Impact of gene expression associated with glucocorticoid-induced transcript 1 (GLCCI1) on severe asthma and future exacerbation

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Summary

Genetic variations in glucocorticoid-induced transcript 1 (GLCCI1) have been associated with the response to corticosteroid treatment. However, the associations of GLCCI1 polymorphisms or gene expression with the prognosis of asthma and pathophysiological factors related to steroid insensitivity remain unclear. We sought to investigate the associations of GLCCI1, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and histone deacetylase 2 (HDAC2) mRNA expression levels and the GLCCI1 rs37973 polymorphism with asthma severity and future exacerbation in patients with asthma. Subjects included 25 patients with severe asthma and 127 patients with nonsevere asthma. mRNA expression levels in peripheral blood mononuclear cells were measured and evaluated as predictors of severe asthma using receiver operating characteristic (ROC) analysis. The hazard ratios of the mRNA expression levels for time to first exacerbation in the 1-year follow-up period were calculated. GLCCI1, Nrf2, and HDAC2 mRNA expression levels were significantly lower in patients with severe asthma than in patients with nonsevere asthma and could predict severe asthma with an area under the ROC curve of 0.68, 0.71, and 0.65, respectively. In contrast, no relationship was found between the GLCCI1 rs37973 polymorphism and severe asthma. The hazard ratios for asthma exacerbation in patients with low GLCCI1, Nrf2, and HDAC2 mRNA expression levels were 3.24 (95% confidence interval, 1.42–7.40), 3.13 (1.37–7.16), and 2.98 (1.22–7.25), respectively. Patients with severe asthma could be distinguished by lower GLCCI1, Nrf2, and HDAC2 mRNA levels in peripheral blood cells, and all of these gene signatures could predict future asthma exacerbations.
Keywords: asthma; gene expression signature; glucocorticoid-induced transcript 1; histone deacetylase 2; nuclear factor (erythroid-derived 2)-like 2
**Introduction**

Asthma is a heterogeneous disease that can be characterized by several different phenotypes.\(^1\) Approximately 5-10% of patients with asthma are classified as having severe asthma with inadequate control despite optimal pharmacological treatment;\(^2,3\) however, the underlying molecular pathogenesis of this phenotype is not fully understood.

Several studies have focused on the relationship between corticosteroid resistance and severe asthma.\(^4\) Corticosteroids are effective in reducing inflammatory gene expression through the activating glucocorticosteroid receptor, which leads to the recruitment of histone deacetylase 2 (HDAC2) to the activating transcription complex. Reductions in HDAC2 expression or activity have been suggested to contribute to steroid resistance, and these reductions are higher in patients with severe asthma.\(^5\) Although the mechanism by which HDAC2 expression is downregulated has not been fully elucidated, oxidative stress is known to contribute to the inactivation of HDAC2.\(^6\) The transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) plays a central role in cellular antioxidant defense systems by positively regulating antioxidant gene expression,\(^7,8\) and its downregulation is correlated with a decrease in HDAC2 activity.\(^9\)

Genetic variations could explain interindividual variability in corticosteroid response among patients with asthma. A previous genome-wide association study showed that the rs37973 polymorphism in the promoter region of the glucocorticoid-induced transcript 1 (GLCCI1) gene was associated with the response to inhaled corticosteroid (ICS) treatment.\(^10\) The minor alleles of these polymorphisms, which decrease GLCCI1 gene expression, contributed to the poor improvement of lung function after 4 to 8 weeks of ICS therapy.\(^10\)
However, it remains unclear whether GLCCI1 polymorphisms or gene expression affects the prognosis of patients with asthma, such as the development of severe asthma and exacerbation. Moreover, little is known about the molecular interaction of GLCCI1 with previously reported pathophysiological factors related to steroid-insensitive and severe asthma.

We hypothesized that a minor allele of the GLCCI1 polymorphism and lower GLCCI1 mRNA expression levels would impact the worsening of clinical outcomes. This study aimed to investigate whether GLCCI1 polymorphisms and gene expression levels could be predictors of severe asthma and future exacerbation.

**Materials and methods**

**Data and clinical variables**

The present study included 152 patients with asthma from a previous study performed at Shizuoka General Hospital. All patients with asthma fulfilled the definition of the Global Initiative for Asthma (GINA). This protocol was approved by the Shizuoka General Hospital Ethics Committee (SGH 15-01-55). All subjects provided written informed consent.

Patients underwent blood sampling, spirometry, and measurement of fractional exhaled nitric oxide (FeNO) on the same day, which is treated as the initial date in the analysis due to the cross-sectional manner. Severe asthma was defined according to the European Respiratory Society/American Thoracic Society guideline: 1) high-intensity treatment
(ICSs ≥1000 μg/day plus a long-acting β₂-agonist (LABA) or treatment with oral corticosteroids or omalizumab) and 2) uncontrolled asthma. The occurrence of exacerbations, defined as worsening of asthma symptoms requiring treatment with systemic corticosteroids for ≥ three days, was recorded during the 1-year follow-up period.

Quantitation of mRNA expression levels

As described in our previous publication, total RNA was isolated from peripheral blood mononuclear cells (PBMCs). Real-time PCR was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using Fast SYBR Green Master Mix (Applied Biosystems). The primers used were as follows: GLCCI1, forward 5′-TGG TAG CCC TTG CTC AAC AG-3′ and reverse 5′-ACC TTC ACT CGC TCA CAT CC-3′; HDAC2, forward 5′-GCC TCA TAG AAT CCG CAT GAC-3′ and reverse 5′-TGT CAT TTC TTC GGC AGT GG-3′; and, Nrf2, forward 5′-CGG TAT GCA ACA GGA CAT TGA G-3′ and reverse 5′-GTT TGG CTT CTG GAC TTG GAA C-3′. Relative mRNA expression levels were normalized to β-actin expression levels. The melt curve analysis confirmed the specificity of the amplicons. Each test sample was analyzed in duplicate, and all samples with a coefficient of variation greater than 5% were retested.

Genetic analysis of the polymorphism

Leukocyte genomic DNA was extracted directly from blood specimens using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), as described in a previous report. The genotype of GLCCI1 (rs37973) was determined using designed primers (forward 5′-TGT
TGA CCC CTG CTA TTC AGT G-3′ and reverse 5′-CAG GAG AAA TGT CTG GAA CGT G-3′) and HotStarTaq DNA polymerase (Qiagen). The amplified PCR products were digested with ApaLI (New England BioLabs, Ipswich, MA, USA) and separated by electrophoresis.

**Statistical analysis**

Categorical values are presented as frequencies and percentages, and continuous variables are expressed as medians and interquartile ranges. We used the Kruskal-Wallis test or the Wilcoxon rank-sum test for comparisons of continuous variables, and the Fisher’s exact test for comparisons of categorical variables. The expression level of each mRNA was expressed as a log2 transformed value and normalized to a mean of zero.

To assess the relationship between the mRNA expression levels and clinical patient characteristics, we used a linear regression model with mRNA expression levels as the dependent variable, and calculated the beta coefficients, corresponding 95% confidence intervals (CIs) and P values based on the Wald test. The Spearman rank correlation coefficient (r) and the test with the null hypothesis: r = 0 were calculated to evaluate the relationship with the mRNA expression levels. Receiver operating characteristic (ROC) curves were drawn to evaluate the suitability of the mRNA expression levels to discriminate patients with severe asthma and nonsevere asthma, and we calculated the area under the ROC curve (AUC) and the 95% CI.

To evaluate whether the mRNA expression levels of *GLCCI1*, *Nrf2*, and *HDAC2* influence to the time to first exacerbation, we used Cox proportional hazards regression models, and the hazard ratio (HR) and 95% CI were estimated. The exacerbation-free rates
were estimated by the Kaplan-Meier method, and we used the log-rank test for comparisons between two groups. The high and low expression group for each mRNA were divided using the cut-off value. The cut-off value for mRNA was determined based on a maximum statistic (minimum p-value) of the chi-squared test, and the corresponding test was performed on cross-table by exacerbation category and a candidate cut-off value, which was derived from the range of expression level of mRNA.

A $p$ value less than 0.05 was considered to indicate statistical significance. We performed all analyses using R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of study subjects

The clinical characteristics of the study subjects have been previously described. The diagnostic criterion for severe asthma was fulfilled by 16.4% (25/152) of the patients with asthma. Patients with severe asthma had significantly lower levels of FEV$_1$/FVC ($p = 0.004$) and %FEV$_1$ ($p < 0.001$) than did those with nonsevere asthma (Supplementary Table 1).

The frequencies of the rs37973 AA, AG, and GG genotypes were 30.9%, 46.1%, and 23.0%, respectively, which did not deviate from Hardy-Weinberg equilibrium (Supplementary Table 2). Among the clinical characteristics, %FEV$_1$ was slightly greater in the GG genotype than in the other genotypes; this difference was only significant in the dominant model (AA/AG vs. GG, $p = 0.026$).
Association between the gene expression levels and clinical variables

Linear regression analysis demonstrated that high-dose ICS therapy was negatively associated with GLCCI1 and Nrf2 mRNA expression levels, neutrophil count was negatively associated with GLCCI1 mRNA expression levels (Table 1). The correlation matrix indicated moderate correlations among GLCCI1, Nrf2, and HDAC2 mRNA expression levels (Fig. 1). There was no significant association of the rs37973 genotype with GLCCI1 mRNA expression levels ($p = 0.881$, Supplementary Fig. 1).

Genetic variables classified into severe and nonsevere asthma

Patients with severe asthma showed significantly lower mRNA expression levels of GLCCI1, Nrf2, and HDAC2 than did those with nonsevere asthma (Fig. 2A). The ROC analysis revealed that the GLCCI1, Nrf2, and HDAC2 mRNA expression levels discriminated between patients with severe asthma and patients with nonsevere asthma (Fig. 2B). The AUCs were 0.68 (95% CI, 0.56–0.79) for GLCCI1, 0.71 (95% CI, 0.60–0.82) for Nrf2, and 0.65 (95% CI, 0.53–0.77) for HDAC2, and these values were not significantly different (GLCCI1 vs. Nrf2, $p = 0.552$; GLCCI1 vs. HDAC2, $p = 0.671$; Nrf2 vs. HDAC2, $p = 0.239$). Next, we evaluated which criteria of severe asthma are more influential in reducing the mRNA expression levels using a multivariate linear regression model. Among the criteria for severe asthma, the high-intensity treatment was more strongly associated with GLCCI1 mRNA expression levels than uncontrolled asthma (Table 1). The allele frequencies of the GLCCI1 rs37973 genotypes were not different between patients with severe asthma and nonsevere asthma ($p = 0.883$, Supplementary Table 1).
Genetic factors predicted future exacerbations

During the 1-year follow-up period, asthma exacerbations occurred in 44.0% (11/25) of patients with severe asthma and 9.4% (12/127) of patients with nonsevere asthma. The Kaplan-Meier curves for the time to first exacerbation divided by 2-category mRNA expression levels are shown in Fig. 3. The clinical characteristics of patients with high and low mRNA expression levels of GLCCI1, Nrf2, and HDAC2 are shown in Supplementary Table 3-5. We found that patients with low mRNA expression of GLCCI1, Nrf2, and HDAC2 had a higher risk for exacerbation (Fig. 3 and Table 2). These relationships between the mRNA expression levels and risk for exacerbations were tended to maintain after adjustment for the presence of severe asthma (Table 2). There was no significant difference in the exacerbation-free rates between the GLCCI1 rs37973 genotypes ($p = 0.53$, Supplementary Fig. 2).

Discussion

The present study demonstrated that lower GLCCI1 mRNA expression levels were associated with a higher risk for severe asthma, whereas no relationship was found between GLCCI1 rs37973 polymorphisms and severe asthma. Moreover, we showed that the mRNA expression levels of Nrf2 and HDAC2 were significantly reduced in patients with severe asthma, and the expression levels of all three genes, which were positively correlated, could predict future asthma exacerbations.
We provide the first report of the association between \textit{GLCCI1} mRNA expression levels and worsening clinical outcomes in patients with asthma. Our study showed that \textit{GLCCI1} mRNA expression levels were negatively associated with ICS dose. Moreover, among the criteria for severe asthma, the high-intensity treatment but not uncontrolled asthma was significantly contributed to the decreased mRNA expression levels of \textit{GLCCI1}. A previous report indicated that the \textit{GLCCI1} rs37973 allele, which decreases \textit{GLCCI1} gene expression levels, attenuated the response to ICS treatment.\textsuperscript{10} Therefore, low \textit{GLCCI1} expression levels may be involved in the molecular mechanisms of steroid insensitivity. Although it was reported that GLCCI1 was upregulated by glucocorticoid treatment in vitro study,\textsuperscript{10} the association of long-term treatment of ICS in asthmatic patients with \textit{GLCCI1} expression levels has not been reported. The role of GLCCI1 in the pathology of asthma should be uncovered in further studies. Previous studies reported that GLCCI1 expression is a marker of early glucocorticoid-induced apoptotic effects on inflammatory cells.\textsuperscript{13,14} Notably, we observed a negative relationship between \textit{GLCCI1} expression levels and blood neutrophil count; a similar trend was observed for blood eosinophil count, although this trend was not significant. These results suggested that the downregulation of GLCCI1 expression may lead to a reduction in apoptosis, which causes residual inflammation in asthma.

In this study, it was interesting that \textit{GLCCI1} mRNA expression levels but not polymorphisms contributed to asthma severity. Tantisira \textit{et al.}\textsuperscript{10} first reported the association of \textit{GLCCI1} polymorphism with ICS response in a genome-wide association study. This result was supported by other studies that showed the influence of the \textit{GLCCI1} rs37973 polymorphism on a decline in FEV$_1$,\textsuperscript{15} and the response to 24 weeks of ICS treatment;\textsuperscript{16}
however, there are controversial results regarding the impact of GLCCI1 polymorphisms on clinical outcomes of asthma. Large cohort studies indicated no association between rs37973 and ICS response\textsuperscript{17,18} or risk for asthma exacerbations\textsuperscript{19}. In this study, GLCCI1 gene expression levels might have been influenced by other pathophysiological factors of asthma rather than by genetics. Therefore, we considered that GLCCI1 gene expression levels might be a more sensitive indicator to predict the pathological progression of severe asthma and future exacerbations.

As with GLCCI1 levels, Nrf2 and HDAC2 levels were significantly reduced in patients with severe asthma, and patients in low expression groups had a higher risk for future exacerbations. Oxidative stress is known to increase in patients with asthma\textsuperscript{20} and is suggested to prompt a reduction in corticosteroid responsiveness\textsuperscript{21}. Nrf2 generally plays a crucial role in antioxidant mechanisms\textsuperscript{7,8}, but it was recently shown to have various effects on the pathogenesis of asthma; activating Nrf2 enhances the stabilization of the airway epithelial barrier\textsuperscript{22} and suppresses the allergic lung inflammation induced by type 2 innate lymphoid cells\textsuperscript{23}, which play a pivotal role in the development of steroid resistance\textsuperscript{24}. Taken together, the data suggest that Nrf2 dysfunction might reflect the progression of airway inflammation. Few studies have focused on the relationship between Nrf2 and severe asthma or asthma exacerbations in patients with asthma\textsuperscript{25}; therefore, our results could support the strategy of treating asthma with Nrf2 activators\textsuperscript{26}. Previous studies with a small number of cases showed a significant reduction in HDAC2 activity in patients with severe asthma compared with those with nonsevere asthma\textsuperscript{5} and in patients with neutrophilic asthma compared with those with eosinophilic asthma\textsuperscript{27}. The reduction in HDAC2 activity was
believed to be related to corticosteroid insensitivity;\textsuperscript{4}) this hypothesis is supported by evidence in this study for an association between attenuated \textit{HDAC}2 expression and increased risks for asthma exacerbation and severe asthma.

We also found a relationship between the mRNA expression levels of \textit{GLCCI1} and those of \textit{Nrf}2 and \textit{HDAC}2. The interaction between \textit{Nrf}2 and \textit{HDAC}2 has been investigated previously,\textsuperscript{9,28} and this study suggested the involvement of \textit{GLCCI1} in the \textit{Nrf}2-\textit{HDAC}2 axis. This finding is essential to understanding the molecular function of \textit{GLCCI1} in patients with asthma. Although further studies are needed to uncover the exact mechanisms of these interactions, previous studies have suggested a common pathway for these three molecules. Oxidative stress is associated with the mechanisms of steroid insensitivity through activation of phosphoinositide 3-kinase (PI3K) \(\delta\), which results in the attenuation of HDAC2 activity.\textsuperscript{6,29} Findings in a mouse model of steroid-insensitive asthma also support the association between PI3K pathway activation and decreases in \textit{Nrf}2 and HDAC2 protein levels.\textsuperscript{30,31} Recently, Kim \textit{et al.} suggested that \textit{GLCCI1} expression in the glomerulus is controlled by the PI3K pathway and that PI3K activation leads to a decrease in \textit{GLCCI1} levels.\textsuperscript{32} These results suggest that the PI3K pathway might be a common pathway affected by \textit{GLCCI1}, \textit{Nrf}2, and HDAC2.

The current study has several limitations. First, we measured the mRNA expression levels in PBMCs. The relationship between mRNA expression in PBMCs and in airway cells or sputum was not evaluated; however, peripheral blood cells are known to play an essential role in the inflammatory process of asthma. Second, the number of patients with severe asthma was not large enough to separate these patients into subgroups based on the several
phenotypes of severe asthma, which could have been done to assess the pathology of severe asthma.\textsuperscript{33)}

In conclusion, we show that patients with severe asthma can be distinguished by lower mRNA levels of *GLCCI1*, *Nrf2*, and *HDAC2* in peripheral blood cells. These decreases in gene expression levels were associated with a higher risk for future asthma exacerbations. Moreover, GLCCI1 might be involved in mechanisms of corticosteroid insensitivity due to associations with Nrf2 and HDAC2.

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**Conflict of Interest**

T. Shirai reports personal fees from AstraZeneca Japan outside the submitted work. The other authors have nothing to disclose.

**Supplementary Materials**

The online version of this article contains supplementary materials.
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Fig. 1. Correlations among the GLCCI1, Nrf2, and HDAC2 mRNA expression levels.
Fig. 2. The relationship between severe asthma and the mRNA expression levels of *GLCCI1*, *Nrf2*, and *HDAC2*. (A) Differences in mRNA expression levels between patients with severe and nonsevere asthma. (B) Receiver operating characteristic (ROC) analysis for the discrimination between severe and nonsevere asthma.
Fig. 3. Kaplan-Meier curves for the time to first exacerbation stratified by high and low mRNA expression levels of GLCCI1, Nrf2, and HDAC2.
Table 1. Univariate and multivariate linear regression analysis of factors influencing GLCCI1, Nrf2, and HDAC2 mRNA expression levels

|                      | GLCCI1                      | Nrf2                      | HDAC2                      |
|----------------------|-----------------------------|---------------------------|----------------------------|
|                      | β (95% CI)                  | p value                   | β (95% CI)                 | p value                   | β (95% CI)                 | p value                   |
| **BMI, body mass index** | -0.557 (-0.954, -0.160)     | 0.007                     | -0.416 (-0.681, -0.171)    | 0.001                     | -0.400 (-0.772, -0.027)    | 0.037                     |
| **Age (per 10 years)** | -0.077 (-0.174, 0.020)      | 0.123                     | -0.050 (-0.113, 0.013)     | 0.124                     | -0.085 (-0.175, 0.005)     | 0.066                     |
| **BMI (per 10 kg/m²)** | -0.094 (-0.396, 0.207)      | 0.541                     | -0.060 (-0.256, 0.136)     | 0.551                     | -0.020 (-0.301, 0.261)     | 0.888                     |
| **Sex, male**        | -0.137 (-0.439, 0.164)      | 0.373                     | -0.015 (-0.211, 0.181)     | 0.880                     | 0.054 (-0.227, 0.324)      | 0.708                     |
| **Atopy**            | 0.117 (-0.230, 0.465)       | 0.569                     | 0.220 (-0.004, 0.443)      | 0.656                     | 0.263 (-0.058, 0.584)      | 0.110                     |
| **Allergy rhinitis** | 0.236 (-0.069, 0.541)       | 0.132                     | 0.113 (-0.086, 0.312)      | 0.266                     | 0.182 (-0.102, 0.407)      | 0.211                     |
| **Pack-years (per 10)** | -0.059 (-0.121, 0.015)     | 0.110                     | -0.012 (-0.059, 0.035)     | 0.618                     | -0.001 (-0.068, 0.067)     | 0.995                     |
| **FEV₁/FVC (per 10%)** | -0.041 (-0.170, 0.087)     | 0.529                     | 0.017 (-0.007, 0.100)      | 0.695                     | -0.042 (-0.161, 0.078)     | 0.496                     |
| **FEV₁, % predicted (per 10%)** | 0.037 (-0.032, 0.107) | 0.297                 | 0.032 (-0.013, 0.077)      | 0.167                     | -0.001 (-0.066, 0.064)     | 0.977                     |
| **FeNO (per 10 ppb)** | -0.018 (-0.057, 0.020)      | 0.353                     | -0.017 (-0.042, 0.009)     | 0.197                     | -0.005 (-0.041, 0.031)     | 0.805                     |
| **Total IgE (per 100 IU/mL)** | -0.006 (-0.017, 0.004)   | 0.239                     | 0.067 (-0.001, 0.141)      | 0.056                     | 0.004 (-0.006, 0.014)      | 0.411                     |
| **Blood eosinophil (per 100 cells/μL)** | -0.040 (-0.084, 0.003) | 0.071                | -0.011 (-0.040, 0.017)      | 0.446                     | -0.026 (-0.067, 0.015)     | 0.210                     |
| **Blood neutrophil (per 100 cells/μL)** | -0.017 (-0.027, -0.006) | 0.004               | -0.005 (-0.012, 0.003)     | 0.220                     | -0.008 (-0.018, 0.003)     | 0.142                     |
| **ICS dose (per 100 μg/day)** | -0.085 (-0.131, -0.039) | <0.001            | -0.046 (-0.077, -0.016)    | 0.003                     | -0.035 (-0.079, 0.010)     | 0.130                     |
| **GINA step (per 1 step increase)** | -0.026 (-0.045, -0.013) | <0.001          | -0.150 (-0.258, -0.043)    | 0.007                     | -0.018 (-0.336, -0.026)    | 0.024                     |
| **Medication**       |                             |                           |                            |                           |                            |                           |
| **ICS dose (per 100 μg/day)** | -0.085 (-0.131, -0.039) | <0.001            | -0.046 (-0.077, -0.016)    | 0.003                     | -0.035 (-0.079, 0.010)     | 0.130                     |
| **Maintenance OCS**  | -0.547 (-1.149, 0.054)      | 0.077                     | -0.431 (-0.820, -0.042)    | 0.031                     | -0.720 (-1.273, -0.160)    | 0.012                     |
| **Anti-IgE therapy** | -0.205 (-0.105, 0.640)      | 0.635                     | -0.348 (-0.895, 0.198)     | 0.213                     | 0.080 (-0.705, 0.866)      | 0.841                     |
| **LABA**             | 0.308 (-0.705, 0.100)       | 0.131                     | -0.153 (-0.412, 0.106)     | 0.249                     | -0.006 (-0.441, 0.302)     | 0.715                     |
| **LAMA**             | -0.666 (-1.407, -0.324)     | 0.002                     | -0.565 (-0.917, -0.214)    | 0.002                     | 0.181 (-0.337, 0.700)      | 0.404                     |
| **LTRA**             | -0.469 (-0.776, -0.162)     | 0.003                     | -0.311 (-0.510, -0.112)    | 0.003                     | -0.301 (-0.591, -0.011)    | 0.044                     |
| **Theophylline**     | -0.385 (-0.836, 0.067)      | 0.097                     | -0.167 (-0.462, 0.128)     | 0.270                     | -0.102 (-0.525, 0.322)     | 0.639                     |
| **Criteria for severe asthma** |                             |                           |                            |                           |                            |                           |
| **High-intensity treatment** | -0.527 (-0.868, -0.186) | 0.003             | -0.339 (-0.561, -0.117)    | 0.003                     | -0.276 (-0.601, 0.046)     | 0.094                     |
| **Uncontrolled asthma** | -0.058 (-0.360, 0.244)     | 0.707                     | -0.017 (-0.365, 0.023)     | 0.086                     | -0.082 (-0.362, 0.198)     | 0.568                     |
| **Multivariate model** |                             |                           |                            |                           |                            |                           |
| **High-intensity treatment** | -0.540 (-0.894, -0.185) | 0.003             | -0.310 (-0.539, -0.080)    | 0.009                     | -0.270 (-0.606, 0.006)     | 0.114                     |
| **Uncontrolled asthma** | 0.049 (-0.256, 0.353)       | 0.755                     | -0.110 (-0.307, 0.087)     | 0.272                     | -0.029 (-0.318, 0.260)     | 0.845                     |
Table 2. Hazard ratios for exacerbation according to GLCCI1, Nrf2, and HDAC2 mRNA expression levels

| Gene expression levels          | GLCCI1     | Nrf2       | HDAC2  |
|--------------------------------|------------|------------|--------|
| Unadjusted                     |            |            |        |
| Low vs. high expression group  | 3.24 (1.42, 7.40) | 3.13 (1.37, 7.16) | 2.98 (1.22, 7.25) |
| Continuous variable (per 2-fold decrease) | 1.46 (0.98, 2.20) | 2.08 (1.11, 3.88) | 1.45 (0.95, 2.21) |
| Adjusted with severe asthma    |            |            |        |
| Low vs. high expression group  | 2.27 (0.96, 5.37) | 2.43 (1.04, 5.62) | 2.24 (0.90, 5.57) |
| Continuous variable (per 2-fold decrease) | 1.23 (0.81, 1.87) | 1.67 (0.83, 3.35) | 1.24 (0.81, 1.88) |

GLCCI1, glucocorticoid-induced 1; HDAC2, histone deacetylase 2; Nrf2, nuclear factor (erythroid-derived 2)-like 2.