

REVIEW ARTICLES

The role of ORMDL proteins, guardians of cellular sphingolipids, in asthma

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Abstract

A family of widely expressed ORM-like (ORMDL) proteins has been recently linked to asthma in genomewide association studies in humans and extensively explored in *in vivo* studies in mice. ORMDL proteins are key regulators of serine palmitoyltransferase, an enzyme catalyzing the initial step of sphingolipid biosynthesis. Sphingolipids play prominent roles in cell signaling and response to stress, and they affect the mechanistic properties of cellular membranes. Deregulation of sphingolipid biosynthesis and their recycling has been proven to support and even cause several diseases including allergy, inflammation, and asthma. ORMDL3, the most extensively studied member of the ORMDL family, has been shown to be important for endoplasmic reticulum homeostasis by regulating the unfolded protein response and calcium response. In immune cells, ORMDL3 is involved in migration and in the production of proinflammatory cytokines. Furthermore, changes in the expression level of ORMDL3 are important in allergen-induced asthma pathologies. This review focuses on functional aspects of the ORMDL family proteins, which may serve as new therapeutic targets for the treatment of asthma and some other life-threatening diseases.

ORMDL family

The ORM1 sequence was firstly mentioned in a study of open reading frames in yeasts (1). Later, analysis of the new data from the human genome project revealed three homologs of ORM1 in the human genome, ORM1-like 1 (ORMDL1), ORMDL2, and ORMDL3 genes (2). However, a conflict in nomenclature of ORMDL proteins has emerged in recent years. Wu et al. (3) explained ORMDL3 as an orosomucoid 1-like 3 gene. This statement, replicated in several other papers (4–10), originated from the fact that both the yeast ORM1 gene and human α 1-acid glycoprotein (orosomucoid 1) gene share the same name, ORM1. However, the amino acid sequence comparison (Fig. 1) shows clearly that human (h) ORMDL3 and orosomucoid 1 belong to different families, sharing only 18% sequence similarity. In contrast, hORMDL3 and yeast ORM1 exhibit 35% similarity. It should be noted that orosomucoid proteins are produced mainly by hepatocytes, which secrete them into the blood, and in this way, they are distributed to other tissues (11). In contrast, ORMDL proteins are ubiquitously expressed transmembrane proteins localized in the endoplasmic reticulum

(ER). Thus, ORM and ORMDL proteins have nothing to do with orosomucoid.*

Human ORMDL1, ORMDL2, and ORMDL3 family members are localized on chromosomes 2q32, 12q13.2, and 17q21, respectively, whereas mouse ORMDL1, ORMDL2, and ORMDL3 are found on chromosomes 1, 10, and 11. Members of the human ORMDL family are proteins of 153 amino acids associated with ER. Furthermore, two processed pseudogenes similar to ORMDL1 and ORMDL2 have been described (2). Recently, a novel splice variant of ORMDL3 was discovered in HeLa cells with 94 amino acids corresponding to the C-terminal part of ORMDL3. This variant showed normal subcellular localization and was expressed in

*Regarding yeast ORM, it is not an abbreviation. Dr. Claus Børsting explained to us that he, while a PhD student in the Karsten Kristiansen's laboratory, found an open reading frame close to the ACB1 gene in *Saccharomyces cerevisiae*. It had no homology to any known gene at that time. Therefore, he had the honor of giving the gene a name. During that time, a PC game called Nibbles was popular, and Dr. Børsting played it with his fellow students. In the game, the players had to control worms that were supposed to collect objects (numbers in their version). At the end, the best player won the title ORM, which is the name for worm in Danish. So, as a champion, Dr. Børsting named the unknown gene *ORM*.

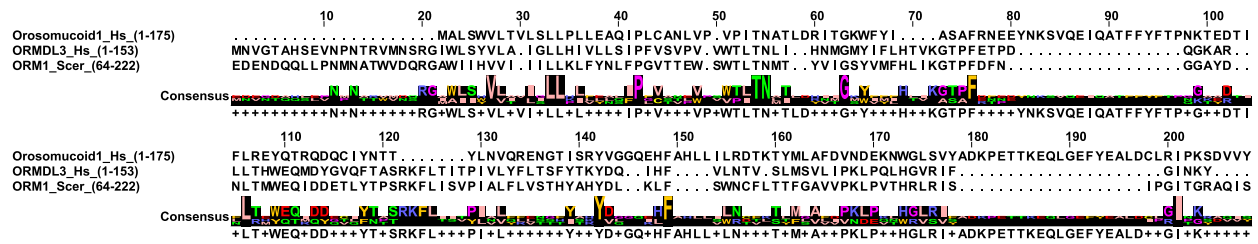


Figure 1 Amino acid sequence alignment of Orosomucoid 1, ORMDL3, and ORM1 proteins. Amino acid sequences for human Orosomucoid 1 (gi.199607822), human ORMDL3 (gi.21165608), and

yeast ORM1 (gi.1323023) were aligned using clustal omega software (107). The alignment was subsequently analyzed by JalView to obtain the consensus sequence (108).

a variety of tissues such as brain, liver, testis, and leukocytes (12). However, the function of this short ORMDL3 protein remains to be determined. Sequence comparison revealed a striking similarity of amino acid sequences of ORMDL proteins in vertebrates, pointing to the evolutionary importance of the ORMDL family members. There is higher similarity (99%) between hORMDL1 and mouse (m)ORMDL1 than between hORMDL1 and hORMDL2 (83%) (2). The comparison of hORMDLs with ORMDL from *Arabidopsis thaliana* and different yeast species showed 30–40% homology. Our own analyses of ORMDL3 sequences in various databases have shown that ORMDL orthologues are found in all examined species (Fig. 2). Interestingly, the ORMDL orthologue is missing in *Caenorhabditis elegans*, but is present in other *Nematode* species. Because ORMDL proteins possess highly conserved motifs present in most of the species, it is likely that ORMDLs are important for cellular functions and that *C. elegans* developed another mechanism to control this function.

Association of ORMDL3 with asthma and other pathologies

Genomewide association studies (GWAS) showed that the ORMDL3 locus is associated with numerous pathologies such as asthma (13), rheumatoid arthritis (14), type 1 diabetes (15, 16), primary biliary cirrhosis (17), ulcerative colitis (18), glioma (19), Crohn's disease (20, 21), allergic rhinitis (22), and ankylosing spondylitis (23). There are less data on association of ORMDL1 and ORMDL2 with diseases. GWAS showed that ORMDL1 is associated with increased bilirubin levels (24). Furthermore, it has been found that polymorphisms in all three ORMDL genes are associated with asthma and that asthmatics exhibit increased ORMDL levels (25). The findings on the role of ORMDL3 in asthma were replicated in studies from different populations (26–28) (Table 1). Further analyses supported the conclusion that genetic elements regulating ORMDL3 expression (Table 2) may contribute to asthma development (7, 13, 22, 25, 29–34).

In these studies, some inconsistencies have been noticed. Thus, Leung and co-workers confirmed that polymorphisms in locus 17q21 are associated with childhood-onset asthma and atopy in Chinese children, but instead of ORMDL3, they found other genes in this region as key players (35).

However, a subsequent study showed that even in the Chinese population polymorphisms in ORMDL3 are associated with asthma in children (36). To deal with this problem, another study on Chinese eastern Han population was conducted in 2014. The data confirmed that a SNP in the ORMDL3 locus on chromosome 17q21 is a risk factor for childhood-onset asthma (37). Conflicting results also appeared about the association of 17q12-21 polymorphisms with increased IgE levels in asthma patients. Yu and co-workers found that in Chinese population rs7216389 asthmatic children exhibit increased levels of IgE (38). On the other hand, several other studies examining different populations found no significant differences in the IgE levels between patients with asthma and healthy controls (28, 39, 40).

Several studies focused on exposure to environmental agents and impact of ORMDL3 on asthma. rs8076131A is a risk allele for developing childhood-onset asthma with stronger impact in children exposed to tobacco smoking in early life (39). 17q12-21 variants have been shown to interact with smoke exposure as a risk factor of developing childhood asthma, but in this study no correlation between the age of asthma onset and ORMDL3 SNPs was seen (41). Data from a study of asthmatics in the British population confirmed SNPs in the 17q12-21 locus as a risk factor for asthma in adults and interactions between the 17q12-21 locus and smoking as a contributing factor to asthma and impaired lung functions (42). The combined data suggested that exposure to tobacco smoking is a risk factor for onset of asthma in both children and adults and that ORMDL3 is involved in these processes.

Enhanced expression of ORMDL3 is accompanied by poorer response to inhaled corticosteroid therapy in patients with asthma (34). Firstly, *in vitro* study of the ORMDL3 promoter revealed that Est-1, p300, and CREB are regulators of ORMDL3 expression and that the levels of these regulators correlated with expression of ORMDL3 in recurrent wheeze patients (43). Later, STAT6 was shown to be a direct regulator of ORMDL3 expression (44), confirming previous findings that both ORMDL3 and STAT6 are associated with asthma (45). Furthermore, the role of chromatin in ORMDL3 expression was evaluated. It has been shown that rs12936231 affects nucleosome formation and thereby expression of the genes in the 17q12 region including ORMDL3



childhood-onset asthma (48). Thus, regulation of ORMDL3 expression in patients with asthma is affected by histone modifications and binding of transcription factors.

A new direction in ORMDL family research was set by a study analyzing polymorphisms in all three human ORMDL

genes. Based on previous findings, different ORMDL3 polymorphisms were associated with different subtypes of asthma (27, 28, 40). The same situation was also observed in the case of ORMDL1 rs5742940, which was significantly associated with atopic asthma, but not with nonatopic subtypes. If confirmed, a new era for ORMDL family research could be started (25).

Table 1 Studies evaluating associations of rs7216389, ORMDL3 expression-altering SNP, with asthma in different populations

Age	Risk	Population	Reference
CO	Atopic asthma	German	(13)
		British	
CO	Atopic asthma	Japan	(40)
CO	Severe asthma	Puerto Rican	(28)
	IgE upregulation		
CO, LO	Asthma	French Canadian	(26)
CO	Asthma	North American of European ancestry	(30)
CO	Asthma	Scottish	(29)
	Exacerbations		
CO	Wheeze	Danish	(31)
	Asthma		
	Exacerbations		
CO	Asthma	African American	(41)
		European American	
CO	Asthma	Chinese	(35)
	IgE not changed		
CO	Asthma	Mexican	(3)
CO	Asthma Severity	Icelandic	(32)
	ORMDL3 expression		
CO	Asthma	German	(7)
	ORMDL3 expression		
LO	Asthma	UK	(42)
CO	Allergic rhinitis	Japanese	(22)

CO, childhood-onset asthma; LO, late-onset asthma.

ORMDLs in regulation of sphingolipid pathway

Sphingolipids represent an important structural component of cell membranes which is instrumental to the regulation of membrane fluidity and other functions. Together with cholesterol, sphingolipids contribute to the formation of the membrane microdomains, called lipid rafts (49). These microdomains provide conformational support for membrane proteins and contribute to the formation of plasma membrane signalosomes (50). The importance of sphingolipid homeostasis in human diseases was established by discoveries demonstrating the accumulation of glucosylceramide in Gaucher's disease (51) and in the pathogenesis of Alzheimer's disease (52). The sphingolipid biosynthesis pathway has been reviewed extensively (53, 54). The entire pathway is depicted in Fig. 3. The key reaction of sphingolipid biosynthesis is mediated by the enzyme serine palmitoyltransferase (SPT). This enzyme catalyzes the chemical reaction in which acyl-CoA is condensed with serine, producing 3-ketosphinganine, which is subsequently reduced to dihydrosphingosine (DHS). Opposite to synthesis is the degradation pathway taking place in lysosomes, on the membranes, and in the nucleus. In this pathway, the fatty acid moiety is cleaved from the ceramide by ceramidase, producing sphingosine, which can be recycled back to ceramide or sphingosine-1 phosphate. In yeasts, the SPT enzyme is active as a heterooligomer formed by LCB1, LCB2, and TSC3 proteins localized to ER membrane. TSC3 protein is an additional activator of the SPT enzyme (55), but no mammalian orthologue has been identi-

Table 2 SNPs associated with changes in ORMDL genes expression. Changes in ORMDL1 and ORMDL3 expression associated with polymorphisms in asthmatics in comparison with nonasthmatics

Gene	SNP	Risk allele	Stimulated with				Unstimulated	Reference
			PHA	HDM	LpA	Ppg		
ORMDL1	rs5742940	A	NS	↑	NS	↑	NS	(25)
ORMDL3	rs7216389	T	-	-	-	-	↑	(32)
			-	↑	-	-	NS	(7)
			-	-	-	-	↑	(22)
	rs2872507	G	-	-	-	-	↑	(34)
	rs12150079	G	-	-	-	-	↑	(22)
	rs3744246	C	-	-	-	-	↑	(22)
	rs4794820	G	-	-	-	-	↑	(33)
	rs8069176	T (nonrisk)						
	rs4795405	T (nonrisk)	↓	↓	↓	NS		(25)
	rs3902920	T (nonrisk)	NS	↓	NS	NS		(25)
	rs8079416	C (nonrisk)	↑	NS	↑	↑		(25)

PHA, phytohaemagglutinin; LpA, lipid A; Ppg, peptidoglycan; NS, nonsignificant change; ↑ - enhanced expression; ↓ - reduced expression; - - data not available.

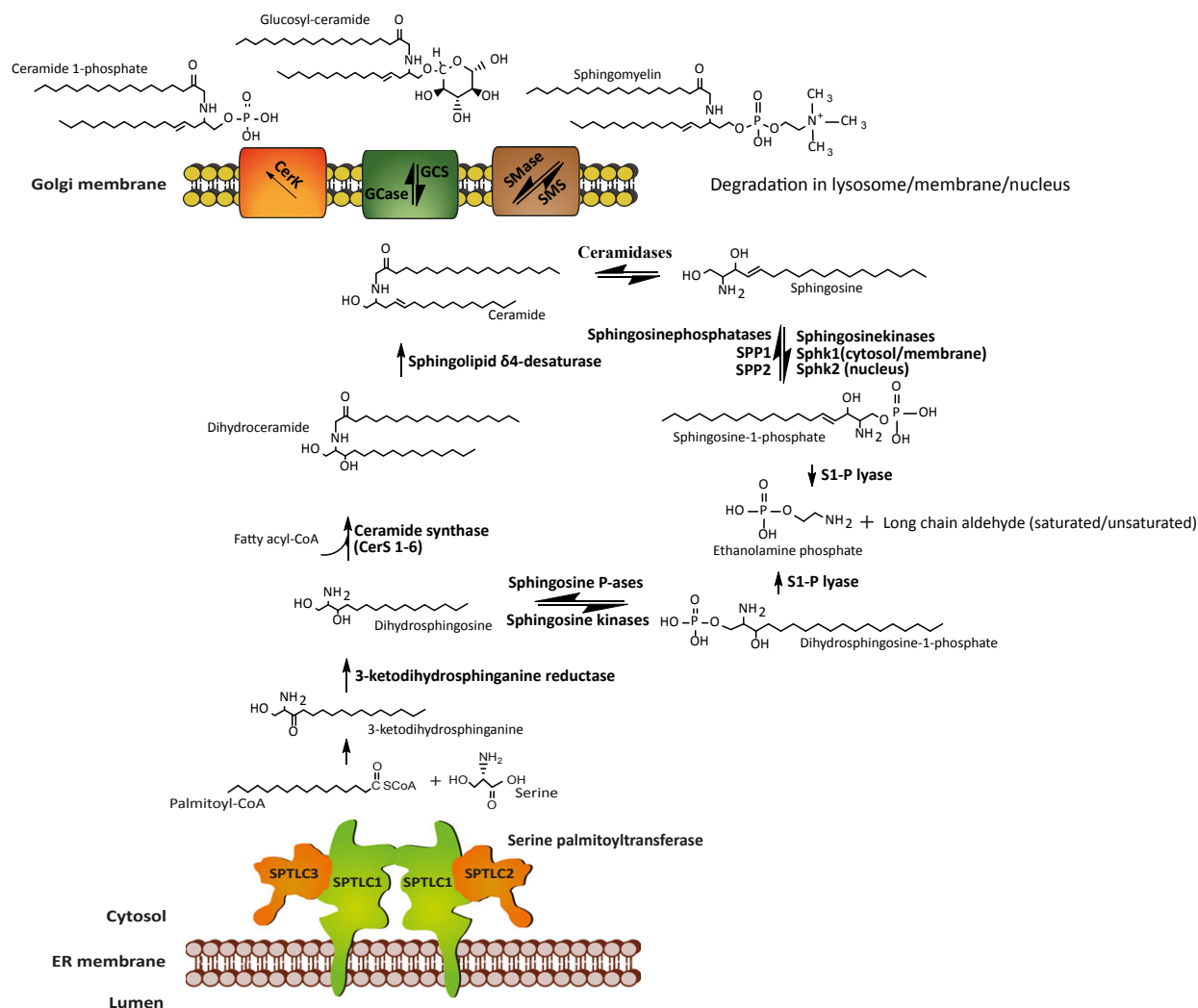


Figure 3 Sphingolipid biosynthesis in vertebrates. Serine palmitoyltransferase (SPT) enzyme catalyzes condensation of acyl-CoA with serine leading to production of 3-ketodihydrosphinganine, which is subsequently reduced to dihydrosphingosine (DHS). DHS can be phosphorylated by sphingosine kinase to DHS 1-phosphate, or it is acetylated by ceramide synthase (CerS) to dihydroceramide. In human, six different CerS are known, each of which uses specific acyl chains, typically with saturated or mono-unsaturated fatty acids with 14 to 26 carbons. After that, dihydroceramide is dehydrogenated by sphingosine δ4-desaturase to ceramide. Upon transport of ceramides from ER to Golgi, ceramides undergo various modifications such as glycosylation resulting in glycosphingolipids,

fied so far. Soon after the discovery of yeast LCBs, human orthologues SPTLC1 and SPTLC2 were described (56–58). In mammals, the SPT complex is composed of three different subunits SPTLC1, SPTLC2, and SPTLC3 acting as an octamer of four SPTLC1 and two SPTLC2 and SPTLC3 (59). An important role in the regulation of SPT enzyme is played by ORM DL family proteins, which are localized to the perinuclear ER membrane (2), where the sphingolipid synthesis is initiated. Although the ORM DL proteins are present in most

phosphorylation resulting in phosphoceramides, and addition of phosphoethanolamine (PEA). Addition of PEA to ceramide produces sphingomyelin. Sphingolipids are molecules undergoing rapid turnover. Complex sphingolipids can be recycled to ceramide by degradation enzymes, for example, sphingomyelinase (SMase), glucosylceraminase (GCase), and sphingosine can be recycled to ceramide, which are important steps in sphingolipid homeostasis. Sphingosine can also be further phosphorylated by sphingosine kinase (SphK1 acting in the cytosol and membrane and SphK2 acting in the nucleus) to sphingosine 1-phosphate. Degradation of sphingosine 1-P is mediated by sphingosine 1-P lyase (S1P lyase) producing phosphoethanolamine and long-chain aldehyde.

of the studied species, they could play different roles in regulation of SPT, as exemplified by ORM DL proteins in non-mammalian and mammalian species.

ORMDL proteins in nonmammalian species

Experiments with yeast cells deficient in either ORM1 or ORM2 showed normal production of sphingolipids. However, cells deficient in both ORM proteins exhibited dramati-

cally increased production of long-chain-based sphingolipids (60, 61). Furthermore, yeast cells deficient in both ORMs showed higher sensitivity to stress induced by exposure to various agents such as dithiothreitol or tunicamycin (2, 60, 62–65). Inhibition of SPT enzyme by low doses of myriocin in *orm1/orm2*-deficient cells resulted in normal growth even in the presence of tunicamycin (60, 61). These findings suggested that yeast ORM proteins are negative regulators of sphingolipid biosynthesis and act through the inhibition of SPT. This conclusion was later confirmed in other studies using cells of various origin, including rice (66), mice (67), and human cell lines (68–71).

Changes in sphingolipid biosynthesis resulting from ORM–SPT interaction take place in response to various conditions such as starving, changes in sphingolipid content (61), plasma membrane status (63), or heat stress (64) (Fig. 4A). Both yeast ORM proteins contain an N-terminal phosphorylation motif bearing serine residues (Fig. 2, red rectangle) representing an optimal consensus phosphoreceptor motif for Ypk1 kinase (72). It has been reported that sphingolipid depletion by myriocin leads to the activation of Ypk1 via the transducer of regulated CREB protein (TORC)2 and subsequent phosphorylation of ORM N-terminal motives and activation of sphingolipid *de novo* synthesis (62). ORM proteins do not respond only to lack of sphingolipids but also to high concentrations of downstream products of sphingolipid biosynthesis. In 2012, Liu and co-workers reported that Npr1 kinase regulates ORM1 phosphorylation in response to the nutrient status of the cell. In the presence of rapamycin, TORC1 inhibitor simulating starving conditions, Tap42-Sit4-PP2A phosphatase dephosphorylated Npr1, resulting in decreased phosphorylation of ORM1 (63).

Rapid and transient accumulation of sphingolipids acting as signaling molecules initiating heat-shock protein translation, gene regulation, and cell cycle arrest suggests that responses to stress require precise temporal regulation mediated by the phosphorylation of ORM1 and ORM2 proteins (64). Regulation of ORM proteins via phosphorylation was supported by experiments in which mutation of N-terminal serines resulted in constitutive inhibition of SPT (64). The findings that ORM1 is phosphorylated by Npr1 kinase and ORM2 by Ypk1 kinase have been confirmed (73). It was shown that TORC1 regulates ORM1 through Npr1 in response to both lack of a nitrogen source and sphingolipid content. On the other hand, TORC2 signals to induce ORM2 phosphorylation in the case of changed plasma membrane status and changes in sphingolipid content (73). Dephosphorylation of ORM proteins and reactivation of sphingolipid synthesis is then mediated by Cdc55-PP2A (64).

On the other hand, it has been shown that sphingolipids are important for male fertility in plants. In *Arabidopsis thaliana*, sphingolipids are required for proper male gametophyte development, and male plants lacking SPT are sterile (74, 75). Rice ORMDL proteins are associated with a temperature-sensitive genetic male sterility phenotype controlled by *tms2*. There are three ORMDL genes in rice, but only one of them is located to the region associated with male sterility phenotype LOC_Os07 g26940 ORMDL. It showed high

expression in plant panicles and anthers and the expression varied based on the temperature corresponding to fertile or sterile conditions. Suppression of LOC_Os07 g26940 ORMDL or all three rice ORMDLs lead to abnormal pollen development resulting in male sterility. In contrast with previous findings in yeasts and mammals, where decreased expression of ORMDLs leads to increased production of sphingolipids and ceramides, in *tms2* sterile plants, where expression of LOC_Os07g26940 ORMDL is decreased, the ceramide levels are also decreased (66). It needs to be determined whether ORMDL-mediated regulation of sphingolipid biosynthesis could play a role in production of gametes, not only in plants, but also in other organisms.

ORMDL proteins in mammalian species

After describing the role of ORM proteins in yeast cell processes, extrapolation to other species seemed obvious (Fig. 4B). However, the yeast N-terminal phosphorylation motif regulating the formation of SPT–ORM complexes was missing in all other organisms tested (2, 60). This indicated that other mechanisms must be used for SPT–ORMDL cross-talk. New insights were brought just recently, when Kiefer and co-workers have found that it is a balance in expression of individual ORMDL family members that regulates the sphingolipid biosynthesis pathway. They induced *de novo* synthesis of ceramides by palmitate. Changes in ceramide production were observed only when expression of all three ORMDL proteins was reduced (68, 70). Cells exposed simultaneously to ORMDL1, ORMDL2, ORMDL3 siRNAs showed increased production of ceramides in comparison with wild-type cells in both studies. Overexpression of individual ORMDL members did not cause a significant decrease in the ceramide content in HEK293 cells. However, in agreement with the siRNA experiment, overexpression of all ORMDL members led to complete blockage of *de novo* ceramide synthesis. On the other hand, a recent study performed with A549 and RAW264.7 cells showed that expression of siRNA for ORMDL1 alone or ORMDL3 alone, but not ORMDL2 alone, can lead to increased ceramide content (67). Furthermore, when expression of all ORMDLs was decreased and ORMDL3 had been re-introduced, the production of ceramides was lowered in comparison with ORMDLs siRNA (67). This contradicts previous findings (68, 70) and supports the theory that it is ORMDL3-mediated inhibition of sphingolipid metabolism that stays behind the ORMDL3-associated asthma phenotype. The levels of ORMDL proteins could also be regulated by the speed of their degradation. A recent study showed that degradation of ORMDL proteins was enhanced in RAW247 macrophages exposed to free cholesterol. The high cholesterol content in ER led to initiation of autophagosome formation and translocation of ORMDL1 to cytoplasmic puncta corresponding to the autophagosome (76).

Structure–function studies showed that ORM and ORMDL proteins tend to form homooligomers and/or heterooligomers (60, 70). Furthermore, formation of the homooligomers was ceramide concentration dependent (77). When

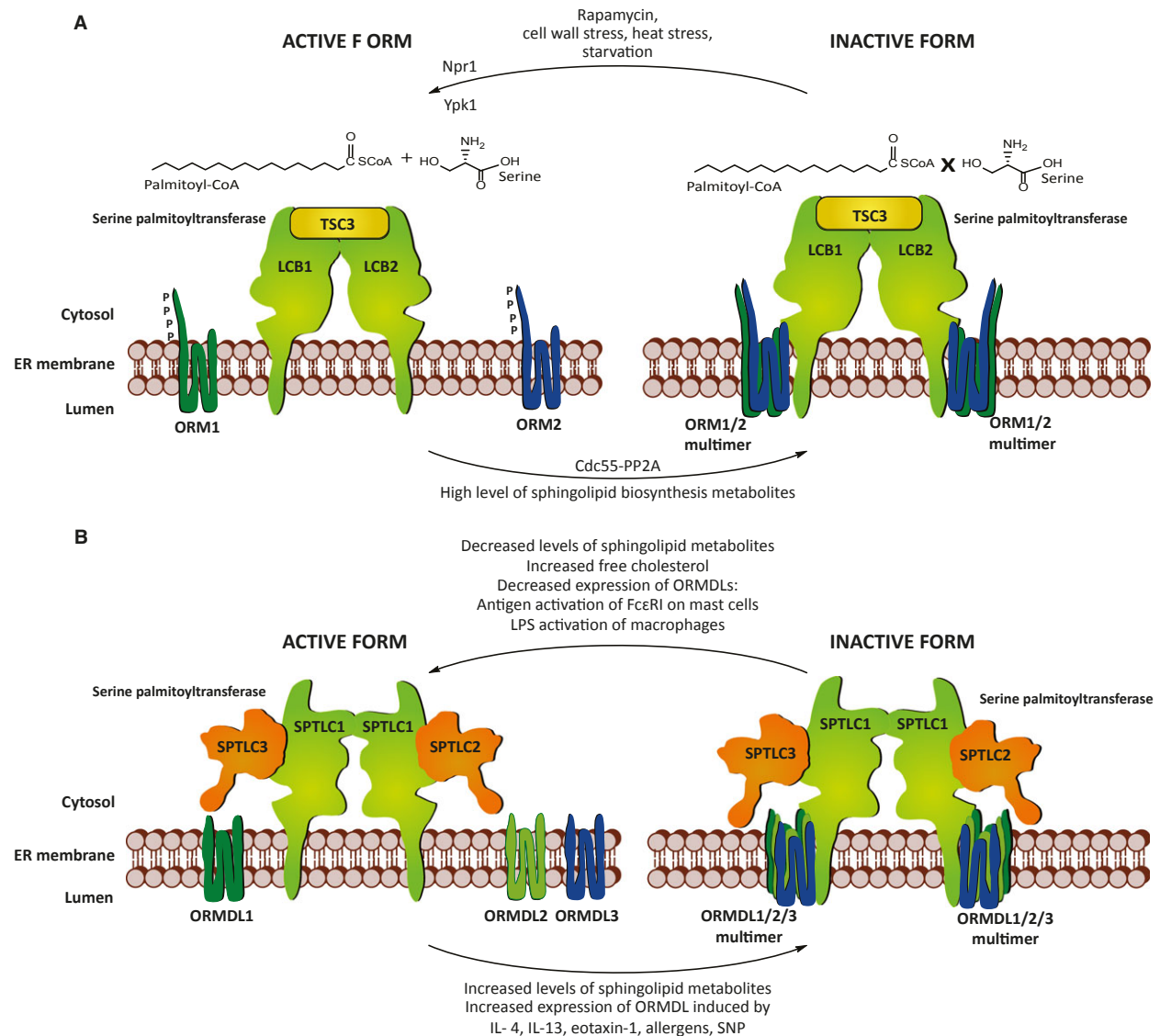


Figure 4 Regulation of serine palmitoyltransferase by ORMDL proteins in yeast and mammals. (A) In yeasts, the serine palmitoyltransferase (SPT) trimer comprises of two catalytic subunits, LCB1 and LCB2, and one regulation subunit TSC3. In active form, ORM proteins are phosphorylated on N-terminal serine-rich motives and dissociated from SPT. When the levels of sphingolipid metabolites are high, ORM proteins get dephosphorylated by Cdc55-PP2A phosphatase, bind SPT, and in this way inhibit *de novo* sphingolipid biosynthesis. When the conditions change back to normal, cells are treated with rapamycin, undergo changes in environmental conditions such as cell wall stress, heat stress or starvation, ORM proteins get phosphorylated by Npr1 and/or Ypk1 kinase, dissociate from SPT, and sphingolipid biosynthesis is restored. (B) In mam-

mals, SPT is an octamer formed by three different subunits SPTLC1 (4×), SPTLC2 (2×), and SPTLC3 (2×) localized to ER membrane with its catalytic core formed in the cytosol. In active state, SPT is free and able to catalyze condensation of serine with palmitoyl CoA as in yeasts. When the cell senses increased levels of sphingolipid metabolites, or the increased expression of ORMDLs is initiated by cytokines (IL-4, IL-13, eotaxin-1) or allergens, ORMDLs bind SPT, presumably in the form of homo- or heterooligomers, and they inhibit its activity and stop *de novo* sphingolipid biosynthesis. When sphingolipid levels in the cell are low, the cell is activated (FcεRI antigen, LPS), or the amount of free cholesterol is high, the concentration of ORMDL proteins is decreased and sphingolipid biosynthesis is reactivated.

C6 ceramide concentration was increased, the affinity of ORMDL1 to ORMDL1 and ORMDL3 to ORMDL3 was significantly decreased, which seems to be analogous to the regulation of yeast ORM proteins, where the presence of high amounts of sphingolipids in the cell led to the phospho-

rylation of ORM proteins (62) and different expression of ORM proteins regulated the formation of SPT–ORM complexes (63). In mammals, similar mechanisms could be involved in the regulation of sphingolipid biosynthesis. In HeLa cells with decreased expression of all three ORMDLs,

of the tested sphingolipids only dihydrosphingosine-1-phosphate and sphingosine-1-phosphate were elevated after high-level C6 ceramide treatment (71). This suggests a mechanism in which the phosphorylation of ceramide precursors by sphingosine kinase 1 and subsequent degradation by sphingosine-1-phosphate lyase keeps ceramides at normal levels at least in the tested time frame of ORMDLs depletion. Macrophages are known to increase the ceramide content after activation with lipopolysaccharide (LPS) (78, 79). The peak in production of ceramides and sphinganine is reached 8 h after LPS exposure (70). After 24 h, the content of ceramides and sphinganine is significantly reduced. Expression of all ORMDL genes followed a similar pattern. The most prominent decrease in the expression of ORMDL1 and ORMDL3 was seen four hours upon LPS triggering and remained decreased further until it was strongly upregulated after 24 h (70). The role of ORMDL3 and SPT in the regulation of sphingolipid biosynthesis was found to be more complicated than originally thought. In cells overexpressing ORMDL3 in mid-levels, inhibition of SPT activity was observed (67). On the other hand, when strong ORMDL3 overexpression was induced, the levels of the studied sphingolipids remained unchanged (68) or even elevated (67). As normal inhibition of *de novo* biosynthesis of sphingolipids was observed, increased recycling of complex ceramides was behind the observed higher levels of sphingolipids in the cell. These hints at the regulation of ORMDL-induced SPT inhibition should be verified in other models. The most valuable model will be inducible knockout mice in single or combined ORMDLs. Almost 13 years had passed since ORMDL human orthologues have been discovered, and yet, no report of ORMDL knockout mice has been published. We can only hypothesize that increased sphingolipid production caused by the lack of ORMDL proteins could lead to damage and subsequently death of ORMDL^{-/-} cells during development.

ORMDLs- γ -secretase cross-talk and asthma

Since the discovery of the γ -secretase function, more than 90 type 1 membrane proteins have been identified as γ -secretase substrates (80, 81). All of these proteins regulate broad variety of cellular processes including development (82), cell adhesion (83), synaptic plasticity (84), and inflammation (85–87). A study focused on the role of ORMDLs role in neuronal cells showed that changes in ORMDL proteins, in the article referred to as adoplins proteins, expression contribute to the regulation of formation of the γ -secretase complex through maturation of nicastrin, a receptor subunit of γ -secretase (88). In this process, immature nicastrin is synthesized on the ER membrane, and then, during transport to the Golgi, it is heavily glycosylated (89). Interestingly, in the case of ORMDL downregulation higher levels of immature nicastrin were observed (88). Similarly, in the absence of presenilin (PS)1 and PS2 proteins, maturation of nicastrin was defective (89). Interestingly, mice deficient in both PS proteins demonstrated increased expression of ORMDL1 and ORMDL2 at the mRNA level and ORMDLs at the protein level (88). Araki and co-authors also showed that ORMDLs

are downregulated in the cells expressing Alzheimer's disease (AD)-associated mutant (I143T or G384A) of PS-1. From these findings, it has been concluded that ORMDL proteins may be involved in various cell processes by affecting function of the γ -secretase complex, which is responsible for cleavage of the broad variety of substrates (90).

As silencing of ORMDL expression leads to abrogated maturation of nicastrin and, therefore, a reduced activity of the γ -secretase (88), one can speculate that in the case of allergen-induced asthma, where ORMDL3 expression levels are increased (6, 67, 91), the activity of γ -secretase complex may be enhanced. To this end, it has been shown that pharmacological inhibition of γ -secretase cleavage of Notch resulted in decreased Th2 cytokine response and suppressed lung inflammation (87, 92, 93). Stronger activity of γ -secretase may result in the increased production of Th2 cytokines, which were shown to stimulate ORMDL3 expression in mice (44, 67, 91). Based on these observations, we propose a mechanism according to which enhanced expression of ORMDL3 in allergen-exposed patients with asthma results in an increased maturation and/or activity and stability of γ -secretase complex. This may then lead to stronger production of Th2 cytokines that further support ORMDL3 expression and promote inflammation in the lung (44, 67, 91). Further investigation of the ORMDL- γ -secretase cross-talk is needed to determine whether the ORMDL- γ -secretase pathway could be a suitable target for the treatment of asthma disease.

The role of ORMDLs in immune response regulation

The finding that ORMDL is a regulator of sphingolipid biosynthesis was a key to understanding multiple roles of ORMDLs in a variety of cellular processes. *De novo* ceramide synthesis is initiated by exposure of cells to different cell stressors including inducers of unfolded protein responses (68), inhibitors of TORC signaling, and various chemotherapeutic agents (94), and cell activators (70). Better understanding of the regulation of sphingolipid biosynthesis pathways is needed to comprehend the origin of diseases in which these pathways are deregulated. An example is a group of severe skin diseases. Terminal differentiation of skin keratinocytes is crucial for normal skin barrier development. Keratinocyte differentiation is dependent on the production of various modified ceramides (95). More subtle abnormalities in the skin barrier function occur in association with common skin diseases such as psoriasis (96) and atopic dermatitis (97). Disruption in the skin barrier leads to a rapid increase in SPT expression resulting in enhanced *de novo* synthesis of sphingolipids (98). Although not proven, it is likely that negative regulators of ORMDLs are involved in these processes as well.

Several studies suggested that ORMDL3 is associated with proinflammatory and autoimmune diseases and has an important role in the immune system functions. ORMDL3 is widely expressed in lymphocytes (5), eosinophils (91), macrophages (4), and lung epithelial cells (6). Furthermore, overexpression of ORMDL3 has been described in patients with different asthma pathologies (7, 13), and enhanced expression

of ORMDL3 was induced by Est-1, p300, CREB (43), STAT6 (44) as well as Th2 cytokines (67, 91). CREB-induced ORMDL3 expression is involved in the retinoic acid receptor pathway activated by all trans-retinoic acid (ATRA), which is PKA dependent (8). ORMDL3 is also an allergen-inducible gene. Enhanced ORMDL3 expression was observed after treatment with ovalbumin (6), or exposure to alternaria (91) or house dust mite (HDM) (67). In mice, interleukin (IL)-3 and eotaxin-1 administration lead to increased ORMDL3 expression (91). A recent study with IL-13 knockout mice described the dependence of ORMDL3 overexpression on IL-13 (67). It has also been shown that IL-4 and IL-13 induction of ORMDL3 expression is STAT6 dependent (44). Very similar regulation of ORMDL3 expression was observed in studies of fatty acid-binding protein 4 (FABP4), which is known to regulate allergic airway inflammation and provides a link between the fatty acid metabolism and asthma phenotype (99). As expected, upregulation of ORMDL3 was observed in adipocytes with FABP4 knock-down (100). As already mentioned, upregulation of ORMDL3 after stimulation with different agents was also observed in patients with asthma depending on SNP genotype (Table 2). The fact that expression of hLCB1 did not change after increased SPT activity indicates that autoregulation of SPT expression is not a major mechanism for the regulation of sphingolipid biosynthesis. It was also shown that the main signal to induce ORMDL expression comes from ceramides rather than long-chain bases (69).

Calcium signaling is one of the most important events in the immunoreceptor-activated immune cells. Overexpression of hORMDL3 in mice was shown to increase expression of sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA)2b, which has been implicated in airway remodeling in asthma (4). On the other hand, no changes in SERCA2b expression were observed in bone marrow-derived mast cells with altered ORMDL3 expression (9). ORMDL3 was shown to alter store-operated calcium entry in HEK293 and in Jurkat T cells (5, 101). In HEK cells, eight amino acids at the N-terminus were pointed out as necessary for ORMDL3 regulation of calcium response (92). However, in a more recent publication the first 16 amino acids of ORMDL3 were shown to play a role in the regulation of Ca^{2+} entry into lymphocytes. Direct interaction of ORMDL3 with SERCA2b was described (101). Furthermore, ORMDL3 was described as the first ER resident protein that modulates Ca^{2+} influx in mitochondria (5). However, ORMDL3 is not involved in Ca^{2+} signaling in all cell types, as indicated by our recent study in which we described similar calcium responses in control cells and cells with ORMDL3 knockdown after activation through the high-affinity IgE receptor (FcεRI) (9).

Based on recent findings, it is clear that the extent of ORMDL3 expression is important for the asthma phenotype. Increased expression of mediators such as interleukins, TNF- α , and chemokines was only observed when ORMDL3 was overexpressed (91). However, mast cells with reduced ORMDL3 expression exhibited increased production of TNF- α , IL-6, and IL-13 (9). This could be explained by increased translocation of NF κ B to the nucleus, reflecting

increased phosphorylation of I κ B by AKT after antigen stimulation (9). A similar role of ORMDL3 in production of proinflammatory cytokines was observed in mice challenged with HDM. Mice overexpressing ORMDL3 showed lung airway remodeling characteristic of asthma, increased area of peribronchial smooth muscles, increased peribronchial fibrosis, increased lung collagen, and mucus expression (4, 67). Mice treated with myriocin, which causes decreased sphingosine synthesis, exhibited airway hyper-responsiveness (AHR) to inhaled methacholine (102). On the other hand, no AHR was observed in myriocin-treated mice in a recent study by Oyeniran and coworkers (67). These inconsistencies could be explained by different models used. However, HDM treatment resulted in increased ORMDL3 expression and augmented levels of ceramides, which led to increased AHR. Moreover, mice challenged with HDM exhibited increased IgE levels in the serum (4). Airway inflammation in allergen-induced asthma was associated with increased cell counts of eosinophils (4, 67), macrophages, CD4 $^{+}$ T cells, and neutrophils (4). The involvement of ORMDL3 in the regulation of airway inflammation was confirmed in other studies, in which reduced expression of ORMDL3 led to reduction of both spreading and trafficking of eosinophils *in vitro* (91). On the other hand, mast cells with decreased ORMDL3 expression migrated strongly toward antigen *in vitro* (9). Similar results were observed *in vivo*, reflecting lower expression of α 4- and β 2-integrins and CD45 (6). Very promising are results with immunosuppression of the asthma phenotype in HMD-challenged mice by immunosuppressive drugs. Increased expression of ORMDL3 after HDM challenge was attenuated when mice were simultaneously treated with a derivative of myriocin, FTY720 (67). In such mice, the ceramide levels were normalized and the AHR response to methacholine and expression of cytokines and chemokines was also decreased. The evidence that the observed asthma phenotype resulted from increased ceramide levels was obtained in experiments in which asthma could be treated with fungal toxin fumonisin B1 (FB1), an inhibitor of ceramide synthase (CerS). HDM-induced ORMDL3 overexpression remained unchanged, but the ceramide levels were normalized, and subsequently, AHR and expression of cytokines and chemokines were decreased. This suggested that FTY720 could be used as a therapeutic drug for the treatment of allergic asthma (4).

Future directions and concluding remarks

Although new knowledge about ORMDL is rapidly accumulating, one should realize that it is far from complete. Extensive attention was paid to transcriptional regulation of the ORMDL expression, but much less to ORMDL degradation, which might be equally important. There are also several poorly explored areas in ORMDL functions, including potential roles of post-translational modifications, miRNAs and other noncoding RNAs, epigenetic regulation of ORMDLs and their interaction partners. As at present numerous sophisticated techniques are available, such as siRNA, CRISPR/Cas, super-resolution and multiphoton microscopy,

mass spectrometry, and next-generation sequencing, our pace of discoveries in ORMDL physiology will be accelerated in the next few years. We need to better understand how changes in the expression of individual ORMDLs contribute to asthma. This involves analysis of individual ORMDLs in various cell types under *in vivo* as well as *in vitro* conditions and after exposure to various stimuli. For better understanding of ORMDL functions, studies should be extended to other organisms as well. For example, there has been only one study about drosophila (d)ORMDL to date. Drosophila expresses only one ORMDL gene encoding a protein with the same length and about 41% homology to human ORMDL1 (2). dORMDL plays a negative role in airway development (103). There is a broad spectrum of genetic and molecular methods allowing to study the complicated developmental processes in Drosophila (104–106). The presence of only one ORMDL gene may unravel new, so far unknown, mechanisms of the regulation of ORMDL action that may

also apply to single ORMDL1, ORMDL2, or ORMDL3. This type of studies will deepen our understanding of the cellular and molecular mechanisms of allergy and other inflammatory diseases and could contribute to the development of new anti-allergy drugs.

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Conflicts of interest

The authors have no conflicting financial interests.

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