Effects of Glucocorticoid-Induced Transcript 1 Gene Deficiency on Glucocorticoid Activation in Asthmatic Mice

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Abstract

Background: Glucocorticoid (GC) is the first-line therapy for asthma, but some asthmatics are insensitive to it. Glucocorticoid-induced transcript 1 gene (GLCCI1) is reported to be associated with GCs efficiency in asthmatics, while its exact mechanism remains unknown.

Methods: A total of 30 asthmatic patients received fluticasone propionate for 12 weeks. Forced expiratory volume in 1 s (FEV₁) and GLCCI1 expression were detected. Asthma model was constructed in wild-type and GLCCI1 knockout (GLCCI1⁻/⁻) mice. Glucocorticoid receptor (GR) and mitogen-activated protein kinase phosphatase 1 (MKP-1) expression were detected by polymerase chain reaction and Western blotting (WB). The phosphorylation of p38 mitogen-activated protein kinase (MAPK) was also detected by WB.

Results: In asthmatic patients, the change of FEV₁ was positively correlated with change of GLCCI1 expression (r = 0.430, P = 0.022). In animal experiment, GR and MKP-1 mRNA levels were significantly decreased in asthmatic mice than in control mice (wild-type: GR: 0.769 vs. 1.000, P = 0.022; MKP-1: 0.493 vs. 1.000, P < 0.001; GLCCI1⁻/⁻: GR: 0.629 vs. 1.645, P < 0.001; MKP-1: 0.377 vs. 2.146, P < 0.001). Hydrocortisone treatment significantly increased GR and MKP-1 mRNA expression levels than in asthmatic groups; however, GLCCI1⁻/⁻ asthmatic mice had less improvement (wild-type: GR: 1.517 vs. 0.769, P = 0.023; MKP-1: 1.036 vs. 0.493, P = 0.003. GLCCI1⁻/⁻: GR: 0.846 vs. 0.629, P = 0.116; MKP-1: 0.475 vs. 0.377, P = 0.388). GLCCI1⁻/⁻ asthmatic mice had more obvious phosphorylation of p38 MAPK than wild-type asthmatic mice (9.060 vs. 3.484, P < 0.001). It was still higher even though after hydrocortisone treatment (6.440 vs. 2.630, P < 0.001).

Conclusions: GLCCI1 deficiency in asthmatic mice inhibits the activation of GR and MKP-1 and leads to more obvious phosphorylation of p38 MAPK, leading to a decremental sensitivity to GCs.

Trial Registration: ChiCTR.org.cn, ChiCTR-RCC-13003634; http://www.chictr.org.cn/showproj.aspx?proj=5926.

Key words: Asthma; Glucocorticoid Receptor; Glucocorticoid-Induced Transcript 1; Glucocorticoids; Mitogen-Activated Protein Kinase Phosphatase-1

INTRODUCTION

Asthma is a chronic airway inflammatory disease that affects approximately 315 million people worldwide.¹ Inhaled corticosteroids (ICS) are the first-line anti-inflammatory therapy of asthma. However, not all asthmatic patients benefit from ICS treatment. In a study by Pignatti,² 22% of patients taking inhaled beclomethasone exhibited a decrease in forced expiratory volume in 1 s (FEV₁) after 12 weeks of therapy. Some asthmatics even experienced worsened symptoms after corticosteroid treatment.³ Studies have revealed that several genes, such as NR3C1,⁴ STIP1,⁵ CRHR1,⁶ and FCER2,⁷ were associated with ICS efficiency of asthmatic patients.

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In 2011, Tantisira et al.[15] revealed that glucocorticoid-induced transcript 1 gene (GLCCI1) functional variant rs37973 was associated with marked attenuation of the response to treatment with ICS through changes in GLCCI1 expression. Soon after that, Izuhara et al.[16] proposed that GLCCI1 variant rs37973 was a risk factor for pulmonary function decline in Japanese patients with asthma receiving long-term ICS treatment. Xu et al.[17] also found that GLCCI1 variant rs37973 was associated with poorer clinical therapeutic response to inhaled glucocorticoids (GCs) in a Chinese asthma population. Rijavec et al.[11] revealed that GLCCI1 rs37973 was associated with short- and long-term treatment response. Brouwer et al.[12] revealed that GLCCI1 variant rs37972 was also related with the dexamethasone responsiveness in bacterial meningitis as was found in asthma. Furthermore, researchers have found that GLCCI1 polymorphism was involved in the GCs response in several diseases, such as graft-versus-host diseases,[13] rheumatoid arthritis,[14] and nephrotic syndrome.[15] The above reports suggest that GLCCI1 plays an important and critical role in the GCs pathway, but the exact function of GLCCI1 is still relatively unknown.

In our previous study, we found that asthmatic patients with rs37973 mutant genotype had diminished ICS response and less improvement in GLCCI1 expression.[16] However, the association between GLCCI1 expression and the response of ICS is only a statistical speculation. In the present study, we hypothesized that GLCCI1 might affect the ICS efficiency in asthmatic patients by influencing the GC pathway. Thus, we investigated the effects of GLCCI1 deficiency on the GC sensitivity in asthmatic mice model and detected the glucocorticoid receptor (GR), mitogen-activated protein kinase phosphatase 1 (MKP-1) expression, and p38 mitogen-activated protein kinase (MAPK) phosphorylation level in the lung tissue of these mice. Our findings demonstrated that GLCCI1 deficiency in asthmatic mice inhibits the activation of GR and MKP-1 and leads to more obvious phosphorylation of p38 MAPK, leading to a decremental sensitivity to GCs.

## Methods

### Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Chinese Ethnic Committee of Registering Clinical Trials and registered in the Chinese Clinical Trial Registry. Informed written consent was obtained from all patients before their enrollment in this study.

### Participants and design

From September 2013 to December 2014, 36 adult asthmatic patients in mild-to-moderate stage were recruited from the Respiratory Clinic of Xiangya Hospital, Central South University in Changsha, Hunan, China. Asthma diagnosis and severity were established according to the Global Initiative for Asthma guidelines, based on the clinical symptoms and spirometry. Exclusion criteria included (1) intermittent status asthma, severe asthma, or acute exacerbation of asthma; (2) occupational asthma or aspirin or nonsteroidal anti-inflammatory drug-related asthma; (3) life-threatening acute asthma exacerbation occurred within 1 year before the test; (4) recent asthma exacerbations requiring hospitalization or oral corticosteroids use; (5) history of significant lung diseases other than asthma; and (6) history of smoking in excess of 10 pack-years. After a 2-week run-in period, all the asthmatics were treated with fluticasone propionate (125 μg, twice a day, Glaxo Wellcome, S.A., UK) for 12 weeks. All patients received salbutamol (100 μg, Glaxo Wellcome, S.A., UK) as needed throughout the run-in and treatment period. No other asthma medications were permitted. Spirometry, blood sample, asthma control test (ACT), and asthma quality of life questionnaire (AQLQ) we collected at enrollment and at the 12th week. The blood sample was used for the detection of blood eosinophil count and GLCCI1 mRNA expression in peripheral blood mononuclear cells (PBMCs).

### Animals and design

Wild-type and GLCCI1 knockout (GLCCI1−/−) C57BL/6N female mice of 6–8 weeks old weighing 18–20 g were bred under specific-pathogen-free conditions in the Experimental Animal Center of Central South University, Changsha, Hunan, China. The details of the construction of the GLCCI1 knockout homozygous mice were displayed in the supplement file. All procedures performed on the animals were in compliance with the Chinese Council of Animal Care Guidelines, approved by the Central South University Animal Care Committee (No. 201603376). The wild-type and GLCCI1−/− mice were randomly divided into three groups (n = 8): control groups (ConWT and ConKO), asthma groups (AsWT and AsKO), and hydrocortisone-treated asthma groups (AsWT + GC and AsKO + GC). The mice were treated as follows: on day 0, day 7, and day 14, the asthmatic mice were sensitized with an intraperitoneal injection of 50 μg of chicken ovalbumin (OVA, Sigma, Grade V, USA) and 2 mg of aluminum hydroxide (Sigma, USA) in 0.2 ml of sterile saline. The control mice were treated with a sterile saline intraperitoneal injection for sham sensitization. The sensitized mice were exposed to 5% OVA (wt/vol) aerosol for 30 min every day from day 21 to day 27, while the control mice were exposed to sterile saline.[17] The hydrocortisone-treated asthmatic mice were handled with intraperitoneal injection of hydrocortisone (3 mg/kg, Lijun, China) every day 1 h before inhalation of 5% OVA aerosol.[18] Mice were sacrificed with 10% chloral hydrate (3.5 ml/kg) within 24 h after the last intervention. The right middle lungs were stored in 4% paraformaldehyde for hematoxylin and eosin (H and E) staining, and the rest were immediately stored in liquid nitrogen for polymerase chain reaction (PCR) and Western blotting (WB). Bronchoalveolar lavage fluid (BALF) was stored in cell preservation liquid for white blood cell counting.

### Measurement of bronchial responsiveness

Pulmonary function was performed in asthmatic patients using the Jaeger Masterscope spirometry system.
(Jaeger, Wurzburg, Germany) according to the American Thoracic Society guideline. All the asthmatic patients had their pulmonary function measured before and after 12 weeks’ treatment of fluticasone propionate. The change of FEV\textsubscript{1} (ΔFEV\textsubscript{1}) was calculated by FEV\textsubscript{1} measured after 12 weeks’ treatment minus FEV\textsubscript{1} measured before treatment.

In vivo airway responsiveness of the mice to methacholine was measured within 24 h after the last OVA challenge using whole-body plethysmography (Buxco Electronics Inc., USA). The mice were challenged for 2 min with aerosolized solutions of methacholine (0, 2.5, 5.0, 10, and 20 mg/ml in normal saline) by an ultrasonic nebulizer, and airway resistance (R\textsubscript{a}) was measured. The results were expressed for each methacholine concentration as the percentage of baseline R\textsubscript{a} values after saline exposure.

Bronchoalveolar lavage

After determination of bronchial responsiveness, the lungs were lavaged in situ with three successive 1 ml volumes of ice-cold phosphate-buffered solution (PBS) instilled by a syringe. The BALF was recovered manually by gentle aspiration with a disposable syringe after each infusion; the recovery rate of BALF was more than 80%. The lavage fluid was centrifuged at 1500 ×g for 10 min at 4°C; the sediment was resuspended in PBS and then stained with Wright–Giemsa. A total of 200 cells were counted for cell classification.

Lung histopathology

The middle lobe of the right lung was fixed in 4% paraformaldehyde for 18–24 h, embedded in paraffin, and the morphological changes of the lung were observed under a light microscope (Leica Microsystems, Wetzlar, Germany).

Western blotting

Lung tissues of the mice were first ground by an electronic tissue grinder (Tiangen, China) and then incubated in RIPA lysis buffer plus 1 mmol/L phenylmethylsulfon fluoride (PMSF) and a protease inhibitor cocktail (Servicebio, China) for 30 min. The homogenates were centrifuged at 15,000 ×g for 15 min at 4°C. The concentration of proteins in the supernatant fractions was determined by BCA Protein Assay Kit (Beyotime, China). Equal amounts of protein (50 μg) of each selected sample were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Separated proteins were transferred to polyvinylidene fluoride membranes after electrophoresis. Membranes were blocked with 5% nonfat dry milk or 5% bull serum albumin (for phosphorylated proteins) dissolved in Tris-buffered saline with Tween-20 buffer for 1 h and then incubated at 4°C overnight with primary antibodies against GLCCI1 (1:500; Hangzhou Goodhere Biotechnology, China), GR (1:1000; Cell Signaling Technology, USA), MKP-1 (1:1000; Merck-Millipore), p38 (1:1000; Cell Signaling Technology), and phosphorylated p38 (1:1000; Cell Signaling Technology) followed by incubation with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The bands were detected by Image Lab system and quantified with densitometry. The results were expressed as the ratio of the mean band density of experimental groups to that of the control group after normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control.

RNA preparation and real-time reverse transcription-polymerase chain reaction

We determined the relative mRNA levels of human GLCCI1, with human GAPDH mRNA as an internal control, using an ABI V77A 7 real-time PCR system (ABI, USA). For the analysis, 4 ml of the collected blood was used for isolation of PBMCs, using Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) according to the manufacturer’s instructions. Total RNA in PBMC of the asthmatic patients and lung tissue of the mice was extracted by TRIzol reagent (Invitrogen, Life technology). After testing the concentration and integrity of RNA by detecting optical density, RNA was reversely transcribed to cDNA according to the manufacturer’s instructions. Primers for GLCCI1, GR, MKP-1, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6), and interleukin-13 (IL-13) used in real-time PCR were designed by primer premier 3.0 (Premier, Canada), compounded by Sangon Biotech (Shanghai, China), and are listed in Table 1. The PCR reactions were carried out in a 10 μl reaction volume containing 1 ng cDNA, 0.2 μmol/L forward primer, 0.2 μmol/L reverse primer, 5 μl × UltraSYBR Mixture, and 3.6 μl RNase-free double-distilled water. PCR conditions were as follows: step 1, 10 min at 95°C; step 2, 5 s at 95°C; and step 3, 34 s at 60°C; with step 2 and step 3 repeated for 40 cycles. The results were expressed as the ratio of the mean threshold cycle (Ct) values of experimental groups to that of the control group after normalization to GAPDH.

Statistical analysis

Statistical analysis of data was performed with SPSS 19.0 software (IBM, USA). All data were displayed as mean ± standard deviation (SD). Spearman’s correlation was used to investigate the relationship between change in FEV\textsubscript{1} and change in GLCCI1 mRNA expression. One-way analysis of variance (ANOVA) followed by Student–Newman–Keuls post hoc test was used to compare the differences among multiple groups for normally distributed variables. Otherwise, Kruskal–Wallis test followed by Mann–Whitney test was used to compare the differences among multiple groups for abnormally distributed variables. P < 0.05 was considered statistically significant.

Results

Subject characteristics

Among the 36 asthmatic patients receiving fluticasone propionate treatment, 3 lost to follow-up, 2 withdrew consent during follow-up, and 1 was withdrawn by clinician for his poor compliance. As a result, 30 patients completed the 12-week
follow-up. Only individuals who completed the treatment were included in the analysis. The blood eosinophil count significantly decreased after fluticasone treatment for 12 weeks ($P = 0.024$). Significant improvement on FEV$_1$, GLCCI1 mRNA expression, ACT and AQLQ were also observed after fluticasone treatment ($P < 0.001$ for all). The characteristics of these 30 asthmatic patients are listed in Table 2.

**Glucocorticoid-induced transcript 1 gene mRNA expression is associated with inhaled corticosteroid efficiency**

We detected the GLCCI1 mRNA expression level in PBMCs of each patient before and after treatment (0 and 12th week). The change of GLCCI1 mRNA expression level ($\Delta$GLCCI1 expression) was calculated by GLCCI1 mRNA expression at the 12th week minus GLCCI1 mRNA expression at the 0 week. $\Delta$FEV1 was calculated by FEV1 at the 12th week minus FEV1 at the 0 week. Spearman’s correlation analysis revealed that $\Delta$FEV1 was well positively correlated with $\Delta$GLCCI1 expression [Figure 1, $r = 0.430$, $P = 0.022$], suggesting that the change of GLCCI1 mRNA expression after GC treatment is associated with ICS efficiency in asthmatic patients. However, the baseline GLCCI1 mRNA expression was not correlated with ICS efficiency [Figure 1, $P > 0.05$]. Meanwhile, we also analyzed the relationship between GLCCI1 mRNA expression and the blood eosinophil count, which found no correlation neither between baseline GLCCI1 mRNA expression and baseline blood eosinophils nor between $\Delta$GLCCI1 expression and change of blood eosinophils (data not shown).

**GLCCI1 deficiency impaired the inhibitory effects of glucocorticoids on lung inflammation**

To find out how GLCCI1 affects the ICS efficiency in asthmatics, we constructed asthma model in both GLCCI1 knockout (GLCCI1$^{-/-}$) and wild-type mice. The RL value in the asthmatic groups (AsWT and AsKO) was significantly elevated compared with the control groups (ConWT and ConKO) [326.00 ± 95.62% vs. 68.83 ± 35.32%, $t = -6.182$, $P = 0.001$ in wild-type group; 359.83% ± 76.03% vs. 82.00% ± 35.39%, $t = -8.115$, $P < 0.001$ in GLCCI1$^{-/-}$ group; Figure 2a]. Administration of hydrocortisone (AsWT + GC and AsKO + GC) significantly decreased the RL value of the asthmatic mice (AsWT and AsKO) [111.17% ± 42.91% vs. 326.00% ± 95.62%, $P = 0.024$]. Meanwhile, we also detected the ACT and AQLQ were also observed after fluticasone treatment for 12 weeks ($P = 0.002$ in wild-type group; 188.89% ± 49.85% vs. 359.83% ± 76.03%, $P = 0.001$ in GLCCI1$^{-/-}$ group; Figure 2a). However, the RL was higher in GLCCI1$^{-/-}$ group than in the wild-type group. The proportion of eosinophils in BALF was elevated in asthmatic groups (AsWT and AsKO) than in control groups (ConWT and ConKO) (0.57% ± 0.07% vs. 0.38% ± 0.11%, $t = -2.934$, $P = 0.026$ in wild-type
group; 359.83% ± 76.03% vs. 68.83 ± 35.32%, $t = -6.182$, $P = 0.001$ in wild‑type group; 326.00% ± 95.62% vs. 111.17% ± 42.91%

**Table 1: Sequences of target genes for real‑time PCR**

| Gene          | Forward primer (5’ to 3’) | Reverse primer (5’ to 3’) |
|---------------|---------------------------|---------------------------|
| GAPDH (mouse) | GGTGTTCTCTCTGGAAGTTCTCTCA | TGGCCAGGTTTCTACTCC         |
| GLCCI1 (mouse)| AGGCCGAACCTCTCTCTGAAGTTCTCT  | GTGACAGTGTTTCTACTCC         |
| GR (mouse)    | GTGATCTCTCCCGTGCAAGTTCTCT  | TACAGCTCCACACGTGAGCTCA     |
| MKP1 (mouse)  | AGGATATGCTTGGAGCTCTTG      | GCTGCGCTTGTGGTGCTCT         |
| IFN-γ (mouse) | ATGACCTCTGACACACTGATCT    | CCACTTCTTTGCGCTGCTCT       |
| TNF-α (mouse) | CCCCCACACTCAGATCATCTTCT    | GCTACGACGTGGCGTCT          |
| IL-13 (mouse) | CCTGCTCTTTGCTGCTCT         | GGCTGCTGCTGCTGCTCT         |
| IL-6 (mouse)  | TAGCTTTCTCTTCTCTTTCT      | TTGGCTTCTGACACTCTTCT       |
| GAPDH (human) | GGTTAAGGTTGAGAGTTCAAGC    | CAAAGGTTGCTGACACCTCA       |
| GLCCI1 (human)| ACTCGGACGACACTCTCA        | ATGGAGGCGTGCACACTCAG       |

**Table 2: Characteristics of the asthmatic patients receiving fluticasone treatment (n = 30)**

| Description | On admission | After treatment | t   | P   |
|-------------|-------------|----------------|-----|-----|
| Age (years), mean ± SD | 42.10 ± 10.20 | –              | –   | –   |
| Gender (men/women), n | 14/16       | –              | –   | –   |
| FEV$_1$, (L) | 2.19 ± 0.60 | 2.39 ± 0.56    | –4.327| <0.001|
| FEV$_1$% predicted | 75.87 ± 11.48 | 83.26 ± 10.29 | –4.376| <0.001|
| GLCCI1 mRNA expression | 0.030 ± 0.015 | 0.068 ± 0.032 | –8.101| <0.001|
| ACT | 15.90 ± 3.57 | 23.67 ± 1.42   | –11.267| <0.001|
| AQLQ | 3.98 ± 0.48 | 4.68 ± 0.31   | –6.863| <0.001|
| Blood eosinophil count (× 10$^3$/L) | 0.36 ± 0.22 | 0.28 ± 0.17 | 2.377| 0.024|

*: Not applicable; SD: Standard deviation; FEV$_1$: Forced expiratory volume in the 1st s; GLCCI1: Glucocorticoid-induced transcript 1 gene; ACT: Asthma control test; AQLQ: Asthma quality of life questionnaire; SD: Standard deviation.
group; 0.61% ± 0.08% vs. 0.37% ± 0.10%, t = −3.688, P = 0.010 in GLCCI1−/− group; Figure 2b]. Administration of hydrocortisone (AsWT + GC and AsKO + GC) significantly decreased the proportion of eosinophils in BALF of the asthmatic mice (AsWT and AsKO) [0.40% ± 0.12% vs. 0.57% ± 0.07%, t = 2.603, P = 0.041 in wild-type group; 0.42% ± 0.12% vs. 0.61% ± 0.08%, t = 2.689, P = 0.036 in GLCCI1−/− group; Figure 2b]. The IFN-γ, TNF-α, IL-6, IL-13, and IL-4 mRNA expression levels in lung tissue are shown in Table 3 and Figure 2c. The IFN-γ, TNF-α, IL-6, IL-13, and IL-4 mRNA expression levels were significantly elevated in asthmatic groups (AsWT and AsKO) than in control groups (ConWT and ConKO) (P < 0.05). Administration of hydrocortisone significantly decreased IFN-γ, TNF-α, IL-6, IL-13, and IL-4 mRNA expression levels in wild-type asthmatic group (P < 0.05), but not in GLCCI1−/− asthmatic group (except for IFN-γ and TNF-α, P < 0.05). The IFN-γ, TNF-α, IL-6, IL-13, and IL-4 mRNA expression levels in GC-treated GLCCI1−/− asthmatic mice (AsKO + GC) were significantly decreased than those in GC-treated wild-type asthmatic mice (AsWT + GC) (P < 0.05). As shown in Figure 3, the asthmatic groups displayed more severe infiltration of inflammatory cells in lung tissue; the infiltration of inflammatory cells was more serious in GLCCI1−/− asthmatic mice than in wild-type asthmatic mice. Subsequent administration of hydrocortisone decreased the severity of inflammation in the lung tissue.

**Glucocorticoid-induced transcript 1 gene deficiency impaired the effects of glucocorticoids on glucocorticoid receptor and mitogen-activated protein kinase phosphatase-1**

We first detected GLCCI1 mRNA and protein expression in the lung tissue of each group of mice. In wild-type mice, the GLCCI1 mRNA and protein expression levels significantly decreased in asthmatic group (AsWT) than those in control group (ConWT) (mRNA: 0.547 ± 0.180 vs. 1.000 ± 0.000, t = 6.170, P = 0.002; protein: 0.350 ± 0.044 vs. 1.000 ± 0.000, t = 32.500, P < 0.001). After administration of hydrocortisone (AsWT + GC), the GLCCI1 mRNA and protein expression levels significantly increased than those in AsWT group (mRNA: 1.419 ± 0.447 vs. 0.547 ± 0.180, t = −4.429, P = 0.001; protein: 1.222 ± 0.062 vs. 0.350 ± 0.045, t = −25.450, P < 0.001). While in GLCCI1−/− mice, GLCCI1 was almost not expressed, neither at the mRNA nor at protein level, indicating that the GLCCI1 gene was successfully knocked out (mRNA: 0.0028 ± 0.0017 in ConKO, 0.0028 ± 0.0017 in AsKO, 0.0032 ± 0.0031 in AsKO + GC; protein: 0.372 ± 0.061 in ConKO, 0.200 ± 0.054 in AsKO, 0.308 ± 0.043 in AsKO + GC). The data are shown in Figure 4.

To evaluate whether GLCCI1 influences the expression of GR and MKP-1 or not, we detected the mRNA and protein levels of GR and MKP-1 in the lung tissue of each group of mice. In wild-type mice, the GR and MKP-1 mRNA and protein expression levels significantly decreased in asthmatic group (AsWT) than those in control group (ConWT) (GR mRNA: 0.769 ± 0.172 vs. 1.000 ± 0.000, t = 3.292, P = 0.022;

![Figure 1](image1.png)

**Figure 1:** The correlation between the change in FEV₁ and GLCCI1 mRNA expression in asthmatic patients (n = 30). (a) Spearman's correlation analysis revealed that change in FEV₁ was well positively correlated with change in GLCCI1 expression after fluticasone treatment for 12 weeks (r = 0.430, P = 0.022). (b) The baseline GLCCI1 mRNA expression was not correlated with ICS efficiency (P > 0.05). FEV₁: Forced expiratory volume in 1 s; GLCCI1: Glucocorticoid-induced transcript 1 gene.

**Table 3: IFN-γ, TNF-α, IL-6, IL-13, and IL-4 mRNA expression in lung tissue**

| Groups     | TNF-α       | IFN-γ       | IL-13      | IL-6       | IL-4       |
|------------|-------------|-------------|------------|------------|------------|
| ConWT      | 1.000       | 1.000       | 1.000      | 1.000      | 1.000      |
| AsWT       | 1.518 ± 0.374* | 2.245 ± 0.627* | 15.711 ± 0.664* | 4.024 ± 0.207* | 2.086 ± 0.795* |
| AsWT + GC  | 1.120 ± 0.105  | 1.228 ± 0.072  | 4.184 ± 2.732  | 1.529 ± 0.455  | 1.173 ± 0.304  |
| ConKO      | 1.017 ± 0.291  | 1.293 ± 0.302  | 1.066 ± 0.090  | 0.779 ± 0.685  | 1.165 ± 0.241  |
| AsKO       | 2.275 ± 0.3431   | 2.904 ± 0.3731   | 15.677 ± 5.5571 | 4.176 ± 2.7441 | 3.000 ± 1.1901 |
| AsKO + GC  | 1.533 ± 0.2433   | 2.199 ± 0.4573   | 9.781 ± 3.246   | 4.096 ± 1.628   | 2.109 ± 0.475   |

*P < 0.05 versus ConWT group, †P < 0.05 versus AsWT group, ‡P < 0.05 versus ConKO group, §P = 0.05 versus AsKO group, ††P = 0.05 versus AsWT + GC group. ConWT: Wild-type control group; AsWT: Wild-type asthmatic group; AsWT + GC: Hydrocortisone-treated wild-type asthmatic group; ConKO: GLCCI1 knockout control group; AsKO: GLCCI1 knockout asthmatic group; AsKO + GC: Hydrocortisone-treated GLCCI1 knockout asthmatic group; n = 6 in each group. TNF-α: Tumor necrosis factor-α; IFN-γ: Interferon-γ; IL-13: Interleukin-13; IL-6: Interleukin-6; IL-4: Interleukin-4; GLCCI1: Glucocorticoid-induced transcript 1 gene.
Figure 2: Effect of GLCCI1 deficiency on lung inflammation in asthmatic mice under glucocorticoid treatment. (a) Airway resistance in each group (n = 6). (b) Cell differential counts in bronchoalveolar lavage fluid (%) (n = 4). (c) The mRNA expression of inflammatory genes in lung tissue (n = 6). *P < 0.05 versus ConWT group, †P < 0.05 versus AsWT group, ‡P < 0.05 versus ConKO group, §P < 0.05 versus AsKO group, ¶P < 0.05 versus ConWT + GC group. ConWT: Wild-type control group; AsWT: Wild-type asthmatic group; AsWT + GC: Hydrocortisone-treated wild-type asthmatic group; ConKO: GLCCI1 knockout control group; AsKO: GLCCI1 knockout asthmatic group; AsKO + GC: Hydrocortisone-treated GLCCI1 knockout asthmatic group; BALF: Bronchoalveolar lavage fluid; TNF-α: Tumor necrosis factor-α; IFN-γ: Interferon-γ; IL-13: Interleukin-13; IL-6: Interleukin-6; IL-4: Interleukin-4; GLCCI1: Glucocorticoid-induced transcript 1 gene.
GR protein: 0.730 ± 0.061 vs. 1.000 ± 0.000, \( t = 9.859, P = 0.001 \).

MKP-1 mRNA: 0.493 ± 0.152 vs. 1.000 ± 0.000, \( t = 8.152, P < 0.001 \);
MKP-1 protein: 0.406 ± 0.053 vs. 1.000 ± 0.000, \( t = 24.968, P < 0.001 \).

After administration of GLCCI1 deficiency on GR, MKP-1 expression and the phosphorylation of p38 MAPK. (a) The GLCCI1, GR, and MKP-1 mRNA expression in lung tissue. Data were shown as mean ± SD (n = 6). (b) The Western blotting analyses of GLCCI1, GR, MKP-1, and p38 MAPK. (c) Relative band density values in b. Data are shown as mean ± SD (n = 5). *P < 0.05 versus ConWT group, †P < 0.05 versus AsWT group, ‡P < 0.05 versus ConKO group, §P < 0.05 versus AsKO group, ||P < 0.05 versus AsWT + GC group. ConWT: Wild-type control group; AsWT: Wild-type asthmatic group; AsWT + GC: Hydroprednisone-treated wild-type asthmatic group; ConKO: GLCCI1 knockout control group; AsKO: GLCCI1 knockout asthmatic group; AsKO + GC: Hydroprednisone-treated GLCCI1 knockout asthmatic group; GLCCI1: Glucocorticoid-induced transcript 1 gene; GR: Glucocorticoid receptor; MKP-1: Mitogen-activated protein kinase phosphatase-1; MAPK: Mitogen-activated protein kinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; SD: Standard deviation.
Glucocorticoid-induced transcript 1 gene deficiency impaired the inhibitory effects of glucocorticoids on p38 mitogen-activated protein kinase pathway

To clarify whether GLCCI1 involved in the p38 MAPK pathway, we evaluated the phosphorylation ratio of the p38. As shown in Figure 4, WB revealed that phospho-p38 (p-p38) was prominent in asthmatic group than in control group, both in wild-type and GLCCI1+−/− asthmatic mice (wild-type: 3.484 ± 0.235 vs. 1.000 ± 0.000, t = −23.613, P < 0.001; GLCCI1+−/−: 9.060 ± 0.313 vs. 4.766 ± 0.177, t = −26.663, P < 0.001). Furthermore, the level of p-p38 in GLCCI1+−/− asthmatic mice was significantly much higher than that in wild-type asthmatic mice (9.060 ± 0.313 vs. 3.484 ± 0.235, t = −31.816, P < 0.001). Administration of hydroprednisone in asthmatic mice significantly decreased the p-p38 both in wild-type and GLCCI1+−/− mice (wild-type: 2.630 ± 0.181 vs. 3.484 ± 0.235, t = 6.434, P < 0.001; GLCCI1+−/−: 6.440 ± 0.160 vs. 9.060 ± 0.313, t = 16.637, P < 0.001), but the level of p-p38 in hydroprednisone-treated GLCCI1+−/− asthmatic group was still significantly higher than that in hydroprednisone-treated wild-type asthmatic group (6.440 ± 0.160 vs. 2.630 ± 0.181, t = −35.223, P < 0.001).

**DISCUSSION**

GCs are the most important anti-inflammatory drugs used in asthma as they inhibit the expression of inflammatory mediators by macrophages and other cells. However, a proportion of asthmatic patients do not benefit from their GC treatment and bear from uncontrolled and worsened condition of asthma. Until now, the mechanism of GCs insensitivity in asthma remains poorly understood.

We have previously found that the GLCCI1 mRNA expression was correlated with asthma susceptibility, but not correlated with asthma severity.[16] In this study, we observed that the change of GLCCI1 mRNA expression was significantly well positively correlated with FEV1 in asthmatic patients treated with ICS in a Chinese Han adult population, which was consistent with the previous study by Tantisira et al.[8] Many researchers have studied the effect of GLCCI1 polymorphism on ICS efficiency,[8,24] lung function,[9] and asthma control of asthmatics.[25] GLCCI1 was identified as a novel pharmacogenetics determinant of the response of asthmatic patients to ICS. Tantisira et al.[8] proposed that GLCCI1 might be an early marker of GC-induced apoptosis as it was a key mechanism through which GCs resolve lymphocytic and eosinophilic inflammation in asthma. However, the exact mechanism of how GLCCI1 affects the ICS response of asthmatics still remains unknown. It is well known that the GC pathway plays a critical role in the GC efficiencies; thus, we hypothesized that GLCCI1 might affect the ICS efficiency of asthmatic patients by influencing the GC pathway.

In 2011, Nishibori et al.[26] discovered that GLCCI1 promotes the normal development and maintenance of podocyte structure and function using GLCCI1 gene knockout zebrafish. As zebrafish is not available for pulmonary research, we constructed GLCCI1 knockout homozygous mice to explore how GLCCI1 affects the ICS response of asthmatics. We determined the role of GLCCI1 on the effects of GCs in asthma by constructing an asthma model in both wild-type and GLCCI1+−/− mice. As expected, both the wild-type and GLCCI1+−/− mice of the allergic asthma model exhibited airway hyperresponsiveness (AHR), eosinophilic airway inflammation, elevated lung immunological cytokines, and infiltration of inflammatory cells. In wild-type asthmatic mice, administration of GC significantly decreased AHR and alleviated airway inflammation. On the other hand, the function of GC in GLCCI1+−/− asthmatic mice was weakened, with less decrease in AHR and less alleviation of airway inflammation, which indicated that GLCCI1 deficiency might lead to GCs inefficiency in asthmatic condition.

The effects of GCs are mediated by GR. GR is a member of the nuclear hormone receptor superfamily of transcription factors.[27] In the absence of GC, GR stays in the cytoplasm in a complex with a number of proteins such as heat shock protein 90 (HSP 90) at an inactivated status. After crossing the cell membrane by passive diffusion, the GC binds to its receptor GR and activates GR by dissociation with HSP 90. As zebrafish is not available for pulmonary research, we constructed GLCCI1 knockout homozygous mice to explore how GLCCI1 affects the ICS response of asthmatics. We determined the role of GLCCI1 on the effects of GCs in asthma by constructing an asthma model in both wild-type and GLCCI1+−/− mice. As expected, both the wild-type and GLCCI1+−/− mice of the allergic asthma model exhibited airway hyperresponsiveness (AHR), eosinophilic airway inflammation, elevated lung immunological cytokines, and infiltration of inflammatory cells. In wild-type asthmatic mice, administration of GC significantly decreased AHR and alleviated airway inflammation. On the other hand, the function of GC in GLCCI1+−/− asthmatic mice was weakened, with less decrease in AHR and less alleviation of airway inflammation, which indicated that GLCCI1 deficiency might lead to GCs inefficiency in asthmatic condition.

The effects of GCs are mediated by GR. GR is a member of the nuclear hormone receptor superfamily of transcription factors.[27] In the absence of GC, GR stays in the cytoplasm in a complex with a number of proteins such as heat shock protein 90 (HSP 90) at an inactivated status. After crossing the cell membrane by passive diffusion, the GC binds to its receptor GR and activates GR by dissociation with HSP 90. The GC-GR complex then migrates to the nucleus, binds to the glucocorticoid response element, and activates the transcription of target genes, including MKP-1,[29] GILZ,[30] and SGK1.[31] Via binding to the receptor, GCs are able to...
inhibit p38 MAPK by inducing MKP-1, which in turn results in de-phosphorylation of p38. MKP-1 is a phosphatase that dephosphorylates and inactivates MAPKs, including p38 MAPK, and inhibits production of pro-inflammatory cytokines, which is critical for the anti-inflammatory functions of GCs. Clark et al. proposed that MKP-1 played a role in the inhibition of p38 MAPK and the consequent destabilization of pro-inflammatory mRNAs by GCs. Keränen et al. found that dexamethasone increased MKP-1 expression and resulted in the suppression of p38 MAPK signaling leading to the inhibition of cytokine production in human bronchial epithelial cells. Thus, they revealed that MKP-1 was an important factor in the therapeutic effects of GCs in the treatment of inflammatory lung diseases. MKP-1 may be involved in GC response since GCs inhibit p38 MAPK in macrophages from MKP1−/− mice but not in those from MKP-1−/− littermates. As a result, MKP-1 has been identified as a marker of responsiveness to GC treatment.

In order to explore how GLCCI1 deficiency leads to GCs inefficiency in asthmatic condition, we detected both the GR and MKP-1 expression in the lung tissue of each group. Interestingly, we found that GCs significantly increase the expression of GR and MKP-1 in wild-type asthmatic mice. Although the GR and MKP-1 expression also increase after GC treatment in GLCCI1−/− asthmatic mice, the GR and MKP-1 mRNA and protein expression levels in GC-treated GLCCI1−/− asthmatic group were significantly less than that in GC-treated wild-type asthmatic group. Bhattacharyya et al. discovered that dexamethasone could induce MKP-1 expression and inhibit p38 MAPK pathway in mice with normal macrophages, but not in mice with conditional deletion of GR in macrophages, suggesting that GR and its activation are essential for GCs to induce MKP-1 expression and inhibit p38 MAPK activity. We then concluded that GLCCI1 deficiency might suppress the activation of GR and inhibit the formation of GC-GR complex, resulting in less induction of MKP-1 expression under GC stimulation. However, the mechanism of how GLCCI1 deficiency suppresses the activation of GR needs further research.

To deeply determine whether p38 MAPK is influenced by GLCCI1 deficiency, we observed the phosphorylation of p38 MAPK and the expression of TNF-α and IL-6 of lung tissue in each group as p38 MAPK pathway mediates the expression of them. We found that the phosphorylation of p38 MAPK was more obvious in GLCCI1−/− asthmatic mice than that in wild-type asthmatic mice. Treatment of GCs decreased the phosphorylation of p38 MAPK, but the phosphorylation level of p38 MAPK in GC-treated GLCCI1−/− asthmatic group was still significantly higher than that in GC-treated wild-type asthmatic group. At the same time, GC intervention significantly decreased the expression of TNF-α and IL-6 in wild-type asthmatic mice, but showed less decrease in GLCCI1−/− asthmatic mice than that in wild-type asthmatic mice. These results indicated that GLCCI1 deficiency resulted in more obvious phosphorylation of p38 MAPK and led to more serious inflammation in the asthmatic model, and GCs had a mild effect on them. There were various indications that MKP-1 deficiency is associated with an increase in the production of many cytokines including TNF-α, IL-6, and IL-1β. It was reasonable to conclude that MKP-1 decreased the production of many cytokines by inhibiting the phosphorylation of p38 MAPK.

In summary, we found that GLCCI1 is closely related to GC efficiency in the clinical trial with 30 asthmatic patients receiving fluticasone treatment. Furthermore, we speculated that GLCCI1 deficiency might inhibit the activation of GR, leading to less improvement of MKP-1 pathway and less inhibition for the pro-inflammatory genes under GCs, which induce poor response to GCs in asthma. GC: Glucocorticoid; GR: Glucocorticoid receptor; GRE: Glucocorticoid response element; HSP 90: Heat shock protein 90; MKP-1: Mitogen-activated protein kinase phosphatase-1; MAPK: Mitogen-activated protein kinase; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; IL-1β: Interleukin-1β.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: Findings from the cross-sectional world health survey. BMC Public Health 2012;12:204. doi: 10.1186/1471-2458-12-204.

2. Pignatti PF. Trends in pharmacogenomics of drugs used in the treatment of asthma. Pharmacol Res 2004;49:343-9. doi: 10.1016/j.phrs.2003.04.002.

3. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Piñeiro A,
Wei LX, et al. Oral montelukast, inhaled beclometasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/ Beclometasone study group. Ann Intern Med 1999;130:487‑95. doi: 10.7326/0003-4819-130-6-19990310-00005.

4. Panek M, Pietras T, Antczak A, Fabijan A, Przemęcka M, Górski P, et al. The N363S and I559N single nucleotide polymorphisms of the h‑GR/NR3C1 gene in patients with bronchial asthma. Int J Mol Med 2012;30:142‑50. doi: 10.3892/ijmm.2012.956.

5. Hawkins GA, Lazarus R, Smith RS, Tantisira KG, Peters DA, Peters SP, et al. The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. J Allergy Clin Immunol 2009;123:1376‑83.e7. doi: 10.1016/j.jaci.2009.01.049.

6. Tantisira KG, Lake S, Silverman ES, Palmer LJ, Lazarus R, Silverman EK, et al. Corticosteroid pharmacogenetics: Association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. Hum Mol Genet 2004;13:1353‑9. doi: 10.1093/hmg/ddh149.

7. Tantisira KG, Silverman ES, Mariani TJ, Xu J, Richter BG, Klanderjan BJ, et al. FCER2: A pharmacogenetic basis for severe exacerbations in children with asthma. J Allergy Clin Immunol 2007;120:1285‑91. doi: 10.1016/j.jaci.2007.09.005.

8. Tantisira KG, Laskey‑Su J, Harada M, Murphy A, Litenjua AA, Himes BE, et al. Genomewide association between GLCCI1 and response to inhaled corticosteroid therapy in asthma. N Engl J Med 2011;365:1173‑83. doi: 10.1056/NEJMoa0911533.

9. Izhuhara Y, Matsumoto H, Kanemitsu Y, Izuhara K, Tohda Y, et al. Effect of aerosolized docosahexaenoic acid in a mouse model of atopic asthma. Int Arch Allergy Immunol 2000;123:327‑32. doi: 10.1159/000053645.

10. Xu Y, Wu H, Xu X, Xu Y, Zhao J, Xie J, et al. GLCCI1 rs37973: A potential genetic predictor of therapeutic response to inhaled corticosteroids in Chinese asthma patients. Medicine (Baltimore) 2017;96:e2944. doi: 10.1097/MD.0000000000002944.

11. Rajeev M, Žávbi M, Lopert A, Flézar M, Koroseč P. GLCCI1 polymorphism rs37973 and response to treatment of asthma with inhaled corticosteroids. J Investig Allergol Clin Immunol 2018;28:165‑71. doi: 10.18176/jiaci.0229.

12. Brouwer MC, van der Ende A, Baas F, de van Beek D. Genetic variation in GLCCI1 and dexamethasone in bacterial meningitis. J Infect 2012;65:465‑7. doi: 10.1016/j.jinf.2012.07.001.

13. O’Meara A, Boukouaci W, Robin M, Xhaard A, Fortier C, Marzais F, et al. Genetic variation in the glucocorticoid‑induced transcript 1 gene are associated with response to glucocorticoid therapy in asthma. Eur Respir J 2015;45:1173‑83. doi: 10.1183/09031936.00034815.

14. Wu XM, Hu CP, Li XZ, Zou YQ, Zou JT, Li YY, et al. Asthma pregnancy alters postnatal development of chromaffin cells in the rat adrenal medulla. PLoS One 2011;6:e20337. doi: 10.1371/journal.

15. Suvanto M, Jahnukainen T, Kestilä M, Jalanko H. Single nucleotide polymorphism in FCER2: A pharmacogenetic basis for severe asthma in children with asthma. J Allergy Clin Immunol 2014;133:587‑9. doi: 10.1016/j.jaci.2013.08.024.

16. Vrijverberg SJ, Tavendale R, Leusink M, Koenderman L, Raaijmakers JA, Postma DS, et al. Pharmacogenetic analysis of GLCCI1 in three North European pediatric asthma populations with a reported use of inhaled corticosteroids. Pharmacogenomics 2014;15:799‑806. doi: 10.2217/pgs.14.137.

17. Nishibori Y, Katayama K, Parikka M, Oddsson A, Nukui M, Hultenby K, et al. GLCCI1 deficiency leads to proteinuria. J Am Soc Nephrol 2011;22:2037‑43. doi: 10.1615/ASN.2010011477.

18. Newton R. Molecular mechanisms of glucocorticoid action: What is important? Thorax 2000;55:603‑13. doi: 10.1136/thorax.55.7.603.

19. Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci 2013;34:518‑30. doi: 10.1016/j.tips.2013.07.003.

20. Clark AR. MAP kinase phosphatase 1: A novel mediator of biological effects of glucocorticoids? J Endocrinol 2003;178:5‑12. doi: 10.1677/joe.0.1780050.

21. Wang JC, Derynck MK, Nonaka DF, Khodabakhsh DB, Haq C, Yamamoto KR, et al. Chromatin immunoprecipitation (ChIP) scanning identifies primary glucocorticoid receptor target genes. Proc Natl Acad Sci U S A 2004;101:15603‑8. doi: 10.1073/pnas.0407081101.

22. Itani OA, Liu KZ, Cornish KL, Campbell JR, Thomas CP. Glucocorticoids suppress interleukin‑1beta‑induced human beta‑defensin 2 mRNA expression: Involvement of p38 MAPK, JNK, MKP‑1, and NF‑κappaB transcriptional factor. J Immunol 2004;172:960‑2. doi: 10.1159/000053645.

23. Abu‑Raya H, Yiu Y, Chen H, Yau CH, Plumeti SM, Silverman ES, et al. Corticosteroid pharmacogenetics: Association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. J Allergy Clin Immunol 2014;133:587‑9. doi: 10.1016/j.jaci.2013.08.024.
GLCCI1基因敲除参与支气管哮喘小鼠糖皮质激素通路活化下调

摘要

背景: 糖皮质激素是支气管哮喘治疗的一线药物，但部分哮喘患者对激素不敏感。研究发现，糖皮质激素诱导的转录基因1（GLCCI1）与哮喘患者糖皮质激素疗效有关，但其具体机制尚不明确。

方法: 30例哮喘患者接受为期12周的丙酸氟替卡松吸入治疗，检测FEV1及GLCCI1表达水平。利用野生型及GLCCI1基因敲除小鼠构建哮喘模型。造模成功后，通过PCR及Western blotting检测肺组织中GR、MKP-1表达水平；Western blotting检测p38 MAPK信号通路的磷酸化程度。

结果: 哮喘患者经丙酸氟替卡松吸入治疗后FEV1改变量与GLCCI1 mRNA表达水平改变量呈正相关（r = 0.430，P = 0.022）。在动物实验中，哮喘小鼠肺组织GR及MKP-1 mRNA表达水平低于对照组（野生型组：GR：0.769 vs. 1.000，P = 0.022；MKP-1：0.493 vs. 1.000，P < 0.001；GLCCI1−/−组：GR：0.629 vs. 1.645，P < 0.001；MKP-1：0.377 vs. 2.146，P < 0.001）。与哮喘组小鼠相比，经氢化泼尼松治疗后的哮喘小鼠GR及MKP-1 mRNA表达水平显著升高，但GLCCI1−/−组升高程度低于野生型组（野生型组：GR：1.517 vs. 0.769，P = 0.023；MKP-1：1.036 vs. 0.493，P = 0.003；GLCCI1−/−组：GR：0.846 vs. 0.629，P = 0.116；MKP-1：0.475 vs. 0.377，P = 0.388）。与野生型哮喘小鼠相比，GLCCI1基因敲除哮喘小鼠p38 MAPK磷酸化水平升高（9.060 vs. 3.484，P < 0.001）。经氢化泼尼松治疗后GLCCI1基因敲除哮喘小鼠p38 MAPK磷酸化水平仍高于野生型哮喘小鼠（6.440 vs. 2.630，P < 0.001）。

结论: GLCCI1基因敲除哮喘小鼠GR及MKP-1表达受抑制，导致p38 MAPK磷酸化，从而导致糖皮质激素敏感性下降。