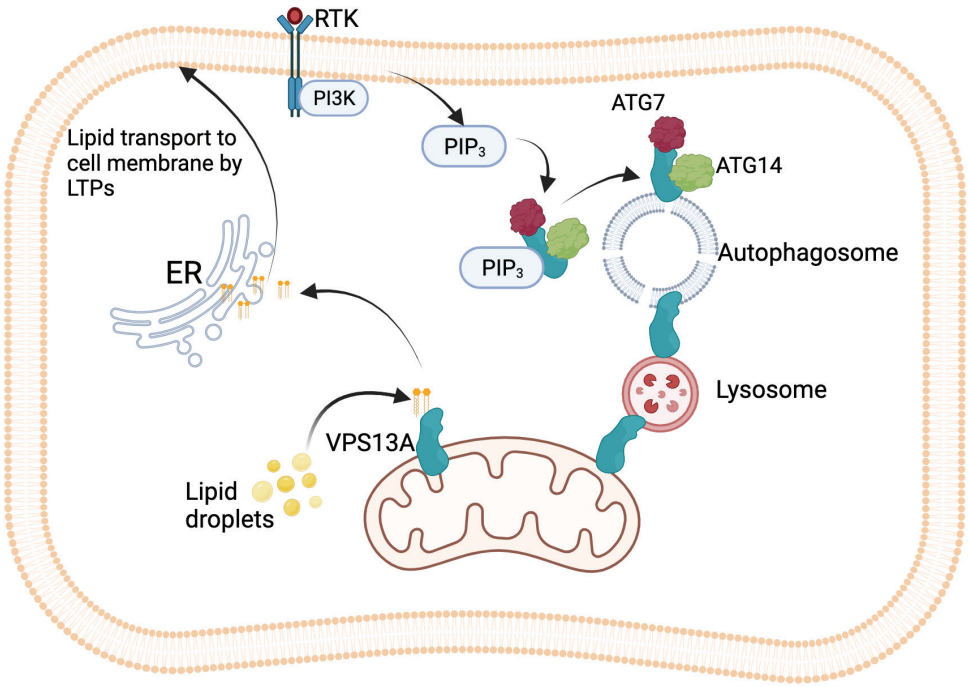

Fig. 2. PotentialVPS13A central role in autophagosome formation and lipid transport. Activation of receptor tyrosine kinase (RTK) causes phosphorylation of p85 moiety of PI3K and this subsequently results in the conversion of Phosphatidylinositol (4,5)-bisphosphate (PIP2) to Phosphatidylinositol (4,5,6)-triphosphate (PIP3). PIP3 likely forms a complex with ATG proteins and VSP13A facilitating autophagosome formation and may also ensure the fusing of lysosome with the autophagosome. More importantly, VPS13A is known to facilitate lipid translocation between mitochondria, the ER, and the cell membrane. Impairment in this axis may result in reduced lipid transport to the cell membrane, resulting in loss of membrane integrity.

a link between VPS13A and autophagic vesicle trafficking during erythroid maturation [59]. Taken together, it seems plausible that targeting Lyn may be beneficial in ChAc. Indeed, therapeutic approaches targeting Lyn kinase have been shown to be promising in ChAc treatment. For instance, nilotinib, a blood-brain barrier-penetrating Lyn kinase inhibitor, has demonstrated improved hematological and neurological outcomes in Vps13a-deficient mice, correlated with enhanced autophagy and reduced neuroinflammation [2].

VPS13A's role in cell survival and apoptosis

The most evident role of VPS13A in cell survival and death in ChAc is linked to calcium signaling. Stimulation of intracellular calcium ion (Ca^{2+}) release and store-operated calcium entry (SOCE) can initiate fluctuations in cytosolic Ca^{2+} levels [Ca^{2+}] [68]. These fluctuations involve the release of intracellular Ca^{2+}, transient activation of SOCE, and subsequent Ca^{2+} removal. These pulsating and short-lived increases in [Ca^{2+}] play a regulatory role in various cellular functions, including the activation of Ca^{2+}-dependent transcription factors and the organization of the actin cytoskeleton. Importantly, unlike sustained increases in [Ca^{2+}], these oscillations do not compromise cell survival. These Ca^{2+} oscillations also influence complex cellular processes like cell cycle progression into the S and M phases [69]. Additionally, [Ca^{2+}] oscillations may contribute to cell survival. Isoforms of ORAI and STIM, which are essential proteins involved in the regulation of calcium signaling in cells are involved in orchestrating the cell tumor growth, survival, and migration as well as in neural stem/progenitor cells [70]. In contrast, sustained increases in [Ca^{2+}] trigger apoptosis [71].

Neurons originating from induced pluripotent stem cells (iPSCs), derived from ChAc patient fibroblasts, exhibit diminished ORAI1 and STIM1 protein levels in comparison to neurons derived from healthy control fibroblasts [70]. As a result, SOCE, partially regulated by ORAI1 and STIM, is inhibited in ChAc neurons, leading to an increased percentage of apoptotic cells [70,72]. Conversely, 24-hour treatment with the antidepressant lithium has demonstrated the reversal of these effects by elevating ORAI1 and STIM1 transcript levels, protein abundance, SOCE, and cell survival. These effects are antagonized by pharmacological inhibition of serum- and glucocorticoid-inducible kinase SGK1 or ORAI1 [73].

Comparable findings were observed in fibroblasts, where ChAc patient fibroblasts exhibit

decreased ORAI1 protein expression compared to healthy control fibroblasts, resulting in reduced SOCE and increased apoptosis [72]. Lithium treatment enhances SOCE and reduces the cell death rate in ChAc fibroblasts, and this effect is countered by pharmacological inhibition of ORAI1. The expression of ORAI1 is positively modulated by the PI3K-dependent serum- and glucocorticoid-inducible kinase SGK1 [73]. In chorein-deficient cells, compromised PI3K activation impairs SGK1 activation, subsequently reducing ORAI1 expression [5]. PI3K signaling is required for the survival of various cell types, including neurons and cancer cell, thus deficiency in chorein expression seem to perturb PI3K signaling coupled with an imbalance in SOCE movement of Ca^{2+} resulting in apoptosis. In support of this, in drug-resistant and poorly differentiated human ZF rhabdomyosarcoma cells, suppression of chorein causes downregulation of PI3K, resulting in reduced ORAI1 expression and SOCE [74]. Increased intracellular Ca^{2+} due to deficient SOCE thus results in increased ratio of anti-apoptotic Bcl-2 to pro-apoptotic Bax levels, mitochondrial depolarization, and caspase 3 activation, a hallmark of caspase-dependent apoptosis [74].

It becomes evident that VSP13A's role in regulating apoptosis is intricately linked to its influence on calcium signaling, primarily through perturbations in the SOCE pathway, which affects the efflux of Ca^{2+}. This modulation encompasses elements such as calcium oscillations and the expression of calcium-associated proteins like ORAI1 and STIM. Understanding these mechanisms sheds light on the intricate interplay between VSP13A and cell survival, providing potential insights for therapeutic interventions. This is particularly relevant in conditions characterized by the dysregulation of apoptosis, including ChAc and specific cancer types.

Physiological consequence of VPS13A mutations in Chorea-acanthocytosis

Mutations in the *VPS13A* gene lead to a lack of chorein, which is involved in the intracellular transport of transmembrane proteins and the sorting of vacuolar proteins (Table 2). For instance, a study reported two novel heterozygous VPS13A pathogenic variants in ChAc. One was a nonsense variant (NM_033305.2: c.8215G>T, p. Glu2739Ter) and the other was a deletion variant in the exons 25–311 [26]. The identified nonsense variant induces premature translation termination, while the deletion variant is anticipated to create a substantial in-frame deletion of amino acid residues within the encoded protein. Both variations are deemed pathogenic, giving rise to the production of malfunctioning proteins. This interference with protein transport and sorting may conceivably contribute to the neurological symptoms manifested in ChAc.

The significance of chorein's function becomes even more apparent when considering the consequences of loss-of-function mutations in the *VPS13A* gene, which are associated with ChAc. These mutations result in a functional deficiency of chorein, leading to a constellation of pathological manifestations. In ChAc, erythrocytes undergo shape changes, manifesting as acanthocytosis, which contributes to the characteristic abnormalities seen in peripheral blood smears [75]. In the central nervous system, neurons face the grim fate of apoptosis, leading to the neurodegeneration observed in ChAc patients [5,70]. This neuronal loss is reflected in the characteristic chorea and associated movement abnormalities seen in the disease.

Genetically engineered mice with chorein knockout exhibited erythrocyte shape changes similar to those seen in ChAc patients [76], thus recapitulating a key feature of the disease. Additionally, these mice display behavioral abnormalities, emphasizing the impact of chorein deficiency on neuronal function and behavior. Collectively, these observations underscore the critical role of chorein in maintaining cellular and neuronal integrity and provide valuable insights into its significance in both physiological processes and the pathogenesis of neurodegenerative diseases like ChAc.

Conclusion and Future Directions

The biological process linking acanthocytosis (abnormal, spiky red blood cells), basal ganglia degeneration, raised creatinine kinase, and monogenetic mutations in protein sorting genes like VPS13A is yet to be elucidated. The loss of cells (atrophy) in certain brain regions, particularly the basal ganglia, is a major cause of the neurological problems seen in people with chorea-acanthocytosis. Acanthocytosis is thought to result from an imbalance of cholesterol and phospholipid on the blood cell membranes, but how this relates to the neurological symptoms of ChAc is unclear. The membranes of red blood cells, like other cell membranes in the body, consist of various lipids, including cholesterol and phospholipids.

Table 2. Mutations in genes implicated in ChAc

Gene	Importance	Function
VPS13A	Mutations in the VPS13A gene cause ChAc.	VPS13A encodes the protein chorein, although its exact function remains unknown. It likely plays a role in intracellular trafficking of lipids and membrane dynamics within cells [17], [32], [35].
CHAC	Mutations in the CHAC gene on chromosome 9q21 are linked to ChAc.	CHAC encodes ChaC glutathione-specific gamma-glutamylcyclotransferase 1; an enzyme involved in glutathione metabolism. Dysregulation may contribute to oxidative stress and neuronal damage. Enzyme believed to contribute to cellular stress response in ChAc [77]

A balance between these lipids is crucial for maintaining the structural integrity and flexibility of the cell membrane. When this balance is disrupted, it can lead to the alteration of the membrane's physical properties. The evidence thus far seems to point at the role of VPS13A in lipid transport and how this influences cell stiffness or softness and subsequent apoptosis or cytolysis in the pathomechanism of ChAc. However, the link between the lipid transport and PI3K pathway and how both play a role in enabling ChAc is yet to be understood. Lack of functional VPS13A may perturb intracellular signalling affecting cell survival via PI3K modulated pathway. This coupled with a compromise in cell membrane integrity due to dysfunctional lipid transport may be a form of two-hit mechanism regulating the pathophysiology of ChAc.

Our review has illuminated the current comprehension of VPS13A's pathophysiological consequences. Nonetheless, several enigmatic facets of this protein's role call for further exploration. To advance our understanding in this domain, future research endeavors should consider:

a) understanding chorein/VPS13A's roles in cellular and intracellular processes, particularly within the nervous system, requires detailed exploration of molecular mechanisms in lipid transport, membrane integrity, and protein trafficking,

b) investigating how VPS13A mutations contribute to neurodegeneration in ChAc, especially in neuronal and glial cells, is crucial for potential therapeutic targets. Identifying reliable ChAc biomarkers, such as lipid profiles or metabolites, is imperative for diagnostic tools and treatment assessments, distinguishing ChAc from similar diseases like McLeod syndrome,

c) exploring the relationship between lipid imbalances, especially cholesterol and phospholipids, and neuronal health is essential for understanding ChAc's pathophysiology.

Developing therapeutic strategies for ChAc, including lipid supplementation, gene therapy, or lipid metabolism modulators, holds promise. Investigating genetic modifiers and epigenetic factors influencing phenotypic variability in ChAc is an intriguing research area. Bridging fundamental science and clinical applications through translational research is essential for developing therapies and clinical trials.

Patient registries and natural history studies are indispensable for tracking disease progression and assessing treatment outcomes, requiring international collaborations for sufficient data. Advanced imaging techniques and biomarker analyses, such as advanced MRI and mass spectrometry, provide intricate insights into the neurological consequences of VPS13A mutations, aiding in identifying affected brain regions. In conclusion, investigating VPS13A's pathophysiological consequences is an evolving field with prospects for innovative diagnostic methods and therapeutic strategies for ChAc.

Conflict of Interest

There is no conflict of interest.

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