

## ORIGINAL ARTICLE

## Asthma and Rhinitis

***IL4R $\alpha$*  and *ADAM33* as genetic markers in asthma exacerbations and type-2 inflammatory endotype**

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**Summary**

**Background:** Genetic markers of susceptibility to asthma exacerbations in adults remain unclear.

**Objective:** To identify genetic markers of asthma exacerbations, particularly in patients with type-2 inflammatory endotype.

**Methods:** In this observational study of patients enrolled in the Kinki Hokuriku Airway disease Conference multicenter study, frequency of exacerbations requiring systemic corticosteroids during 2 years after enrolment and associated risk factors was determined. For genetic marker analysis, interleukin-4 receptor  $\alpha$  (*IL4RA*) rs8832 and a disintegrin and metalloprotease 33 (*ADAM33*) S\_2 (rs528557), T\_1 (rs2280091), T\_2 (rs2280090), and V\_4 (rs2787094) variants were included.

Elevated serum periostin levels at enrolment ( $\geq 95$  ng/mL, defined as type-2 inflammatory endotype) were considered in the analysis.

**Results:** Among 217 patients who were successfully followed up for 2 years after enrolment, 60 patients showed at least one asthma exacerbation during the 2 years. Airflow limitation (%FEV<sub>1</sub> <80%) and recent exacerbations but not genetic variants were identified as risk markers of exacerbations. A total of 27 patients showed type-2 inflammatory endotype (serum periostin  $\geq 95$  ng/mL at enrolment) and subsequent exacerbations; risk factors in these patients were airflow limitation (odds ratio, 6.51; 95% confidence interval (CI): 2.37–18.6;  $P=.0003$ ), GG genotype of *IL4RA* rs8832 (odds ratio, 4.01; 95% CI: 1.47–11.0;  $P=.007$ ), and A allele of *ADAM33* T\_2 (odds ratio, 2.81; 95% CI: 1.05–7.67;  $P=.04$ ) by multivariate analysis. In addition, GG genotype of *IL4RA* rs8832 was associated with type-2 endotype, whereas A allele of *ADAM33* T\_2 was associated with mixed type of eosinophilic/type-2 and neutrophilic inflammations.

**Conclusions and Clinical Relevance:** *IL4RA* and *ADAM33* variants may be risk markers of asthma exacerbations in type-2 inflammatory endotype. Precise endotyping may facilitate the identification of genetic risk markers of asthma exacerbations.

#### KEYWORDS

asthma, genetics, clinical immunology

## 1 | INTRODUCTION

Treatment of asthma has recently improved remarkably with inhaled corticosteroids and other controllers. However, some patients experience repeated exacerbations despite optimal treatment, which can result in poor quality of life, poor lung function, and subsequent exacerbations. Previous studies revealed several clinical risk factors for asthma exacerbations<sup>1</sup> such as poor lung function,<sup>2,3</sup> poor asthma control,<sup>4</sup> excess eosinophilic inflammation,<sup>5</sup> and recent exacerbations.<sup>6</sup> However, the genetic background of asthma exacerbations that would be inherently heterogeneous, particularly in adults, remains unclear.

Asthma is a complex disease with multiple clinical phenotypes and endotypes. Nevertheless, type-2 cytokines, such as interleukin (IL)-4 and IL-13, often play important roles in driving inflammatory processes towards type-2 high endotype and may modify pathophysiology of other asthma endotypes.<sup>7</sup> Therefore, several genetic variants of *IL4*, *IL13*, and their receptors as well as related pathways were investigated by candidate gene analysis approaches in the context of atopy and asthma pathophysiology<sup>8</sup>. Although genetic markers of exacerbations are reported by a limited number of studies on adult asthma, assessment of IL-4 receptor (*IL4R*) variants yielded inconsistent results,<sup>9,10</sup> which might reflect the heterogeneity of asthma exacerbations. However, a recent pharmacogenetics study on pitrakinra, a recombinant form of IL-4 that blocks IL-4R $\alpha$  complexes and inhibits downstream signalling, reported that certain variants of *IL4R $\alpha$*  (*IL4RA*) located on chromosome 16 were associated with efficient reduction in exacerbations with pitrakinra treatment,<sup>11</sup> raising the possibility that in certain

patients with asthma, *IL4RA* variants, and downstream signalling involving IL-4R $\alpha$  complexes might contribute to disease pathogenesis, particularly in exacerbations.

We and others recently established that serum periostin is a robust and relatively stable<sup>12,13</sup> marker of eosinophilic/type-2 severe asthma.<sup>14–17</sup> In addition, serum periostin, which is partially regulated by its coding gene *POSTN*,<sup>15</sup> could be utilized as a companion diagnostic parameter for anti-IgE (omalizumab)<sup>18</sup> and anti-IL-13 (lebrikizumab)<sup>19,20</sup> monoclonal antibody therapies. Thus, in this well-characterized cohort, wherein we previously investigated the role of serum periostin, we examined the effects of a variant of *IL4RA* on asthma exacerbations with consideration of type-2 inflammatory endotype, which was defined by high serum periostin levels. In addition, we examined the effects of variants of a disintegrin and metalloprotease 33 (*ADAM33*), a robust asthma susceptibility gene located on chromosome 20p13,<sup>8,21–23</sup> on asthma exacerbations. This investigation was based on our preliminary findings showing that a variant of *ADAM33* was associated with mixed type of eosinophilic/type-2 and neutrophilic inflammations,<sup>24</sup> another important endotype of severe asthma.<sup>25–27</sup> Some of the results have been previously reported in the form of an abstract.<sup>24</sup>

## 2 | METHODS

### 2.1 | Study design and patients

This was a follow-up, observational study on asthma exacerbations in 224 patients recruited from nine facilities belonging to the Kinki Hokuriku Airway disease Conference (KiHAC) where patients with

asthma are treated by experts. The details of this cohort were previously reported.<sup>15</sup> In brief, patients were enrolled if they had been treated with inhaled corticosteroids for more than 4 years at the time of enrolment. Patients who smoked more than 10 pack-years or were smokers in the year prior to enrolment and those with other respiratory 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 UMIN00002414). Written informed consent was obtained from all participants.

As a validation study to demonstrate an association between ADAM33 T<sub>2</sub> variant and blood granulocyte counts, an independent cohort of patients with asthma was examined (see Supplemental Methods for details).

## 2.2 | Measurements

On enrolment in the KiHAC study, patients underwent medical history-taking, including Asthma Control Test™ (ACT) and pulmonary function and blood tests. Blood analysis included serum levels of eosinophilic cation protein (ECP), IgE, periostin (Shino-Test, Kanagawa, Japan), IL-6, IL-17, high sensitivity C reactive protein (hsCRP), and genotyping. The details were previously reported.<sup>15</sup> The treatment step at enrolment was determined according to the Global Initiative for Asthma (GINA) 2010.<sup>28</sup> Poor asthma control was defined as an ACT score of <20. Type-2 inflammatory endotype was defined as a serum periostin level of ≥95 ng/mL at enrolment.<sup>15,26,29</sup> After enrolment, the frequency of asthma exacerbations requiring systemic corticosteroids during the 2-year study period was examined.

## 2.3 | Genotyping

In this study, *IL4RA* rs8832<sup>11</sup> and *ADAM33* S<sub>2</sub> (rs528557), T<sub>1</sub> (rs2280091), T<sub>2</sub> (rs2280090), and V<sub>4</sub> (rs2787094) single nucleotide polymorphisms (SNPs)<sup>30</sup> were examined. Among several *IL4RA* variants, rs8832 was focused in this study, because this variant was proposed as an excellent predictive marker for responses to anti-IL-4Rα receptor therapy.<sup>11</sup> Previously determined *POSTN* rs3829365,<sup>15</sup> a variant of periostin coding gene, was used for the statistical analysis of serum periostin levels. Genotyping was performed using TaqMan® genotyping assay according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan). Analysis was performed using Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems). The Japanese in Tokyo (JPT) data from the 1000 Genomes Project (release 17, www.1000genomes.org) was used for assessment of linkage disequilibrium among the four *ADAM33* SNPs.

## 2.4 | Statistical analysis

Statistical analyses were performed using JMP® version 11 (SAS Institute, Tokyo, Japan) and IBM SPSS Statistics version 19 (IBM, Armonk, New York, NY, USA). Comparison between two or more groups was performed using the  $\chi^2$ , Fisher's exact, or Wilcoxon rank sum test, as appropriate. For multivariate logistic regression analysis,

non-genetic variables with *P* values of ≤1 and genetic factors with *P* values of <.05 on the univariate analysis were included. The Jonckheere-Terpstra test was used for the trend analysis between genetic variants and clinical indices.<sup>31,32</sup> Data were presented as means±SD. *P* values of <.05 were considered significant.

## 3 | RESULTS

### 3.1 | Risk factors in patients with asthma exacerbations independent of type-2 inflammatory endotype

Of a total of 224 patients enrolled in the KiHAC study, seven were excluded from this study because of failure to follow-up. There were no significant differences in the clinical characteristics between these subjects and the entire cohort (data not shown). Among the remaining 217 patients, 60 (28%) had exacerbations at a frequency of 3.5±7.4 (median, 2) times within 2 years after enrolment, and 83 subjects (38%) showed type-2 inflammatory endotype (serum periostin ≥95 ng/mL). Details of patient characteristics, categorized according to the presence or absence of subsequent asthma exacerbations, are presented in Table 1. GINA treatment step 5, poor asthma control, airflow limitation (FEV<sub>1</sub> <80% predicted), recent exacerbations, and higher blood neutrophil counts were associated with the presence of exacerbations within 2 years after enrolment (Table 1). As assessed using multivariate analysis, the presence of exacerbations was associated with airflow limitation and recent exacerbations within 6 months (Table 2). None of the assessed *IL4RA* or *ADAM33* variants were associated with presence of exacerbations.

Genotype frequencies of *IL4RA* rs8832 were as follows: AA 67 (31%), AG 109 (50%), and GG 41 (19%). Patients with *IL4RA* rs8832 GG genotype showed type-2 inflammatory endotype more frequently than those with *IL4RA* rs8832 AA/AG genotypes (54% vs 35%, *P*=.03; Table S1), and elevated serum periostin in patients with rs8832 GG genotype was observed in the absence of *POSTN* rs3829365 GG that was previously shown to be associated with elevated serum periostin levels (Figure 1).<sup>15</sup> Specific *ADAM33* variants and their frequencies are shown in Table S2, and the linkage disequilibrium among the four *ADAM33* SNPs is shown in Figure S1. There were no associations between genotype frequencies of *IL4RA* rs8832 and the four *ADAM33* SNPs. All genotyped data were in Hardy-Weinberg equilibrium.

### 3.2 | Risk factors in patients with type-2 inflammatory endotype and subsequent asthma exacerbations

A simple comparison between exacerbators and non-exacerbators did not reveal any genetic risks pertaining to exacerbators. However, we hypothesized that genetic deviation may become apparent when type-2 inflammatory endotype was included in the analysis.

First, when risks of *IL4RA* rs8832 or *ADAM33* T<sub>2</sub> for asthma exacerbations were assessed while including the effect of type-2

**TABLE 1** Characteristics of patients categorized according to asthma exacerbation during the 2 y after enrolment<sup>a</sup>

|                                                       | Subsequent exacerbation (–)<br>n=157 | Subsequent exacerbation (+)     |                             | P value for type 2 vs non-type 2 | P value for exacerbation (+) vs exacerbation (–) |
|-------------------------------------------------------|--------------------------------------|---------------------------------|-----------------------------|----------------------------------|--------------------------------------------------|
|                                                       |                                      | Non-type 2 <sup>b</sup><br>n=33 | Type 2 <sup>b</sup><br>n=27 |                                  |                                                  |
| Sex, male/female, n                                   | 43/114                               | 2/31                            | 7/20                        | .07                              | .07                                              |
| Age at enrolment, y                                   | 61.3±14.2                            | 64.9±12.4                       | 64.8±10.8                   | .81                              | .13                                              |
| Age at onset, y                                       | 41.8±19.4                            | 45.5±17.4                       | 40.1±17.2                   | .17                              | .60                                              |
| BMI, <25 kg/m <sup>2</sup> /≥25 kg/m <sup>2</sup> , n | 118/39                               | 20/13                           | 24/3                        | .02                              | .86                                              |
| Smoking history, never/ex, n                          | 125/32                               | 30/3                            | 20/7                        | .10                              | .70                                              |
| GINA Treatment step <sup>c</sup> , 1–4/5, n           | 150/7                                | 27/6                            | 21/6                        | .75                              | <.001                                            |
| Asthma control <sup>d</sup> , well/poor, n            | 145/12                               | 25/8                            | 20/7                        | 1.0                              | .001                                             |
| FEV <sub>1</sub> , % of predicted                     | 100±21                               | 97±23                           | 83±23                       | .02                              | .009                                             |
| FEV <sub>1</sub> , ≥80%/<80% of predicted, n          | 132/25                               | 26/7                            | 11/16                       | .004                             | <.001                                            |
| Recent exacerbation <sup>e</sup> , –/+ , n            | 150/7                                | 20/13                           | 20/7                        | .41                              | <.001                                            |
| Serum IgE, IU/mL                                      | 186(67 469)                          | 84(12 295)                      | 324(161 513)                | .004                             | .47                                              |
| Atopic predisposition <sup>f</sup> , –/+ , n          | 49/108                               | 7/26                            | 7/20                        | .76                              | .37                                              |
| Blood eosinophils, /μL                                | 313±383                              | 265±176                         | 424±418                     | .41                              | .32                                              |
| Blood neutrophils, /μL                                | 3603±1263                            | 4278±1931                       | 4367±1930                   | .99                              | .01                                              |
| Serum periostin, ng/mL                                | 92±39                                | 68±20                           | 128±28                      | -                                | .40                                              |
| Serum periostin <sup>b</sup> , low/high, n            | 101/56                               | 33/0                            | 0/27                        | -                                | .22                                              |
| Serum ECP, μg/L                                       | 15±32                                | 10±9                            | 20±29                       | .04                              | .66                                              |
| IL4RA rs8832, AA/AG/GG, n                             | 55/73/29                             | 5/27/1                          | 7/9/11                      | <.001                            | .85                                              |
| ADAM33 T <sub>2</sub> , GG/AG/AA, n                   | 114/40/3                             | 26/6/1                          | 13/11/3                     | .04                              | .32                                              |

BMI, body mass index; FEV<sub>1</sub>, forced expiratory volume in 1 s; ECP, eosinophil cationic protein.

Data at enrolment are presented unless otherwise stated. Data are presented as means±SD, except for serum IgE, presented as median (range).

<sup>a</sup>Asthma exacerbations requiring systemic corticosteroids.

<sup>b</sup>Determined as low or non-type-2, determined by a serum periostin level of <95 ng/mL at enrolment and as high or type-2, with a serum periostin level of ≥95 ng/mL.

<sup>c</sup>According to the Global Initiative for Asthma (GINA) 2010.<sup>28</sup>

<sup>d</sup>Patients with Asthma Control Test™ score <20 were considered as poorly controlled and those with ACT score ≥20, well controlled.

<sup>e</sup>The presence of exacerbations during the preceding 6 mo before enrolment requiring systemic corticosteroids.

<sup>f</sup>Considered atopic when one or more specific IgE antibodies against common inhaled allergens were positive.

endotype, significant positive interactions were observed between type-2 endotype and *IL4RA* rs8832 GG genotype ( $P=.01$ ) (Figure 2A) or A allele of *ADAM33* T<sub>2</sub> ( $P=.01$ ) (Figure 2B). Frequencies of patients with *IL4RA* rs8832 GG genotype (Table S3a) or with A allele of *ADAM33* T<sub>2</sub> (Table S3b) according to type-2 endotype and the presence of exacerbations are shown in the Supplementary data.

Second, a total of 27 patients showed type-2 endotype and subsequent exacerbations. Multivariate analysis in the entire population revealed that *IL4RA* rs8832 GG and A allele of *ADAM33* T<sub>2</sub> were significant risk markers in patients with type-2 endotype and subsequent exacerbations (Table 3) (group D vs the remaining in Figure S2). Other variants of *ADAM33* S<sub>2</sub>, T<sub>1</sub>, and V<sub>4</sub> did not show any associations (data not shown).

### 3.3 | *ADAM33* variant in association with blood neutrophilia in eosinophilic/type-2 inflammation

The A allele of *ADAM33* T<sub>2</sub> may have played a role in severe asthma by augmenting mixed type of eosinophilic/type-2 and

neutrophilic inflammation.<sup>24</sup> When the analysis included all patients of the cohort, *ADAM33* T<sub>2</sub> A allele (AA or AG genotype,  $n=64$ , 29%) was significantly associated with higher blood neutrophil counts ( $P=.01$ ) and serum IL-6 levels ( $P=.02$ ) at enrolment (Table S4). The association between A allele of *ADAM33* T<sub>2</sub> and higher neutrophil counts was emphasized in patients with type-2 inflammatory endotype ( $n=83$ ,  $P=.009$ ) and those with blood eosinophilia (≥250/μL) at enrolment ( $n=99$ ,  $P=.007$ ) (Figure 3A). In addition, when risks of *ADAM33* T<sub>2</sub> for asthma exacerbations were assessed while including the effect of blood eosinophilia, there was a significant positive interaction between blood eosinophilia and A allele of *ADAM33* T<sub>2</sub> ( $P=.04$ ), which was not observed for *IL4RA* rs8832 (data not shown). Univariate analysis identified *ADAM33* T<sub>2</sub> A allele ( $P=.05$ ), but not *IL4RA* GG genotype, as a risk marker for subjects with both blood eosinophilia and subsequent exacerbations.

Finally, the association between A allele of *ADAM33* T<sub>2</sub> and neutrophil counts was verified in an independent cohort of patients with asthma. Among 234 recruited patients, 130 were included in the validation study. The analysis showed that for all patients ( $n=130$ , 90 females,

**TABLE 2** Logistic regression analysis to determine factors associated with asthma exacerbation during 2 y after enrolment<sup>a</sup>

|                                                       | Multivariate analysis<br>Odds ratio (95% CI) | P value |
|-------------------------------------------------------|----------------------------------------------|---------|
| Female gender                                         | 1.91 (0.83, 4.89)                            | .15     |
| GINA treatment step 5 <sup>b</sup>                    | 1.97 (0.54, 6.92)                            | .29     |
| Poor asthma control <sup>c</sup>                      | 0.98 (0.30, 2.99)                            | .98     |
| FEV <sub>1</sub> , <80% predicted                     | 2.37 (1.07, 5.17)                            | .03     |
| Recent exacerbation <sup>d</sup>                      | 6.57 (2.41, 19.5)                            | <.001   |
| Blood neutrophil counts,<br>by 2000/ $\mu$ L increase | 1.38 (0.84, 2.27)                            | .20     |
|                                                       | $R^2=.16$                                    | <.001   |

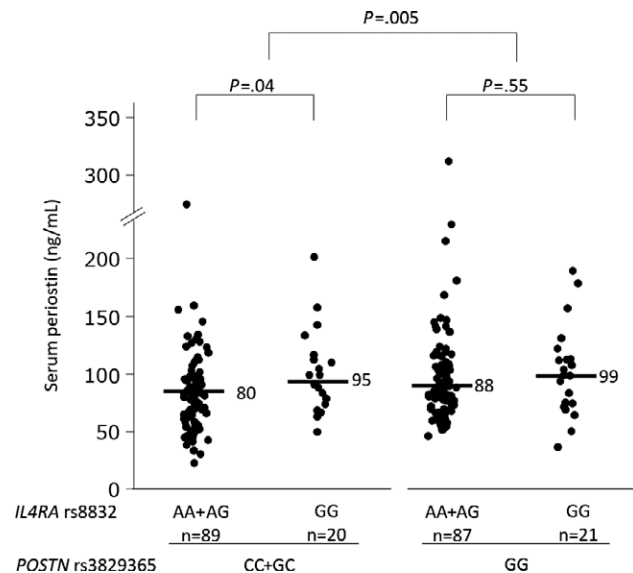
FEV<sub>1</sub>, forced expiratory volume in 1 s; CI, confidence interval.

<sup>a</sup>Asthma exacerbations requiring systemic corticosteroids during the 2 y after enrolment.

<sup>b</sup>According to the Global Initiative for Asthma (GINA) 2010.<sup>28</sup>

<sup>c</sup>As defined by Asthma Control Test™ score <20.

<sup>d</sup>The presence of exacerbations during the preceding 6 mo before enrolment requiring systemic corticosteroids.

**FIGURE 1** Serum periostin levels at enrolment stratified according to *IL4RA* rs8832 and *POSTN* rs3829365 genotypes. Line and number, median

mean age,  $55 \pm 12$  years), blood neutrophil counts ( $3244 \pm 945/\mu\text{L}$ ) were not associated with A allele of *ADAM33* T<sub>2</sub> ( $P=.14$ ). However, for patients with blood eosinophilia ( $\geq 250/\mu\text{L}$ ) ( $n=52$ ), those with A allele of *ADAM33* T<sub>2</sub> showed significantly higher blood neutrophil counts than those with *ADAM33* GG genotype ( $P=.007$ ) (Figure 3B).

## 4 | DISCUSSION

To the best of our knowledge, this is the first report showing that *IL4RA* rs8832 GG genotype and A allele of *ADAM33* T<sub>2</sub> (rs2280090) were genetic markers of asthma exacerbations in adult

patients with type-2 inflammatory endotype of asthma, which was defined as a high serum periostin level of  $\geq 95$  ng/mL. Furthermore, *IL4RA* rs8832 GG genotype was associated with type-2 inflammatory endotype, and A allele of *ADAM33* T<sub>2</sub> was associated with mixed type of eosinophilic/type-2 and neutrophilic inflammations, an endotype of the most severe form of asthma.

A simple comparison between exacerbators and non-exacerbators in this study confirmed well-known risk factors of exacerbation, which were GINA treatment step 5, poor asthma control, airflow limitation, and a recent exacerbation. On multivariate analysis, airflow limitation and a recent exacerbation were independent risks for subsequent exacerbations. Besides these clinical characteristics, however, no genetic markers were identified in this analysis. This finding was not surprising given the limited number of studies demonstrating genetic markers of asthma exacerbation in adults; this is in contrast with studies on childhood asthma. In children, in addition to candidate gene analyses,<sup>10</sup> two recent genome-wide association studies identified *CTNNA3* and *SEMA3D*<sup>33</sup> as well as *CDHR3*<sup>34</sup> as susceptibility genes of asthma exacerbations. We also examined the susceptibility genes of asthma exacerbations, that is *CDHR3* rs6967330 and *ORMDL3* rs7216389, and their association with exacerbations. However, the two polymorphisms were not associated with exacerbations and type-2 inflammation either defined by high serum periostin or blood eosinophilia in this cohort (data not shown). The difficulty in identification of genetic markers of susceptibility to asthma exacerbations in adults may be because of disease heterogeneity.<sup>35</sup> When type-2 inflammatory endotype was included into the analysis, the effects of *IL4RA* and *ADAM33* variants on asthma exacerbations became apparent in this study.

IL-4 and IL-13 are key players in asthma pathophysiology, including IgE production, Th2 cell differentiation, mast cell activation, airway hypersecretion, and airway remodelling. Type I and type II IL-4R share a common  $\alpha$  subunit, to which IL-13 has affinity as well. The complex comprising IL-13 and its specific receptor, IL-13R $\alpha$ 1, recruits IL-4R $\alpha$  to form a high affinity complex<sup>36</sup> and activates downstream signalling. Therefore, genetic variants of *IL4RA* have been repeatedly examined in candidate gene analyses of asthma pathophysiology,<sup>8</sup> and several molecules targeting IL-4R $\alpha$  have been developed.<sup>11,37</sup> Wenzel et al. reported that minor alleles at *IL4R* E375A (rs1805011) and Q551R (rs1801275) were significantly associated with severe asthma exacerbations, requiring intubation or intensive care.<sup>9</sup> However, other cohorts of patients with milder exacerbations showed a protective effect of the minor allele at E375A (rs1805011), which suggested that the direction of the gene effect on exacerbations might differ among patients with differing asthma or exacerbation severity.<sup>10</sup> However, a recent pharmacogenetics study by Slager et al. revealed that *IL4RA* rs8832 GG genotype was associated with an efficient reduction in exacerbations in patients treated with pitrakinra that blocks IL-4 and IL-13 signalling by preventing assembly of the IL-4R $\alpha$  complex.<sup>11</sup> These findings support our results that *IL4RA* rs8832 GG genotype was a genetic marker for asthma exacerbations in patients with type-2 inflammatory endotype.

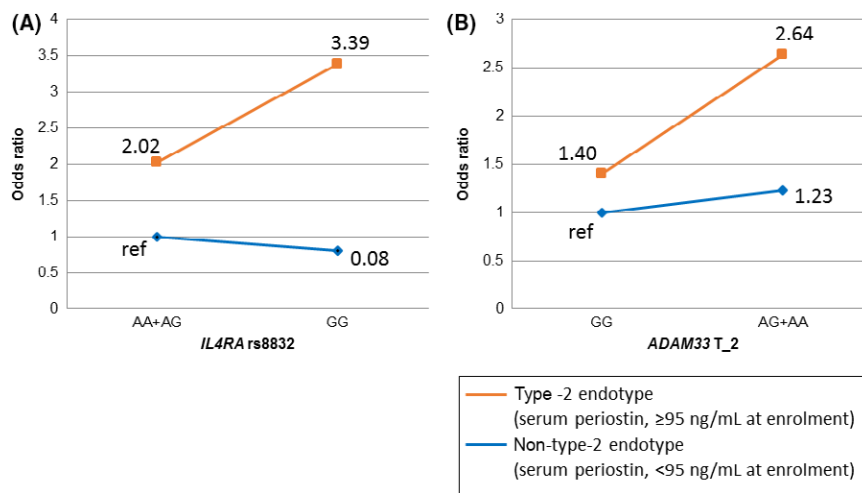


**FIGURE 2** (A) Risks of asthma exacerbations according to *IL4RA* rs8832 GG genotype by type-2 endotype.  $P=.02$  for the main effect of type-2 endotype and  $P=.01$  for interaction of *IL4RA* rs8832 GG genotype by type-2 endotype. (B) Risks of asthma exacerbations according to *ADAM33* T\_2 A allele by type-2 endotype.  $P=.05$  for the main effect of type-2 endotype and  $P=.01$  for interaction of *ADAM33* T\_2 A allele by type-2 endotype. ref, reference

**TABLE 3** Risks of patients with type-2 inflammatory endotype<sup>a</sup> and subsequent asthma exacerbations<sup>b</sup>

|                                                    | Univariate model    |         | Multivariate model  |         |
|----------------------------------------------------|---------------------|---------|---------------------|---------|
|                                                    | Odds ratio (95% CI) | P value | Odds ratio (95% CI) | P value |
| Body mass index, $\geq 25$ kg/m <sup>2</sup>       | 3.01 (0.87, 10.4)   | .10     | 0.29 (0.06, 1.04)   | .06     |
| GINA treatment step 5 <sup>c</sup>                 | 3.89 (1.34, 11.3)   | .02     | 1.73 (0.36, 7.65)   | .48     |
| Poor asthma control <sup>d</sup>                   | 2.98 (1.12, 7.91)   | .05     | 0.98 (0.20, 4.04)   | .98     |
| FEV <sub>1</sub> , $< 80\%$ of predicted           | 7.18 (3.05, 16.9)   | $<.001$ | 6.51 (2.37, 18.6)   | $<.001$ |
| Recent exacerbations <sup>e</sup>                  | 2.98 (1.12, 7.91)   | .05     | 2.04 (0.48, 7.98)   | .32     |
| Serum IgE levels <sup>f</sup> , IU/mL              | 1.82 (1.02, 3.40)   | .05     | 1.88 (0.90, 4.13)   | .10     |
| Blood neutrophil counts, by 2000/ $\mu$ L increase | 1.63 (1.02, 2.61)   | .03     | 0.90 (0.47, 1.71)   | .74     |
| <i>IL4RA</i> rs8832, GG                            | 3.67 (1.55, 8.67)   | .006    | 4.01 (1.47, 11.0)   | .007    |
| <i>ADAM33</i> T_2, A allele                        | 3.02 (1.33, 6.85)   | .01     | 2.81 (1.05, 7.67)   | .04     |
|                                                    |                     |         | $R^2=.26$           | $<.001$ |

FEV<sub>1</sub>, forced expiratory volume in 1 s; CI, confidence interval.

<sup>a</sup>Determined as serum periostin level  $\geq 95$  ng/mL at enrolment.

<sup>b</sup>Asthma exacerbations requiring systemic corticosteroids during the 2 y after enrolment.

<sup>c</sup>According to the Global Initiative for Asthma (GINA) 2010.<sup>28</sup>

<sup>d</sup>As defined by Asthma Control Test<sup>TM</sup> score  $< 20$ .

<sup>e</sup>The presence of exacerbations during the preceding 6 mo before enrolment requiring systemic corticosteroids.

<sup>f</sup>Log-transformed.

*IL4RA* rs8832 is located in the 3' untranslated regions, where RNA stability and/or processing is affected; however, the functional consequence of this variant remains unknown. In this study, the rs8832 GG genotype was associated with elevated serum periostin levels, which suggested that this genotype may lead to a gain-of-function effect in *IL-4R $\alpha$*  signalling. This speculation may also be supported by findings from our subpopulation analysis where exhaled nitric oxide was measured ( $n=121$ ). In that analysis, the frequency of *IL4RA* rs8832 GG genotype in patients with high levels of both exhaled nitric oxide ( $\geq 25$  ppb) and serum periostin ( $\geq 95$  ng/mL) was significantly higher than that in the remaining patients (35% vs 15%) ( $P=.03$ ).<sup>38</sup> Although *IL4RA* rs8832 GG genotype appeared to be protective against exacerbations in patients with non-type-2 endotype (Table S3a), the result of trend analysis did not support a potential protective effect of G allele against exacerbations (Figure S3). Future

studies are needed to further elucidate genetic markers of asthma pathogenesis in patients with non-type-2 endotype.

*ADAM33* belongs to a disintegrin and zinc-dependent metalloprotease family and exhibits a complex organization that includes eight domains: signal sequence, prodomain, catalytic, disintegrin, cysteine rich, epidermal growth factor like, transmembrane, and cytoplasmic. A recent study revealed that in *Adam33*-null mice, airway remodelling and inflammation after allergen challenge were both suppressed, suggesting an essential role of *ADAM33* in asthma pathophysiology.<sup>39</sup> Expression of *ADAM33* is up-regulated in airway smooth muscles, fibroblasts,<sup>21,40</sup> and less frequently airway epithelium<sup>41-43</sup> of patients with asthma. Its expression is also up-regulated with stimulation by IL-4<sup>43,44</sup> and IL-13,<sup>44</sup> which may support the potential role of *ADAM33* variant in eosinophilic/type-2 inflammatory endotype observed in this study. In addition, current

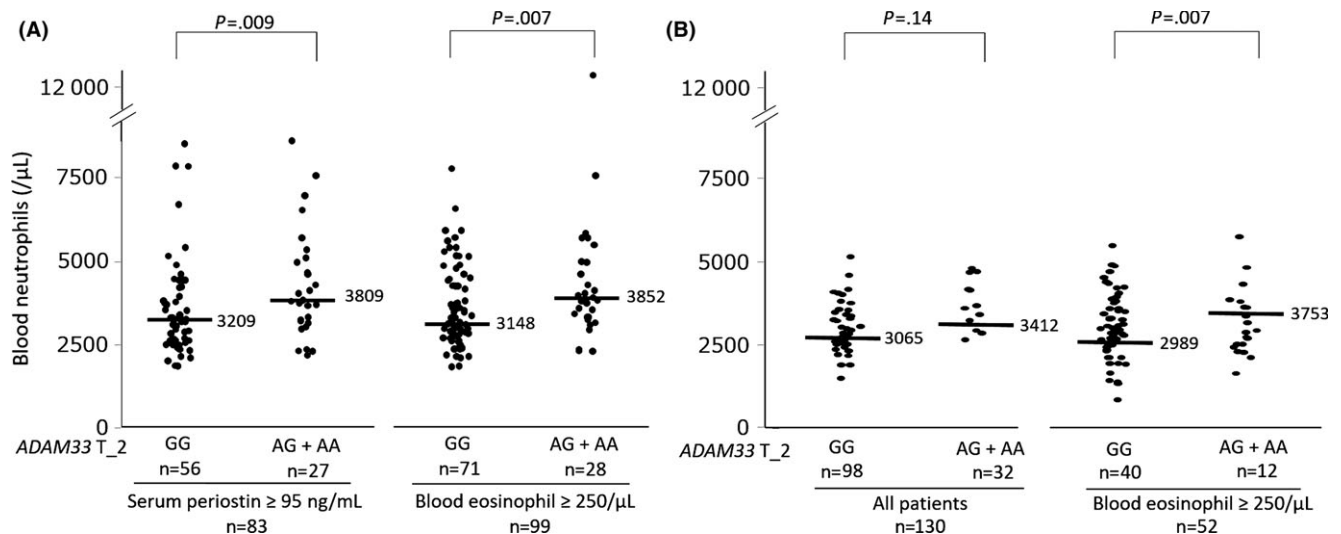


**FIGURE 3** (A) The number of blood neutrophils at enrolment according to ADAM33 T<sub>2</sub> genotypes in patients with type-2 endotype (serum periostin, ≥95 ng/mL at enrolment) or eosinophilic inflammation (≥250/μL at enrolment). Line and number, median. (B) The number of blood neutrophils stratified according to ADAM33 T<sub>2</sub> genotypes in patients of a replication study. Data of entire population and those of eosinophilic (≥250/μL) population are presented. Line and number, median

associations between ADAM33 T<sub>2</sub> variant and increased risks of asthma exacerbations are consistent with previous findings on ADAM33 T<sub>2</sub> variant in association with increased asthma susceptibility<sup>21</sup> and lung function decline in asthma.<sup>30</sup> Associations between ADAM33 T<sub>2</sub> variant and mixed-type inflammation should be further highlighted because this endotype is associated with the most severe and treatment-resistant asthma, which requires the development of novel and non-steroidal therapeutics. The exact mechanisms underlying these associations remain unknown; however, the soluble form of ADAM33 (sADAM33) may be involved. sADAM33, which is increased in bronchoalveolar fluid of patients with asthma,<sup>41</sup> is efficiently released by transforming growth factor-β<sub>2</sub>, is catalytically active, promotes angiogenesis in the human lung tissue,<sup>45</sup> and induces airway remodelling, which may provide the “soil” for type-2 inflammation and airway hyperresponsiveness.<sup>39</sup> Potential interactions between ADAM33 and α catenin, an important protein in airway epithelial barrier function,<sup>46</sup> may also contribute to susceptibility to asthma exacerbation. Given that ADAM33 T<sub>2</sub> variant encodes the cytoplasmic tail of ADAM33, it is plausible that this variant might be vulnerable to shedding, leading to release of sADAM33 by the loss of its anchor. Further studies are necessary to demonstrate associations of ADAM33 T<sub>2</sub> variant with sADAM33 levels, exacerbations, and mixed types of inflammation in asthma.

This study has several limitations. First, the associations between the two genes and patients with type-2 endotype and subsequent exacerbations were not confirmed in an independent cohort and the sample size was insufficient to conclusively demonstrate associations between genotypes and endotypes. However, in addition to the well-characterized cohort, we conducted a validation study in an independent population with asthma and confirmed the association between A allele of ADAM33 T<sub>2</sub> variant and a mixed type of inflammation in patients who did not regularly use systemic

corticosteroids. In addition, a mixed type of inflammation should be confirmed in sputum samples<sup>25,47</sup> but was also reported in blood samples of patients with severe asthma,<sup>26,27 47,48</sup> albeit increased blood neutrophils may merely reflect the use of systemic corticosteroids.<sup>27</sup> Second, causes of exacerbations such as viral infection and allergen exposure and inflammatory subtypes at times of exacerbation were not examined. However, the close relationship between vulnerability to viral infection and allergic or type-2 endotype may exempt information on causes of exacerbations. ADAM33 was involved in viral infection-induced pulmonary inflammation, which was evident by the expression of IL-4 and IL-13.<sup>49</sup>

In conclusion, *IL4RA* rs8832 GG genotype and A allele of ADAM33 T<sub>2</sub> may be genetic markers of exacerbations in patients with asthma with type-2 inflammatory endotype. Precise endotyping of heterogeneous patients with asthma may facilitate the identification of genes driving the disease in these subtypes.

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## CONFLICTS OF INTEREST

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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