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Original article

# ***ABHD2* deficiency aggravates ovalbumin-induced airway remodeling through the PI3K/Akt pathway in an animal model of chronic asthma**

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## **Abstract**

Airway remodeling is a major pathological characteristic of chronic obstructive pulmonary disease (COPD). This study aimed to investigate the effect of *Abhd2* deficiency on ovalbumin (OVA)-induced airway remodeling and inflammation *in vivo*. *Abhd2*-deficient mice were used to establish an OVA-induced asthma model. Lung tissues were analyzed using hematoxylin and eosin (HE) staining, Masson staining, immunohistochemistry, quantitative reverse transcription-polymerase chain reaction (qRT-PCR), and western blotting were used to determine the role of *Abhd2* in the regulation of OVA-induced airway remodeling and inflammation. Our findings revealed that the RNA expression of inflammatory factors, including IL-1 $\beta$ , IL-6, IL-4, and IL-13, was significantly increased in OVA-induced *Abhd2*<sup>Gt/Gt</sup> asthmatic mice. The expression of IFN- $\gamma$  was decreased significantly in OVA-induced *Abhd2*<sup>Gt/Gt</sup> asthmatic mice. The protein expression of airway remodeling factors, including  $\alpha$ -SMA, type I collagen, and Ki67, was also increased in OVA-induced *Abhd2*<sup>Gt/Gt</sup> asthmatic mice compared to that in OVA-induced wild-type (WT) mice. Additionally, *Abhd2* deficiency promoted the expression of p-Akt in tissues of the asthma model. These results suggest that *Abhd2* deficiency exacerbates airway remodeling and inflammation through the PI3K/Akt pathway in chronic asthma.

**Keywords:** *Abhd2*, airway remodeling, inflammation, PI3K/Akt

## Introduction

Airway remodeling is an important feature in chronic obstructive pulmonary disease (COPD) and asthma, and is strongly associated with the progressive nature and severity of airflow obstruction (Adeloye et al. 2015, Bateman et al. 2008). Once airway remodeling occurs, clinical treatment becomes significantly more challenging. Current treatments primarily focus on relieving respiratory symptoms; however, the progressive deterioration of lung function due to airway remodeling cannot be counteracted (Barnes et al. 2015). Therefore, novel therapeutic strategies and drugs are urgently required to prevent airway remodeling in patients with COPD and asthma.

Major pathological changes in airway remodeling include airway smooth muscle (ASM) hyperplasia, subepithelial fibrosis with deposition of abnormal extracellular matrix components in the basement membrane, subepithelial basement membrane thickening, goblet cell hyperplasia and mucus hypersecretion (Barnes 2016). Severe airway remodeling, especially smooth muscle hyperplasia and hypertrophy that lead to airway obstruction and hyperresponsiveness, does not respond to medication in patients with asthma and COPD (Boulet 2018). Thus, it is crucial to explore effective methods to control airway remodeling and investigate the underlying molecular mechanisms.

The  $\alpha/\beta$  hydrolase domain-containing protein 2 gene (*Abhd2*), a member of the  $\alpha/\beta$  hydrolase superfamily, plays multiple roles in maintaining lung structural integrity (Morissette et al. 2022). *Abhd2* was identified in a genetic screen for human emphysematous tissue, and encodes a protein containing an alpha/beta hydrolase fold, that serves as a catalytic domain for a wide range of enzymes (Jin et al. 2009). Previous studies have shown that mouse *Abhd2* is expressed in airway smooth muscle cells (ASMCs), monocytes, macrophages, airway epithelial cells, and vascular smooth muscle cells (VSMCs), and enhanced macrophage infiltration and apoptosis of alveolar type II epithelial cells have been observed in *Abhd2*-deficient mice (Miyata et al. 2008). Our previous study using *Abhd2*-deficient mice created using gene trap mutagenesis revealed that derangement of the alveolar phospholipid metabolism can induce emphysema and that *Abhd2* plays a critical role in maintaining lung structural integrity (Kumar et al. 2016). Moreover, the *Abhd2* genetic rs12442260 variant contributes to COPD susceptibility in the Chinese Han population, and its interaction with former smoking status is associated with COPD risk in the general population (Liu et al. 2015). These findings suggest that *Abhd2* is involved in the development of emphysema and COPD. However, no studies have

yet been conducted on airway remodeling by *Abhd2* in allergic mouse models.

Phosphatidylinositol 3-kinase (PI3K)/Akt plays a key role in the pathogenesis of airway remodeling by regulating cell proliferation, growth, differentiation, apoptosis, cell size, metabolism, and motility (Li et al. 2020, Pan et al. 2020). Additionally, the PI3K/Akt pathway is involved in the proliferation of ASMCs (Yan et al. 2021). In a rat asthma model, mesenchymal stem cell transplantation alleviated airway remodeling by inhibiting the PI3K/Akt signaling pathway in a rat asthma model (He et al. 2020). However, the involvement of various components of the PI3K/Akt signaling pathway in asthma inflammation and airway remodeling remains to be elucidated.

The present study aimed to explore the effects of *Abhd2* deficiency on airway remodeling and inflammation in an ovalbumin (OVA)-induced asthma model. Our results show that *Abhd2* deficiency promoted the expression of  $\alpha$ -SMA, type I collagen, and inflammatory factors in an OVA-induced asthmatic mouse model. The expression of p-Akt also increased in the tissues of the asthma model. These data suggest that *Abhd2* modulates airway remodeling in chronic asthma through the PI3K/Akt signaling pathway. This suggests that *Abhd2* can be used as a new therapeutic target for the management of asthmatic airway remodeling.

## Materials and Methods

### Animals

The *Abhd2*<sup>Gt/Gt</sup> gene-disrupted mouse line was established as described previously (Miyata et al. 2008). Genotyping was performed using polymerase chain reaction (PCR) with tail genomic DNA as a template, based on a previously described method (Jin et al. 2009). Female C57BL/6 mice weighing 18–22 g and aged 6–8 weeks were used in this study. All animals were maintained in a pathogen-free environment at the Animal Center of the Experimental Center of the Fourth Affiliated Hospital of Harbin Medical University (Harbin, China). The mice were housed in a light- and temperature-controlled room with free access to deionized drinking water and standard chow. All experiments were approved by the local animal care committee of the Fourth Affiliated Hospital of Harbin Medical University Center (HRBMUEC20200116).

### Airway remodeling models

C57BL/6 and *Abhd2*<sup>Gt/Gt</sup> mice were randomly divided into four groups of eight mice each: wild type (WT)+phosphate-buffered saline (PBS) (C57BL/six

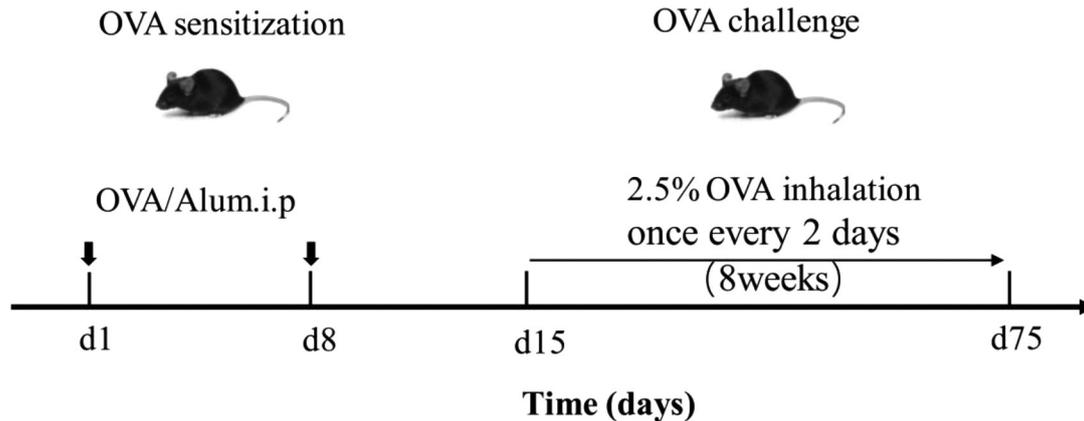


Fig. 1. Mice model of ovalbumin (OVA)-induced airway remodeling.

mice +PBS), WT+OVA (C57BL/six mice +OVA), *Abhd2*<sup>Gt/Gt</sup> +PBS, and *Abhd2*<sup>Gt/Gt</sup> +OVA (*Abhd2*<sup>Gt/Gt</sup> mice +OVA) groups.

Briefly, the mice in the WT+OVA and *Abhd2*<sup>Gt/Gt</sup> +OVA groups were sensitized on days 1 and 8 through intraperitoneal (i.p.) injection of 20 µg OVA (Grade V, Sigma-Aldrich, USA) adsorbed onto 2 mg aluminum hydroxide (Thermo, USA) in 200 µL sterilized PBS once daily. Starting on day 15, the mice were placed in a 0.5 m<sup>3</sup> atomized inhalation box (West Sussex P0229SL, Boehringer Ingelheim) and exposed to aerosolized 2.5% OVA in saline that was atomized through a jet atomization generator (VMD, Shanghai Yuyan Instrument Inc.). This process was performed for 30 min once every 2 d for 8 weeks. Mice in the WT+PBS and *Abhd2*<sup>Gt/Gt</sup> +PBS groups were sensitized and exposed to the same volume and frequency of PBS instead of OVA. A schematic of the experiment is shown in Fig. 1.

#### Hematoxylin and eosin (HE) staining

HE staining was performed as previously described (Li et al. 2020). Briefly, lung tissues from the left lobe were fixed with 4% paraformaldehyde for 24 h and embedded in paraffin. Tissue sections (4 µm) were stained with HE to observe pulmonary histopathological changes. The total area of the airway wall (Wat) was assessed using Image-Pro Plus 6.0, and the perimeter of the basement membrane (Pbm) was used to normalize the Wat. The ratio of Wat to Pbm (Wat/Pbm) was used to evaluate airway structural remodeling.

#### Periodic acid-Schiff (PAS) staining

Goblet cell hyperplasia was examined using AB-PAS staining. All slides were assessed using light microscopy at ×200 magnification. Bronchioles with a 150–200 µm internal diameter were selected

in a blinded manner, observed, and photographed. The area of AB-PAS staining was measured using Image-Pro Plus software. The AB-PAS staining area/Pbm ratio in the airway epithelium was quantified.

#### Masson trichrome staining

Paraffin-embedded tissue sections were stained with Masson's trichrome to assess peribronchial collagen deposition. Sections were examined using a light microscope (200× magnification) to observe changes in collagen fibrosis, airway inflammation, and airway remodeling. On each slide, a minimum of ten bronchioles with an inner diameter of 150–200 µm were selected. The collagen fibers were blue upon Masson's trichrome staining. Quantitative analysis of the collagen deposition area was performed using Image-Pro Plus 6.0. The Wat and airway smooth muscle (Wam) were determined using morphometric analysis of the transverse sections. The BI2000 Medical Image Analysis System was used to evaluate airway remodeling.

#### Immunohistochemistry analysis

Lung tissue sections were deparaffinized in xylene and rehydrated using a graded ethanol series. They were boiled in 3% H<sub>2</sub>O<sub>2</sub> to quench endogenous peroxidases. After antigen retrieval and sealing with 5% goat serum, sections were incubated with *Abhd2* antibody (1:100, ab230417; ABCAM), α-SMA antibody (1:600, PA5-78716; Thermo Scientific), COL1A1 (1:300, PA5-29569; Thermo Scientific), and Ki67 (1:300, ab15580; ABCAM) at 4°C overnight, followed by incubation with horseradish peroxidase (HRP)-linked secondary antibody (31460 Thermo Scientific) for 30 min at 25°C. A DAB solution (8801-4965-72, Thermo Scientific) was used for the chromogenic reactions. Sections were observed using a microscope. The stained areas were assessed using Image-Pro Plus.

Table 1. Primer sequences used for quantitative RT-PCR.

Gene Name	Primer Sequence (5'3')	Product Length (bp)
TGFβ1	F: GTGTGGAGCAACATGTGGAACCTCTA R: TTGGTTCAGCCACTGCCGTA	143
IL-1β	F: ACCTTCCAGGATGAGGACATGA R: CTAATGGGAACGTCACACACCA	116
IL-6	F: GGCCTTCCCTACTTCACAAG R: ATTTCCACGATTTCCAGAG	126
IL-4	F: ATGGATGTGCCAAACGTCCT R: AAGCCCGAAAGAGTCTCTGC	166
IL-13	F: ATGAGTCTGCAGTATCCCG R: CCGTGGCAGACAGGAGTGTT	269
IFN-γ	F: CGGCACAGTCATTGAAAGCC R: TGCATCCTTTTCGCCCTTGC	268
MUC5AC	F: CAGCATCATCAACAGCGAAAC R: TAGTCACAGAACAGTGGGCAGA	216
β-Actin	F: TGTGCCATCTACGAGGGCTAT R: TTTGATGTCACGCACGATTTCC	155

The  $\alpha$ -SMA staining area/Pbm ratio was calculated to evaluate airway remodeling.

#### Western blotting

Lung tissues were dissolved in total protein lysis buffer (R0011; Solarbio Beijing) supplemented with a protease inhibitor (T8031; Solarbio, Beijing), phosphatase inhibitor (P1260; Solarbio, Beijing), and phenylmethylsulphonyl fluoride (PMSF, P8340; Solarbio, Beijing). The homogenates were centrifuged at 12000 rpm at 4°C for 30 min to obtain supernatants. Protein concentrations were assessed using the bicinchoninic acid (BCA) assay (12002231, Bio-Rad, Hercules, CA, USA) and then suspended in 5× sample buffer (P0015, Beyotime Biotechnology, Shanghai) at 4:1 and boiled for 15 min. The protein was loaded (50 µg per lane) onto 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene difluoride (PVDF) membranes (88585, Thermo Scientific). The membranes were blocked at room temperature with 5% defatted milk for 2 h, and incubated overnight at 4°C with the appropriate primary antibodies against MMP2 (1:1000, PA5-16504; Thermo Scientific),  $\alpha$ -SMA (1:200, PA5-78715; Thermo Scientific), p-Akt (1:2000, 44-621G; Thermo Scientific), Akt (1:1000, MA5-14916; Thermo Scientific), and  $\beta$ -action (1:10000, PA1-183; Thermo Scientific). The bands were washed thrice for 10 min with Tris-buffered saline containing Tween-20 (TBST). After incubation with HRP-conjugated goat anti-rabbit IgG (1:10000, 31460, Thermo Scientific) for 2 h, the membranes were probed using an ECL system (Millipore, MA, USA). Quantification

of band densitometry was performed using ImageJ (NIH, Bethesda, MD, USA).

#### Real-time quantitative PCR

Total RNA was isolated from lung tissues using TRIzol Reagent (Invitrogen, Shanghai, China), according to the manufacturer's protocol. mRNA was reverse-transcribed using a Revert Aid First Stand cDNA Synthesis kit (Thermo Fisher, Beijing, China). Real-time PCR was performed using the SYBR Premix Ex Taq (Takara Bio, Dalian, China). The primers used for PCR analysis are shown in Table 1. The  $2^{-\Delta\Delta C_t}$  method was used to calculate the relative expression. All PCR reactions were performed in triplicate.

#### Statistical analysis

Statistical analyses were performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). All data are expressed as the mean  $\pm$  standard deviation (SD) of at least three separate repeated experiments. Differences between groups were analyzed using one-way analysis of variance (ANOVA) or Dunnett's test. Statistical significance was set at  $p < 0.05$ .

## Results

### Low expression of *Abhd2* in OVA-induced airway remodeling mice

To investigate the expression of *Abhd2* in OVA-induced airway remodeling mice, we compared the changes in *Abhd2* protein levels between OVA-induced allergic airway remodeling mice (WT+OVA group) and the

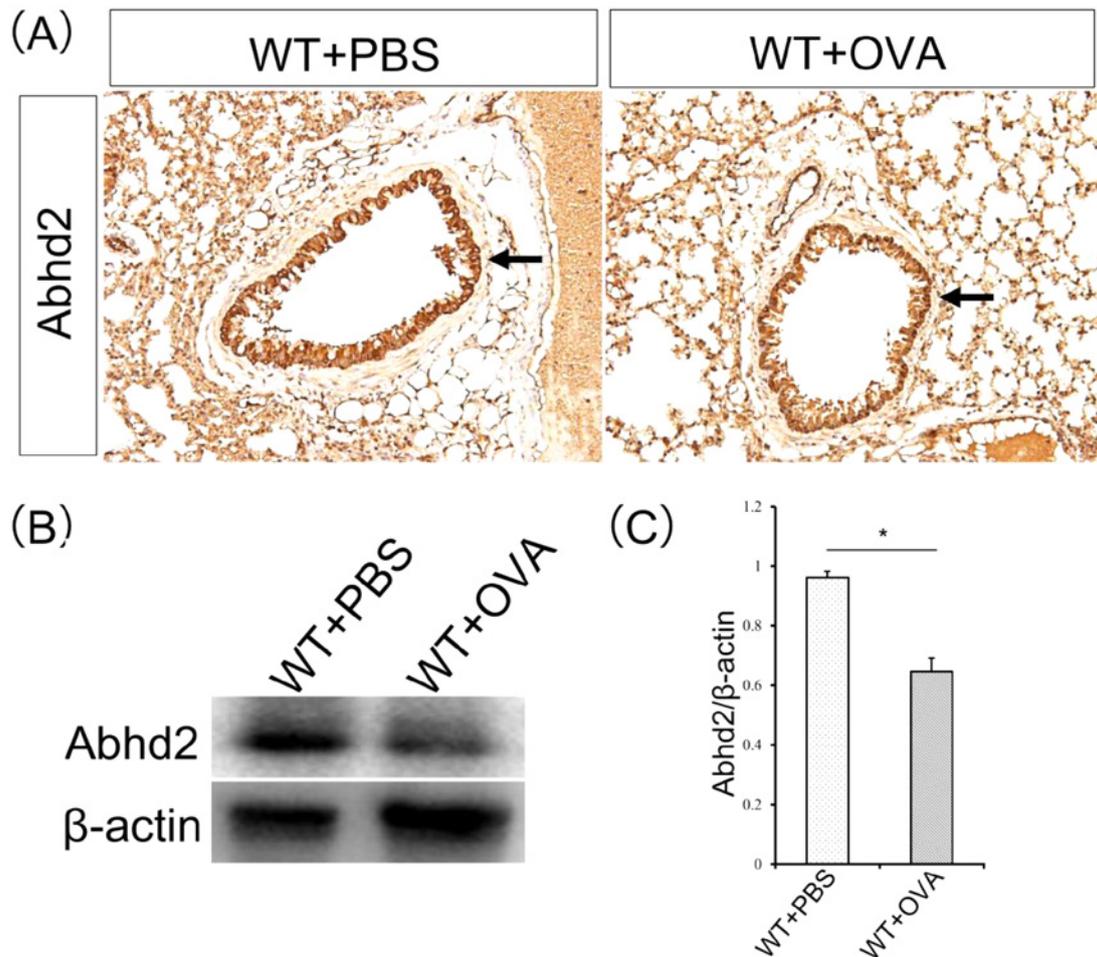


Fig. 2. Expression of *Abhd2* in OVA-induced airway remodeling mice (A) Immunohistochemistry of *Abhd2* in lung tissue sections (magnification,  $\times 100$ ): (B) Western blots of *Abhd2* and  $\beta$ -actin in mouse lung tissues. (C) Corresponding densitometric analyses of the protein bands of *Abhd2* and  $\beta$ -actin. Graphs display a summary of two to three independent experiments. Data are expressed as the mean  $\pm$  standard error of the mean (SEM). \*  $p < 0.05$  indicates significant differences. WT – wild type; PBS – phosphate-buffered saline

WT+PBS group using immunohistochemistry in lung tissues. The immunohistochemical results showed that *Abhd2* was widely expressed in the lung tissue, especially in the airway epithelium, airway smooth muscle, vascular smooth muscle and lung interstitium (for example, the alveolar wall) in both groups (Fig. 2A). In the WT+OVA group, the expression of *Abhd2* protein decreased in the airway epithelium, airway smooth muscle, vascular smooth muscle and lung interstitium (Fig. 2A). Western blotting was used to analyze the protein levels of total *Abhd2*. As illustrated in Fig. 2B and C, we observed that *Abhd2* was significantly decreased in the WT+OVA group compared to that in the WT+PBS group.

#### ***Abhd2* deficiency exacerbated OVA-induced airway inflammation in mice**

To examine the effects of *Abhd2* deficiency on the persistence of airway inflammation, we compared inflammatory cell recruitment and cytokine levels in the

lung tissues. As shown in Fig. 3A and B, the levels of peribronchial and perivascular inflammatory cell infiltration were significantly higher in the OVA group than in the control group. *Abhd2* deficiency significantly exacerbated inflammatory cell infiltration in the OVA group.

The cytokines IL-1 $\beta$ , IL-6, IL-4, IL-13 and IFN- $\gamma$  were detected in the lung tissues using reverse transcription (RT)-PCR to assess the nature of the cytokines in the WT+OVA and *Abhd2*<sup>Gt/Gt</sup>+OVA groups (Fig. 3C–F). The expression levels of IL-1 $\beta$ , IL-6, IL-4, and IL-13 increased significantly in the *Abhd2*<sup>Gt/Gt</sup>+OVA group compared to those in the WT+OVA group ( $p < 0.05$ ). *Abhd2* deficiency significantly increased the levels of these cytokines in lung tissues (Fig. 3C–E). The changes in IFN $\gamma$  were in contrast to the above cytokine results (Fig. 3F). These results indicate that *Abhd2* deficiency significantly increased inflammatory cell recruitment and cytokine levels in lung tissues.

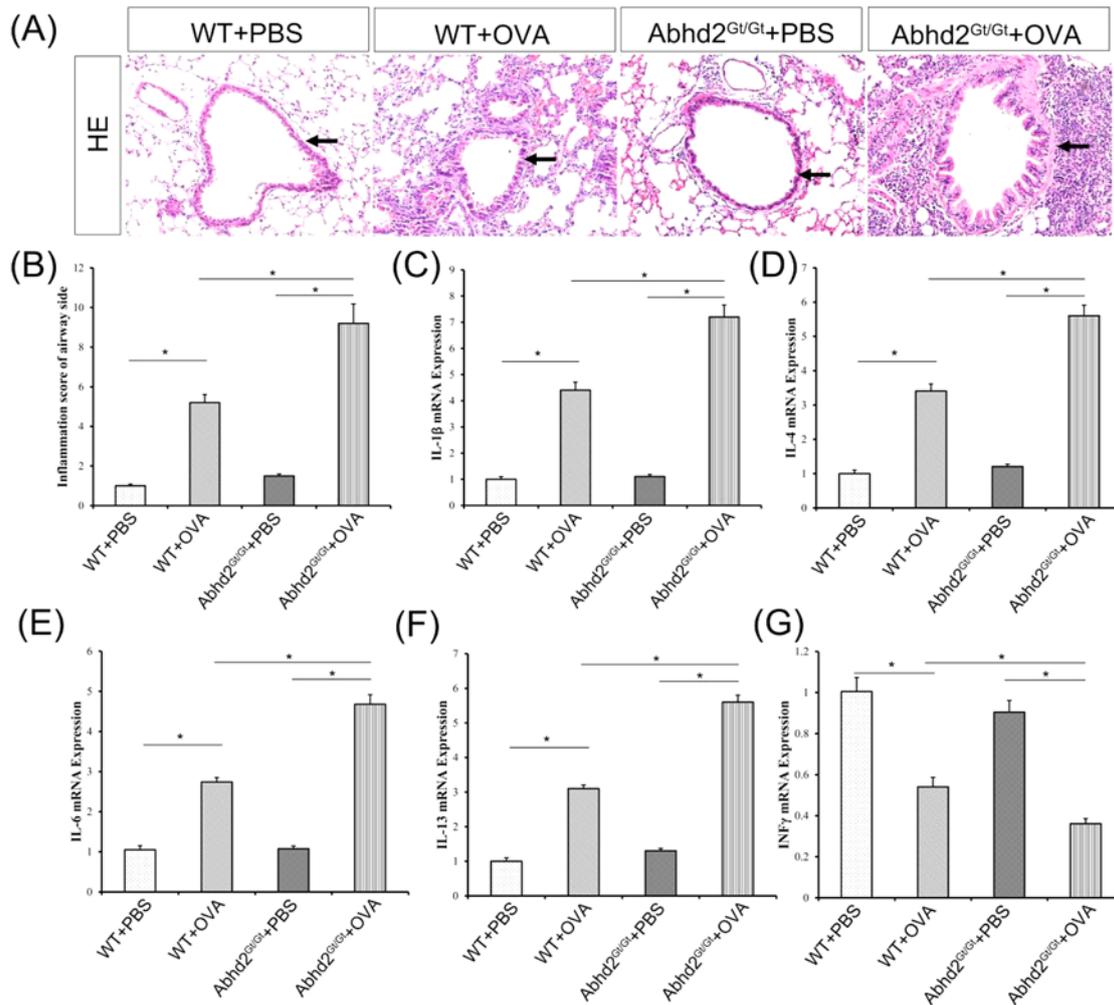


Fig. 3. Effect of *Abhd2* deficiency on airway inflammation in OVA-induced airway remodeling mice (A) Representative photomicrographs of HE staining lung sections (magnification,  $\times 100$ ). (B) Inflammation score of the airway and airway side in mice of each group. The expression of (C) IL-1 $\beta$ , (D) IL-4, (E) IL-6, (F) IL-13, and (G) IFN- $\gamma$  mRNA in mouse lung tissues were evaluated using RT-qPCR. Graphs display a summary of two to three independent experiments. Data are expressed as the mean  $\pm$  SEM. \*  $p < 0.05$  indicates significant differences.

### *Abhd2* deficiency aggravated OVA-induced goblet cell hyperplasia and airway mucus hypersecretion

Airway epithelial goblet cell metaplasia is a primary feature of airway remodeling, and MUC5AC is a major marker of airway goblet cell metaplasia and mucus hypersecretion (Bodas et al. 2021, Jia et al. 2021). As shown in Fig. 4A and B, the percentage of PAS-positive epithelial cells also significantly increased in the OVA-induced *Abhd2* deficient mice group compared to that in the WT+OVA group, consistent with the trend of the mRNA expression of MUC5AC in mice lung tissues (Fig. 4C).

### *Abhd2* deficiency aggravated airway smooth muscle remodeling

Masson's staining was used to evaluate the effect of *Abhd2* deficiency on histopathological changes

in lung tissues. The airway wall thickness (Wat,  $\mu\text{m}^2$ ) and smooth muscle layer (Wam,  $\mu\text{m}^2$ ) were measured and standardized to the perimeter of the basement membrane (Pbm). As shown in Fig. 5A, B, and C, normal bronchi were found in the lung tissue of the control group, without thickening of the lumen. The lung tissue from the OVA group exhibited bronchiolar wall thickening, airway smooth muscle thickening, epithelial cell damage, and impaired partial alveolar cavity integrity compared to that of the control group. However, in the *Abhd2*<sup>Gt/Gt</sup>+OVA group, the above pathological changes were significantly exacerbated compared to those in the WT+OVA group.

To investigate the effect of *Abhd2* deficiency on OVA-induced airway smooth muscle hyperplasia, immunohistochemistry was used to detect  $\alpha$ -SMA expression in the peribronchial area. As presented in Fig. 5A and D, immunohistochemical analysis

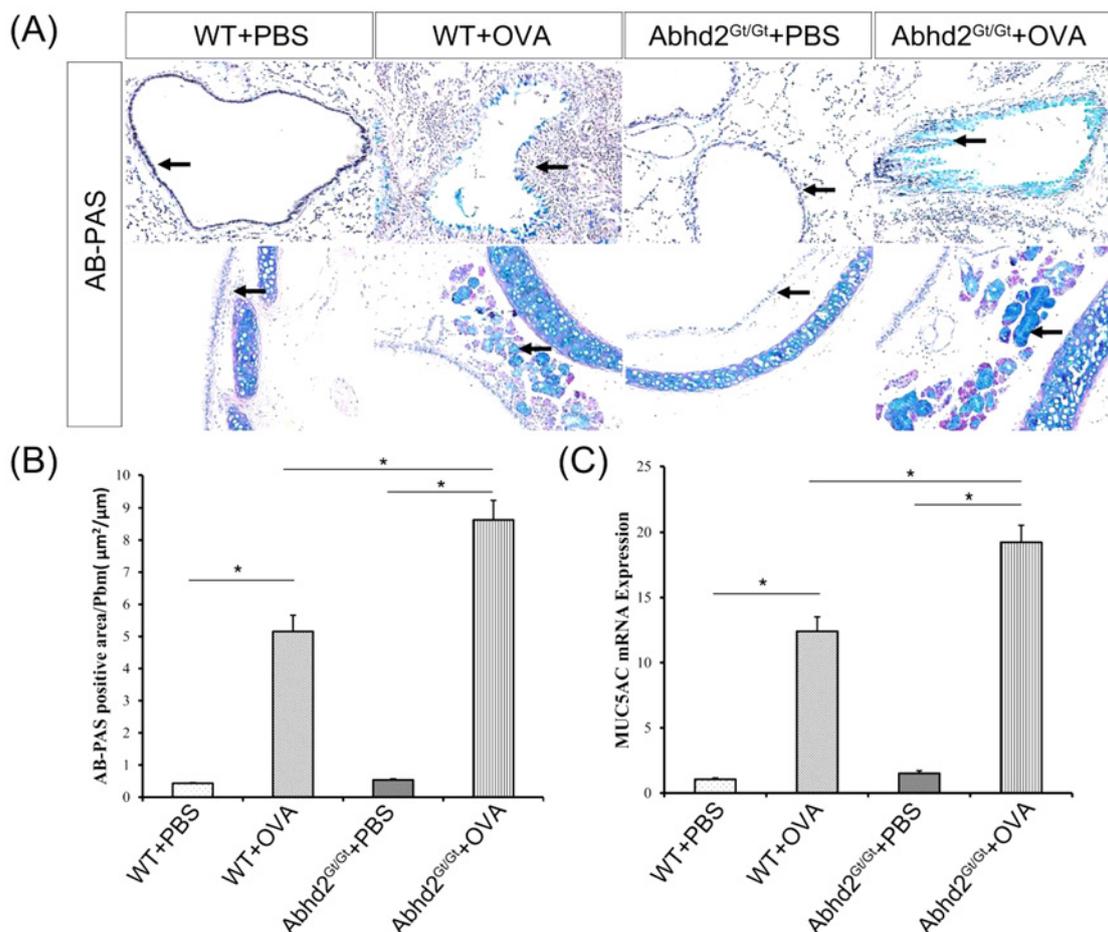


Fig. 4. Effect of *Abhd2* deficiency on goblet cell hyperplasia and airway mucus hypersecretion in OVA-induced airway remodeling mice (A) Representative photomicrographs of PAS-stained lung sections (magnification,  $\times 100$ ). (B) The PAS-positive area per total area was measured. (C) The expression of MUC5AC mRNA in mouse lung tissues was evaluated by RT-qPCR. Graphs display a summary of two to three independent experiments. Data are expressed as the mean  $\pm$  SEM. \*  $p < 0.05$  indicates significant differences.

demonstrated that  $\alpha$ -SMA staining in the OVA group was significantly increased compared to that in the control group. The  $\alpha$ -SMA stained area was significantly increased in the Abhd2<sup>Gt/Gt</sup>+OVA group compared to that in the WT+OVA group. In addition, as shown in Fig. 5E and F, the protein level of  $\alpha$ -SMA measured using western blotting was also markedly upregulated in the OVA group, whereas *Abhd2* deficiency promoted the above increase of  $\alpha$ -SMA expression in the Abhd2<sup>Gt/Gt</sup>+OVA group.

#### *Abhd2* deficiency aggravated airway fibrosis and collagen deposition

To investigate the effect of *Abhd2* deficiency on type I collagen and MMP2 expression, the expression of type I collagen was determined using immunohistochemistry and that of MMP2 using western blotting (Fig. 6). As shown in Fig. 7A, B, E, and F, the protein levels of type I collagen and MMP2 in the OVA group were significantly higher than those in the control group, whereas both type I collagen and MMP2 expres-

sion in the Abhd2<sup>Gt/Gt</sup>+OVA group were significantly higher than those in the WT+OVA group.

The effect of *Abhd2* deficiency on cell proliferation was evaluated by analyzing the expression of TGF- $\beta$ 1 and Ki67 in mice. As shown in Fig. 7A, C, and D, Ki67 protein and TGF- $\beta$ 1 mRNA expression were significantly higher in the WT+OVA group than in the WT+PBS group. However, *Abhd2* deficiency promoted an increase in the above indicators in the Abhd2<sup>Gt/Gt</sup>+OVA group compared with those in the WT+OVA group.

#### *Abhd2* deficiency promoted the activation of phosphor-Akt in an airway remodeling model

The *Akt* signaling pathway is a classical pathway involved in airway remodeling. To determine whether *Abhd2* affects airway remodeling through the *Akt* pathway, western blotting was used to analyze the protein levels of total *Akt* and its phosphorylation level. As illustrated in Fig. 7A and B, we observed that the phosphorylation of *Akt* was significantly elevated

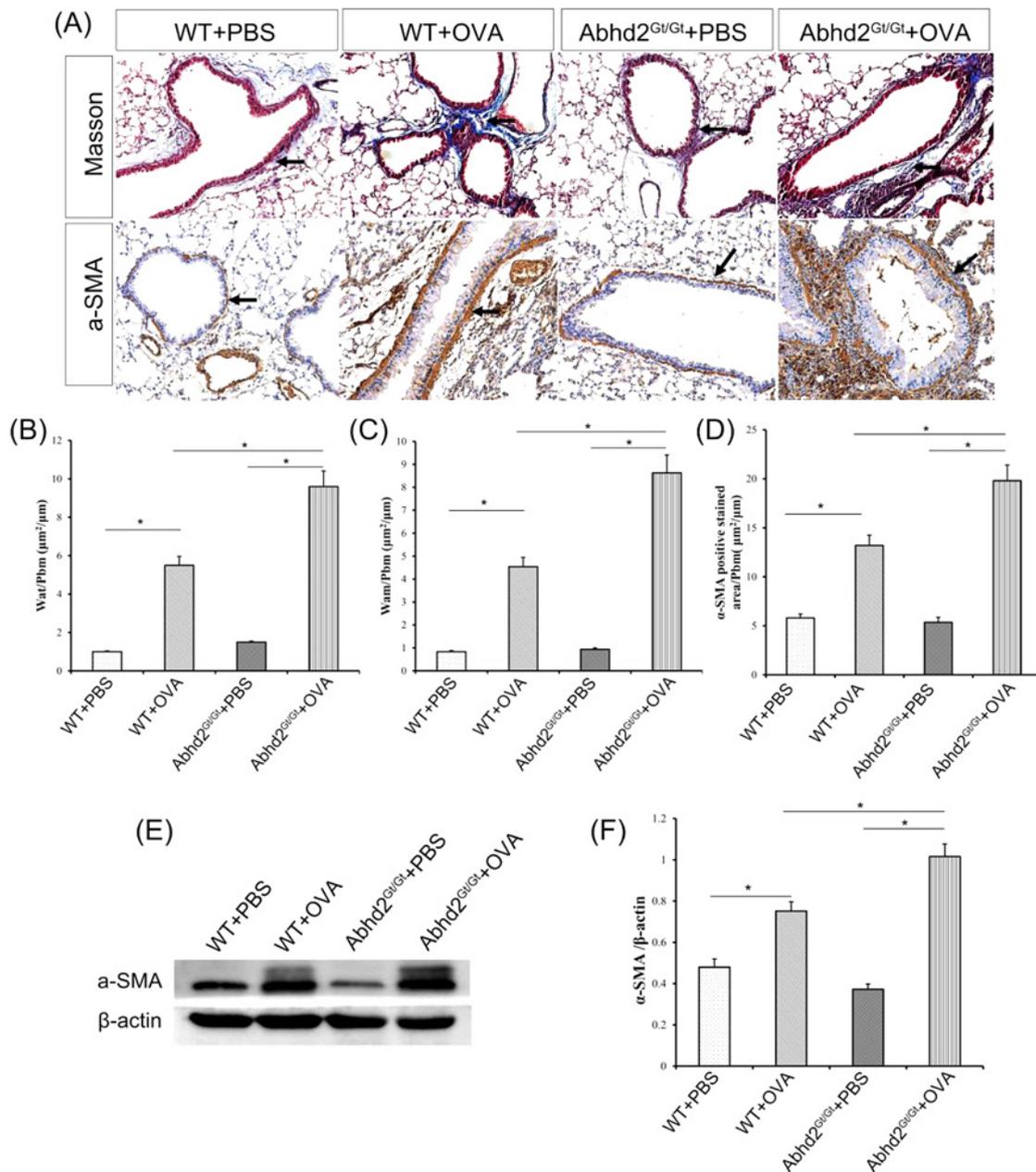


Fig. 5. Effect of *Abhd2* deficiency on histopathological changes in OVA-induced airway remodeling mice (A) Representative photomicrographs of Masson staining and immunohistochemical staining of  $\alpha$ -SMA lung sections (magnification,  $\times 100$ ). The levels of (B) Wat/Pbm and (C) Wam/Pbm were measured using Masson staining. (D) The  $\alpha$ -SMA-positive area per total area was measured. (E) Western blots of  $\alpha$ -SMA in mouse lung tissues. Corresponding densitometric analyses of the protein bands of (F)  $\alpha$ -SMA/ $\beta$ -actin. Graphs display a summary of two to three independent experiments. Data are expressed as the mean  $\pm$  SEM. \*  $p < 0.05$  indicates significant differences.

in OVA-treated mice compared to that in the control group. Subsequently, OVA-stimulated *Abhd2* deficient mice exhibited higher levels of phosphorylated Akt than the WT+OVA group, whereas the total Akt level remained unchanged.

## Discussion

Airway remodeling is the main pathological feature of asthma and COPD, and leads to a progressive decline in lung function and disease progression (Hartley et al. 2016). In the present study, we established an OVA-induced mouse airway remodeling model. Airway inflammation is a primary characteristic of COPD (Nakawah et al. 2013). Excessive inflammatory cell infiltration was shown using HE staining and

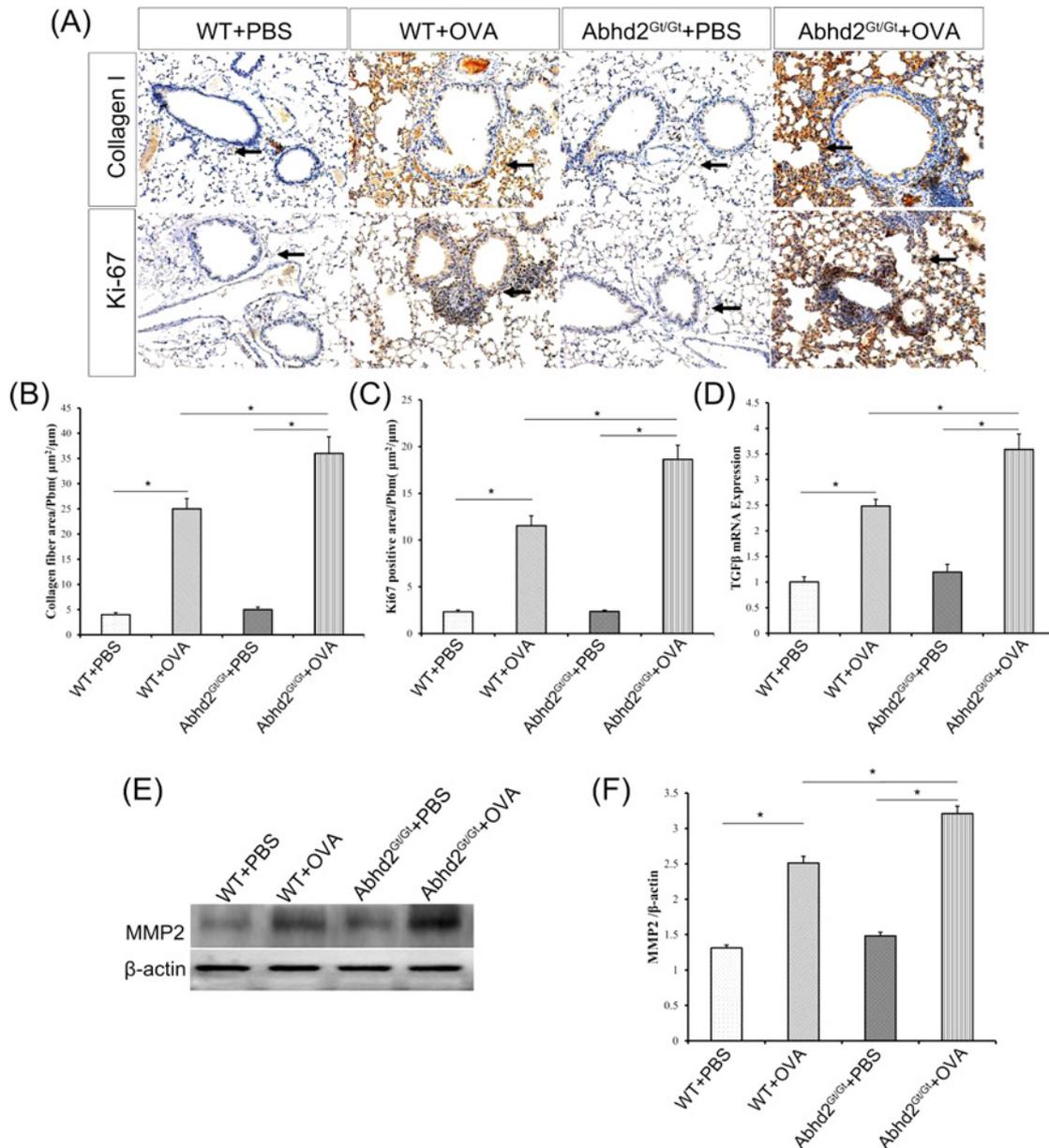


Fig. 6. Effect of Abhd2 deficiency on collagen deposition and cell proliferation in OVA-induced airway remodeling mice (A) Representative photomicrographs of immunohistochemical staining of type I collagen and MMP2 in lung sections (magnification,  $\times 100$ ). The (B) type I collagen and (C) MMP2 positive area per total area were measured. (D) Expression of TGF- $\beta 1$  mRNA in mouse lung tissues were evaluated using RT-qPCR. (E) Western blots of MMP2 in mouse lung tissues. Corresponding densitometric analyses of the protein bands of (F) MMP2/ $\beta$ -actin. Graphs display a summary of two to three independent experiments. Data are expressed as the mean  $\pm$  SEM. \*  $p < 0.05$  indicates significant differences.

excessive inflammatory factor secretion was determined using RT-PCR in Abhd2<sup>Gt/Gt</sup>+OVA airway remodeling mice. The expression levels of IL-1 $\beta$ , IL-6, IL-4, and IL-13 increased significantly and the expression of IFN $\gamma$  was decreased in the Abhd2<sup>Gt/Gt</sup>+OVA group. Infiltrating inflammatory cells, including neutrophils, macrophages, T lymphocytes, and abnormally secreted inflammatory factors in the airway may lead to airway remodeling by promoting airway smooth muscle proliferation, extracellular matrix metalloproteinase overexpression, and collagen deposition

(Gueders et al. 2006). IL-1 $\beta$  is a pro-inflammatory cytokine that promotes type 2-low neutrophilic asthma (Woodruff et al. 2009). IL-4 and IL-13 secreted by Th2 cells are central mediators of asthma (Hinks et al. 2021). IL-6 is a biomarker of systemic inflammation that is increased in the serum and airways of patients with asthma (Neveu et al. 2010). IFN- $\gamma$  can improve the symptoms of allergic asthma by promoting the secretion of IL-27 (Mei et al. 2019). Abhd2 deficiency aggravates the degree of this airway inflammation by regulating the expression of IL-1 $\beta$ , IL-6, IL-4,

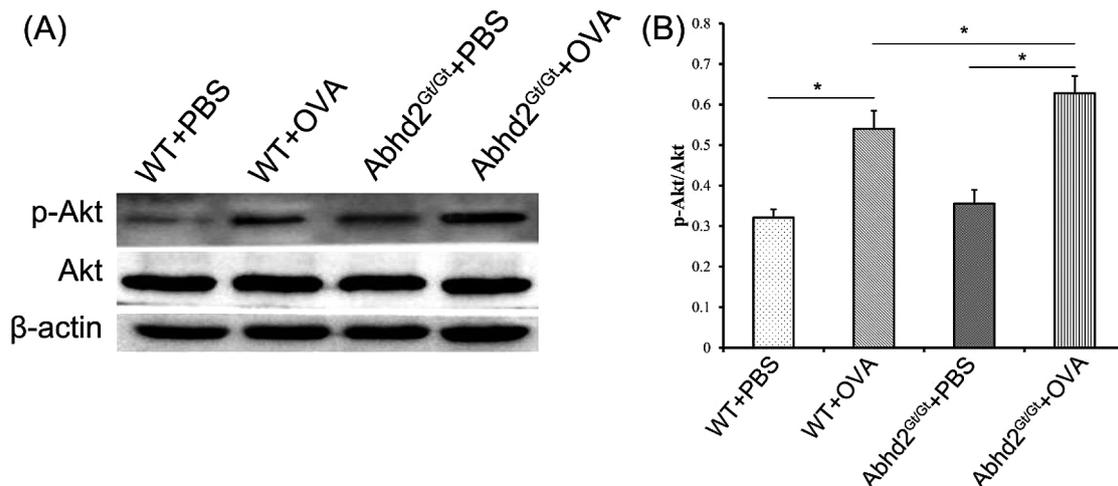


Fig. 7. Western blot analysis of the protein expression levels of p-Akt and Akt in mouse lung tissues. (A) Western blots of p-Akt and Akt in mouse lung tissues. Corresponding densitometric analyses of the protein bands of (B)p-Akt/ Akt. Graphs display a summary of two to three independent experiments. Data are expressed as the mean  $\pm$  SEM. p, phosphorylated. \*  $p < 0.05$  indicates significant differences.

IL-13, and IFN- $\gamma$ . Additionally, *Abhd2* deficiency increases the thickening of the airway basement membrane and promoted the overdeposition of collagen around the airway wall in a mouse model of OVA-induced airway remodeling.

Airway smooth muscle remodeling is an important factor influencing the severity of COPD and asthma in the pathology of airway remodeling (Damera et al. 2009). Overexpression of  $\alpha$ -SMA is a hallmark of airway smooth muscle remodeling (Jin et al. 2009, Matoba et al. 2018). In the present study, we analyzed  $\alpha$ -SMA expression to examine the effects of *Abhd2* deficiency on airway remodeling. Compared to the control group,  $\alpha$ -SMA expression was upregulated in the OVA group. This result shows that *Abhd2* deletion promoted OVA-induced upregulation of  $\alpha$ -SMA.

Airway subepithelial fibrosis and collagen deposition are the main features of airway remodeling. MMPs are the major rate-limiting enzymes that regulate the extracellular matrix metabolism (Wang et al. 2018). MMP2; in particular, they regulate multiple collagen degradations and subepithelial fibrosis in the airways. These results suggest that *Abhd2* deficiency promotes the OVA-induced upregulation of MMP and type I collagen. We also investigated the effect of *Abhd2* deficiency on cell proliferation ability by evaluating the expressions of TGF- $\beta$ 1 and Ki67 in mice. It is well known that TGF- $\beta$ 1 has a crucial role in airway remodeling by inducing fibroblast proliferation and differentiation into myofibroblasts, and Ki67 is one of the representative proliferation markers extensively used to evaluate the proliferation ability of cells (Evasovic et al. 2019, Lanng et al. 2019). Clinically, Ki67 is primarily used to label proliferating cells (Joyce et al. 1996). In the present study, *Abhd2* deficiency enhanced

the expression of TGF- $\beta$ 1 and Ki67 in mice with OVA-induced airway remodeling.

The PI3K/Akt signaling pathway plays a crucial role in the pathological process of airway remodeling in COPD and asthma (Gutor et al. 2022). It is activated by inflammatory factors such as IL-1 $\beta$ , IL-6 and TGF- $\beta$  (Li et al. 2021). The Akt phosphorylation is a hallmark of PI3K activation (Revathidevi et al. 2019). The PI3K/Akt pathway is broadly involved in a range of cellular processes such as cell differentiation and proliferation, inflammation, metabolism, and apoptosis (Miricescu et al. 2021). In the present study, we found that the *Abhd2* deficiency group showed higher protein expression of p-Akt, suggesting that *Abhd2* deficiency promotes airway remodeling and may result from enhanced activity of the p-Akt pathway. However, a limitation of this study is that the effect of *Abhd2* on the PI3K/Akt signaling pathway was not evaluated *in vitro*, and whether *Abhd2* affects the PI3K/Akt signaling pathway in lung structural cells was not evaluated.

In conclusion, this study demonstrates that *Abhd2* deficiency promotes OVA-induced airway remodeling and may exacerbate airway remodeling and inflammation through the PI3K/AKT pathway. Our findings provide new experimental evidence that *Abhd2* is a potential candidate for future airway remodeling therapy.

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