

Letter to the Editor

ORMDL deregulation increases stress responses and modulates repair pathways in *Drosophila* airways

To the Editor:

Genome-wide association studies found that childhood asthma is highly associated with polymorphisms in a locus on chromosome 17q21, especially with the gene orosomucoid 1-like 3 (*ORMDL3*).¹ Carriers of the asthma risk allele have increased *ORMDL3* expression.² *ORMDL3* is 1 of 3 members of the ORMDL family in mammals; it encodes an endoplasmic reticulum transmembrane protein that is implicated in diverse cellular processes. Elucidation of *ORMDL3*'s functional relevance in asthma pathogenesis has been hindered by extensive similarities between all 3 ORMDL proteins (ORMDL1-3) as well as their strongly overlapping expression patterns.

We used the fruit fly *Drosophila melanogaster* as a model,³ which has only a single ORMDL gene, to investigate the physiological and pathophysiological relevance of this asthma

susceptibility gene (Fig 1, A). Because flies have a very simple airway system and completely lack adaptive immunity, they permit an unveiled view on ORMDL3's role for the airway epithelial biology and innate immunity. The focus on airway epithelia that is inherent to the *Drosophila* system is consistent with recent findings that impute a central role in orchestrating asthma development to this cell population.⁴

To mimic the situation found in human carriers of the risk allele, we induced a mild ORMDL overexpression specifically in the airway epithelium, using the Gal4/UAS expression control system.⁵ Overexpression of ORMDL in the airway epithelia induced modifications of the airway structure, especially in the terminal cells, which are the functional equivalents of the lung's alveoli (Fig 1, B-E). Terminal cells of ORMDL-overexpressing animals had a reduced number of branches and the individual branches were smaller if compared with terminal cells from matching control animals (Fig 1, D and E). The reduced arborizations and thus the smaller respiratory surface of terminal airway cells are assumed to result in a reduced oxygen-uptake capacity.

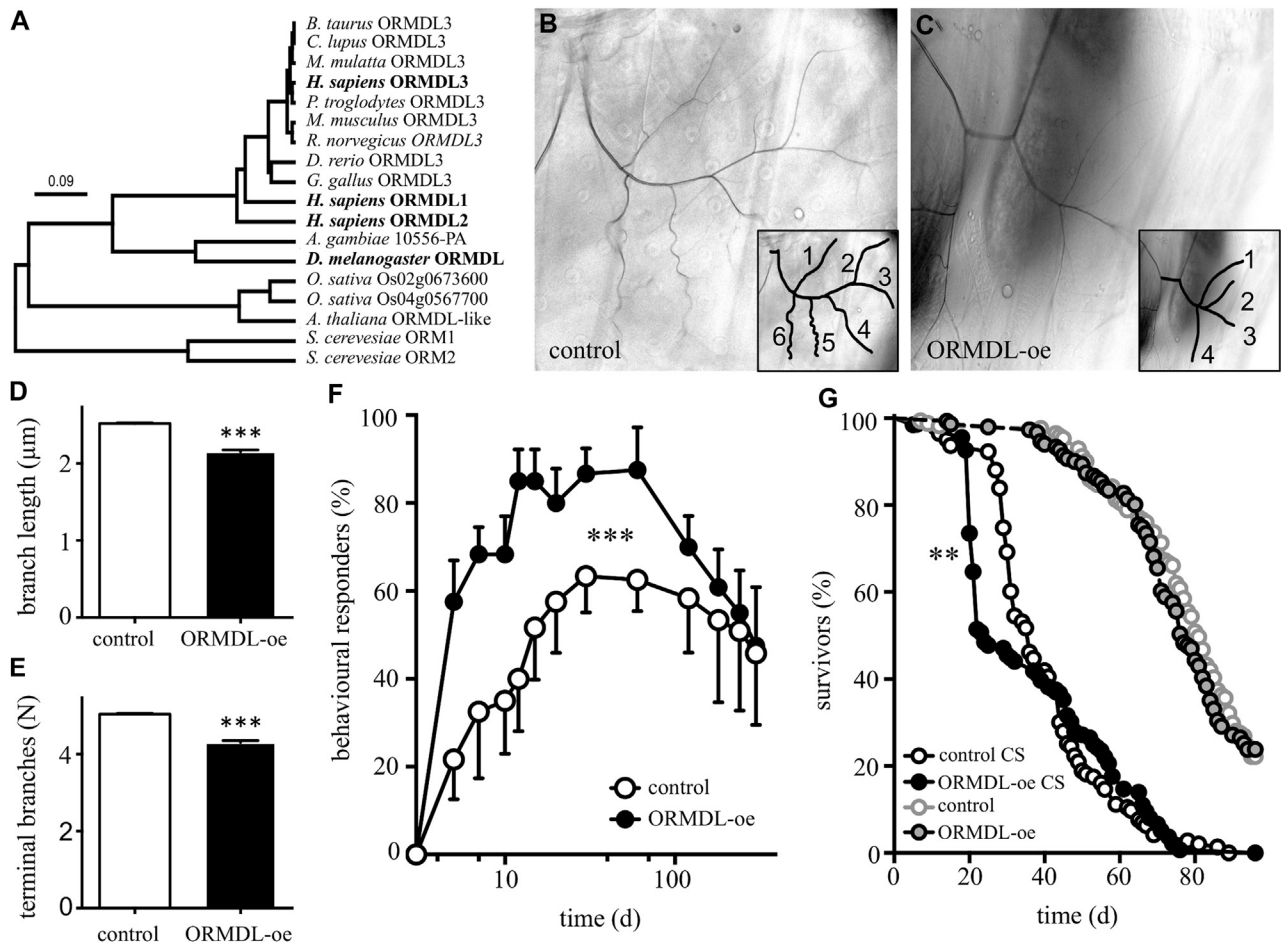


FIG 1. A, Phylogenetic tree of ORMDL proteins. ORMDL overexpression in the airways results in structural changes in terminal airway branches. B-E, Insets show the terminal branches highlighted in black. Quantification of lengths (Fig 1, D) and numbers (Fig 1, E) of terminal branches ($n > 10$). F, ORMDL overexpression increases the behavioral response to hypoxia ($n > 4$) and reduces survival after CS exposure ($n > 135$, G). * $P < .05$, ** $P < .01$, and *** $P < .005$.

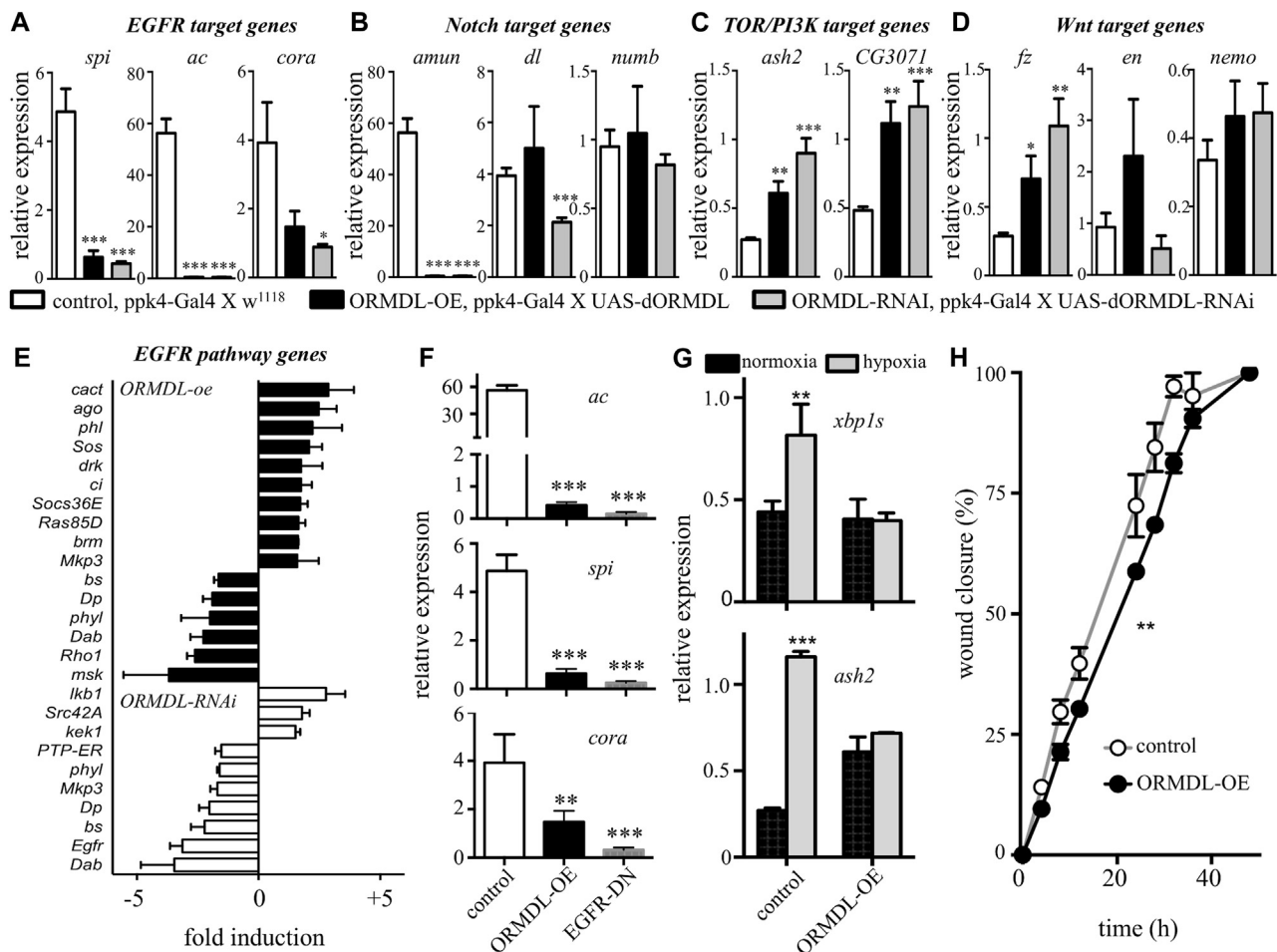


FIG 2. Quantitative expression analysis of target genes of significantly enriched signaling pathways and (A-D) pathway analysis of EGFR signaling (microarray data, $n = 3$) (E). F, Comparison of ORMDL overexpression and EGFR-DN overexpression on transcription of canonical pathway genes. G, Expression levels of stress responsive genes after ORMDL overexpression. H, Wound-closure assay in BEAS-2B cells overexpressing ORMDL3 ($n > 5$). * $P < .05$, ** $P < .01$, and *** $P < .001$.

To test whether structural impairments induced by ORMDL overexpression have functional consequences, we confronted *Drosophila* larvae with hypoxia (5% O_2) and measured their behavioral response. Usually larvae are burrowed into the medium, but they leave it in response to oxygen deprivation; the kinetics of this reaction represents a quantifiable parameter of a response to hypoxia. Larvae with increased ORMDL levels in their airway epithelium exhibited a statistically significantly stronger and more rapid response if compared with matching controls (Fig 1, F). The increased behavioral response toward hypoxia correlates well with the anticipated reduced oxygen-uptake capacity in ORMDL-overexpressing animals. Moreover, we observed in ORMDL-overexpressing animals that the HIF-1 α subunit Sima shows partial nuclear translocation even in normoxia, which is not seen in control animals.

For the *ORMDL3* risk allele, a gene by environment interaction with cigarette smoke was observed, suggesting that genetically determined *ORMDL3* overexpression alters the effect of cigarette smoking (CS) on asthma development.⁶ Although ORMDL overexpression in the airway epithelium had per se no influence on lifespan, animals exposed to daily doses of cigarette smoke showed a reduced lifespan in both ORMDL-overexpressing flies

and matching controls. Interestingly, ORMDL-overexpressing flies had a significantly lower median lifespan than did matching controls if exposed to daily doses of CS (Fig 1, G). The higher sensitivity toward CS implies that increased ORMDL (and ORMDL3) expression in airway epithelial cells reduces the ability to cope with chronically applied airborne stressors.

To identify molecular signatures and pathways that are modulated by the deregulation of ORMDL expression in the *Drosophila* airway epithelium, we performed transcriptome studies, focusing on significantly enriched processes and pathways. To obtain a more comprehensive picture, we used tracheae with reduced ORMDL expression (*ppk4-Gal4xUAS-ormdl-RNAi*) in addition to ORMDL-overexpressing tracheae (*ppk4-Gal4xUAS-ormdl*). KEGG pathway analyses showed that the epidermal growth factor receptor (EGFR), Notch, PI3K, and Wnt signaling pathways were enriched in these samples. To assess changes in the activities of these signaling pathways in response to the deregulation of ORMDL expression, we quantified the expression of canonical pathway target genes by quantitative RT-PCR (Fig 2, A-D). Expression of the EGFR pathway target genes *spitz* (*spi*), *achete* (*ac*), and *coracle* (*cora*) was massively downregulated either by overexpression or

RNAi-mediated gene silencing, showing that signaling via this pathway is reduced substantially (Fig 2, A). For the Notch pathway, a more complex response was observed. Although the expression of *amun* was downregulated by both types of interventions, only RNAi-mediated gene silencing reduced the expression of *delta* (*dl*) (Fig 2, B). However, the expression of the stress response–related TOR/PI3K pathway target genes *ash2* and *CG3071* was higher after ORMDL deregulation, indicative of an overall increased activity of this pathway (Fig 2, C). The Wnt pathway activity was also increased in response to the deregulation of ORMDL expression (Fig 2, D).

We chose the EGFR pathway for an in-depth analysis because of 3 reasons: 1) it showed the most significant changes in signaling activity, 2) this signaling pathway is known to be decisive for the fate of the airway epithelium, and 3) the activity of EGFR signaling in the epithelium has been associated with severe asthma.⁷ Transcript levels of a great number of EGFR pathway genes were modified by the deregulation of ORMDL expression, further supporting the observation that the 2 systems are connected (Fig 2, E). To evaluate whether mild ORMDL overexpression induced responses, we compared expression levels of EGFR pathway genes in control airways with those overexpressing ORMDL and those expressing a dominant negative version of the EGFR. Because the chosen genes are expected to be canonical EGFR target genes, their expression should be under the control of this signaling pathway. ORMDL overexpression reduced the expression of the EGFR target genes close to the levels observed in airway epithelia with dysfunctional EGFR signaling (Fig 2, F). Our observation of reduced epithelial EGFR signaling induced by ORMDL overexpression is consistent with the hypothesis that a defective epithelial repair caused by impaired EGFR signaling is tightly linked to asthma development.⁷ To test whether the deregulation of ORMDL expression affects the cellular stress response,⁸ we measured transcript levels of stress responsive genes under control (normoxia) and stressed (hypoxia) conditions (Fig 2, G). Hypoxia increased the transcript levels of *xbp1s*, the spliced version of the central endoplasmic reticulum-stress protein *xbp1*, and those of the PI3K target gene *ash2* statistically significantly. In contrast, ORMDL overexpression in airway epithelia abolished the cell's ability to respond to stressors adequately (Fig 2, G). To test whether the assumption that ORMDL overexpression impairs epithelial repair thus promoting the development toward an asthmatic phenotype, we performed a simple wound repair assay using cultured human lung epithelial cells (BEAS-2B). This scratch assay was performed with control BEAS-2B cells (transfected with an empty vector) and those transfected with an *ORMDL3* overexpression vector (Fig 2, H). The wound repair assay demonstrated that ORMDL3-overexpressing BEAS-2B cells show a delay in wound closure, implying that their repair capacity is reduced.

In conclusion, we found that increased epithelial ORMDL expression, which mimics the situation found in carriers of the risk allele, changes cellular response characteristics in a way that promote asthma development. ORMDL overexpression changes 1) terminal airway structures, 2) increases the susceptibility to stressors such as CS and hypoxia, 3) abolishes the airway

epithelial's ability to react adequately to stressors, and 4) reduces the repair capacities of the epithelium substantially. We hypothesize that these ORMDL overexpression-induced changes are of central importance for the observed phenotypes that collectively lead to an increased susceptibility to acquire asthma.

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