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# Dexamethasone-Induced *FKBP51* Expression in Peripheral Blood Mononuclear Cells Could Play a Role in Predicting the Response of Asthmatics to Treatment with Corticosteroids

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**Abstract**
*Background* Corticosteroids (CSs) are the preferred anti-inflammatory therapy for the treatment of asthma, but the responses of asthmatics to CSs are known to vary. It has thus become important to discover reliable markers in predicting responses to CSs.
*Methods* We performed time-series microarrays using a murine model of asthma after a single dose of dexamethasone, based on the assumption that the gene showing a greater change in response to CSs can also be a potential marker for that finding. We then evaluated the clinical meaning of the gene discovered in the microarray experiments.
*Results* We found that the expression of FK506 binding protein 51 gene (*FKBP51*) in lung tissue markedly increased after dexamethasone treatment in a murine model of asthma. We then measured dexamethasone-induced *FKBP51* expression in peripheral blood mononuclear cells (PBMCs) in asthmatics. Dexamethasone-induced *FKBP51* expression in PBMCs was significantly higher in severe asthmatics compared with mild-to-moderate asthmatics treated with inhaled CSs. In addition, we found that dexamethasone-induced *FKBP51* expression in PBMCs was inversely correlated with improvement in lung function after treatment with orally administered prednisolone in six steroid-naive asthmatics.
*Conclusion* Dexamethasone-induced *FKBP51* expression in PBMCs may be a reliable and practical biomarker in predicting the response to CSs in asthmatics.

**Keywords** Asthma · glucocorticoids · peripheral blood mononuclear cells · FKBP51

**Abbreviations**

| | |
|---|---|
| CSs | Corticosteroids |
| FKBP51 | FK506 binding protein 51 |
| GR | Glucocorticoid receptor |
| PBMCs | Peripheral blood mononuclear cells |
| PBS | Phosphate-buffered saline |

## Introduction

Current guidelines recommend corticosteroids (CSs) as the preferred anti-inflammatory therapy for the treatment of asthma [1, 2]. Previous studies have demonstrated marked variability in response to CSs [3–5], however, and thus it is important to investigate markers of predicting the response of asthmatics to CSs. Some investigators have proposed

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potential candidates [6, 7] for those markers, but they need to be confirmed in larger studies.

In the present study, we assumed that the gene showing a greater change in response to CSs can be a potential marker of predicting the responsiveness to CSs. To elucidate this marker, we utilized hypothesis-free methods: time-series microarrays using a murine model of asthma after a single dose of dexamethasone. Through this animal study, we found that a 51-kDa FK506 binding protein, coded by *FKBP51*, could be a potential candidate in predicting the responsiveness to CSs and evaluate its clinical implications using human samples.

## Methods

### Time-Series Microarrays

To generate a murine model of asthma, six female BALB/c mice (6–8 weeks of age) purchased from The Jackson Laboratory were sensitized twice intraperitoneally with 75 μg of ovalbumin (Sigma, St. Louis, CA, USA) plus 2 mg of aluminum hydroxide (alum, Sigma) and then challenged intranasally with 50 μg of ovalbumin (see Supplementary Figure 1). For the case group ($n$=3), dexamethasone (2.5 mg/kg) was administrated intraperitoneally at 24 h after the last challenge of ovalbumin. Through a preliminary experiment, we confirmed that 2.5 mg/kg of dexamethasone administered in a single intraperitoneal injection significantly suppressed airway inflammation and non-specific airway hyperresponsiveness (data not shown). Phosphate-buffered saline (PBS) was administrated to the control mice ($n$=3). Mice were then killed at 1, 6, and 12 h after dexamethasone and PBS treatment. Right lung homogenates from the case and control mice were each pooled into one group to eliminate any individual variation, and triplicated experiments were done. Total RNA was extracted from lung homogenates using trizol reagent (Invitrogen, Carlsbad, CA, USA) as recommended by manufacturer's protocol. After trizol purification, RNA was repurified with chloroform. Following chloroform extraction, total RNA was precipitated in isopropanol, washed with ethanol, suspended in nuclease-free water, and stored at −70°C until used for cDNA synthesis. RNA quality and quantity were determined by the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). We used the GeneChip® mouse genome 430 2.0 Array from Affymetrix (Santa Clara, CA, USA), which allows the comprehensive analysis of genome-wide expression on a single array (45,000 probe sets to analyze the expression level of over 39,000 transcripts and variants from over 34,000 well-characterized mouse genes). Microarray analysis was performed as follows: Target labeling for the expression analysis was performed for 1 μg total RNA per sample according to the manufacturer's protocol (Affymetrix). The GeneChips® were automatically stained and washed in a fluidics station as recommended by the manufacturer. The scanning and the analysis were done using the Affymetrix GeneChip Operating Software (v. 1.2). The quality of each sample was first evaluated by visual inspection of the distribution of genes in graphs, and by calculating sample similarity values as correlation coefficients. The value distribution of each array was normalized for fair across-chip comparison using robust multi-array average. Two-way analysis of variance was applied to determine the differentially expressed sets of genes. Self-organizing map clusterings were applied to the differentially expressed genes.

This animal study protocol was approved by the International Animal Care and Use Committee of the Seoul National University, Seoul, Republic of Korea.

### Study Subjects

To evaluate the clinical implications of *FKBP51* expression changes which was identified as a potential candidate gene from time-series microarrays, a total of 29 asthmatics were enrolled at Seoul National University Hospital, Seoul, Republic of Korea. A diagnosis of asthma was made when a subject with symptoms of dyspnea or wheezing showed reversible airway obstruction as detailed by the guidelines of the National Institute of Health [1]. Exclusion criteria included: a smoking history of >10 pack years and positive abnormality on simple chest radiography. Twenty-four of the enrolled asthmatics (on-treatment group) received the standard treatment recommended by the current guidelines and showed stable lung function [variability of forced expiratory volume in one second (FEV1) of ≤5%] and no acute asthma exacerbation [hospitalizations, unscheduled visits for worsening asthma symptoms, or significant reductions in FEV1 (≥20% and 500 ml from baseline FEV1 value) requiring orally administered corticosteroid treatment for at least 3 days] for at least 3 months before enrollment. Asthma severity was determined based on lung function and the medication use index needed to obtain control, as previously described [8, 9]. Peripheral blood was drawn for analysis without cessation of their current medication. Six of the enrolled asthmatics (treatment-naive group) visited our clinic first with dyspnea and decreased lung functions (FEV1 predicted value ≤80%), had no history of exposure to anti-asthmatic medication, and were diagnosed with asthma based on positive bronchodilator response. They were treated with short-term systemic steroids for the prompt relief of their symptoms (orally administered prednisolone 15 mg, twice a day for 7 days). Peripheral blood was drawn before and just after treatment for analysis. For controls, 27 healthy subjects were recruited by elicitation and had no history of allergic

**Table I** Characteristics of enrolled subjects (on-treatment group)

| Characteristics | Asthmatics | | Controls ($n$=27) |
|---|---|---|---|
| | Mild to moderate ($n$=11) | Severe ($n$=13) | |
| Age, mean (±SD) | 59.3 (±14.7) | 61.3 (±12.4) | 28.6 (±6.7)[a] |
| Male, *n* (%) | 5 (45.4) | 5 (38.5) | 12 (44.4) |
| FEV1 pred. % (±SD) | 93.2 (±11.2) | 69.3 (±21.1) | 98.1 (±16.3) |
| Medication | | | |
| Inhaled corticoglucocorticoids[b] [mean μg (±SD)] | 455.9 (± 153.4)[c] | 1,046.4 (±256.7)[c] | – |
| Methylxanthine, *n* (%) | 1 (9.1)[c] | 9 (69.2)[c] | – |
| Leucotriene modifier, *n* (%) | 0 (0)[c] | 10 (76.9)[c] | – |

[a] Significantly different between controls and mild to moderate asthmatics, and between controls and severe asthmatics ($P$<0.001)

[b] Beclomethasone equivalent dose

[c] Significantly different between mild to moderate and severe asthmatics ($P$<0.001)

disease and no respiratory symptoms, and showed negative results in methacholine bronchial provocation tests. All the subjects in this study provided written informed consent, and the study protocol was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Republic of Korea (Table I).

### Measurement of Dexamethasone-Induced *FKBP51* Expressions in PBMCs

It is reported that the glucocorticoid-induced *FKBP51* mRNA increase in peripheral blood mononuclear cells (PBMCs) can detect sensitivity to glucocorticoid [10–12]. Isolated PBMCs were resuspended to a concentration of $1\times10^6$ cells/ml in RPMI 1640 medium, supplemented with 10% DCC-stripped FCS, 4 mM L-glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin. PBMCs were divided onto a 48-well plate into in 1.0-ml aliquots ($1\times10^6$ cells/ml). Following an overnight pre-incubation to minimize the effects of endogenous glucocorticoids, dexamethasone was added to an end volume of 1 ml/well. After 24-h incubation, total RNA was isolated using TriPure reagent (Roche Applied Science, Mannheim, Germany) and reverse transcribed using MMLV-RT RNase H Minus, Point Mutant (Promega, Madison, WI, USA), according to the manufacturer's protocols. The synthesized cDNA was subsequently diluted 40 times in RNase-free water for use in real-time PCR. Real-time PCR experiments were performed with the LightCycler device (Roche Applied Science), using the DNA Master SYBR-green I kit (Roche Applied Science, Mannheim, Germany). As suggested in a previous study [10], expression levels of *FKBP51* in $10^{-7}$ M dexamethasone were used for the comparison.

### Statistical Analysis

Results are expressed as mean±SEM. The results were assessed by paired two-tailed tests. A $p$ value lower than 0.05 was considered significant.

## Results

### Time-Series Microarrays

Transcript profiling was performed separately on triplicated case groups (0, 1, 6, and 12 h) and their time-matched PBS-treated control groups, generating a total of 24 microarray data sets. To identify dexamethasone-related changes in global gene expression, two-way ANOVA was conducted on the dose- and time-response data, and 209 genes were

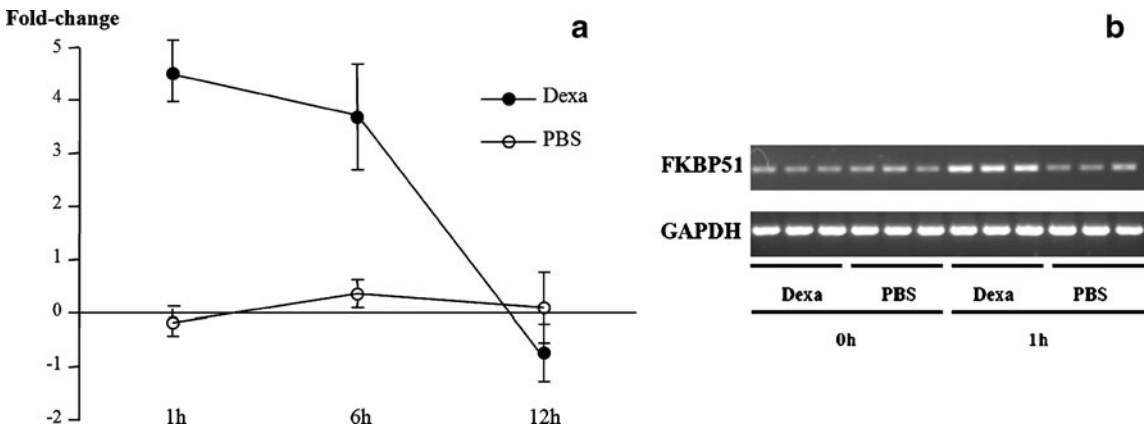


**Fig. 1** Change of *FKBP51* expressions in lung tissue after dexamethasone treatment. **a** Fold changes (microarray data). The *y*-axis represents the fold change in expression (dexamethasone-treated vs. PBS-treated) on a log2 scale. **b** Confirmatory results of RT-PCR (*Dexa* dexamethasone)

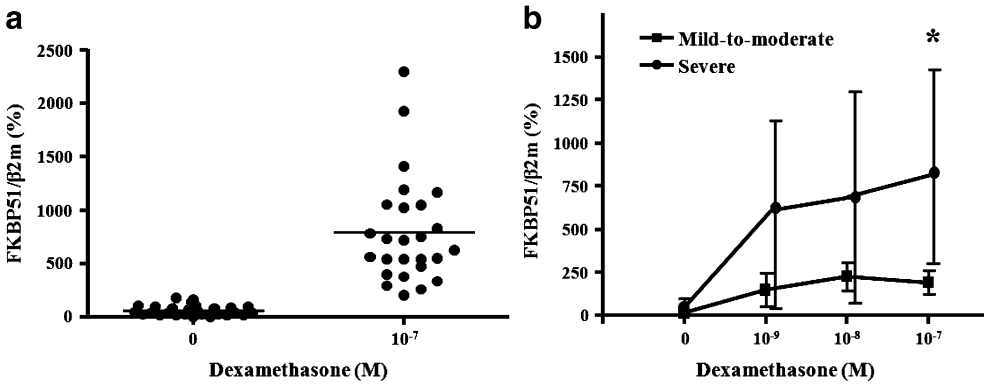


**Fig. 2** Dexamethasone-induced *FKBP51* expressions in PBMCs obtained from controls and asthmatics. **a** *Bars* represent average controls. **b** *Circles* represent mild-to-moderate asthmatics, and *rectangles* represent severe asthmatics

identified as being both time- and dose- dependent with statistical significance ($P<0.01$). After removal of the unidentified genes, 194 known genes were selected and clustering analysis was done. Genes were divided into four clusters according to the similarity of their expression patterns by dose and duration of treatment (see Supplementary Figure 2 and Table 1). After careful review of the gene function and literature, we selected *FKBP51*, which was highly induced by dexamethasone at 1 h of treatment (Fig. 1). The 51-kDa FK506 binding protein coded by *FKBP51* is a chaperone protein of the glucocorticoid receptor (GR) complex, which stabilizes GR in the cytoplasm before it combines with hormones [13]. The overexpression of *FKBP51* appears to inhibit GR signaling by impairing nuclear translocation [14] and reducing hormonal binding [15] of GR, contributing to glucocorticoid resistance syndrome [16]. Moreover, a recent report demonstrated that corticosteroid treatment of airway epithelial cells in asthmatics markedly up-regulated expression of *FKBP51* [17]. We also confirmed the changes in *FKBP51* expression by RT-PCR (Fig. 1).

*FKBP51* Expressions in PBMCs Obtained from Asthmatics

Interestingly, induction of *FKBP51* mRNA by $10^{-7}$ M dexamethasone in PBMC obtained from controls showed a wide variation (Fig. 2a). For asthmatics, significant differences in a dexamethasone-induced *FKBP51* expression in PBMCs at concentration of $10^{-7}$ M were observed (Fig. 2b). Figure 3 shows the relationship between % FEV1 increase after treatment with 7-day prednisolone and baseline dexamethasone ($10^{-7}$ M) induced *FKBP51* expressions in PBMCs in six treatment-naive asthmatics. Significantly, % FEV1 increase was inversely correlated to baseline *FKBP51* induction ($R=-0.821$, $P=0.045$).

## Discussion

Although CSs are highly effective in treating asthma by improving airway hyperresponsiveness, reducing the eosinophilic inflammation, and suppressing the expression of multiple inflammatory genes in the airways [18], the individual response to CSs is known to vary widely [19]. Responsiveness to CSs is, in part, related to the nature and severity of underlying inflammation. Sputum eosinophilia [20] and exhaled nitric oxide [7] have been suggested as predictors of response to CSs. Some asthmatics have refractory asthma, however, and display evidence of eosinophilic airway inflammation despite treatment with high doses of CSs [21], Indeed, CSs-resistant asthma is well characterized [18, 22]. We hypothesized that responsiveness to CSs is not only associated with the asthma-specific trait (for example, nature of inflammation) but also associated with the subject-specific trait, which is determined by an underlying genetic background. Based on this hypothesis, we performed time-series microarrays using a murine model of asthma to discover the gene showing a greater change in response to CSs, which can be a potential marker in the prediction of response.

Similar to the observation by Woodruff et al. [17], we found that dexamethasone treatment markedly up-regulated

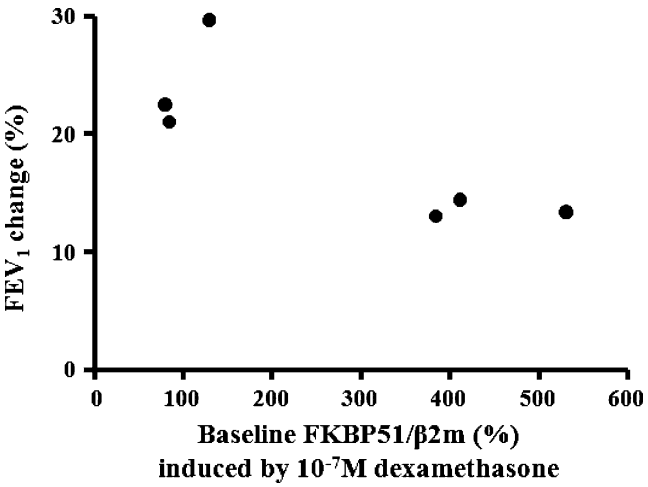


**Fig. 3** Correlation between % FEV1 increase after 7-day prednisolone treatment and baseline dexamethasone ($10^{-7}$ M) induced *FKBP51* expressions in six treatment-naive asthmatics

expression of *FKBP51* on lung tissue in a murine model of asthma. FK506 binding protein coded by *FKBP51* is an immunophilin chaperone protein, a subunit of the multi-protein CSs receptor "aporeceptor" complex that resides in the cytoplasm before hormone binding. On association with an agonist such as dexamethasone, CSs receptor dissociates from the chaperone complex, translocates to the nucleus, and modulates transcription [14]. *FKBP51* overexpression appears to inhibit CSs receptors signaling by impeding nuclear translocation and reducing hormone binding [15, 23]. These observations suggested that FK506 binding protein may serve as a negative feedback. Along with this, *FKBP51* overexpression may contribute to CS resistance syndrome in primates [16, 24].

Interestingly, in the present study, we found that dexamethasone-induced *FKBP51* expression in PBMCs varied widely in healthy controls, which implied that there must be individual variations in intrinsic susceptibility to CSs treatment. Moreover, dexamethasone-induced *FKBP51* expression in PBMCs in severe asthmatics was significantly higher compared with mild-to-moderate asthmatics. The use of a larger dose of inhaled CSs in severe asthmatics was a possible reason for this, as it is known that *FKBP51* expression is also induced by CSs themselves [11, 12]. All the asthmatics enrolled in this study, however, were treated by inhaled CSs, and it is known that systemic absorption of inhaled CSs is essentially negligible [25]. Therefore, the difference observed between severe and mild-to-moderate asthmatics might come from differences in intrinsic properties, rather than from the difference in doses of inhaled CSs.

It was reported that baseline expression of *FKBP51* in epithelial cells of asthmatics correlated with lung function response to fluticasone, an inhaled corticosteroid [17]. Likewise, we observed that changes in FEV1 in response to systemic CSs treatment was inversely correlated to dexamethasone-induced *FKBP51* expression in PBMCs in asthmatics. It is true that the main pathology resides in the airway epithelium of asthmatics, but it is impractical to evaluate dexamethasone-induced *FKBP51* expression on airway epithelium obtained from bronchoscopic biopsy before initiation of treatment. As mentioned earlier, however, it is well known that the glucocorticoid-induced *FKBP51* mRNA increase in PBMC can be used to determine sensitivity to glucocorticoids [10–12]. Therefore, dexamethasone-induced *FKBP51* expression in PBMCs may be a practical biomarker in predicting the responses of asthmatics to CSs.

Drug response is a complex phenotype [26, 27], and thus nature of disease, related genes, environmental factors, and their interactions should be considered together. We are not of the opinion that dexamethasone-induced *FKBP51* expression in PBMCs entirely predicts the response to CSs in asthmatics, but rather prefer to view it as a complementary and practical tool to use clinically. We sincerely hope that our results are confirmed in a successive and large-scale study.

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