Pulmonary ORMDL3 is critical for induction of *Alternaria*-induced allergic airways disease

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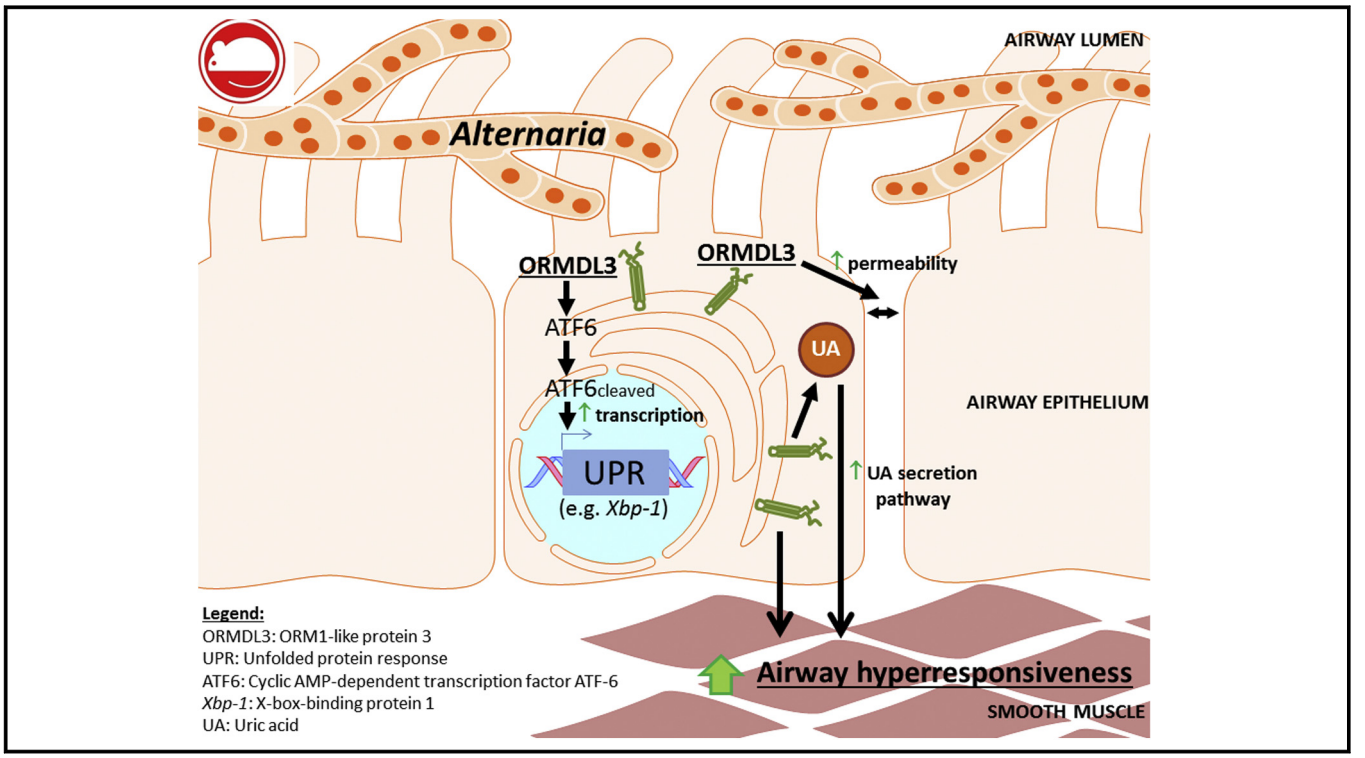
GRAPHICAL ABSTRACT



Background: Genome-wide association studies have identified the ORM (yeast)-like protein isoform 3 (*ORMDL3*) gene locus on human chromosome 17q to be a highly significant risk factor for childhood-onset asthma.

Objective: We sought to investigate *in vivo* the functional role of ORMDL3 in disease inception.

Methods: An *Ormdl3*-deficient mouse was generated and the role of ORMDL3 in the generation of allergic airways disease to the fungal aeroallergen *Alternaria alternata* was determined. An adeno-associated viral vector was also used to reconstitute ORMDL3 expression in airway epithelial cells of *Ormdl3* knockout mice.

Results: *Ormdl3* knockout mice were found to be protected from developing allergic airways disease and showed a marked decrease in pathophysiology, including lung function and airway eosinophilia induced by *Alternaria*. *Alternaria* is a potent inducer of cellular stress and the unfolded protein response, and ORMDL3 was found to play a critical role in driving the activating transcription factor 6–mediated arm of this response through *Xbp1* and downstream activation of the endoplasmic reticulum–associated degradation pathway. In addition, ORMDL3 mediated uric acid release, another marker of cellular stress. In the knockout mice, reconstitution of *Ormdl3*

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transcript levels specifically in the bronchial epithelium resulted in reinstatement of susceptibility to fungal allergen–induced allergic airways disease.
Conclusions: This study demonstrates that *ORMDL3*, an asthma susceptibility gene identified by genome-wide association studies, contributes to key pathways that promote changes in airway physiology during allergic immune responses. (J Allergy Clin Immunol 2017;139:1496-507.)

Key words: *ORMDL3, asthma, unfolded protein response, uric acid,* Alternaria

Asthma encompasses a range of pulmonary disease phenotypes commonly defined by a type 2 inflammatory response to airborne allergen concomitant with airway hyperresponsiveness (AHR).[1] Although the underlying etiologies for asthma are incompletely understood, it is recognized that the epithelial barrier plays a central role in asthma pathogenesis and that genetic predisposition can be pivotal for asthma development.[2] A pioneering genome-wide association study (GWAS) identified single nucleotide polymorphisms (SNPs) on chromosome 17q21 that were strongly linked to asthma.[3] The same SNPs were also found to be associated with increased expression of transcripts of the *ORM-1* like protein 3 (*ORMDL3*) gene.[3] However, the functional role of ORMDL3 in asthma has not as yet been fully elucidated, and it is not known how this molecule contributes to disease pathophysiology.

ORMDL3 is expressed in various tissues, including the lung where it is predominantly localized to airway epithelial cells in response to *Alternaria* or ovalbumin challenge.[4,5] It is a transmembrane protein located in the endoplasmic reticulum (ER)[4] where physiologically it acts to negatively regulate sphingolipid synthesis.[6] Intriguingly, it has been demonstrated that reduction of sphingolipid synthesis with pharmacological compounds increases AHR in mice and human bronchial tissue.[7] However, in a murine model of house dust mite–induced allergic airways disease (AAD), elevated levels of ORMDL3 in response to allergen correlate with increased ceramide production.[8] Previous studies with mice universally overexpressing ORMDL3 have demonstrated that these mice spontaneously develop increased AHR and airway remodeling, which precedes elevated type 2 pulmonary inflammation.[9]

ER stress can be induced on perturbation of luminal Ca^{2+} levels within the ER, consequently decreasing the efficiency of correct protein folding.[10] Accumulation of nascent or misfolded protein in the ER lumen leads to activation of the unfolded protein response (UPR).[11,12] Available data indicate that ORMDL3 modulates the UPR although the pathways involved may be tissue specific. Bone marrow–derived macrophages from *Ormdl3* transgenic mice have increased activation of the activating transcription factor (ATF) 6 pathway of the UPR,[9] similar to previous observations using lung epithelial A549 cells transfected with *ORMDL3 in vitro*.[5] In these systems, expression of ORMDL3 is positively correlated with expression of sarco-endoplasmic reticulum Ca^{2+} ATPase 2b (SERCA2b), a downstream target of the ATF6 pathway.[13] In HEK293 and Jurkat cells transfected with *Ormdl3*, the data illustrate that ORMDL3 binds to and inhibits SERCA, thereby impeding entry of cytosolic Ca^{2+} into the ER lumen and initiating the UPR.[14] However, within this experimental setting, the main impact of ORMDL3 is on the double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK)/eIF2α arm of the UPR.

In the present study, *Ormdl3*-deficient mice were generated to investigate the role of ORMDL3 in determining the pulmonary response to allergen. In addition, we have reconstituted ORMDL3 specifically in the bronchial epithelium of these knockout (KO) mice to ascertain the specific contribution of epithelial ORMDL3. These mice reveal that ORMDL3 is pivotal in the generation of fungal allergen–induced AHR via modulation of cellular stress pathways. Our data reinforce the findings of asthma GWAS, placing ORMDL3 as an important mediator in the development of allergen-induced AHR.

Abbreviations used
AAD: Allergic airways disease
AAV: Adeno-associated viral vector
AHR: Airway hyperreactivity
ATF: Activating transcription factor
BALF: Bronchoalveolar lavage fluid
EGFP: Enhanced green fluorescent protein
ER: Endoplasmic reticulum
GWAS: Genome-wide association study
IRE: Inositol requiring ER-to-nucleus signal kinase
KO: Knockout
ORMDL: ORM-1 like protein
PERK: Double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase
SERCA2b: Sarco-endoplasmic reticulum Ca^{2+} ATPase 2b
SNP: Single nucleotide polymorphism
UK: United Kingdom
UPR: Unfolded protein response
WT: Wild-type
Xbp1: X-box binding protein 1

METHODS

Generation of *Ormdl3* KO mice

Two separate *Ormdl3* alleles were used in the study. The first was generated by Dr. Y Zhang at the Mary Lyon Centre, MRC Harwell, and the second was made and kindly donated by Merck Research Laboratories. Both constructs contained LoxP sites flanking exons 1 to 4 of *Ormdl3*, the translation initiation site of the gene being contained within exon 2. Initially, conditional alleles were generated by Flp-mediated removal of selection cassettes. The targeting strategies for both lines were similar; however, the Merck construct contained dual Neo and Puro selection cassettes, whereas the Harwell allele contained a single Neo selection cassette (see Fig E1 in this article's Online Repository at www.jacionline.org). At Merck, targeting constructs were micro-injected into embryonic stem cells from C57/Bl6NTac mice and complete *Ormdl3* KOs were subsequently generated by Cre-mediated recombination, using globally expressed Cre-driver mouse lines.[15] At Harwell, targeting constructs were micro-injected into ES cells from R1 129 ES cells and *Ormdl3* KOs were subsequently generated by Cre-mediated recombination, using Tg(ACTB-cre) 3Mrt mice (Infrafrontier; accession no. EM:06107). These *Ormdl3* KO mice were subsequently crossed on to a C57BL/6J background (10 generations). A similar response to allergen was observed in both strains. The MERCK *Ormdl3* KO line (*Ormdl3* KO^{Mer}) was used for experiments where mice were treated as adults. Experiments which required in-house breeding at Imperial College were performed using the *Ormdl3* KO line generated by Dr Y Zhang (*Ormdl3* KO^{Har}).

Construction of enhanced green fluorescent protein and *Ormdl3*-enhanced green fluorescent protein adeno-associated viral vectors

An *Ormdl3*-enhanced green fluorescent protein (*EGFP*) (N-terminal tag) gene fusion was constructed and cloned into plasmid pZac2.1 under control of the