ORIGINAL RESEARCH

# Regulator of G-Protein Signaling 2 Repression Exacerbates Airway Hyper-Responsiveness and Remodeling in Asthma

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## Abstract

G protein–coupled receptors (GPCRs) are important regulators of cell functions in asthma. We recently reported that regulator of G-protein signaling (RGS) 2, a selective modulator of $G_q$-coupled GPCRs, is a key regulator of airway hyper-responsiveness (AHR), the pathophysiologic hallmark of asthma. Because RGS2 protein levels in airway cells were significantly lower in patients with asthma compared with patients without asthma, we further investigated the potential pathological importance of RGS2 repression in asthma. The human *RGS2* gene maps to chromosome 1q31. We first screened patients with asthma for *RGS2* gene promoter single-nucleotide polymorphisms (SNPs) and found significant differences in the distribution of two RGS2 SNPs (A638G, rs2746071 and C395G, rs2746072) between patients with asthma and nonasthmatic subjects. These two SNPs are always associated with each other and have the same higher prevalence in patients with asthma (65%) as compared with nonasthmatic subjects (35%). Point mutations corresponding to these SNPs decrease RGS2 promoter activity by 44%. The importance of RGS2 down-regulation was then determined in an acute IL-13 mouse model of asthma. Intranasal administration of IL-13 in mice also decreased RGS2 expression in lungs by ~50% and caused AHR. Although naive RGS2 knockout (KO) mice exhibit spontaneous AHR, acute IL-13 exposure further increased AHR in RGS2 KO mice. Loss of RGS2 also significantly enhanced IL-13–induced mouse airway remodeling, including peribronchial smooth muscle thickening and fibrosis, without effects on goblet cell hyperplasia or airway inflammation in mice. Thus, genetic variations and increased inflammatory cytokines can lead to RGS2 repression, which exacerbates AHR and airway remodeling in asthma.

**Keywords:** asthma; airway hyper-responsiveness; airway remodeling; RGS2; polymorphisms

Airway hyper-responsiveness (AHR) is exaggerated constriction of the airways in response to various bronchoconstrictor stimuli, including G protein–coupled receptor (GPCR) agonists such as acetylcholine and histamine (1). It is a key diagnostic criterion of asthma, and decreased AHR is associated with improved asthma control (2). AHR is due in part to hyper-reactivity of airway smooth muscle (ASM), but other factors, including airway remodeling, contribute significantly (2–5). Airway remodeling typically includes thickening of the subepithelial extracellular matrix due to increased deposition of

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## Clinical Relevance

This study shows for the first time an association between *regulator of G-protein signaling (RGS) 2* gene polymorphisms and asthma. It also suggests that both genetic variations and increased inflammatory cytokines can contribute to pulmonary RGS2 repression, which may increase the risk of developing airway hyper-responsiveness and remodeling, key pathologic features of severe asthma. Thus, increased RGS2 expression could provide a new therapy for treatment of severe asthma.

collagens and other extracellular matrix proteins, increased airway smooth muscle mass, and excessive mucus production from goblet cells (6–8). These pathologic features lead to the airway wall thickening and luminal narrowing that characterize the chronic irreversible AHR of severe asthma (7, 8). Currently, antiinflammatory glucocorticoids and bronchodilator $\beta_2$-adrenoceptor agonists are the most important therapies for treating patients with asthma (9). Although glucocorticoids can potentially slow the progression of airway remodeling in asthma, there are no treatments that can reverse airway remodeling. Thus, elucidating the mechanisms underlying airway remodeling may reveal new therapeutic targets.

GPCRs are important regulators of multiple cell types involved in asthma (10). Numerous agonists for bronchoconstrictor $G_q$-coupled GPCRs are present or up-regulated in the airway during allergic inflammation (11, 12). Regulator of G-protein signaling (RGS) proteins act just downstream from the point of GPCR signaling convergence, such that one type of RGS protein can potentially inhibit responses from multiple types of GPCRs acting through the same signaling cascade (13). Druey and Panettieri have shown that deletion of the RGS5 gene led to enhanced constriction of mouse lung slices (14) and promoted AHR in mice (15). Panettieri also reported that increased RGS4 in ASM correlates with disease severity in asthma and that RGS4 stimulates ASM cell proliferation but inhibits bronchoconstriction (16). We and others recently reported that RGS2, a selective inhibitor of $G_q$-coupled GPCR signaling (17), is a key regulator of AHR and that RGS2 knockout (KO) mice exhibit spontaneous AHR (18, 19). RGS2 protein levels in airway cells were significantly lower in subjects with asthma compared with subjects without asthma (18) and could be synergistically increased by corticosteroids plus $\beta_2$-adrenoceptor agonists (19). However, how RGS2 is down-regulated and whether RGS2 repression contributes to the development of AHR and airway remodeling in asthma remains unknown.

In the present study, we investigated the mechanisms and potential pathological importance of RGS2 repression in asthma. RGS2 gene polymorphisms have been identified and may reduce RGS2 expression in hypertensive patients (20, 21). We found a significant increase in the distribution of two single-nucleotide polymorphisms (SNPs) in the promoter region of the RGS2 gene in patients with asthma as compared with nonasthmatic subjects. Point mutations corresponding to these SNPs decreased RGS2 promoter activity by 44%, suggesting that RGS2 may be genetically repressed in some patients with asthma. We also examined whether the T helper type (Th) 2 inflammatory cytokine IL-13, which is necessary and sufficient to induce many pathologic features of asthma (22–24), could affect RGS2 expression. We found that intranasal administration of IL-13 in mice decreased RGS2 expression in lungs by ~50%. Thus, our results suggest that both genetic variations and inflammatory cytokines can lead to RGS2 repression. The loss of RGS2 expression increases susceptibility of mice to develop AHR and airway remodeling associated with chronic severe asthma. Further investigation of the molecular mechanisms of RGS2 repression in asthma could identify new approaches to target RGS2 expression in airways to ameliorate AHR and airway remodeling in human asthma.

## Materials and Methods

### Selection of Subjects and Sequence Analysis of the RGS2 Gene

The study was approved by the Creighton University Institutional Review Board as previously described (18). We enrolled 16 normal subjects without history of asthma as controls and 16 treatment-naive subjects with mild-to-moderate asthma and significant AHR. Genomic DNA was extracted from monocytes using DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Genomic DNA samples of 24 subjects with asthma and 24 nonasthmatic subjects were purchased from BioServe Biotechnologies (Beltsville, MD). The two RGS2 gene SNPs (SNP1, A638G, rs2746071 and SNP2, G395C, rs2746072) in these DNA samples were genotyped by sequence analysis.

### Luciferase Reporter Constructs and Assays

The human RGS2 promoter fragment (−757/−1) was amplified and cloned into pGL3-Basic luciferase reporter vector from human genomic DNAs as described (25). Point mutations corresponding to SNP1 and SNP2 were generated using a QuikChange mutagenesis kit (Stratagene, La Jolla, CA). RGS2 promoter activity was assayed in human ASM cells using the dual luciferase assay kit (Promega, Fitchburg, WI) (25).

### Acute IL-13 Murine Model of Asthma

All studies were approved by the Institutional Animal Care and Use Committee at Creighton University and were in accordance with the GlaxoSmithKline Policy on the Care, Welfare and Treatment of Laboratory Animals. Anesthetized wild-type (WT) and RGS2 KO C57BL/6J mice (18) were administered intranasal saline or IL-13 (5 μg/d) for 3 days as described previously (26).

### Measurement of AHR

Mice were ventilated at a breathing rate of 140 breaths/min, and the lung resistance ($R_L$) and dynamic compliance ($C_{dyn}$) responses to increasing concentrations of nebulized methacholine (0–50 mg/ml) were recorded using a FinePointe system (Buxco, St. Paul, MN) (18, 26).

### Inflammatory Cell Numbers and Cytokine Levels in Bronchoalveolar Lavage Fluid

Bronchoalveolar lavage (BAL) fluid was collected by lavaging mouse lungs with 1 ml saline twice via a tracheal catheter (27). Leukocytes in the BAL fluid were stained and counted under a microscope (27). Cytokines/chemokines in the BAL fluid were measured using a Mouse Cytokine/Chemokine

Magnetic Bead Panel Immunoassay Kit (Millipore, Billerica, MA). Active and total TGF-β1 levels were measured using TGF-β1 Single Plex Kit (Millipore) before and after acidification of the BAL fluid.

### Staining and Histopathology of Lung

Parasagittal sections of paraffin-embedded mouse lung were immunostained for α–smooth muscle actin (18), trichrome stained for collagen, hematoxylin and eosin stained for inflammatory infiltrate, and periodic acid–Schiff (PAS) stained for mucus-secreting goblet cells (28).

### Measurement of RGS2 Protein Expression and Collagen Deposition in Mouse Lung

Mouse lung was minced in liquid nitrogen. RGS2 protein levels in minced lung tissue were analyzed by Western blot as described (18). The amount of collagen in minced lung tissue was measured using a Sirius red total collagen detection kit (Chondrex, Redmond, WA) according to the manufacturer's instructions. A collagen standard was used for calibration.

### Data Analysis

Data are expressed as means ± SE. Groups were compared using Student's *t* test for unpaired observations or two-way ANOVA with the Bonferroni correction where there were multiple comparisons. A probability level of $P < 0.05$ was statistically significant.

## Results

### RGS2 Gene Polymorphisms Repress Its Promoter Activity

We screened patients with asthma for sequence variations in the RGS2 gene promoter regions and found significant differences in distribution of two SNPs (SNP1, A638G, rs2746071 and SNP2, G395C, rs2746072) between patients with asthma and nonasthmatic subjects (Figures 1A and 1B). These two SNPs are associated with each other. In 40 nonasthmatic subjects, only 14 samples (35%) had both SNP1 and SNP2, versus 26 samples (65%) in 40 patients with asthma ($P = 0.0073$). All SNPs found in nonasthmatic subjects are heterozygous (A/G in SNP1 and G/C in SNP2) and carry the WT allele. In contrast, three patients with asthma were found to carry homozygous mutants (G/G in SNP1 and C/C in SNP2). The single point mutation corresponding to SNP1 decreased the RGS2 promoter activity by ~27%, whereas double mutations corresponding to SNP1 and SNP2 decreased the RGS2 promoter activity by 44% ($P < 0.01$) (Figure 1C).

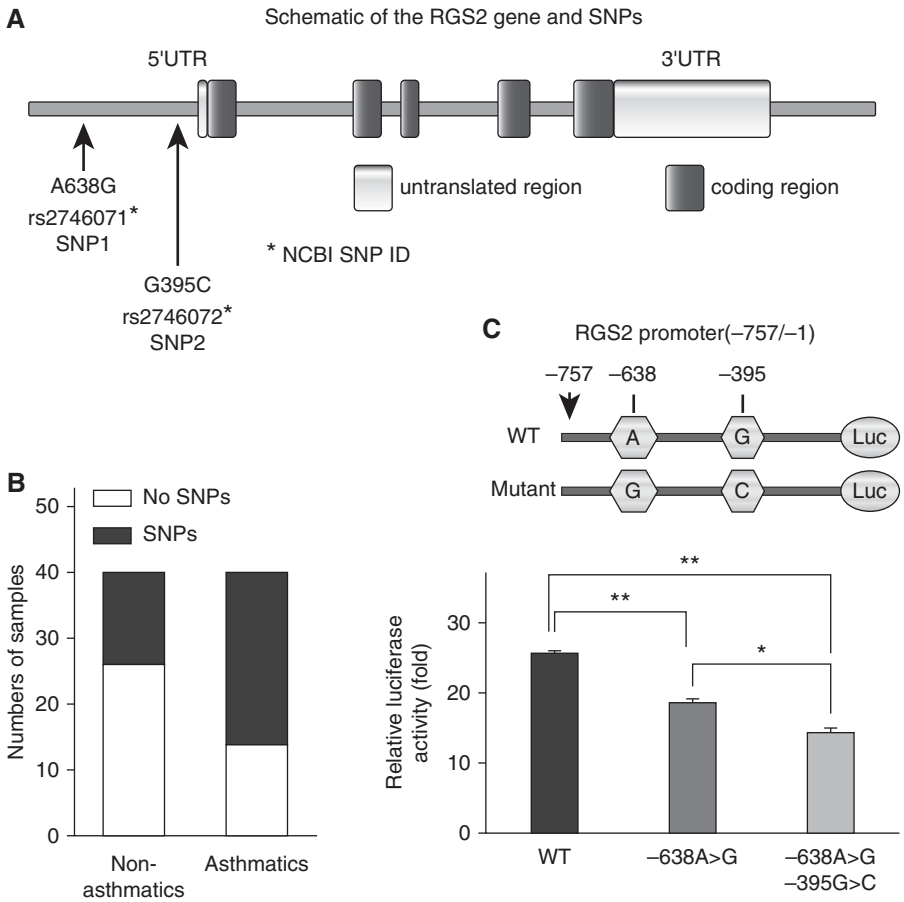


**Figure 1.** Polymorphisms and promoter activity of the *regulator of G-protein signaling (RGS) 2* gene in patients with asthma. (*A*) Diagram of the *RGS2* gene and single-nucleotide polymorphisms (SNPs). (*B*) Association of RGS2 polymorphisms with asthma. Two common SNPs, SNP1 (A638G) and SNP2 (G395C), were genotyped in 40 nonasthmatic subjects and 40 patients with asthma. Data were analyzed using a $\chi^2$ test, which gave a *P* value of 0.0073. (*C*) *RGS2* gene polymorphisms repress promoter activity. Human airway smooth muscle (ASM) cells were transfected with pGL3-luciferase (Luc) plasmids driven by SV-40 or the RGS2 wild-type (WT) or mutant promoter corresponding to SNP1 and SNP2. Results are presented as fold increase compared with pGL3 containing a SV-40 promoter. Data are means ± SE of three independent experiments in triplicate done in two primary ASM cell lines. $^*P < 0.05$ and $^{**}P < 0.01$ between groups. NCBI, National Center for Biotechnology Information; UTR, untranslated region.

### Acute IL-13 Exposure Down-Regulated Lung RGS2 Expression and Caused AHR in WT Mice

Increased IL-13 plays a central role in the development of AHR in allergic asthma. Intranasal administration of murine IL-13 into WT mice (Figure 2A) decreased RGS2 protein expression in mouse lung tissue by ~50% (Figure 2B, *inset*) and caused AHR, as reflected by higher $R_L$ (Figure 2B) and lower $C_{dyn}$ (Figure 2C).

### Loss of RGS2 Increased Susceptibility of Mice to Develop AHR

RGS2 KO mice exhibited spontaneous AHR as compared with WT mice, consistent with our previous report (18). Loss of RGS2 also exacerbated IL-13–induced AHR, reflected by further augmented $R_L$ and reduced $C_{dyn}$ in RGS2 KO mice as compared with WT mice (Figures 2B and 2C).

### Loss of RGS2 Did Not Alter IL-13–Induced Airway Inflammation in Mice

An increase in total cells and eosinophils was observed in WT and RGS2 KO mice after IL-13 treatment ($P < 0.01$) (Figures 3A and 3B). However, no differences were

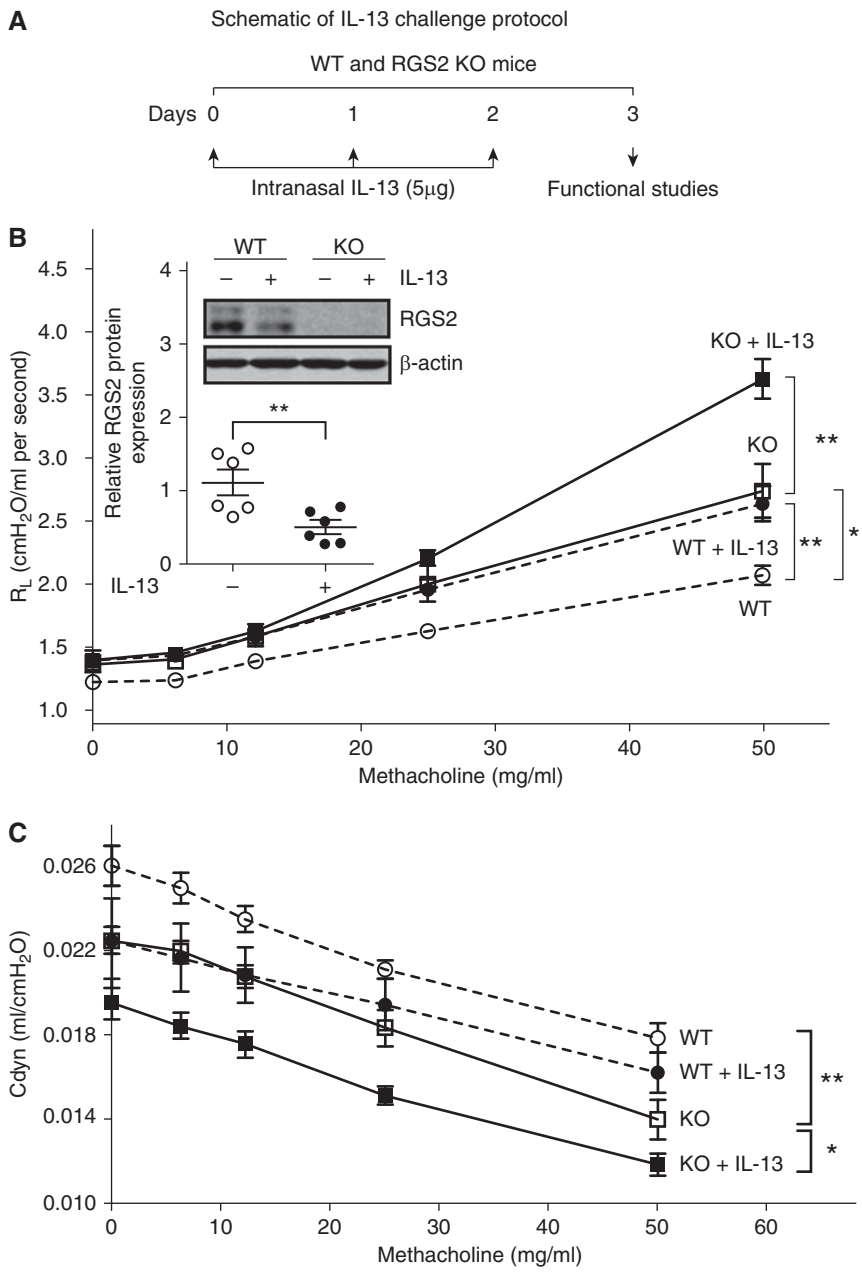


**Figure 2.** Loss of RGS2 increases susceptibility of mice to acute IL-13–induced airway hyperresponsiveness (AHR). (*A*) Diagram of IL-13 challenge protocol. AHR was determined by direct measurement of lung resistance ($R_L$) and dynamic compliance ($C_{dyn}$) using a Buxco FinePointe apparatus. Changes in $R_L$ (*B*) and $C_{dyn}$ (*C*) of WT (*circle*) and RGS2 knockout (KO) (*square*) mice without (*open circle* or *open square*) or with IL-13 challenge (*solid circle* or *solid square*) in response to increasing concentrations of nebulized methacholine. Data are mean ± SE ($n$ = 8–11 mice per group). *$P < 0.05$ and **$P < 0.01$ between groups. *Inset*: Representative Western blot of RGS2 protein expression in lung homogenates of WT and RGS2 KO mice without or with IL-13 challenge, analyzed by measuring band density and normalized by β-actin. Data are means ± SE ($n$ = 6 mice per group). **$P < 0.01$ between WT mice without and with IL-13 challenge.

observed in total cells or eosinophils in BAL fluid between WT and RGS2 KO mice. Hematoxylin and eosin staining of lung sections showed similar peribronchial and perivascular inflammatory infiltrates in both groups of mice after IL-13 treatment (Figure 3C). In addition, WT and RGS2 KO mice showed significant and comparable elevations in total and active TGF-β1 in the BAL fluid after IL-13 treatment, whereas no changes were observed in levels of IL-2, IL-4, IL-5, or IL-12 (Figure 3D). The total TGF-β1 levels in mouse lung homogenates were also measured. Again, IL-13 caused equal increase in lung TGF-β1 levels in WT and RGS2 KO mice (*see* Figure 1 in the online supplement).

### Loss of RGS2 Exacerbated IL-13–Increased Peribronchial Smooth Muscle Thickness but Not Goblet Cell Numbers in Mice

The area of peribronchial α–smooth muscle actin immunostaining in RGS2 KO mice was significantly greater than in WT mice (2.1 ± 0.1 versus 0.9 ± 0.1 $\mu m^2/\mu m$ circumference of bronchiole; $P < 0.01$) (Figure 4A). IL-13 treatment increased the thickness of the peribronchial smooth muscle layer in WT mice (0.9 ± 0.1 versus 1.6 ± 0.1; $P < 0.05$) and in RGS2 KO mice (2.1 ± 0.1 versus 4.5 ± 0.2; $P < 0.01$) (Figure 4A). Lung sections of WT and RGS2 KO mice exposed to IL-13 showed an increased number of PAS-positive epithelial cells (goblet cells) compared with that in control animals (Figure 4B). However, the percentage of PAS-positive epithelial cells observed in lung sections of IL-13–treated RGS2 KO mice (38.9 ± 1.6%) was similar to that of IL-13–treated WT mice (41.4 ± 2.6%).

### Increased Subepithelial Fibrosis and Collagen Deposition in RGS2 KO Mice

The area of peribronchial trichrome staining for collagen was significantly larger in RGS2 KO mice compared with WT mice (11.0 ± 0.8 versus 4.5 ± 0.4 $\mu m^2/\mu m$ circumference of bronchiole; $P < 0.01$) (Figures 5A and 5B). IL-13 increased the area of peribronchial trichrome staining in both WT and RGS2 KO mice, but the area of trichrome staining in IL-13–treated RGS2 KO mice was still significantly greater than in IL-13–treated WT mice (23.3 ± 2.1 versus 11.4 ± 1.1; $P < 0.01$). Total lung collagen assays showed increased lung collagen in RGS2 KO mice compared with WT mice (115.4 ± 12.7 versus 38.3 ± 6.5 μg per 100 mg of lung tissue; $P < 0.01$) (Figure 5C). This elevated collagen was further increased to 329.6 ± 23.6 μg per 100 mg of lung tissue after IL-13 treatment. In contrast, lung collagen in IL-13–treated WT mice was only 105.8 ± 12.1 μg per 100 mg of lung tissue.

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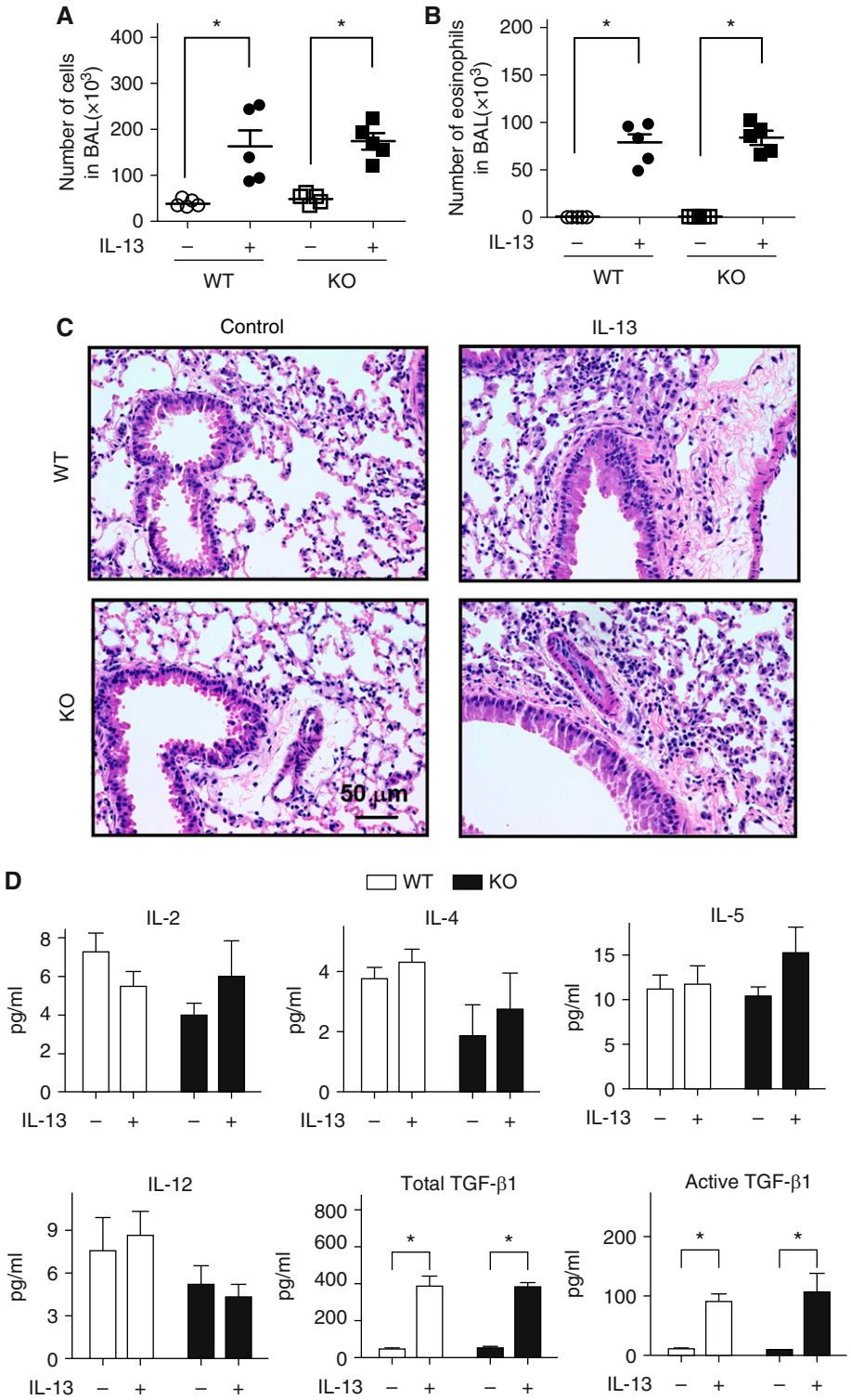


**Figure 3.** Loss of RGS2 does not alter IL-13–induced airway inflammation in mice. Total inflammatory cell numbers (*A*) and eosinophils (*B*) in bronchoalveolar lavage (BAL) of WT and RGS2 KO mice without or with IL-13 challenge were counted ($n = 5$ mice per group). (*C*) Hematoxylin and eosin–stained lung sections from control and IL-13–treated WT and RGS2 KO mice. (*D*) Levels of cytokine IL-2, IL-4, IL-5, IL-12, and total and active transforming growth factor (TGF)-β1 in the BAL of WT and RGS2 KO mice without or with IL-13 challenge. Data are means ± SE ($n = 5$ mice per group). *$P < 0.01$ between groups.

## Discussion

We previously reported that expression of RGS2 was reduced in airway cells of human asthma patients and that naive RGS2 KO mice spontaneously display AHR, supporting RGS2 as a key regulator of AHR (18). RGS2 gene polymorphisms have been identified in hypertensive patients and might reduce RGS2 expression (20, 21). The human RGS2 gene maps to chromosome 1q31 (29) and SNPs at this region were strongly associated with asthma in children (30). Thus, we first screened patients with asthma for RGS2 gene promoter SNPs and found significant differences in the distribution of two RGS2 SNPs (A638G, rs2746071 and C395G, rs2746072) between patients with asthma and nonasthmatic subjects. These two SNPs are always associated with each other, and both have a 1.8-fold higher prevalence in patients with asthma (65%) as compared with nonasthmatic subjects (35%). Although an association of these two RGS2 SNPs with suicide in the Japanese population has been reported (31), to our knowledge, our study is the first report of an association between RGS2 gene polymorphisms and asthma. The point mutation corresponding to SNP1 decreased RGS2 promoter activity, and additional mutation corresponding to SNP2 further decreased RGS2 promoter activity. Thus, both of these SNPs might contribute to repression of the RGS2 gene expression seen in patients with asthma. Furthermore, because heterozygous RGS2 KO mice exhibit spontaneous AHR (19), partial repression of RGS2 due to its genetic variations in patients should be sufficient to trigger AHR in the absence of inflammation, which could contribute to non–Th2-dependent asthma development (32). A limitation of these findings is that the sample size used for the genetic association study was relatively small. We are currently collecting more patient samples to further investigate the regulation of RGS2 gene expression by polymorphisms.

The Th2 inflammatory cytokine IL-13 is necessary and sufficient to induce many pathologic features of asthma (22–24). We previously showed that targeting downstream effectors of GPCRs attenuated IL-13–induced AHR in mice (26). We found that intranasal administration of

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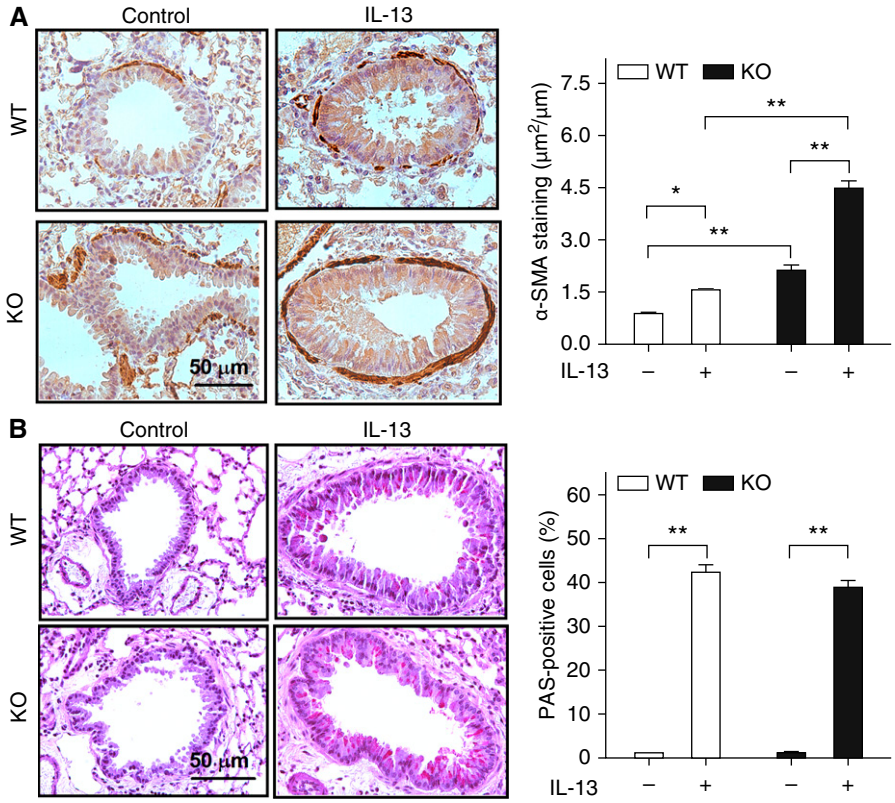


**Figure 4.** Loss of RGS2 exacerbates IL-13–increased peribronchial smooth muscle thickness but not goblet cell numbers in mice. (*A*) Representative α-smooth muscle actin (α-SMA) staining images of ASM cells in WT and RGS2 KO mice without (Control) or with IL-13 challenge (*left*). The area of α-SMA staining was expressed per μm length of basement membrane of bronchioles (*right*). (*B*) Representative periodic acid–Schiff (PAS) staining images for airway goblet cells (*left*). Results are expressed as percentage of PAS-positive cells per bronchiole (*right*), calculated from the number of PAS-positive cells divided by the number of epithelial cells. Data are means ± SE (15 bronchioles from five mice). $^{*}P < 0.05$ and $^{**}P < 0.01$ between groups.

IL-13 decreased RGS2 expression in mouse lungs by ∼50% and caused AHR in mice *in vivo*. The expression of RGS2 is known to be dynamically regulated and responsive to the cell's microenvironment (33–35). We previously reported that RGS2 was also selectively down-regulated in lung tissues of an ovalbumin-sensitized/challenged murine model of asthma (18). Because RGS2 functions as a key negative regulator of AHR (18, 19), it is likely that down-regulation of RGS2 is one mechanism underlying the development of AHR in IL-13 and ovalbumin models of asthma. Thus, our study suggests that increased Th2 cytokines such as IL-13 might contribute to the repression of RGS2 gene expression seen in patients with asthma.

The pathological importance of RGS2 down-regulation was determined in an acute IL-13 mouse model of asthma. Consistent with our previous report (18), naive RGS2 KO mice exhibit spontaneous AHR. However, *in vivo* IL-13 treatment caused additional AHR in RGS2 KO mice, which was much higher than that of IL-13–treated WT mice. Because there were no differences in inflammatory cell numbers or key Th1 and Th2 cytokines in the BAL and lung tissue of RGS2 KO mice compared with WT mice, augmented airway inflammation is unlikely to be a major contributor to increased AHR in RGS2 KO mice. This caused us to speculate that the loss of RGS2 may augment other AHR-promoting factors.

Airway wall remodeling, including goblet cell hyperplasia in airway epithelium, increased peribronchial ASM mass, and airway fibrosis, has been recognized as an important factor contributing to AHR and asthma severity. We found that IL-13 markedly increased airway mucus-producing goblet cells in mice, as reported by others (36). However, no differences were observed between WT and RGS2 KO mice, suggesting that goblet cell hyperplasia is unlikely to be a major contributor to augmented AHR in IL-13–treated RGS2 KO mice. We previously showed that loss of RGS2 enhanced mitogenic stimulation of ASM growth in culture (18). Consistent with these *in vitro* data, the thickness of the peribronchial smooth muscle layer in RGS2 KO mice was much greater than in WT mice. IL-13 treatment of WT mice caused an increase in thickness of the peribronchiolar smooth muscle layer to a level comparable to that in untreated RGS2 KO mice. Moreover, IL-13 treatment of RGS2 KO mice caused an approximate doubling in size of the smooth muscle layer from its already elevated baseline. Thus, loss of RGS2 further increases IL-13–induced airway smooth muscle remodeling, which may contribute to augmented AHR observed in IL-13–treated RGS2 KO mice.

Subepithelial fibrosis, the result of deposition of extracellular matrix, primarily collagens, also contributes to airway remodeling and AHR (36, 37). Indeed, treatment of mice with IL-13 increased the subepithelial collagen deposition in WT and RGS2 KO mice to the same extent (3-fold) above the control deposition. However, with or without IL-13 treatment, the amount of collagen deposition in lungs of RGS2 KO mice was much greater than that of WT mice, suggesting that loss of RGS2 increases subepithelial fibrosis in lungs of mice. In addition, loss of RGS2 reduced basal airway compliance and augmented IL-13–induced reduction of airway compliance, an indication of reduced airway distensibility, presumably due to increased collagens. This result is consistent with the clinical observation that decreased airway compliance correlates inversely with increases in subepithelial fibrosis in patients with asthma (38–40). The increase in airway fibrosis observed in IL-13–treated RGS2 KO mice is most likely attributable to increased fibroblast activation and differentiation. A previous study suggested that RGS2 functions as a negative regulator of angiotensin II–induced fibroblast responses in the myocardium (41). By analogy, it seems likely that loss of RGS2 could augment pulmonary fibroblast responses, leading to exacerbated airway fibrosis and AHR in mice, but this has yet to be proved.

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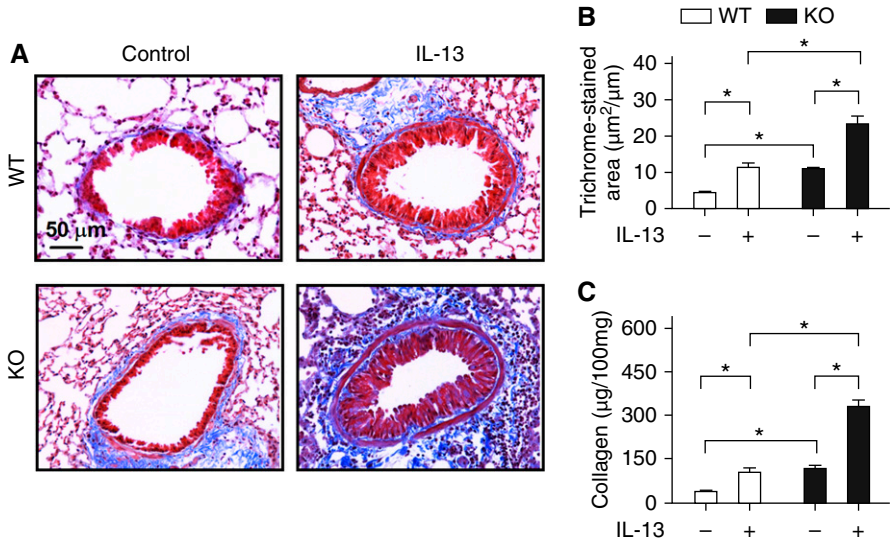


**Figure 5.** Loss of RGS2 increases IL-13–induced peribronchial fibrosis in mice. (*A*) Representative trichrome staining images for collagen in parasagittal sections of lung from WT and RGS2 KO mice without (Control) or with IL-13 challenge. (*B*) The area of trichrome staining was expressed per micrometer length of basement membrane of bronchioles. Data are means ± SE (35 bronchioles from five mice). (*C*) Total lung collagen content was measured by the Sirius red collagen assay. Results are expressed as micrograms of collagen per 100 mg of lung tissue. Data are means ± SE (five lungs from five mice). $^*P < 0.01$ between groups.

AHR and airway remodeling are key factors associated with a decline in lung function in patients with severe asthma, but the relationship between AHR and airway remodeling is poorly defined. Our data suggest that a combination of increased smooth muscle mass and fibrosis most likely explains the augmented AHR in RGS2 KO mice exposed to IL-13. Loss of RGS2 therefore increases susceptibility of mice to development of key features of severe asthma, suggesting the pathological importance of RGS2 repression in the development and progression of asthma. Thus, targeting RGS2 expression could be an attractive therapeutic approach to attenuate the development of AHR, airway smooth muscle hyperplasia, and airway fibrosis in patients with severe asthma. Our study also shows that genetic variations can lead to the RGS2 repression seen in patients with asthma. Asthma has been the focus of a sizeable number of genetic studies (42, 43). Our study identified a significant increase of two common SNPs in the RGS2 gene in patients with asthma, which could reduce RGS2 gene expression. Further clinical investigation could lead to development of these two SNPs of RGS2 as a novel biomarker for early identification of patients who have increased risk to develop AHR and asthma. In addition, current therapies for treating patients with asthma, including $\beta_2$-adrenoceptor agonists and corticosteroids, target the hypercontractile and inflammatory components of asthma. RGS2 regulates ASM contraction and loss of RGS2 leads to hypercontractility of ASM cells (18, 19). Our study is the first to suggest that increases in the Th2 cytokine IL-13 can down-regulate pulmonary RGS2 expression *in vivo*. Because "Th2-mediated" airway inflammation is a major feature of many human patients with asthma, our studies provide a new and exciting link among airway inflammation, hypercontractility, and remodeling seen in chronic persistent asthma. Furthermore, understanding the mechanisms and specific cell types (i.e., airway smooth muscle, epithelium, or fibroblasts) in which Th2-ctyokines down-regulate RGS2 expression could provide new avenues for the treatment of patients with chronic persistent asthma. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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