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Impaired nuclear translocation of the glucocorticoid receptor in corticosteroid-insensitive airway smooth muscle in severe asthma

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Abstract

Rationale—Patients with severe asthma (SA) are less responsive to the beneficial effects of corticosteroid (CS) therapy and relative CS insensitivity has been shown in airway smooth muscle cells (ASMC) from SA patients.

Objectives—We investigated whether there was a defect in the actions of the glucocorticoid receptor (GR) underlying the ability of CS to suppress the inflammatory response in ASMC of patients with SA. ASMC from healthy subjects (n=10), severe (n=8) and non-severe asthma (N-SA; n=8) were cultured from endobronchial biopsies.

Measurements and Main Results—GR expression in ASMC from SA and N-SA was reduced compared to that from healthy subjects by 49% (p<0.01). While baseline levels of nuclear GR were similar, GR nuclear translocation induced by dexamethasone (10⁻⁷ M) in SA was 60% of that measured in either healthy or N-SA. TNF- α induced greater NF- κ B (p65) mRNA expression in ASMC from SA (5.6 vs 2.0-fold; p<0.01), whereas baseline and TNF α -induced nuclear translocation and dexamethasone-mediated suppression of p65 expression were similar between groups. Dexamethasone, while not modulating TNF α -induced p65 nuclear translocation, attenuated p65 recruitment to the CCL11 promoter in the healthy and N-SA group, but this suppressive effect was impaired in SA.

Conclusions—Decreased GR expression with impaired nuclear translocation in ASMC, associated with reduced dexamethasone-mediated attenuation of p65 recruitment to NF κ B-dependent gene promoters, may underlie CS insensitivity of severe asthma.

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Author Contributions: P-JC planned and performed all the experiments and wrote the first draft. CM supervised the laboratory studies and, with JB, grew the smooth muscle cells from bronchial biopsies. NS performed additional experiments and JZ the immuno-histochemistry. PKB and KFC devised the study, discussed the findings, and finalized the manuscript.

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Keywords

airway smooth muscle; asthma; corticosteroid insensitivity; glucocorticoid receptor; nuclear translocation; CCL11

INTRODUCTION

Asthma is a chronic disease characterised by airway inflammation, hyper-responsiveness and remodelling(1). A proportion of asthma patients do not achieve adequate asthma control, despite taking oral corticosteroids (CS) and β -adrenergic agonists leading to frequent hospital admissions and use of emergency services. These patients, referred to as having severe or refractory asthma, are relatively insensitive to the therapeutic benefits of CS as demonstrated in lung macrophages(2) and airway smooth muscle cells(3) (ASM) by the lesser suppressive effect of dexamethasone on induced release of proinflammatory cytokines and on induced proliferation of ASMs compared to cells from non-severe asthma or healthy subjects. In patients with severe asthma, ASM mass is increased in the airways(4, 5) contributing to increased thickening and narrowing of the airways(4) and bronchial hyper-responsiveness(6). ASMs can synthesise cytokines and growth factors and express cell-surface molecules that allow them to interact with the extracellular matrix and inflammatory cells, and may play a central role in orchestrating the inflammatory response within the bronchial wall(7, 8).

The anti-inflammatory effects of corticosteroids are mediated through the glucocorticoid receptor (GR), a ligand-activated transcription factor that modulates both inflammatory and anti-inflammatory gene expression. Following corticosteroid binding to GR, GR dissociates from chaperone proteins and rapidly translocates into the nucleus, where GR either binds to specific glucocorticoid-responsive elements (GRE) on DNA to enhance transcription of anti-inflammatory genes, or represses transcription of pro-inflammatory genes by interaction with inflammatory transcription factors, such as nuclear factor kappa-B (NF- κ B) and activated protein-1 (AP-1). Changes in the phosphorylation pattern of GR as a consequence of its activation leads to many alterations of its function(9). Six serine residues have been identified as phosphorylation targets in the human GR(10) and phosphorylation of GR at serine 211 (Ser211) has been associated with ligand binding, nuclear translocation and transcriptional activation(9).

NF- κ B is of paramount importance in asthmatic inflammation(11). Upon cellular stimulation, NF- κ B translocates to the nucleus and mediates gene transcription. NF- κ B consists of hetero- or homodimers of the DNA-binding Rel family of proteins of which the p65 subunit is ubiquitously expressed and confers transcriptional regulation(12). The NF- κ B binding sites in the promoters of the pro-inflammatory genes such as CCL11 (eotaxin) and CXCL8 have been identified(13) and recruitment of the p65 subunit of NF- κ B to the CCL11 promoter in lung epithelial cells is attenuated by dexamethasone(14). Moreover, we have shown that the suppressive effect of dexamethasone on TNF α -induced CCL11 and CXCL8 release is impaired in ASM of severe asthma compared to non-severe asthma(3).

Insensitivity to CS may be attributed to a reduced ability of GR to bind to DNA or to an increase in the expression of pro-inflammatory transcription factors, such as NF- κ B and AP-1(15). CS insensitivity of ASMC from severe asthma patients is associated with a greater p38 mitogen activated protein kinase (MAPK) activation induced by TNF α (3), similar to findings in alveolar macrophages from severe asthma where p38 MAPK inhibition reversed CS insensitivity(2)(16). In this study, we hypothesised that the regulation of GR and NF- κ B (p65), in terms of their expression, nuclear translocation and recruitment to gene promoters, is perturbed in ASMC of patients with severe asthma compared those of patients with non-severe and healthy subjects. Some of the results of these studies have been previously reported in the form of an abstract (Po- Jui Chang et al. Eur Respir J 2012; 40: Suppl. 56, 615s)

MATERIALS AND METHODS

More details are provided in the Supplementary Material.

Subject characteristics (Table 1)

Severe asthma patients were on high-dose inhaled corticosteroids and sometimes on additional daily oral corticosteroid therapy to achieve a level of mild-to-moderate persistent asthma (17, 18). Patients with non-severe asthma used inhaled beclomethasone (0-1000 μ g/day or equivalent) with good control of asthma. Current and ex-smokers of > 5 pack-years were excluded. All patients gave informed consent to participate in this study approved by the local Ethics Committee. They underwent fiberoptic bronchoscopy during which endobronchial biopsies were obtained.

ASMC isolation, culture and activation

Bronchial biopsies were cut into small pieces (<1 mm²), and transferred to 6-well culture plates. At confluence, cells were harvested and split into larger flasks at each passage. ASMC were identified by the characteristic “hill and valley” morphology, and by their expression of calponin, smooth muscle α -actin and myosin heavy chain in more than 95% of the cells(19). Cells were plated in 6-well culture plates for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or in 75 cm²-flasks for Western Blot and chromatin immunoprecipitation (ChIP assay). At 90% confluence, cells were serum-starved for 24 hours, and stimulated with dexamethasone and/or TNF- α for times as indicated. Cells were used at passage 4 or 5.

Determination of mRNA expression

Total RNA was isolated using RNeasy Mini Kit (Qiagen, Crawley, UK) and reverse-transcribed with random primers and AMV reverse transcriptase (Promega, Southampton, UK) using manufacturer’s instructions. cDNA was quantified by RT-qPCR (Rotor Gene 3000; Corbett Research, UK) using SYBR Green PCR Master Mix Reagent (Qiagen) and gene-specific primer sets for CCL11, p65 and 18S (Qiagen).

Chromatin immunoprecipitation (ChIP) assay

Cells were fixed in 1% formaldehyde for 10 minutes and DNA fragmented by sonication (5 × 10 second pulses). After adding ChIP dilution buffer, 4 µg of antibody was added to pre-cleared chromatin solution overnight. Antibody/DNA complexes were captured, washed, eluted, and reverse cross-linked. Both the DNA and input fractions were purified by phenol/chloroform wash and ethanol precipitation. The precipitated DNA was resuspended and quantitative PCR was performed using primers for the CXCL11 promoter region.

Western Blotting

40 µg of protein from each sample was loaded for electrophoresis. For protein from whole cell lysates, the transfer membrane was incubated with a rabbit anti-phospho-GR antibody, followed by anti-rabbit-HRP antibody. Antibody-bound proteins were visualized by ECL or ECL plus (GE Healthcare, CT, USA). The membrane was then re-probed with a rabbit anti-total GR antibody or with mouse anti-β-actin monoclonal antibody to control for protein loading. For proteins from cytoplasmic or nuclear extracts, the membrane was incubated with rabbit anti-GR p65 ant, and with α-tubulin or TBP (to control for protein loading). Relevant band intensities were quantified by scanning densitometric analysis.

Statistical analysis

Repeated measures analysis of variance (ANOVA) with Dunnett multiple comparison test was used for intra-group analysis. Mann-Whitney test or Kruskal-Wallis test with Dunn's multiple comparison was used to compare results between the groups. $p < 0.05$ was taken as significant.

RESULTS

Participant characteristics

Severe asthmatics had a longer duration of asthma, used higher doses of ICS, and had a lower FEV₁ (% predicted) and increased obstruction (as determined by a reduced FEV₁/FVC% ratio) compared to non-severe asthmatics. Six out of eight patients with severe asthma were on a daily dose of oral prednisolone (Table 1).

Expression and nuclear translocation of GR

Baseline GR expression, measured by Western blot, was lower in ASMC of severe asthma (Kruskal-Wallis, post hoc test: $p < 0.01$) and non-severe asthma (Kruskal-Wallis, post hoc test: $p < 0.05$) compared to healthy subjects, and there was no difference between non-severe and severe asthma (Fig. 1A). Dexamethasone-induced nuclear translocation of GR in ASMC was confirmed by Western blot (Supplemental Fig. E1) and dexamethasone did not influence GR expression up to four hours post-stimulation (Fig. 1B). To compare nuclear translocation induced by dexamethasone in ASMC, cells were stimulated with dexamethasone (10^{-7} M) for 30 minutes to 4 hours. The quantity of nuclear GR at baseline was similar among ASMC of healthy subjects and non-severe and severe asthma (Fig. 2A). In ASMC of healthy subjects and non-severe asthma, dexamethasone induced a 3-fold increase in nuclear GR abundance at 30 minutes, which was maintained at one hour post-

stimulation, followed by a gradual decrease to 50% of induced levels at the 4 hour time point. In contrast, in ASMC of patients with severe asthma, nuclear GR was induced by less than 2-fold which was significantly less than that observed in healthy subjects and patients with non-severe asthma (Fig. 2B).

In order these translate our *in vitro* ASMC culture findings to that in the lung we examined GR expression *ex vivo*, in a limited number of biopsies from healthy subjects, and non-severe and severe asthmatics, using immuno-histochemistry. We analysed the relative percentage of GR positive nuclei in airway smooth muscle bundles (Figure E2A-C). We show that, in support of our *in vitro* data, that there are fewer GR positive nuclei in severe asthmatic biopsies compared to non-severe asthmatics (Kruskal-Wallis; post hoc test: $p < 0.05$; Fig.E2D) and normal subjects (Kruskal-Wallis; post hoc test: $p < 0.05$).

Phosphorylation of GR at serine 211 by dexamethasone

To investigate the effect of dexamethasone on phosphorylation of GR at the serine residue 211 (Ser211), ASMC of healthy subjects were stimulated with dexamethasone (10^{-6} M) for 5 minutes to 4 hours. GR phosphorylation was induced by dexamethasone at 30 minutes ($p < 0.01$), which further increased (5.2-fold) at one hour and was maintained at 4 hours (Fig. 3A; $p < 0.001$). To compare dexamethasone-induced phosphorylation of GR at Ser211 between groups, cells were stimulated with dexamethasone (10^{-6} M) for 2 hours. In ASMC of healthy subjects, dexamethasone induced a 6-fold increase in GR phosphorylation at Ser211, and this induction was similar in ASMC from patients with severe and non-severe asthma (Fig. 3B-C).

Nuclear translocation of p65 and effect of dexamethasone

At 24 hours, TNF- α induced a ~2-fold increase in p65 mRNA expression in ASMC of healthy subjects and non-severe asthmatics compared to 5.6-fold in severe asthma, (Fig. E3A; $p < 0.01$). TNF- α -induced nuclear translocation of p65 was also examined (Fig. E3B). The total quantity of nuclear p65 (Fig. E3C) and the time-course of TNF α -induced p65 nuclear abundance in ASMC of patients with non-severe and severe asthma and of healthy subjects (Fig. E3D) was the same. In ASMC of healthy subjects, dexamethasone suppressed TNF α -induced p65 protein expression by 21.3% at 24 hours ($p < 0.05$; Fig. E4A) and similar effect was observed in ASMC of non-severe asthma ($p < 0.001$) and severe asthma ($p < 0.01$). TNF α -induced p65 mRNA was inhibited in a concentration-dependent manner in all three groups (Fig. E4B), with maximal suppression of ~50% by dexamethasone at 10^{-7} and 10^{-6} M ($p < 0.01$, respectively). We also investigated the effect of dexamethasone on nuclear translocation of p65 induced by TNF- α in ASMC of healthy subjects (Fig. E4C) and non-severe asthma (Fig. E4D). TNF- α increased the nuclear abundance of p65, but this was not affected by dexamethasone.

Effect of dexamethasone on p65 recruitment to the CCL11 promoter

Having previously demonstrated that dexamethasone attenuates the recruitment of p65 to the fractalkine gene promoter in lung epithelial cells(20), we investigated the effect of dexamethasone on induced p65 recruitment to the gene promoter of CCL11 in ASMC. Cells were pretreated with dexamethasone (10^{-7} M) for 2 hours and then stimulated with TNF- α

(10 ng/mL) for one hour. In ASMC of healthy subjects, TNF α -induced an 11.4-fold increase in the recruitment of p65 ($p < 0.001$), which was suppressed by dexamethasone by 52.7% (Fig. 4A; $p < 0.05$). This effect was similar in ASMC of patients with non-severe asthma, where dexamethasone suppressed TNF α -induced p65 recruitment by 51.4% (Fig. 4B). In contrast, the induced p65 recruitment to the gene promoter was not attenuated by dexamethasone in ASMC of severe asthma (Fig. 4C). We also compared the effect of dexamethasone on p65 recruitment to the CCL11 (eotaxin) gene promoter in ASM cells from patients with SA and NSA. Our results show a significant difference in the percentage reduction in p65 recruitment mediated by dexamethasone between NSA and SA, ($52 \pm 12\%$ vs $13 \pm 8\%$, respectively; $p < 0.05$).

DISCUSSION

In the light of our recent observation of corticosteroid insensitivity in ASM of patients with severe asthma(3), we report that GR expression in severe and non-severe asthma is reduced compared to that in the healthy subjects. Furthermore, dexamethasone-induced GR nuclear translocation is attenuated in severe asthma compared of that in either the healthy or non-severe asthma. Baseline levels of nuclear GR were similar in the three groups. TNF α -induced greater p65 mRNA expression in severe asthmatics whereas baseline and TNF α -induced nuclear translocation and dexamethasone-mediated suppression of p65 expression were similar in all groups. Dexamethasone, while not modulating TNF α -induced p65 nuclear translocation, attenuated p65 recruitment to the CCL11 promoter in the healthy and non-severe asthma. However, this suppressive effect was impaired in severe asthma. Thus, decreased GR expression with impaired nuclear translocation in ASMC, associated with reduced dexamethasone-mediated attenuation of p65 recruitment to gene promoters, underlies the mechanism of corticosteroid insensitivity in severe asthma and this may contribute to the chronic inflammation observed in this disease (see Figure 5).

A reduction in GR may account for the failure of corticosteroid suppression of serum-induced ASMC proliferation in asthma(3, 21). In contrast, equivalent GR expression in ASMC of non-severe and severe asthma is supported by reports of similar levels of mRNA expression of either GR α or GR β in peripheral lymphocytes of severe or moderate asthma(22) and similar protein and mRNA expression of both GR α and GR β in peripheral blood mononuclear cells (PBMC) of steroid-dependent well-controlled asthma(23). The abundance of GR protein is reduced by dexamethasone at 24 hours post-stimulation, indicative of a down-regulatory effect of glucocorticoids on GR expression. GR mRNA is negatively regulated by glucocorticoids(24), which could be attributed to their inhibitory effects at the GRE, AP-1, NF- κ B and cAMP response element-binding (CREB) regulatory motifs located in the promoter of GR(25). Alternatively, glucocorticoids may post-transcriptionally regulate the expression of GR, perhaps via destabilization of the GR mRNA(26). Ligand-activated GR protein could also be removed upon prolonged exposure to glucocorticoids by the proteasome-ubiquitin degradation pathway(27).

Nuclear translocation of GR is the critical step in corticosteroid-mediated anti-inflammatory effects. Based on our observation that total GR expression is not influenced by dexamethasone, we surmised that the increase in nuclear GR abundance is a direct

consequence of GR nuclear translocation. However, the reduced dexamethasone-induced nuclear translocation of GR in ASMC of severe asthma, which extends the observation from peripheral blood mononuclear cells(28, 29) to airway resident cells, could underlie the mechanism of corticosteroid insensitivity in severe asthma.

Our study shows a reduction in GR expression in ASMC of both severe and non-severe asthma and yet corticosteroid insensitivity is only observed in the former. CS insensitivity is influenced by many factors, not only the number of GR but also by the affinity of GR and by interference with the glucocorticoid signalling pathways(30). The low affinity state of GR is achieved through binding of HSP70, HSP40 and HSP70–HSP90 organizing protein (HOP) (31), while a switch to a high affinity state occurs on binding of HSP90 and p23. A reduced HSP90:GR ratio has been reported in PBMC from patients with glucocorticoid-resistant asthma(32). Increased expression of the co-chaperone FKBP51, reported in PBMC of severe asthma, could also reduce ligand affinity of GR(33). Transcriptional activity of GR can also be impaired via direct interaction with cytokine-induced proinflammatory transcriptional factors such as NF- κ B, which prevents GR:GRE binding, which is increased in severe asthma.

The defective GR nuclear translocation in corticosteroid-insensitive severe asthma may be partly attributed to hyper-phosphorylation of GR(29), and this can also contribute to impaired effect of corticosteroids as a result of either decreased GR-GRE binding(34) or failure to suppress histone acetyltransferase activity induced by inflammatory stimuli(28). GR phosphorylation can be mediated by mitogen-activated protein kinases (MAPK) p38, c-Jun N-terminal kinase (JNK), or extracellular signal-regulated kinase (ERK)(30). We have reported heightened p38 MAPK activity in alveolar macrophages(35) and ASMCs(3) of severe asthma, and in addition, we have shown that inhibition of p38 MAPK leads to reversal of corticosteroid insensitivity in ASM and alveolar macrophages from patients with severe asthma(3, 16). This raises the possibility that p38 inhibition may improve corticosteroid responsiveness in severe asthma by reversal of defective nuclear translocation of GR, as demonstrated in an IL2- and IL4-induced corticosteroid-resistant T-cells(36). Phosphorylation of GR at serine 211 has been proposed as a marker for the transcriptional potential of GR(37). Absence of differential degrees of phosphorylation, at Ser211 between the asthmatic groups, indicates that this residue may not directly contribute to corticosteroid insensitivity in severe asthma.

TNF α -induced expression of the p65 and p50 components of NF- κ B is suppressed by dexamethasone in ASMC of healthy subjects at both protein and mRNA levels(38), which we now demonstrate in ASMC of asthmatic patients. However, whereas corticosteroid insensitivity is displayed in ASMC of severe asthma, in terms of impaired suppression of the NF κ B-dependent genes such as CCL11 and CXCL8(3), the suppressive effect of dexamethasone on p65 expression is similar between the normal and asthma groups. This suggests that reduced response to corticosteroids in severe asthma does not extend to impaired suppression of total NF- κ B expression.

The inability of dexamethasone to inhibit induced p65 nuclear translocation is consistent with report in the literature in both ASMC(14) and in human lung epithelial cells(20). It is

also reported that dexamethasone does not attenuate either TNF α -induced NF- κ B DNA binding or NF κ B-mediated reporter activity in ASMC(39). However, TNF α -induced p65 recruitment to the CCL11 promoter in AMSC from healthy subjects, is attenuated by dexamethasone. This is associated with reduced acetylation of histone H4, resulting in condensation of chromatin and subsequent hindered access of p65 to DNA(14). However, this effect of dexamethasone is impaired in ASMC from severe asthma, and this suggests a possible mechanism by which corticosteroids fail to suppress NF κ B-mediated inflammatory genes, such as CCL11 and CXCL8, in ASMC from severe asthma(3). These results provide a better mechanistic understanding of corticosteroid insensitivity observed in airway smooth muscle cells obtained from patients with severe asthma. We have shown an attenuation of the suppression of p65 recruitment by dexamethasone associated with a reduced translocation of GR from the cytoplasm to the nucleus in airway smooth muscle cells from severe asthmatics compared to non-severe asthmatics. This observation is likely to explain the poor therapeutic effects of corticosteroid therapy in patients with severe asthma, where the magnitude of the reduction in p65 recruitment to pro-inflammatory gene promoters caused by corticosteroids determines their effects in controlling asthmatic inflammation.

Decreased GR expression with impaired nuclear translocation and subsequent inability to suppress p65 recruitment to the gene promoters contribute to the defective corticosteroid suppression of NF- κ B-mediated chemokine expression in ASMC of severe asthma. These mechanisms may underlie CS insensitivity in severe asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scientific Knowledge on the Subject

Expression of the glucocorticoid receptor (GR) in airway smooth muscle cells (ASMC) of patients with severe asthma and non-severe asthma is reduced compared to that of healthy subjects. GR nuclear translocation induced by dexamethasone in ASMC of severe asthma is 40% lower than that in non-severe asthma or healthy subjects. Dexamethasone attenuates NF- κ B recruitment to the CCL11 promoter in ASMC of healthy subjects and non-severe asthma but this effect is impaired in severe asthma.

What This Study Adds to the Field

Reduced expression of the glucocorticoid receptor with impaired nuclear translocation and subsequent inability to suppress recruitment of pro-inflammatory transcription factor to gene promoters contribute to corticosteroid insensitivity in airway smooth muscle of severe asthma.

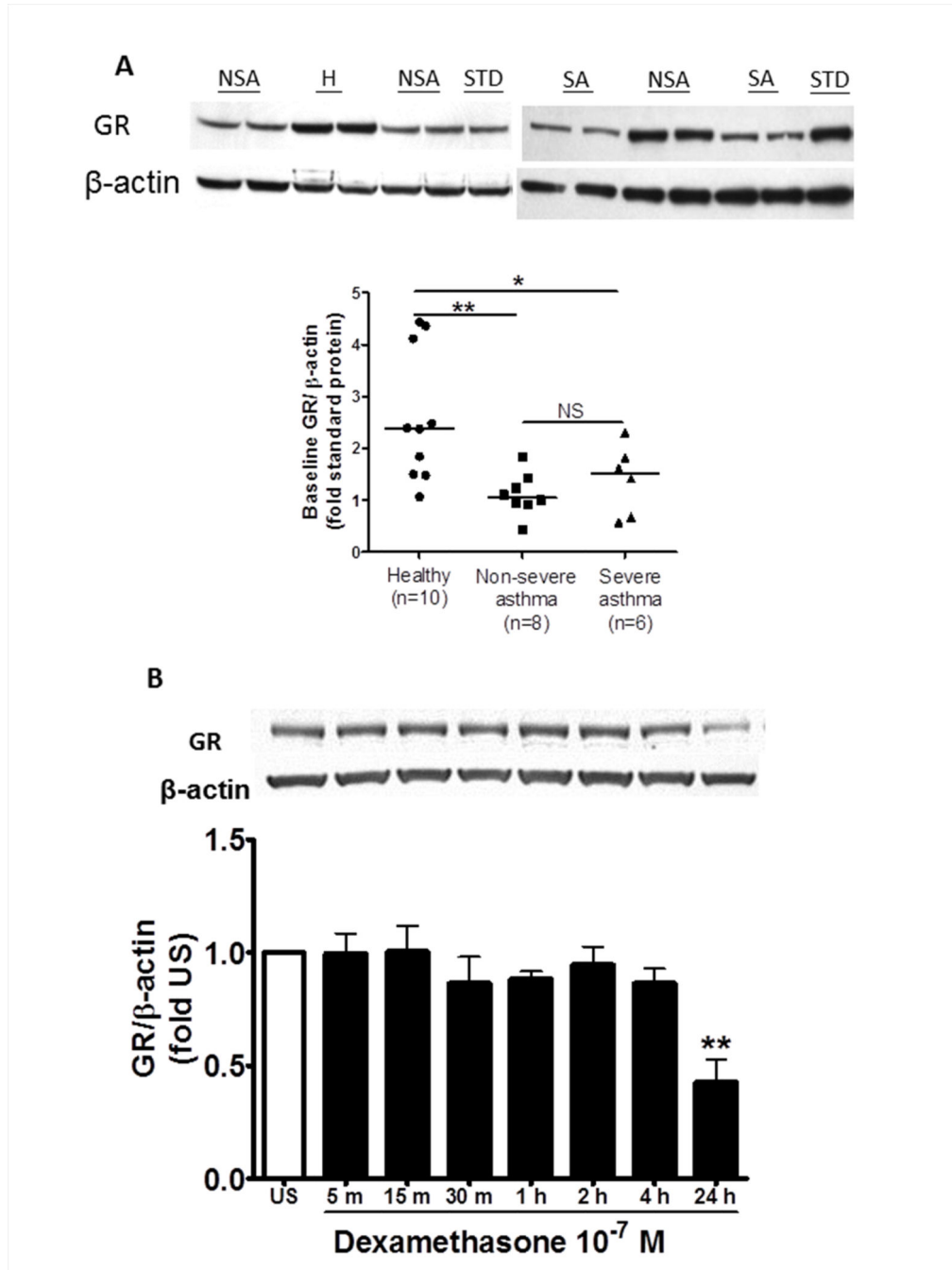


Figure 1. Comparison of GR expression at baseline and effect of dexamethasone on GR expression

(A) Baseline GR expression in ASMC of healthy subjects and asthma patients. After serum starvation for 24 hours, GR and β-actin protein in whole cell lysates from ASMC of healthy subjects and non-severe and severe asthma patients were measured in duplicate along with a standard (STD) protein by Western Blot followed by densitometric analysis. Representative blots are shown. Further analysis shows the comparison of baseline GR expression between the non-severe and severe asthma patients. Horizontal lines represent median. Kruskal-

Wallis, post hoc test: $**p < 0.01$, $*p < 0.05$. (B) Effect of dexamethasone on GR expression. ASMC were treated with dexamethasone (10^{-7} M) for times indicated. GR and β -actin protein in the whole cell lysates were measured by Western Blot followed by densitometric analysis. Bars represent mean \pm SEM from 3 ASMC of healthy subjects. $** p < 0.01$ vs unstimulated (US).

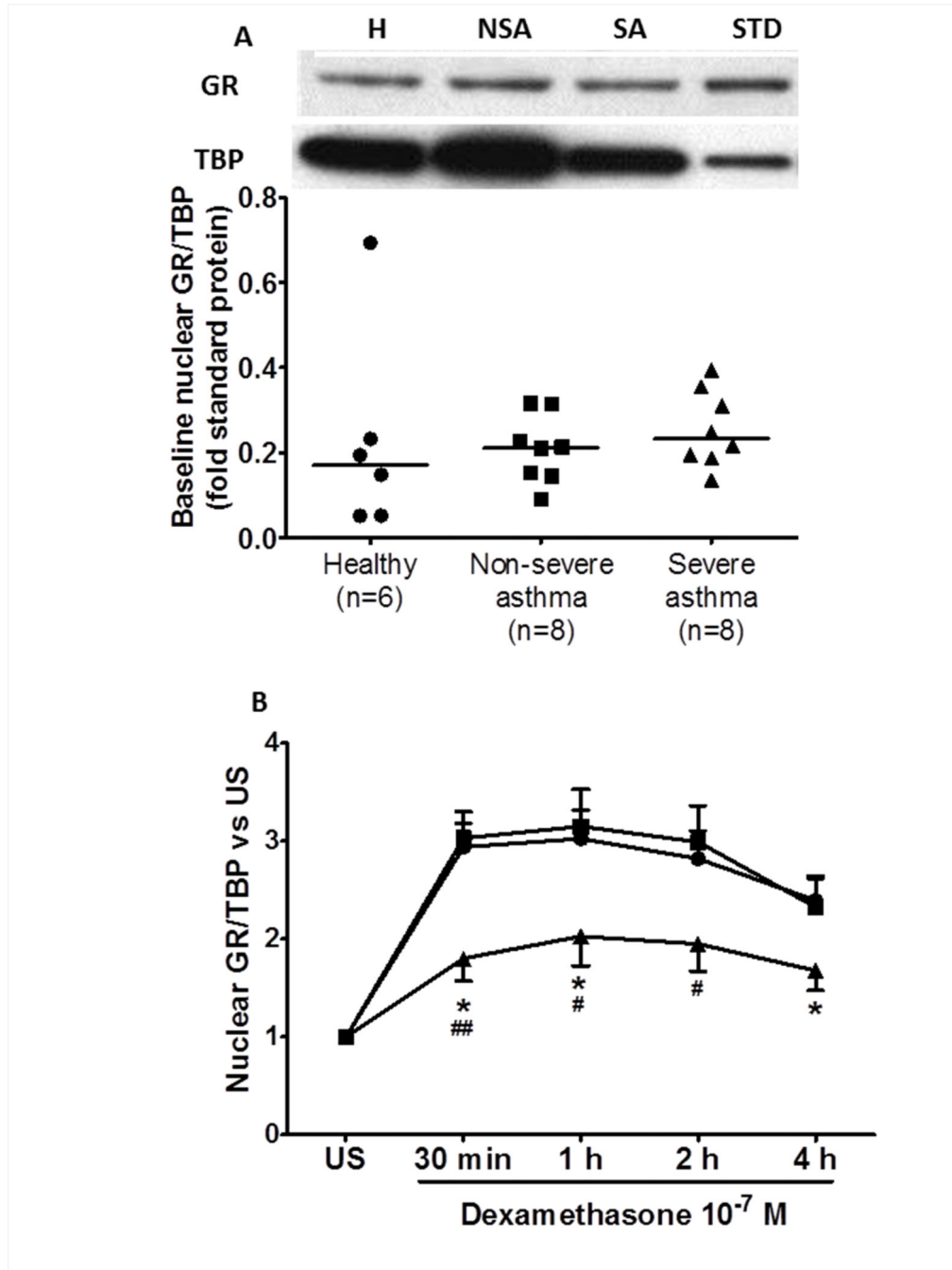


Figure 2. Impaired nuclear translocation of GR in ASMC of severe asthma

(A) Nuclear GR expression at baseline. After serum starvation for 24 hours, GR and TBP protein in nuclear extracts from ASMC of healthy subjects and non-severe and severe asthma patients were measured in duplicate along with a standard (STD) protein by Western Blot followed by densitometric analysis; representative blot is shown. Horizontal lines represent median. (B) Dexamethasone-induced nuclear translocation of GR. ASMC of healthy subjects (●, n=8) and non-severe (■, n=8) and severe asthma (▲, n=8) patients were treated with dexamethasone (10^{-7} M) for times indicated, GR and TATA-box binding

protein (TBP) in the nuclear extracts, as well as a standard (STD) protein, was assessed by Western blot followed by densitometric analysis. Points represent mean \pm SEM. * $p < 0.05$ vs healthy subjects. # $p < 0.05$, ## $p < 0.01$ vs non-severe asthma.

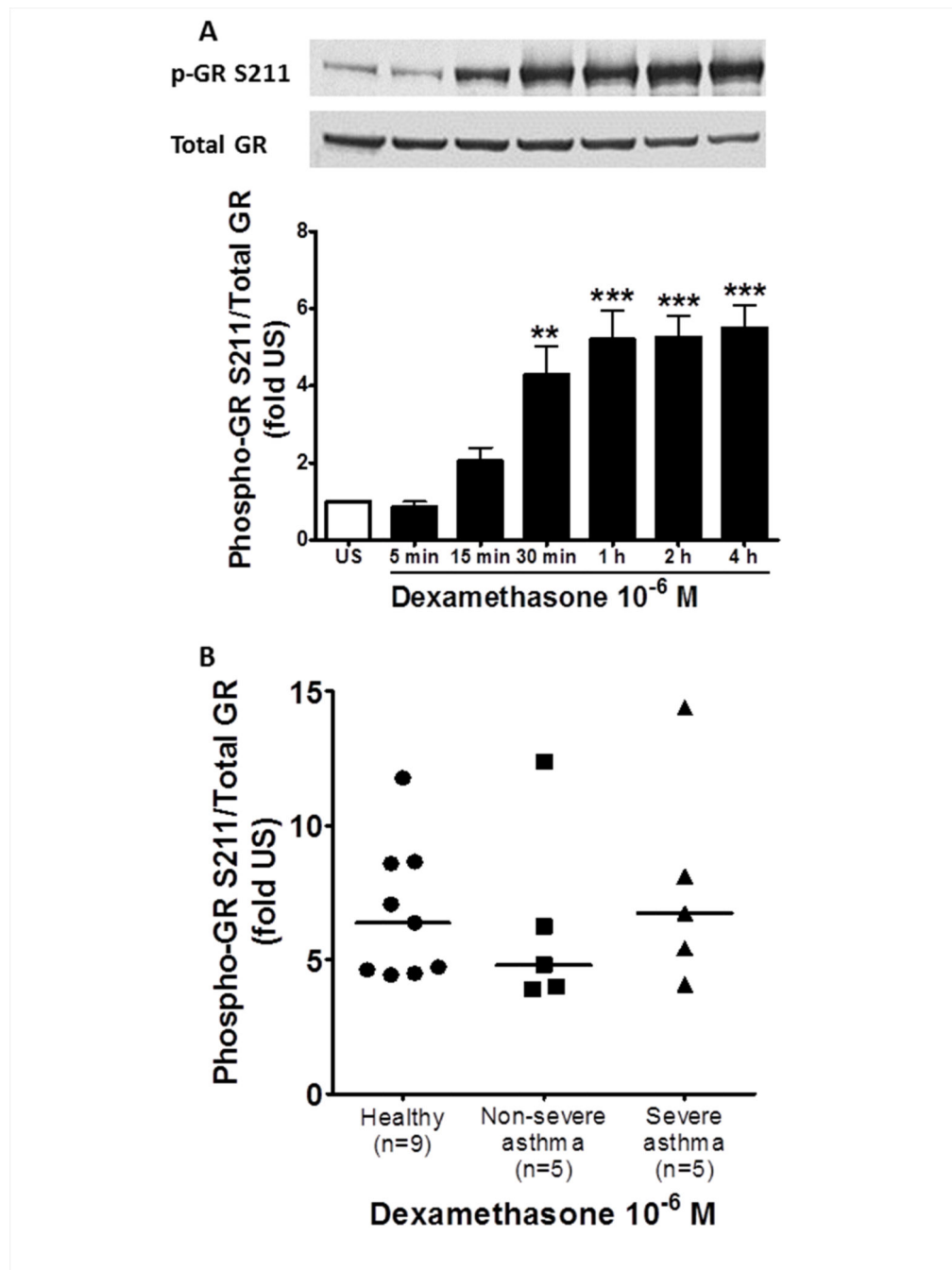


Figure 3. Comparison of dexamethasone-induced GR phosphorylation at serine 211

(A) Time-dependent, dexamethasone-induced, phosphorylation of GR Ser211. After serum starvation for 24 hours, ASMC of healthy subjects were treated with dexamethasone (10^{-6} M) for times indicated. A representative blot is shown. Bars represent mean \pm SEM from 3 ASMC of healthy subjects. ** $p < 0.01$, *** $p < 0.001$ vs unstimulated (US). (B) Comparison of GR Ser211 phosphorylation. ASMC of healthy subjects and non-severe and severe asthma patients were treated with dexamethasone (10^{-6} M) for 2 hours. Phosphorylated

(Ser211) and total GR protein in whole cell lysates were measured by Western blot followed by densitometric analysis. Horizontal lines represent median.

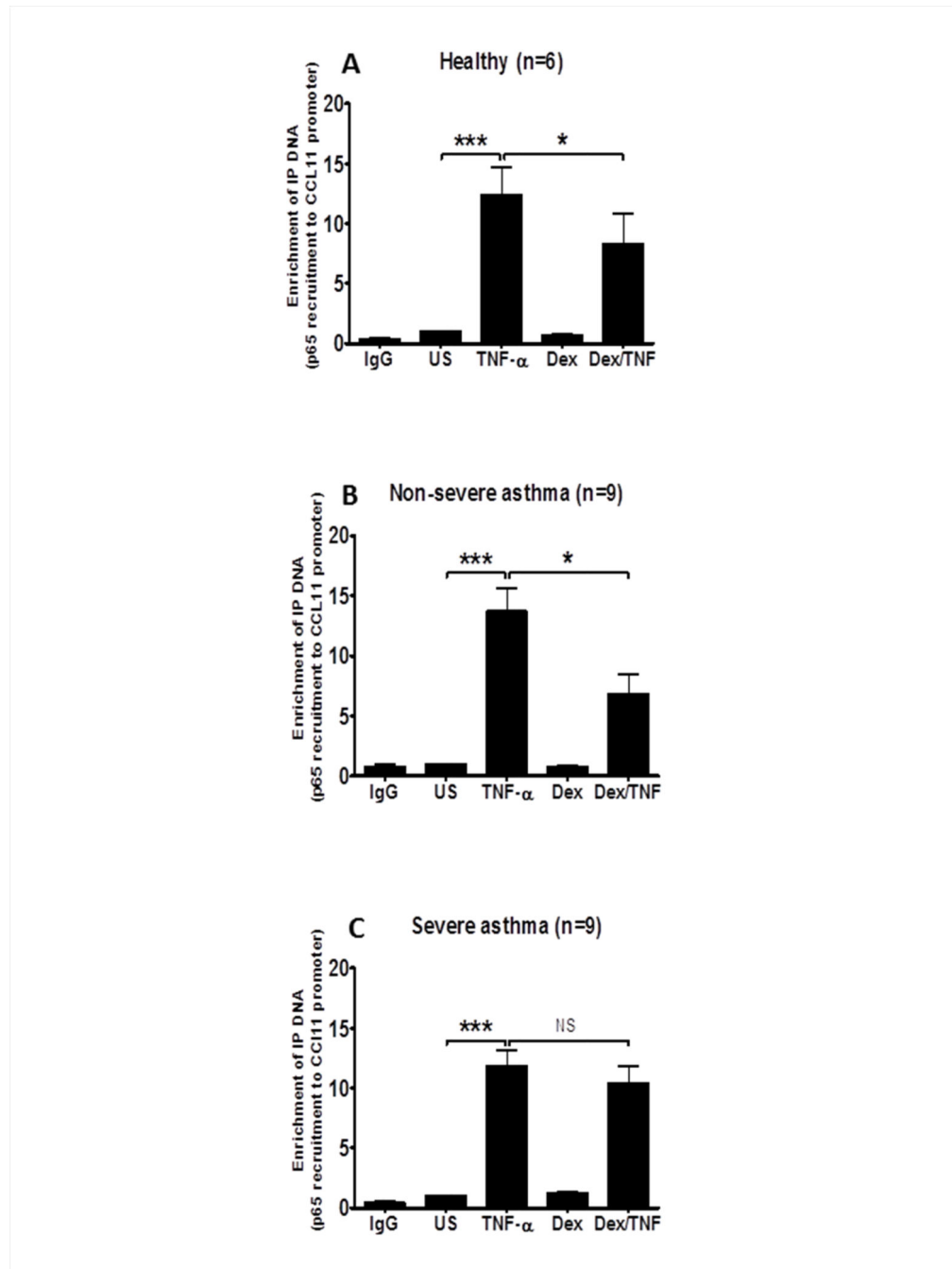


Figure 4. Impaired dexamethasone mediated attenuation of TNF α induced p65 recruitment to the CCL11 promoter in ASMC of severe asthma

After serum starvation for 24 hours, ASMC were pre-treated with dexamethasone (10^{-7} M) for 2 hours and stimulated with TNF- α (10 ng/mL) for 1 hour. p65 recruitment to the CCL11 promoter in ASMC of (A) healthy subjects and (B) non-severe and (C) severe asthma patients was measured by ChIP assay. Bars represent mean \pm SEM. US= unstimulated, Dex=dexamethasone and NS=not significant. * $p < 0.05$, *** $p < 0.001$.

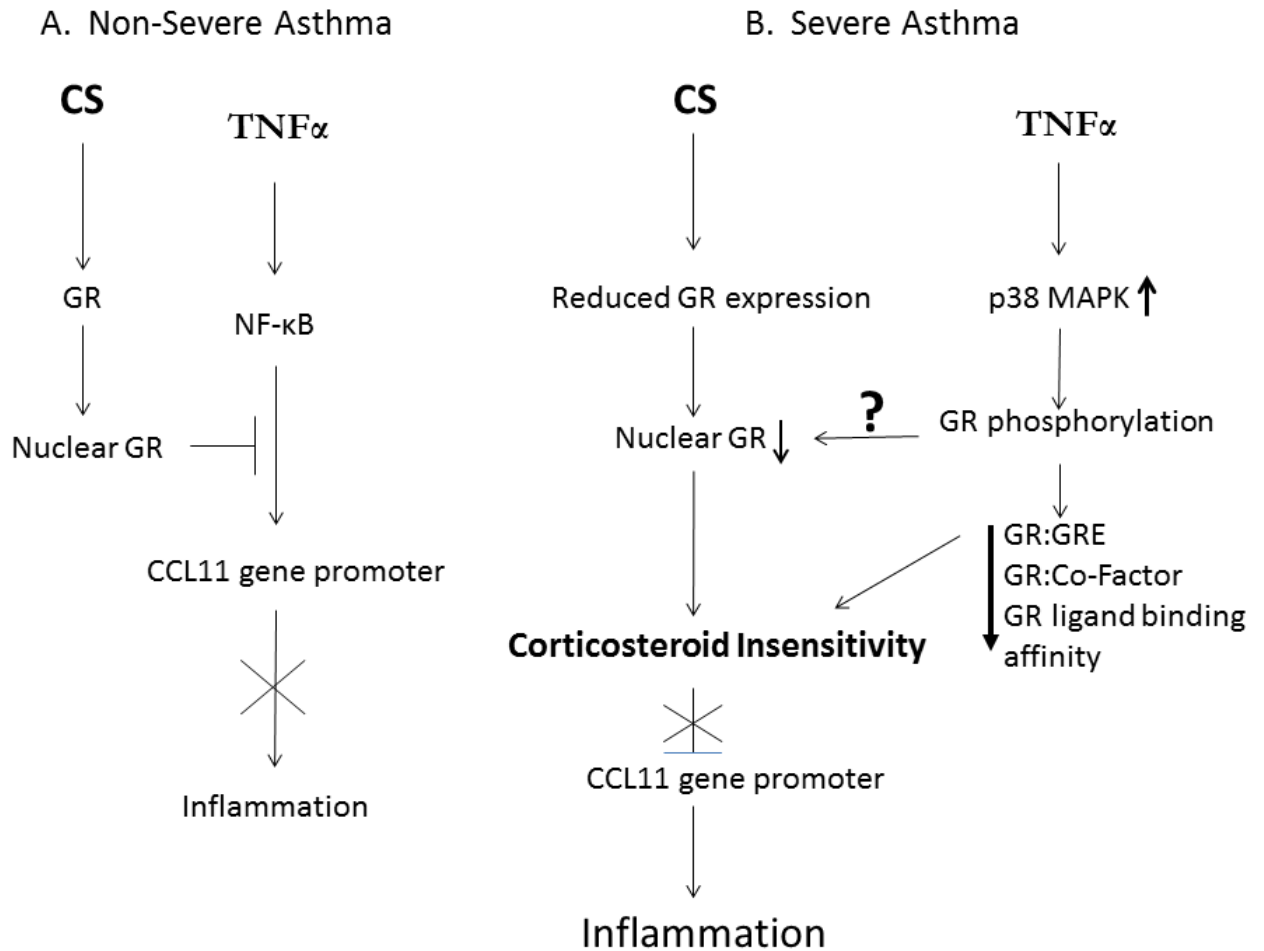


Figure 5. Model of corticosteroid insensitivity in severe asthma

In non-severe asthma (A) TNF α -induced inflammation is suppressed by CS through GR-mediated attenuation of NF- κ B recruitment to the CCL11 promoter. In severe asthma (B) reduced GR expression and impaired nuclear translocation results in corticosteroid insensitivity and an inability to inhibit inflammation. p38 MAPK-mediated phosphorylation of GR may also affect GR nuclear translocation

Table 1

Subject characteristics

	Healthy Controls	Non-severe Asthma	Severe Asthma
Number	10	9	9
Age, years	39.0 ± 11.3	31.8 ± 16	41.4 ± 12
Gender, F/M	5/5	4/5	5/4
Duration of asthma, years	N/A	18.2 ± 3.8	25.7 ± 9.7 *
Inhaled corticosteroid dose, µg BDP equivalent	N/A	622 ± 290	2160 ± 466 *
Atopy (n) [§]	3	6	5
Receiving oral prednisolone (n)	N/A	N/A	6
FEV ₁ , L	3.15 ± 1.860	2.96 ± 0.46	2.0 ± 0.47 **
FEV ₁ , % predicted	91.0 ± 15.6	81.5 ± 8.3	61.59 ± 4.9 ***
FEV ₁ /FVC, %	77.2 ± 3.9	75.0 ± 3.1	69.0 ± 5.9 *
β-agonist reversibility [#] , %	N/A	24.0 ± 3.5	31.0 ± 4.11 *
PC ₂₀ , mg/mL	> 16	2.7 ± 0.104	0.331 ± 0.13 (4/9)

BDP, beclomethasone dipropionate; FEV₁, forced expiratory volume in 1s; FVC, forced vital capacity; PC₂₀, provocative concentration of metacholine causing a 20% fall in FEV₁; N/A: not applicable.

[§] Defined as positive skin prick tests to one or more common aeroallergens.

[#] Measured as percent increase in FEV₁ after 400 µg salbutamol.

* p < 0.05

*** p < 0.001 vs non-severe asthma.