

GLCCI1 variant accelerates pulmonary function decline in patients with asthma receiving inhaled corticosteroids

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Complete List of Authors:	Yumi, Izuhara; Graduate School of Kyoto University, Respiratory Medicine Matsumoto, Hisako; Graduate School of Kyoto University, Respiratory Medicine Kanemitsu, Yoshihiro; Graduate School of Kyoto University, Respiratory Medicine Izuhara, Kenji; Saga Medical School, Division of Medical Biochemistry, Department of Biomolecular Science Tohda, Yuji; Kinki University Faculty of Medicine, Department of Respiratory Medicine and Allergology Horiguchi, Takahiko; Fujita Health University Second Educational Hospital, Respiratory Internal Medicine Kita, Hideo; Takatsuki Red Cross Hospital, Respiratory Medicine Tomii, Keisuke; Kobe City Medical Center General Hospital, Respiratory Medicine Otsuka, Kojiro; Graduate School of Kyoto University, Respiratory Medicine Fujimura, Masaki; Kanazawa University Graduate School of Medicine, Respiratory Medicine, Cellular Transplantation Ohkura, Noriyuki; Kanazawa University Graduate School of Medicine, Respiratory Medicine, Cellular Transplantation Tomita, Katsuyuki; Kinki University Faculty of Medicine, Department of Respiratory Medicine and Allergology Yokoyama, Akihito; Kochi University, Hematology and Respiratory Medicine Ohnishi, Hiroshi; National Jewish Health, Pediatrics; Kochi University, Hematology and Respiratory Medicine Nakano, Yasutaka; Shiga University of Medical Science, Division of Respiratory Medicine, Department of Internal Medicine Oguma, Tetsuya; Shiga University of Medical Science, Division of Respiratory Medicine, Department of Internal Medicine Hozawa, Soichiro; Hiroshima Allergy and Respiratory Clinic, Respiratory Medicine Nagasaki, Tadao; Graduate School of Kyoto University, Respiratory Medicine Niimi, Akio; Kyoto University, Respiratory Medicine Mishima, Michiaki; Kyoto University, Respiratory Medicine

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***GLCCII* variant accelerates pulmonary function decline in patients with asthma receiving inhaled corticosteroids**

Yumi Izuhara MD^{1*}, Hisako Matsumoto MD, PhD^{1,2*}, Yoshihiro Kanemitsu MD^{1,2}, Kenji Izuhara MD, PhD³, Yuji Tohda MD, PhD^{2,4}, Takahiko Horiguchi MD, PhD^{2,5}, Hideo Kita MD, PhD^{2,6}, Kazunobu Kuwabara MD, PhD^{2,6}, Keisuke Tomii MD, PhD^{2,7}, Kojiro Otsuka MD, PhD^{1,2,7}, Masaki Fujimura MD, PhD^{2,8}, Noriyuki Ohkura MD^{2,8}, Katsuyuki Tomita MD, PhD^{2,4}, Akihito Yokoyama MD, PhD^{2,9}, Hiroshi Ohnishi MD, PhD^{2,9}, Yasutaka Nakano MD, PhD^{2,10}, Tetsuya Oguma MD, PhD^{2,10}, Soichiro Hozawa MD, PhD^{2,11}, Tadao Nagasaki MD¹, Isao Ito MD, PhD¹, Tsuyoshi Oguma MD¹, Hideki Inoue MD¹, Tomoko Tajiri MD¹, Toshiyuki Iwata MD¹, Junya Ono BS¹², Shoichiro Ohta MD, PhD¹³, Mayumi Tamari MD, PhD¹⁴, Tomomitsu Hirota DDS, PhD¹⁴, Tetsuji Yokoyama MD, PhD¹⁵, Akio Niimi MD, PhD^{1,2,16} and Michiaki Mishima MD, PhD^{1,2}

¹Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

²Kinki Hokuriku Airway disease Conference (KiHAC), Osaka, Japan

³Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan

⁴Department of Respiratory Medicine and Allergology, Faculty of Medicine, Kinki University, Osaka, Japan

⁵Department of Respiratory Internal Medicine, Fujita Health University Second Educational Hospital, Aichi, Japan

⁶Department of Respiratory Medicine, Takatsuki Red Cross Hospital, Osaka, Japan

⁷Department of Respiratory Medicine, Kobe City Medical Center General Hospital,
Hyogo, Japan

⁸Department of Respiratory Medicine, Cellular Transplantation Biology, Kanazawa
University Graduate School of Medicine, Kanazawa, Japan

⁹Department of Hematology and Respiratory Medicine, Kochi University, Kochi, Japan

¹⁰Division of Respiratory Medicine, Department of Internal Medicine, Shiga University
of Medical Science, Shiga, Japan

¹¹Hiroshima Allergy and Respiratory Clinic, Hiroshima, Japan

¹²Shino-Test Corporation, Kanagawa, Japan

¹³Department of Laboratory Medicine, Saga Medical School, Saga, Japan

¹⁴Laboratory for Respiratory and Allergic Diseases, Core for Genomic Medicine
Center for Integrative Medical Sciences, Institute of Physical and Chemical Research
(RIKEN), Yokohama, Kanagawa, Japan

¹⁵Department of Health Promotion, National Institute of Public Health, Wako, Saitama,
Japan

¹⁶Department of Medical Oncology and Immunology, Nagoya City University School of
Medical Sciences, Aichi, Japan

*YI and HM equally contributed to this work.

Correspondence should be addressed to Hisako Matsumoto MD, PhD

Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University
53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

E-mail: hmatsumo@kuhp.kyoto-u.ac.jp

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Conflicts of Interest

Yumi Izuhara, Hisako Matsumoto, Yoshihiro Kanemitsu, Yuji Tohda, Takahiko Horiguchi, Hideo Kita, Kazunobu Kuwabara, Keisuke Tomii, Kojiro Otsuka, Masaki Fujimura, Noriyuki Ohkura, Katsuyuki Tomita, Hiroshi Ohnishi, Yasutaka Nakano, Tetsuya Oguma, Soichiro Hozawa, Tadao Nagasaki, Isao Ito, Hideki Inoue, Tomoko Tajiri, Toshiyuki Iwata, Junya Ono, Shoichiro Ohta, Mayumi Tamari, Tomomitsu Hirota, Tetsuji Yokoyama, Akio Niimi and Michiaki Mishima have nothing to disclose.

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Abstract

Background: In steroid-naïve patients with asthma, several gene variants are associated with a short-term response to inhaled corticosteroid (ICS) treatment; this has mostly been observed in Caucasians. However, not many studies have been conducted for other ethnicities. Here we aimed to determine the relationship between the annual decline in forced expiratory flow volume in one second (FEV₁) and the variant of the glucocorticoid-induced transcript 1 gene (*GLCC1*) in Japanese patients with asthma receiving long-term ICS treatment, taking into account the effect of high serum periostin levels, a known association factor of pulmonary function decline and a marker of refractory eosinophilic/Th2 inflammation.

Methods: In this study, 224 patients with asthma receiving ICS treatment for at least 4 years were enrolled. The effects of single nucleotide polymorphisms (SNPs) in *GLCC1*, stress-induced phosphoprotein 1 (*STIP1*) and *T* gene on the decline in FEV₁ of 30 mL·yr⁻¹ or greater were determined.

Results: Besides the known contributing factors, i.e. the most intensive treatment step, ex-smoking, and high serum periostin levels (≥95 ng/mL), the GG genotype of *GLCC1* rs37973, and not other SNPs, was independently associated with a decline in FEV₁ of 30 mL·yr⁻¹ or greater. When patients were stratified according to their serum periostin levels, the GG genotype of rs37973 was significantly associated with blood eosinophilia (≥250/μL) in the high serum periostin group.

Conclusions: A *GLCC1* variant is a risk factor of pulmonary function decline in Japanese patients with asthma receiving long-term ICS treatment. Thus, *GLCC1* may be associated with response to ICS across ethnicities.

(Word count 252)

Introduction

Inhaled corticosteroids (ICS) are the primary medication in asthma treatment because they can efficiently reduce cytokine production from structural and inflammatory cells (1) (2) (3) and induce apoptosis of inflammatory cells, particularly eosinophils (4) (5) (6). Clinically, ICS treatment has improved asthma control, reduced hospital admission caused by asthma exacerbation (7, 8) and attenuated pulmonary function decline (9). However, despite ICS treatment, there is a subset of patients who demonstrate a greater decline in pulmonary function or sustained eosinophilia (10, 11). These conditions are considered to reflect steroid insensitivity, and mechanisms as well as their genetic backgrounds have been extensively studied (12).

Several gene variants involved in the molecular pathway of the glucocorticoid response may be associated with changes in pulmonary function after 4 to 12 weeks of ICS administration in patients with asthma. In a candidate gene association analysis, single nucleotide polymorphisms (SNPs) of stress-induced phosphoprotein 1 gene (*STIP1*) were associated with functionally better responses to ICS (13). Similarly, genome-wide association studies (GWAS) revealed that SNPs of the glucocorticoid-induced transcript 1 (*GLCCI1*) (14) and *T* genes (15) were associated with changes in pulmonary function after ICS administration. For *GLCCI1*, rs37973 was identified as a functional SNP (14) that may reduce apoptosis of inflammatory cells, leading to a diminished clinical response to ICS. However, these associations were studied mainly in Caucasians and need to be confirmed in other ethnicities.

In a cohort study of the Kinki Hokuriku Airway disease Conference, we recently reported that high serum periostin levels are associated with greater decline in pulmonary function in Japanese patients with asthma receiving long-term ICS treatment

(11). Periostin is an IL-13-inducible matricellular protein that may play a key role in the chronicity of allergic inflammation *via* mechanisms that differ from those involved in the reduction of inflammatory cell apoptosis (16). In the airways, periostin is derived from epithelial cells (17, 18) and several mesenchymal cells (19) and is a marker of remodeling (20) and refractory eosinophilic/Th2 inflammation (21). In the present study, taking the effect of high serum periostin levels into account, we investigated the associations between the annual decline in pulmonary function and the *GLCCI1* variant as well as other aforementioned SNPs in the same cohort population receiving ICS treatment.

Method

Patients

Subjects and Measurement

Adult patients with stable asthma were recruited from 9 institutions belonging to the Kinki Hokuriku Airway disease Conference, where asthma specialists manage patients, as described previously (11). At enrollment, all patients received ICS treatment for 4 years or more and underwent 3 or more pulmonary function tests when they were stable on their daily medications. The first pulmonary function test was conducted at least 1 year after the commencement of ICS treatment and at ≥ 25 years of age. Patients who had smoked more than 10 pack-years, smoked in the past 1 year or had other pulmonary diseases were excluded. This study was approved by the ethics committee of each participating institution and was registered in the UMIN Clinical Trials Registry (Registry ID UMIN000002414). Written informed consent was obtained from all participants.

At enrollment, patients underwent a workup that included spirometry and blood tests. Serum eosinophil cationic protein (ECP) levels were determined by ImmunoCAP ECP (Phadia, Tokyo, Japan), and serum periostin levels were measured using enzyme-linked immunosorbent assay at Shino-test (Kanagawa, Japan). As described previously (11), a cutoff value of 95 ng/mL was used to define the high serum periostin group on the basis of receiver operating characteristic curve analysis to discriminate patients with asthma suspected of refractory Th2 inflammation from healthy subjects.

Selection of SNPs

SNPs for *GLCCI1* (rs37973) (14), *T* gene (rs3127412) (15) and *STIP1* (rs1011219, rs6591838) were selected (13) because they were previously reported to be associated with changes in pulmonary function 4 to 12 weeks after the commencement of ICS treatment in Caucasian patients with asthma.

Genotyping

Genotyping was performed using a TaqMan genotyping assay according to the manufacturer’s instructions (Applied Biosystems, Tokyo, Japan) and analyzed using Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems). One patient was not genotyped because of poor DNA quality.

Statistical method

Statistical analyses were performed using JMP version 10 (SAS Institute Inc., Tokyo, Japan) and IBM SPSS Advanced Statistics 19 (SPSS Inc, Tokyo, Japan). Annual changes in FEV₁ (Δ FEV₁) were estimated for each subject by fitting a least-square regression line to all the available data points of the subjects. The effects of variables on Δ FEV₁ were estimated using a generalized linear mixed model adjusted for sex, height, age at enrollment and FEV₁ at the first measurement. In this model, the institutions were included as random effects. Similarly, effects on the dichotomous data of an annual decline in FEV₁ of 30 ml·yr⁻¹ or greater (22) were estimated with a generalized linear mixed model. For correlation analysis, the Spearman rank-correlation test was used. Comparisons of variables were performed using the Kruskal–Wallis test or χ^2 test, as applicable. P values of ≤ 0.05 were considered significant.

Result

Patient characteristics and *GLCCII* rs37973 frequency

The characteristics of the study population were previously described (11). The average number of measurements of FEV₁ and follow-up period were 16.2 times and 8.0 years, respectively. Among the studied SNPs, only rs37973 of *GLCCII* was associated with the annual decline in FEV₁ of 30 mL·yr⁻¹ or greater. None of other clinical indices including treatment step and asthma control status significantly deviated depending on the genotype of rs37973 (Table 1). rs37973 frequency was as follows: AA (n = 69, 30.1%), AG (n = 114, 51.1%) and GG (n = 40, 17.9%). All genotyped data were in Hardy–Weinberg equilibrium.

Associations between *GLCCII* rs37973 and decline in FEV₁ of 30 mL·yr⁻¹ or greater

The frequency of patients who showed an annual decline in FEV₁ of 30 mL·yr⁻¹ or greater for each rs37973 genotype was as follows: AA (n = 11), AG (n = 28) and GG (n = 13). When the decline in FEV₁ was analyzed as a continuous variable, only high serum periostin levels (≥ 95 ng/mL), but not the rs37973 genotype, was associated with the annual decline in FEV₁. Meanwhile, the GG rs37973 genotype tended to correlate with an annual decline in FEV₁ of 30 mL·yr⁻¹ or greater by univariate analysis (estimated effect 0.87, 95% confidence interval -0.11 to 1.85, P = 0.08) and showed a significant association with the decline by multivariate analysis (P = 0.047), independent from high serum periostin levels, ex-smoking and treatment step 5 defined by the Global Initiative for Asthma 2010 guidelines (23) (Table 2).

Effect of *GLCCI1* rs37973 on eosinophilic inflammation in high periostin milieu

When patients were analyzed altogether, rs37973 was not associated with other clinical indices, including blood eosinophil counts (Table 1). When patients were stratified into two groups according to their serum periostin levels, in the high serum periostin (≥ 95 ng/mL) group, the number of G alleles of rs37973 was weakly but significantly associated with serum ECP levels ($R_s = 0.25$, $P = 0.024$), marginally correlated with blood eosinophil counts ($R_s = 0.21$, $P = 0.057$) (Fig 1A) and significantly associated with the frequencies of patients with blood eosinophilia ($\geq 250/\mu\text{L}$) (24) ($R_s = 0.27$, $p = 0.013$) (Fig 1B). In addition, serum total IgE levels were marginally associated with the number of G alleles of rs37973 ($R_s = 0.21$, $P = 0.054$). Such associations were not observed in the low serum periostin group (< 95 ng/mL).

Associations between *STIP1* and *T* gene with decline in FEV_1 of $30 \text{ mL}\cdot\text{yr}^{-1}$ or greater

Other studied SNPs, i.e. SNPs of *STIP1* (rs1011219, rs6591838) and *T* gene (rs3127412) did not associate with annual change in FEV_1 or decline in FEV_1 of $30 \text{ mL}\cdot\text{yr}^{-1}$ or greater (Table E1).

Discussion

In this study, we confirmed that *GLCCII* rs37973 was associated with the response to ICS treatment in Japanese patients with asthma. Furthermore, to the best of our knowledge, this is the first study that showed *GLCCII* rs37973 contributed to the annual decline in FEV₁ of 30 mL·yr⁻¹ or greater in Japanese patients with asthma receiving long-term ICS treatment. The GG rs37973 genotype was an independent risk factor for the decline, in addition to high serum periostin levels (≥ 95 ng/mL), the most intensive treatment step and ex-smoking, as previously reported (11). Furthermore, in patients with high serum periostin levels, the number of G alleles of rs37973 was significantly associated with serum ECP levels and the frequencies of patients with blood eosinophilia ($\geq 250/\mu\text{L}$). The coexistence of high serum periostin levels and the GG rs37973 genotype may thus increase the risk of decline in FEV₁ of 30 mL·yr⁻¹ or greater and eosinophilia in patients receiving long-term ICS treatment.

Insensitivity to ICS is an important issue in asthma management, which may lead to refractory eosinophilia, poor asthma control and deterioration of airflow limitation. Mechanisms such as genetic backgrounds that are involved in the molecular glucocorticoid response have thus been extensively examined (13) (15, 25). *GLCCII* that maps to chromosome 7p21.3 was identified as a novel pharmacogenetic determinant of the response to ICS in GWAS using a family-based screening algorithm (14). Tantisira et al. report that the G allele of the functional SNP rs37973 in *GLCCII* is a risk factor for poor improvement in FEV₁ 4 to 8 weeks after the commencement of ICS treatment in patients with asthma. The exact role of *GLCCII*, previously known as *glucocorticoid-induced gene 18 (GIG18)*, remains unknown, but its expression is induced by glucocorticoids (26); it may affect glucocorticoid-induced apoptosis of

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inflammatory cells (4) (5) (6) (27). Furthermore, the G allele of rs37973 downregulates *GLCCII* expression (14) and is presumed to reduce the apoptosis of inflammatory cells. In agreement with the findings by Tantisira et al. and after further analysis, we first demonstrated that the GG rs37973 genotype was an independent risk factor for annual decline in FEV₁ of 30 mL·yr⁻¹ or greater in patients with asthma receiving long-term ICS treatment. Considering that high serum periostin levels, but not the rs37973 genotype, were associated with a continuous variable of ΔFEV₁, the impact of the *GLCCII* variant on the decline in pulmonary function may be small on its own, as was shown in a recent report that failed to replicate the results by Tantisira et al. (28). Nonetheless, the combined *GLCCII* variant and high serum periostin level data may be important in the management of patients with asthma receiving ICS treatment, as discussed below.

Eosinophils are prone to apoptosis, immediately following steroid treatment (5) (6), and the counts and footprints of apoptosis are inversely proportional (4, 25). However, such an association may not be extrapolated to ICS treatment conditions (25). Based on the postulation that the *GLCCII* variant may reduce apoptosis of inflammatory cells (14), the GG rs37973 genotype was expected to be associated with higher eosinophil counts. However, in the present study, when the entire population was analyzed, blood eosinophil counts were not associated with the *GLCCII* polymorphism; in contrast, when patients were stratified into the high serum periostin group, the GG rs37973 genotype was significantly associated with blood eosinophilia and high serum ECP levels. Shoda et al. recently demonstrated that steroid treatment did not inhibit TGF-β-induced periostin production by fibroblasts and IL-4/IL-13-induced periostin production by microvascular endothelial cells (19), whereas IL-4/IL-13-induced

periostin production by fibroblasts (19) (20) or airway epithelial cells (17) was steroid sensitive. These findings suggest that high serum periostin levels may reflect the summation of steroid insensitivity of structural cells and remnants of TGF- β or IL-4/IL-13-producing cells in the airways. Such results may validate our stratification by serum periostin levels to assess the inflammatory background of the *GLCCII* variant in the ICS-treated condition. Finally, for patients with high serum periostin levels and the GG genotype, treatment with biologics such as anti-IL-5 antibody (29) and anti-IL-13 antibody (30) may be recommended rather than increasing ICS doses or adding systemic corticosteroids. Further interventional studies are necessary.

Our study has several limitations. First, we did not examine the initial responsiveness to ICS in this population. However, long-term effects of ICS are also important as preventing loss of pulmonary function is one of the goals of asthma control (31). Second, the number of participants was small for a genetic study to explain the phenotypic differences, so additional studies with more subjects are required. Despite these limitations, the association between the *GLCCII* variant and longitudinal decline in FEV₁ in Japanese patients with asthma would be of great importance and may provide routes for future studies.

In conclusion, the *GLCCII* variant may be a pharmacogenetic determinant of the response to long-term ICS treatment in patients with asthma.

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Author contributions

Author Contributions: Y.I. analyzed and interpreted the data, and wrote the draft. H.M. conceived and designed the study, recruited patients, collected, analyzed and interpreted the data, and wrote and edited the manuscript. Y.K. recruited patients, collected, and analyzed the data. K.I. measured serum level of periostin, offered data of healthy control samples, and critically revised the manuscript. Y.T. contributed to the design of the study, recruited patients, acquired the funding, and critically revised the manuscript. Ta.H., H.K., K.K., Ke.T., K.O., M.F., N.O., Ka.T., A.Y., H.O., Y.N., Te.O., S.H. and A.N. contributed to the design of the study, recruited patients, collected data, and critically revised the manuscript. T.N., I.I., Ts.O., H.I., T.T. and T.I. collected data, and revised the manuscript. J.O. and S.O. measured serum level of periostin and offered data of healthy control samples, and revised the manuscript. T.M., To.H., and T.Y. contributed to the interpretation of the genomic data, and critically revised the manuscript. M.M. provided overall supervision, and critically revised the manuscript.

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Table 1. Patient characteristics according to the rs37973 genotype of *GLCCI1*

	rs37973		
	AA (n = 69)	AG (n = 114)	GG (n = 40)
Sex (males/females), n	19/50	22/92	12/28
Age at enrolment, years	60.6 (14.8)	63.0 (13.3)	63.1 (12.8)
Age at asthma onset, years	38.7 (21.3)	43.3 (17.4)	44.0 (19.3)
Smoking history (never), %	77	84	78
Atopic predisposition, %	72	68	73
ICS daily maintenance dose, µg	512 (339)	533 (318)	513 (279)
Treatment step (2/3/4/5), %	16/32/46/6	14/24/53/9	20/28/40/12
Asthma Control Test [®] , points	22.8 (3.3)	22.4 (3.7)	23.1 (3.2)
History of admission due to asthma, %	35	36	33
Use of systemic steroids due to asthma exacerbations during the recent 6 months, %	9.0	13.2	15.0
Adherence to medication*, %	84.1	87.7	87.5
Allergic rhinitis, %	58	59	55
FEV ₁ , % predicted	93.0 (20.1)	100.5 (23.3)	96.5 (22.1)
Blood eosinophils/µL	304 (376)	321 (350)	342 (372)
Blood neutrophils/µL	3568 (1267)	3901 (1655)	3883 (1376)
Serum total IgE, IU/mL	582 (0–16000)	647 (0–16000)	623 (0–8610)
Serum ECP, µg/L	13.5 (17.5)	15.0 (35.6)	17.6 (25.4)
Serum periostin, ng/mL	90.6 (33.1)	93.4 (41.2)	93.1 (38.6)
Annual changes in FEV ₁ , mL·yr ⁻¹	-4.4 (30.1)	-9.6 (30.2)	-9.1 (50.9)

Results are presented as mean ± SD with the exception of total IgE where the results are presented as median (range).

Date at enrollment are presented unless otherwise stated.

$P > 0.05$ for all characteristics according to the Kruskal–Wallis or χ^2 test

ICS: inhaled corticosteroid, ECP: eosinophil cationic protein, FEV₁: forced expiratory flow volume in one second

* The frequency of patients who never or seldom forgot to take ICS or other medications

For Peer Review

Table 2. Estimated effects of clinical indices and *GLCCI1* rs37973 on the decline in FEV₁ of 30 mL·yr⁻¹ or greater

Multivariate analysis			
	Estimates	95% C.I.	P value
Treatments step [*] , 5 vs. 2–4	1.14	0.02 to 2.27	0.047
History of admission due to asthma	0.72	-0.08 to 1.52	0.08
Chronic sinusitis	0.56	-0.22 to 1.33	0.16
Smoking history, ex [†] vs. never	1.01	0.04 to 1.98	0.041
Periostin group [‡] , high vs. low	0.87	0.11 to 1.62	0.025
GG rs37973 genotype	1.10	0.02 to 2.18	0.047

Estimated effects were adjusted for sex, height, age at enrollment, and FEV₁ at the first measurement.

C.I.: confidence interval, FEV₁: forced expiratory flow volume in one second

^{*} according to the Global Initiative for Asthma 2010 guidelines (23)

[†] 10 or fewer pack-years

[‡] Patients were classified into two groups based on their serum periostin levels: high ≥95 ng/mL and low <95 ng/mL (11)

Figure legends

Fig 1. Blood eosinophil counts according to the *GLCCII* rs37973 genotype in A) the high serum periostin (≥ 95 ng/mL) and B) low serum periostin (< 95 ng/mL) groups. *Line*, median; *box*, 25th–75th percentile; whiskers, 10th and 90th percentiles; outliers, below 10th and above 90th percentiles.

Fig 2. The frequencies of blood eosinophilia ($\geq 250/\mu\text{L}$) (24) according to the *GLCCII* rs37973 genotype in A) the high serum periostin (≥ 95 ng/mL) and B) low serum periostin (< 95 ng/mL) groups. The black bar indicates blood eosinophilia, and the grey bar indicates no blood eosinophilia ($< 250/\mu\text{L}$).

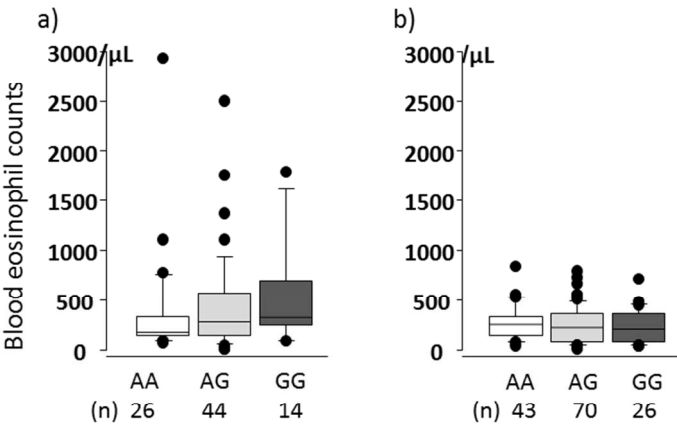


Fig 1

254x190mm (96 x 96 DPI)

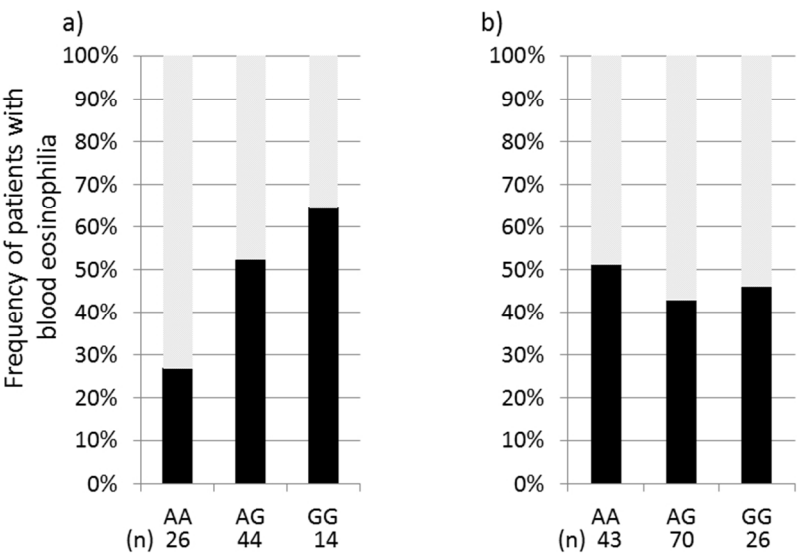


Fig 2

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Online supplement

GLCCII variant accelerates pulmonary function decline in patients with asthma receiving inhaled corticosteroids

Yumi Izuhara MD^{1*} Hisako Matsumoto MD, PhD^{1,2*}, Yoshihiro Kanemitsu MD^{1,2}, Kenji Izuhara MD, PhD³, Yuji Tohda MD, PhD^{2,4}, Takahiko Horiguchi MD, PhD^{2,5}, Hideo Kita MD, PhD^{2,6}, Kazunobu Kuwabara MD, PhD^{2,6}, Keisuke Tomii MD, PhD^{2,7}, Kojiro Otsuka MD, PhD^{1,2,7}, Masaki Fujimura MD, PhD^{2,8}, Noriyuki Ohkura MD^{2,8}, Katsuyuki Tomita MD, PhD^{2,4}, Akihito Yokoyama MD, PhD^{2,9}, Hiroshi Ohnishi MD, PhD^{2,9}, Yasutaka Nakano MD, PhD^{2,10}, Tetsuya Oguma MD, PhD^{2,10}, Soichiro Hozawa MD, PhD^{2,11}, Tadao Nagasaki MD¹, Isao Ito MD, PhD¹, Tsuyoshi Oguma MD¹, Hideki Inoue MD¹, Tomoko Tajiri MD¹, Toshiyuki Iwata MD¹, Junya Ono BS¹², Shoichiro Ohta MD, PhD¹³, Mayumi Tamari MD, PhD¹⁴, Tomomitsu Hirota DDS, PhD¹⁴, Tetsuji Yokoyama MD, PhD¹⁵, Akio Niimi MD, PhD^{1,2,16} and Michiaki Mishima MD, PhD^{1,2}

¹Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

²Kinki Hokuriku Airway Conference (KiHAC), Osaka, Japan

³Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan

⁴Department of Respiratory Medicine and Allergology, Faculty of Medicine, Kinki University, Osaka, Japan

⁵Department of Respiratory Internal Medicine, Fujita Health University Second Educational Hospital, Aichi, Japan

⁶Department of Respiratory Medicine, Takatsuki Red Cross Hospital, Osaka, Japan

⁷Department of Respiratory Medicine, Kobe City Medical Center General Hospital, Hyogo, Japan

⁸Department of Respiratory Medicine, Cellular Transplantation Biology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

⁹Department of Hematology and Respiratory Medicine, Kochi University, Kochi, Japan

¹⁰Division of Respiratory Medicine, Department of Internal Medicine, Shiga University of Medical Science, Shiga, Japan

¹¹Hiroshima Allergy and Respiratory Clinic, Hiroshima, Japan

¹²Shino-Test Corporation, Kanagawa, Japan

¹³Department of Laboratory Medicine, Saga Medical School, Saga, Japan

¹⁴Laboratory for Respiratory and Allergic Diseases, Core for Genomic Medicine

Center for Integrative Medical Sciences, Institute of Physical and Chemical Research (RIKEN), Yokohama, Kanagawa, Japan

¹⁵Department of Health Promotion, National Institute of Public Health, Wako, Saitama, Japan

¹⁶Department of Medical Oncology and Immunology, Nagoya City University School of Medical Sciences, Aichi, Japan

*YI and HM equally contributed to this work.

Correspondence should be addressed to Hisako Matsumoto MD, PhD

Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University

53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

E-mail: hmatsumo@kuhp.kyoto-u.ac.jp

Table E1. Frequency of 3 SNPs and analysis results with the use of dominant and recessive models for annual change in FEV₁ and frequency of rapid decliners

Gene SNP	Genotype	N (%)	Allelic	N (%)	Annual change in FEV ₁ , mL·yr ⁻¹		Frequency of rapid decliners*			
					Mean ± SD	p value		%	p value	
						Dominant [†]	Recessive [‡]		Dominant	Recessive
<i>STIP1</i> rs1011219	GG	162 (73)	G	372 (83)	-8.5 ± 35.9	0.82	0.60	24.1	0.87	0.31
	AG	48 (22)	A	74 (17)	-7.9 ± 33.4			25.0		
	AA	13 (5)			-0.3 ± 21.8			7.7		
<i>STIP1</i> rs6591838	AA	145 (65)	A	356 (80)	-8.4 ± 34.5	0.59	0.57	22.1	0.67	0.37
	AG	66 (30)	G	90 (20)	-8.4 ± 37.1			28.8		
	GG	12 (5)			0.7 ± 22.4			8.3		
<i>T gene</i> rs3127412	TT	169 (76)	T	390 (87)	-6.5 ± 36.6	0.68	0.77	23.1	0.80	0.25
	CT	52 (23)	C	56 (13)	-12.6 ± 27.6			23.1		
	CC	2 (1)			-5.7 ± 46.3			50.0		

* Rapid decliners are defined as patients who showed a decline in FEV₁ of 30 mL or greater per year.

[†] Assuming that heterozygotes have the same increased risk as minor homozygous genotypes.

[‡] Assuming that heterozygotes have no increased risk.