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# The Duffy Antigen Receptor for Chemokines (DARC) Regulates Asthma Pathophysiology

**David G. Chapman, Ph.D**[1,4], **Edward B. Mougey, Ph.D**[5], **Jos L. Van der Velden, Ph.D**[2], **Karolyn G. Lahue, BS**[2], **Minara Aliyeva, MD**[1], **Nirav Daphtary, MS**[1], **Katherine L. George, BS**[5], **Sidra M. Hoffman, Ph.D**[2], **Robert W. Schneider, BS**[2], **Russell P Tracy, Ph.D**[1,3], **George S. Worthen, MD**[6], **Matthew E. Poynter, Ph.D**[1], **Stephen P Peters, MD, Ph.D**[7], **John J. Lima, Pharm. D**[5], **Yvonne MW Janssen-Heininger, Ph.D**[2], and **Charles G. Irvin, Ph.D**[1]

[1]Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont, United States

[2]Department of Pathology, University of Vermont College of Medicine, Burlington, Vermont, United States

[3]Department of Biochemistry, University of Vermont College of Medicine, Burlington, Vermont, United States

[4]Woolcock Institute of Medical Research, Sydney Medical School, University of Sydney, NSW, Australia

[5]Nemours Pharmacogenetics Center, Nemours Children's Clinic, Jacksonville, Florida, United States

[6]Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, Pennsylvania, United States

[7]Wake Forest School of Medicine, Section on Pulmonary, Critical Care, Allergy & Immunologic Diseases, Winston-Salem, NC

## Abstract

**Background—**The Duffy Antigen Receptor for Chemokines (DARC) is an atypical receptor that regulates pro-inflammatory cytokines. However, the role of DARC in asthma pathophysiology is unknown.

**Objective—**To determine the role of DARC in allergic airways disease in mice, and the association between *DARC* single nucleotide polymorphisms (SNPs) and clinical outcomes in patients with asthma.

**Methods—**Mice with targeted disruption of the *Darc* gene ($Darc^{\Delta E2}$) or WT mice were challenged over three weeks with house dust mite (HDM) antigen. Allergic airways disease was

Corresponding Author: David Chapman, PhD, Vermont Lung Center, Health Sciences Research Facility, 149 Beaumont Avenue, Burlington, Vermont, United States 05405. Tel: +1 (802) 656-0375, Fax: +1 (802) 656-8926, David.Chapman@uvm.edu.

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assessed 24 hours and 7 days following the final challenge. Additionally, associations between *DARC* SNPs and clinical outcomes were analyzed in a cohort of poorly-controlled asthmatics.

**Results—**Total airway inflammation following HDM did not differ between *Darc*$^{\Delta E2}$ and WT mice. At 24 hours, *Darc*$^{\Delta E2}$ mice had increased airway hyperresponsiveness; however, at 7 days airway hyperresponsiveness had completely resolved in *Darc*$^{\Delta E2}$ but persisted in WT mice. In poorly-controlled asthmatics, DARC SNPs were associated with worse asthma control at randomization and subsequent increased risk of healthcare utilization (odds ratio 3.13(1.37–7.27), p=0.0062).

**Conclusions and Clinical Relevance—**Our animal model and human patient data suggest a novel role for DARC in the temporal regulation in asthma pathophysiology and symptoms.

### Keywords

Duffy Antigen Receptor for Chemokines; Airway Hyperresponsiveness; Asthma

## Introduction

The chronic inflammatory environment in asthma is characterized by chemokines interacting with G-protein-coupled receptors on target leukocytes (1). However, four atypical chemokine receptors have been described which lack the structural components to induce downstream signaling (2) and are thought to regulate inflammatory responses by acting as chemokine decoys or scavengers. One such atypical receptor is the Duffy Antigen Receptor for Chemokines (DARC) which strongly binds many inflammatory chemokines of both the CXC and CC subfamilies (3). Highlighting the importance of DARC to cytokine homeostasis, a single nucleotide polymorphism (SNP) in *DARC* accounts for 20% of the variability in serum CCL2 in humans (4). Despite the substantial role of DARC as a promiscuous regulator of inflammatory chemokines, its role in asthma is largely unknown.

A role for DARC in asthma pathophysiology was suggested by the association of a SNP in *DARC* with asthma prevalence in African descendants (5). Although any causal link has yet to be determined, DARC is expressed on several cells relevant to asthma such as erythrocytes, lung endothelial cells, alveolar epithelial cells (6) and airway smooth muscle (ASM) cells (7). Erythrocyte DARC internalizes circulating cytokines which prevents the interaction between cytokines and leukocytes (8). In contrast, endothelial DARC promotes chemokine transcytosis and subsequent leukocyte migration into organs (9). Although the function of DARC in airway epithelial cells is presently unclear, *Darc* expression is upregulated in airway epithelium in response to acute airway inflammation (10). Global ablation of *Darc* in mice reduced airway neutrophilia during acute lung injury (11–13); however, whether DARC influences the severity of asthma, and by what mechanisms, has yet to be determined.

Due to the role of DARC in pro-inflammatory chemokine regulation and the contribution of other decoy receptors to resolution of inflammation (14), we hypothesized that a loss of *Darc* function would exaggerate severe allergic airways disease and prolong resolution of pathophysiology. Therefore we determined, in a mouse model of allergic airways disease,

the effect of genetic deletion of DARC on the severity and resolution of asthma pathophysiology. In order to establish the clinical translatability of our findings to human asthma we determined whether *DARC* single nucleotide polymorphisms (SNPs) were associated with clinical outcomes in a clinical trial of patients with severe asthma.

## Materials and Methods

### Animals and animal study design

Mice in which a 90bp region of the *Darc* gene locus had been eliminated from exon II and bred on a C57Bl\6J background (*Darc* $^{\Delta E2}$ mice) (15) were maintained as a breeding colony under pathogen-free conditions. Deletion of the 90kb region leads to loss of normal DARC mRNA expression and a substantially reduced affinity of erythrocytes to CXCL8 and CCL2 (15). Wild-type C57Bl\6J mice (WT) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Mice were studied at eight weeks of age and all experiments were approved by the Institutional Animal Care and Use Committee at the University of Vermont (#11-029).

### House dust mite (HDM) antigen administration

Mice were administered 50ug of intranasal HDM (Greer Laboratories, Lenoir, NC, USA) in sterile PBS (1 mg protein/mL) or PBS alone on 15 occasions over three weeks (Figure 1A). Mice were evaluated 24 hours or 7 days following the final instillation. Airway mechanics were assessed by the forced oscillation technique as Newtonian resistance, a measure of airway resistance, tissue damping, a measure of tissue resistance, and tissue elastance, a measure of lung stiffness (13). Lungs were lavaged with 1.0ml of PBS and centrifuged at 1200xg for 5 minutes to isolate cells. The pellet was re-suspended for total and differential cell counts and the supernatant used for quantification of cytokines. Lung samples were taken for protein lysates, RNA extraction and analysis of collagen content. Additional methodological details are provided in the Online Supplement.

### Human genomic analyses

Eleven *DARC* SNPs (5–15% frequency) were identified in the CEU cohort of the 1000 Genomes dataset (16) available at www.1000genomes.org (10 of which were present in our sample). A twelfth SNP, rs12075, was included due to its association with circulating pro-inflammatory cytokines (4). Tag SNPs were interrogated by TaqMan genotyping in 169 poorly controlled White asthmatic patients who participated in the LODO ALA-ACRC clinical trial (17). See Online Supplement for a description of the study population, prevalence of the analyzed SNPs in the current population (Table E1) and schematic of the study design (Figure E1). Clinical severity was assessed as contact with healthcare providers due to asthma (total contacts), unscheduled visits to asthma clinics and asthma control (ACQ (18) and Asthma Symptom Utility Index, ASUI (19)). The study was approved by the Western Institutional Review Board and local IRB approval was obtained from all individual sites. Written informed consent was obtained from all patients.

### Statistical analysis

Animal data were evaluated using two- or three-way ANOVA (AHR data) with Tukey post-hoc comparisons or Kruskal-Wallis with Dunn post-hoc comparisons. Data are presented as mean ± SEM or median ± IQR and p values < 0.05 were regarded as statistically significant (JMP® Pro 10, SAS Institute Inc., Cary, NC, USA). Associations between DARC SNPs and continuous clinical outcomes were analyzed by general linear modeling assuming an additive model, controlling for age, BMI, and gender (SPSS release 22.0.0.1). Reported means are inverse Box-Cox transformed (95% confidence intervals). Associations between SNPs and incident phenotypes were analyzed by Fisher's Exact test assuming a dominant model, coding individuals who experienced one or more incidents as 1. Analyses were stratified by race, and where required, by treatment. The Bonferroni corrected p-value for significance was 0.0045–0.0063 depending on the number of SNPs included in each analysis.

## Results

### Increased *DARC* expression in lung epithelium in mice with allergic airways disease

*Darc* mRNA (Figure 1B) and protein expression (Figure 1C) in lung homogenates was significantly increased at 24 hours following HDM challenge. The increased levels of both tended to persist at 7 days (p = 0.08 and 0.06, respectively). Although the increase in mRNA due to HDM challenge was reduced at 7 days compared to 24 hours (p = 0.001) there was no difference in protein expression between time points (p = 0.52). Immunofluorescence staining indicated that the increase in DARC was partly explained by increased expression in the airway epithelium, as evidenced by co-localization of DARC and Club Cell Secretory Protein (CCSP, Figure 1D).

### DARC promotes the resolution of HDM-induced neutrophilic airway inflammation

The increase in total leukocytes in BALF following HDM challenge did not differ between WT and $Darc^{\Delta E2}$ mice at either 24 hours or 7 days (Figure 2A). Similarly, the increase in individual cell differentials following HDM challenge at either time-point did not differ between WT and $Darc^{\Delta E2}$. The exception was neutrophil levels at 7 days which were increased in $Darc^{\Delta E2}$ compared to WT mice suggesting that DARC contributes to the resolution of neutrophilic inflammation.

To determine the effect of Darc ablation on the immune phenotype we measured gene expression of $Th_1$, $Th_2$ and $Th_{17}$ associated cytokines in lung samples (Figure E2). *Il-4* and *Il-13* were increased by HDM but were not different between $Darc^{\Delta E2}$ and WT mice. Similarly, *Il-17A* did not differ between $Darc^{\Delta E2}$ and WT mice. The $Th_1$-associated cytokine interferon-γ did was not altered by HDM in either group. These findings suggest a $Th_2$ immune phenotype that was unaltered by DARC.

To determine the effect of DARC on chemokines levels during allergic airways disease we measured chemokines in BAL which encompassed high (CCL11, CXCL1, CCL2, CCL5,), moderate (CXCL10, CXCL9) and low (CCL3, CCL4) DARC affinity (3, 20). Although HDM increased BAL levels of CXCL10, CCL2 and CXCL9 at 24 hours these did not differ

between WT and *Darc*$^{\Delta E2}$ mice (Figure E3A). In contrast, there were trends for increased CXCL1, CXCL2 and CCL5 at 24 hours in *Darc*$^{\Delta E2}$ mice; however, increased levels were also observed for CXCL1, CXCL2 and CCL2 in *Darc*$^{\Delta E2}$ PBS mice relative to WT mice (~2 fold). Indeed, the significant genotype factor but non-significant interaction factor suggests that the increase in cytokines in *Darc*$^{\Delta E2}$ mice was a consequence of increased baseline levels rather than an exaggerated response to HDM. These differences in cytokine levels were no longer apparent at 7 days, except a small increase in CXCL10 in HDM-challenged *Darc*$^{\Delta E2}$ mice (Figure E3B).

## DARC regulates the severity and resolution of HDM-induced airway hyperresponsiveness

We next determined the role of DARC in the severity and resolution of AHR (Figure 3). Baseline respiratory mechanics after vehicle control (saline) were similar between WT and *Darc*$^{\Delta E2}$ mice. At 24 hours following HDM, AHR measured by central airway resistance and tissue resistance did not differ between *Darc* $^{\Delta E2}$ and WT mice (Figure 3A and B). However, AHR as measured by tissue elastance was increased in HDM-challenged *Darc* $^{\Delta E2}$ mice compared to WT mice (Figure 3C). At 7 days, AHR measured by central airway resistance tended to be reduced in HDM-challenged *Darc* $^{\Delta E2}$ mice compared to WT mice, although this did not reach statistical significance (Figure 3A). However, AHR measured by both tissue resistance and tissue elastance was significantly reduced in *Darc* $^{\Delta E2}$ compared to WT mice (Figure 3B and C). Taken together, the presence of DARC reduced AHR during peak inflammation but maintained AHR despite resolution of inflammation.

## DARC does not alter HDM-induced airway remodeling

Mucous hyperplasia following HDM challenge was similar between WT and *Darc* $^{\Delta E2}$ mice measured histologically (Figure 4A and B) and by evaluation of *Muc5ac* and *Gob5* mRNA (Figure 4C and D). The increase in soluble collagen and *Collagen 1* mRNA following HDM challenge also did not differ between WT and *Darc*$^{\Delta E2}$ mice at either time-point (Figure 4E and G). There was no effect of HDM on airway smooth muscle, as measured by *α-smooth muscle actin* mRNA, in either WT or *Darc*$^{\Delta E2}$ mice (Figure 4F). Taken together these findings suggest that DARC does not alter airway remodeling in allergic airways disease.

## DARC SNPs are associated with symptoms and healthcare utilization in patients with asthma

The potential clinical relevance of our *in vivo* animal findings were explored by analyzing the associations between twelve common (5–15% frequency) *DARC* SNPs and clinical outcomes in patients with asthma, stratified by race (Table 1 and E2). In poorly controlled White asthmatics, rs12042349 was associated with worse asthma control at randomization as measured by ACQ (major homozygote (MM) = 3.14 (2.99–3.30), heterozygote (mM) = 3.75 (3.36–4.22), p=0.0023) with a trend towards an association with ASUI (MM=0.75 (0.72–0.77), Mm=0.67 (0.57–0.71), p=0.0062). After 24 weeks of treatment, Whites with at least one copy of rs35333710 tended to have an increased risk of requiring contact with a healthcare provider for asthma (odds ratio 3.13 (1.37–7.27), p=0.0062). When the association with rs35333710 was stratified by treatment, the odds ratios for requiring contact with a healthcare provider for asthma were 4.67(0.93–23.37), p=0.061, 2.34(0.58–9.50),

p=0.34, and 2.71(0.64–11.53), p=0.20 for placebo, montelukast, and theophylline treatments, respectively.

## Discussion

By combining an animal model and human genetic data we herein report the novel role for the Duffy Antigen Receptor for Chemokines (DARC) in asthma pathophysiology. Our animal model highlights the temporally complex interaction between DARC and asthma; DARC reduced AHR and cytokine levels during the height of airway inflammation but promoted the persistence of AHR during the resolution phase. Consistent with the effect of DARC in the animal model, DARC SNPs were associated with worse asthma control and healthcare utilization in poorly-controlled asthmatics. Interestingly, these associations were only evident in patients on ICS and/or LABA, highlighting the novel potential effect of DARC genotype on treatment response.

Our human data reveals a complex and potentially important role for genetic alterations of *DARC* in the manifestation of asthma symptoms. In White poorly controlled asthmatics, ~75% of which were prescribed ICS and LABA, rs12042349 was related to increased asthma symptoms at randomization. Importantly, the magnitude of the increase in ACQ associated with having one copy of rs12042349 was greater than the minimal clinically important difference (21). Similarly, increased asthma symptoms as assessed by total contacts with healthcare providers due to asthma was associated with rs35333710 in those patients continuing on their pre-study treatment (ICS or ICS+LABA). In contrast, there was no association in patients on additional montelukast or theophylline. Taken together, these findings may reveal a novel interaction between select *DARC* SNPs and the efficacy of ICS and/or LABA in controlling asthma symptoms. However, further research is required to verify this interaction and determine whether non-ICS treatments confer particular benefits in patients with specific *DARC* SNPs.

The present findings strengthen the role of DARC as a promiscuous, but specific, scavenger of inflammatory chemokines. Our findings of increased *DARC* mRNA and protein expression during allergic airways disease are consistent with the increased *DARC* gene expression in bronchial epithelial cells of steroid-naïve asthmatics compared to non-asthmatics (Gene Expression Omnibus repository number GSE23611 www.ncbi.nlm.nih.gov/projects/geo (22)). This suggests that DARC upregulation may be a protective mechanism to maintain chemokine homeostasis during allergic airways disease. Consistent with findings in models of acute lung injury (10, 13, 23, 24), loss of DARC activity in our murine model was associated with increased levels of CXCL1, CXCL2, CCL2 and CL5 at 24 hours; all known to have high affinity for DARC in humans (3) and mice (20). This suggests that loss of DARC function likely alters chemokine homeostasis which promotes an exaggerated chemokine response during allergen challenge.

Surprisingly, the effect of DARC on pro-inflammatory cytokines did not translate into differences in overall airway inflammation. However, this lack of effect of DARC status on total inflammation is consistent with findings in patients with acute lung injury (23) and endotoxemia (25). There are conflicting reports from animal models as to the effect of

DARC on airway inflammation following acute lung injury with both increased (15, 24) and decreased (10, 11) airway neutrophilia. Subtle differences in time points exist between these studies and conflicting findings may reflect a role for DARC in the resolution of airway neutrophilia, as reported in the present study. Our findings suggest a complex temporal interaction between DARC, chemokines and neutrophil recruitment. At 24 hours, chemokines associated with neutrophil recruitment were increased in BAL from mice lacking DARC and we speculate that this translated into the delayed resolution of airway neutrophils at 7 days. It is likely that in WT mice, airway epithelial DARC functions to sequester circulating chemokines thereby reducing the signal for neutrophil migration. Without this function of DARC, airway epithelial cells are unable to act as a "chemokine sink" and thus neutrophil migration into the airways is extended ie delayed resolution. Given DARC's role in regulating airway neutrophilia, and it may be that DARC plays a more substantial role in allergic airways disease characterized by exaggerated neutrophilia, such as that seen with transfer of $T_H17$ cells (26). These neutrophilic models are refractory to corticosteroids and may contribute further mechanistic insight into the association between DARC SNPs and asthma symptoms in asthmatics who were poorly controlled despite treatment. Alternatively, loss of DARC function may simply equate to reduced competition binding which allows increased interaction of cytokines with other receptors. CXCR2 binds CXCL1 and CXCL2, mediating the activation and migration of neutrophils into the airways (27). Despite no change in CXCR2 mRNA in *Darc*$^{\Delta E2}$ lung homogenates in the present study (data not shown), it is possible that reduced receptor competition led to increased chemokine binding with CXCR2 and thus delayed resolution of neutrophilia.

Our animal model provides important clues as to the mechanisms underlying the association between DARC SNPs and asthma symptoms in humans. Firstly, DARC is unlikely to affect asthma severity through effects on airway inflammation or immune phenotype. Indeed, the effect on neutrophils was rather small and conflicts with previous findings suggesting that neutrophils promote AHR (28, 29). Although recent *in vitro* findings suggest that loss of DARC promotes CXCL1-induced ASM proliferation (7) we did not detect an effect of DARC on *α-smooth muscle actin* mRNA or any other markers of airway remodeling. Although we cannot rule out that the relatively short exposure period (three weeks) may be insufficient to detect an effect of DARC on airway remodeling, we were able to detect an effect of AHR at this time-point. Furthermore, a lack of effect on airway remodeling is consistent with findings in other diseases associated with structural remodeling (30, 31). In contrast, the loss of DARC function led to more severe AHR during peak allergen responses suggesting DARC may contribute to worse bronchoconstriction following allergen exposure. This is consistent with increased symptoms, assessed by questionnaire and healthcare utilisation due to asthma, in patients with DARC SNPs. Although the exact mechanisms by which DARC alters AHR remain unknown, it is possible that they are mediated by increased chemokine levels. For example, CXCL1 has been shown to induce airway smooth muscle contraction (32) and promote mast cell recruitment to airway smooth muscle (33), both of which could increase the extent of bronchoconstriction. However, further research is required to determine whether the loss of DARC alters airway smooth muscle function or contributes to AHR and asthma symptoms via other mechanisms.

The present study uniquely combines a murine model of allergic airways disease and a long-term clinical trial of severe asthmatics to examine the potential role of DARC in the temporal manifestation of asthma. Nonetheless, the present study does have some limitations. Firstly, the LODO population was predominantly White and therefore underpowered to determine associations between clinical outcomes and DARC SNPs in Blacks. Therefore findings cannot be generalized to Blacks in which DARC is known to be associated with asthma prevalence (5) and subsequent analyzes specifically in this population are needed to determine the relevance of DARC SNPs on asthma symptoms in Blacks. As such, we did not include the predominant DARC SNP in Blacks, rs2814778, as its frequency in our population was 0–0.5% in Whites and 70–80% in Blacks. However, rs2814778 completely ablates erythrocyte DARC with no effect on DARC on other cells, such as endothelial and epithelial cells. Therefore our mouse model, in which *Darc* was ablated in all cell types, is less applicable to the effect of rs2814778 in Blacks. In contrast, rs12075 in European descendants causes a 50% minor allele frequency for each of the two principal antigens, Fya and Fyb. This SNP alters the level of DARC activity on erythrocytes and non-erythrocyte cells uniformly to produce three phenotypes of DARC activity; DARC which binds chemokines with “high activity”, “moderate activity” (heterozygous) or “low activity”. Thus the global ablation of *Darc* in mice would seem to be most analogous to the “low activity” phenotype in Whites. While the effects of other *DARC* SNPs on DARC function are unknown, the present finding that DARC contributes to asthma severity is most likely only applicable to White patients with asthma.

Through a combination of animal and human data we suggest that DARC plays a role in regulating the severity and resolution of asthma pathophysiology. Our findings in mice suggest that DARC alters the severity and resolution of AHR. These findings are complimented by our human analyses in which select DARC SNPs are associated with worse asthma control and symptoms. Interestingly, our data unveils an important gene-treatment interaction in which select *DARC* SNPs are associated to worse clinical outcomes only in patients prescribed ICS/LABA. Despite the underlying mechanisms remaining unclear, our study suggests that assessment of *DARC* status may provide the ability to discriminate those uncontrolled patients whom may gain the most benefit from alternative treatment strategies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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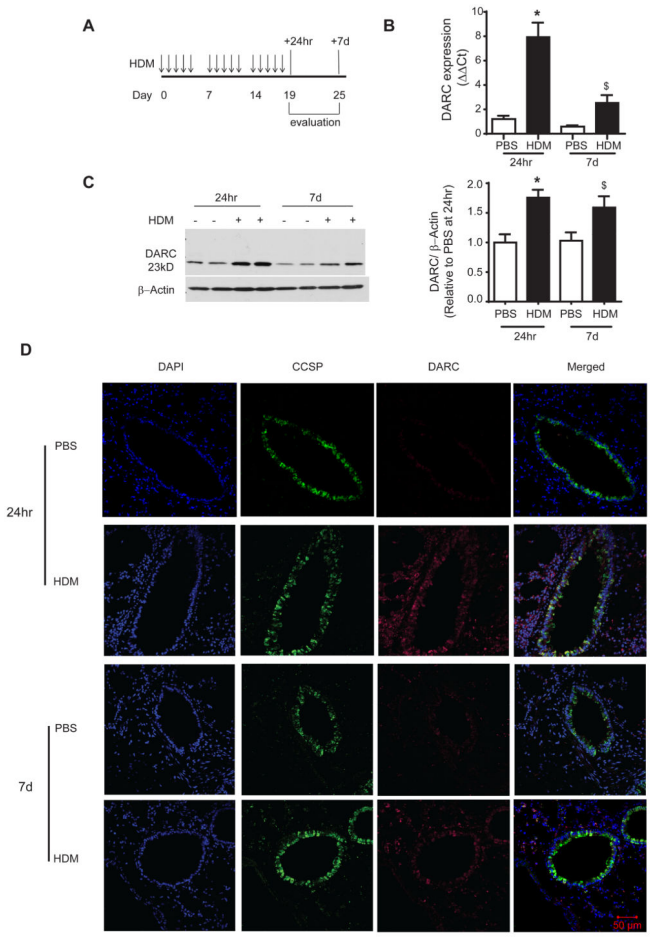


**Figure 1.**

Increase in DARC expression in alveolar epithelial cells in wild type mice 24 hours and 7 days following house dust mite (HDM) challenge. (A) Mice were challenged with 15 intranasal instillations of HDM over three weeks and evaluated at 24 hours and 7 days following the final instillation. DARC (B) *mRNA* and (C) protein expression was increased in lung homogenates at both 24 hours and 7 days. Lung tissue sections were stained using an antibody for DARC (*red*) and Club Cell Secretory Protein (CCSP, green), as a marker of airway epithelial cell co-localization. Data are presented as mean ± SEM of 8–9 mice/group. ANOVA with Tukey post-hoc comparisons: * $p < 0.05$ and $ $p \leq 0.08$ vs respective PBS.

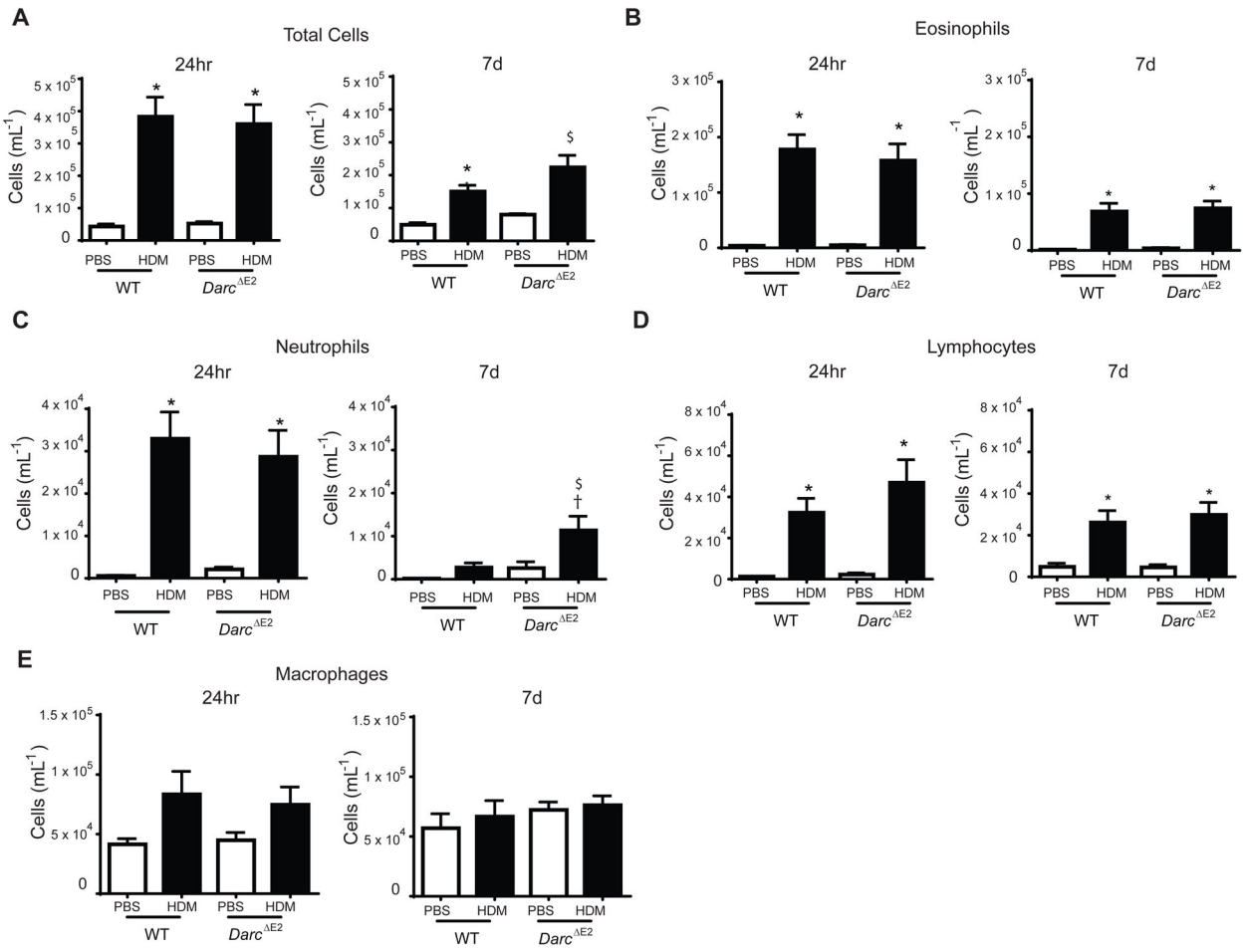


**Figure 2.**

Airway inflammation measured in bronchoalveolar lavage from WT and D*arc*$^{\Delta E2}$ evaluated 24 hours and 7 days following house dust mite challenge. Shown are (A) total cell counts, (B) eosinophils, (C) neutrophils, (D) lymphocytes and (E) macrophages. Data are presented as mean ± SEM of 7–10/group except for D*arc*$^{\Delta E2}$ PBS at 7days (n = 4). ANOVA with Tukey post-hoc comparisons: * $p < 0.05$ and $ $p = 0.06$ vs respective PBS, † $p < 0.05$ WT HDM vs *Darc*$^{\Delta E2}$ HDM.

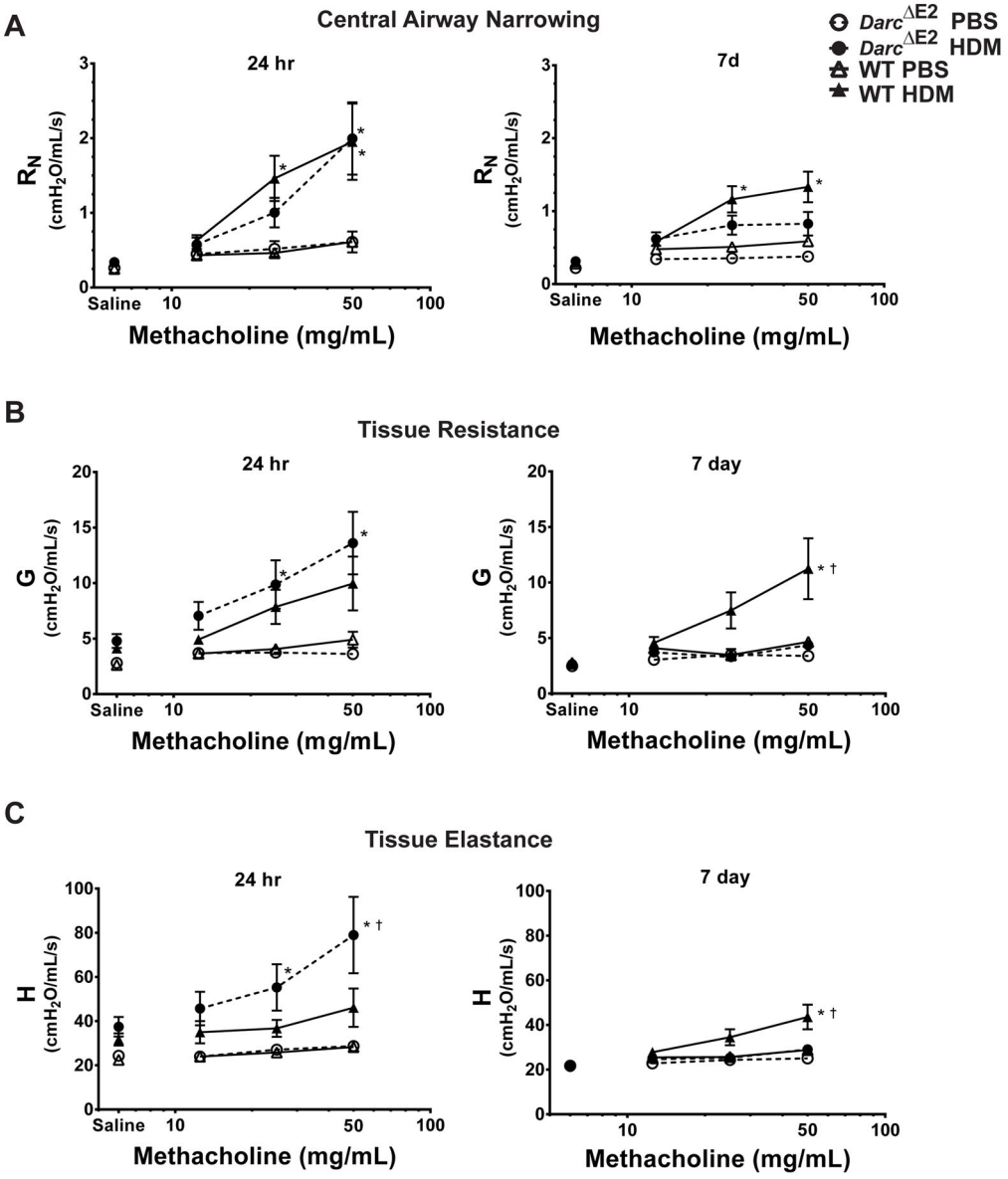


**Figure 3.**

DARC regulates the severity and resolution of airway hyperresponsiveness. Respiratory mechanics were measured by the forced oscillation technique following administration of nebulized PBS control and three doses of methacholine. Respiratory impedance was partitioned into measures of (A) Newtonian resistance (Rn), a measure of central airway resistance, (B) tissue dampening (G), a measure of tissue resistance, and (C) tissue elastance (H), a measure of the stiffness of the lung. The response to methacholine at each dose was quantified as the average of the three peak measurements for each parameter. Data are presented as mean ± SEM of 7–10/group except for *Darc* $^{\Delta E2}$ PBS at 7days (n = 4). ANOVA with Tukey post-hoc comparisons: * $p < 0.05$ vs genotype PBS, $^{\dagger}$ $p < 0.05$ WT HDM vs *Darc* $^{\Delta E2}$ HDM.

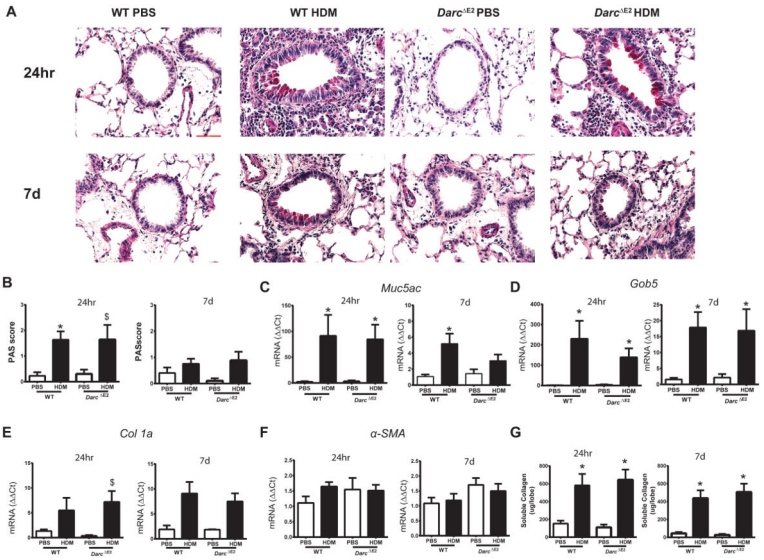


**Figure 4.**

Airway remodeling in WT and *Darc* $^{\Delta E2}$ evaluated 24 hours and 7 days following house dust mite challenge. (A) Periodic Acid-Schiff staining of airway mucus (magnification: 40x, red scale bar = 50μm) and (B) quantification of staining intensity determined by two blinded investigators. Quantification of mRNA levels of (C) *Muc5ac*, (D) *Gob5, (E) Collagen 1* and (F) *α-smooth muscle actin*, α–SMA (G) in lung tissue homogenates by q-PCR. Results are presented as fold change compared to PBS controls. (G) Soluble collagen measured by the Sircol assay. Data are presented as mean ± SEM of 7–10/group except for *Darc*$^{\Delta E2}$ PBS at 7days (n = 4). *Collagen 1A, Muc5AC, Gob5* and *α-smooth muscle actin* mRNA data were log transformed for statistical analyses. ANOVA with Tukey post-hoc comparisons: * $p < 0.05$ vs respective PBS, $^{\$}$ $p \leq 0.08$ vs respective PBS.

## Table 1

Associations of single nucleotide polymorphisms in DARC with clinical outcomes in White asthmatics

| | LODO (n = 169) | | |
|---|---|---|---|
| **SNP** | **ASUI randomization (V2) #** | **ACQ randomization (V2) #** | **Sum Total contacts at 24 wks (V6)** |
| rs41313908 | 0.93 | 0.068 | 1.0 |
| rs35333710 | 0.92 | 0.69 | 0.0062 |
| rs140772227 | 0.79 | 0.97 | 0.55 |
| rs3027016 | 0.23 | 0.11 | 0.59 |
| rs34599082 | 0.31 | 0.73 | 0.41 |
| rs12075 | 0.42 | 0.037 | 0.029 |
| rs36007769 | 0.53 | 0.74 | 0.27 |
| rs12042349 | 0.0062 | **0.0023** * | 0.42 |
| rs111444457 | NA | NA | NA |
| rs139415758 | 0.77 | 0.31 | 1.0 |
| rs2073090 | 0.0050 | 0.044 | 0.45 |

* Corrected p-value below requirement for significance (0.0045–0.0063 depending on number of SNPs analyzed).

# Data non-normally distributed and Box-Cox transformed.

Contacts = contact, whether in person or other means, with healthcare providers due to asthma, ACQ = Asthma control questionnaire, LODO = Low Dose theophylline vs montelukast study (17)