



Contents lists available at ScienceDirect



The CC16 A38G polymorphism is associated with asymptomatic airway hyper-responsiveness and development of late-onset asthma

Natsuko Taniguchi, MD^{*}; Satoshi Konno, MD^{*}; Takeshi Hattori, MD^{*}; Akira Isada, MD^{*}; Kaoruko Shimizu, MD^{*}; Kenichi Shimizu, MD^{*}; Noriharu Shijubo, MD[†]; Shau-Ku Huang, PhD[‡]; Nobuyuki Hizawa, MD[§]; and Masaharu Nishimura, MD^{*}

^{*} First Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan

[†] Department of Respiratory Medicine, JR Sapporo Hospital, Sapporo, Japan

[‡] Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland

[§] Division of Respiratory Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

ARTICLE INFO

Article history:

Received for publication May 28, 2013.

Received in revised form August 3, 2013.

Accepted for publication August 12, 2013.

ABSTRACT

Background: Clara cell secretory protein (CC16) is expressed primarily in the respiratory tract and is a potent anti-inflammatory agent that protects the airway from inflammation. The associations of the A38G polymorphism in this gene with asymptomatic airway hyper-responsiveness (AHR), which is considered a risk factor for future asthma in adults, and the development of adult-onset asthma are unclear.

Objective: To evaluate the association of the CC16 A38G polymorphism with asymptomatic AHR in healthy young adults and the development of adult-onset asthma and the association between plasma CC16 level according to this genotype and asymptomatic AHR.

Methods: Nonspecific AHR was measured in 154 asymptomatic, young, healthy adults using a continuous methacholine inhalation method. The cumulative dose values of inhaled methacholine measured at the inflection point at which respiratory conductance started to decrease (Dmin) were used as an index of AHR. Case–control analysis was performed for the association between this polymorphism and the development of asthma in 1,086 unrelated Japanese subjects (504 subjects with asthma and 582 healthy subjects).

Results: The 38AA + AG genotype was associated with lower Dmin values and lower plasma CC16 levels ($P = .012$ and $.020$). There was a significant positive correlation between Dmin values and plasma CC16 levels ($P = .012$). In the case–control study, the 38AA + AG genotype was significantly associated with late-onset asthma (onset at >40 years; odds ratio, 1.63; $P = .016$).

Conclusion: These results suggest that the CC16 A38G polymorphism may play a role in asymptomatic AHR and contribute to the development of late-onset asthma.

© 2013 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Asthma is a common chronic respiratory disease characterized by reversible airflow obstruction and airway hyper-responsiveness (AHR). Recently, asthma has been described as a clinical syndrome that can be caused by different pathologies rather than a single disease.¹ Several approaches for clustering asthma have suggested that age of onset is a determinant that can be used to identify certain asthma phenotypes and their underlying specific pathogenesis.²

In contrast to childhood-onset asthma, asthma that develops during adulthood is more heterogeneous, and more complex

environmental factors appear to be involved in its pathogenesis.³ Although the role of genetic predisposition in adult-onset asthma is less clear than in atopic childhood-onset asthma, specific host genetic factors may be important in the development of adult-onset asthma. Notably, the authors previously reported that a functional polymorphism on the promoter region of the *CCL5/RANTES* gene and tissue factor gene were associated with adult late-onset asthma in a Japanese population.^{4,5}

Nonspecific AHR, which is objectively defined by an abnormal response to a nonspecific stimulus such as histamine or methacholine, is thought to be determined by several genetic and environmental factors and is an important feature of asthma. Although AHR is often accompanied by respiratory symptoms, it can occur without any clinical respiratory symptoms, which is called asymptomatic AHR. Although the clinical significance of asymptomatic AHR and its relation to asthma is not fully understood, prospective

Reprints: Satoshi Konno, MD, First Department of Medicine, Hokkaido University School of Medicine, Kita-15 Nishi-7 Kita-Ku, Sapporo 060-8638, Japan; E-mail: satkonno@med.hokudai.ac.jp.

Disclosures: Authors have no disclosures.

studies have suggested that asymptomatic AHR is a risk factor for future asthma in adults.^{6,7} Recent large prospective surveys conducted in Europe also have shown that the presence of AHR in a young adult is a significant risk factor for the development of new-onset asthma.⁸

Clara cell secretory protein (CC16), also known as secretoglobin 1A1 (SCGB1A1), CC10, CCSP, and uteroglobin, is expressed primarily in the respiratory tract and is a potent anti-inflammatory agent that protects the airway from inflammation.⁹ Mice that are deficient in CC16 expression exhibit a higher level of allergic airway inflammation through augmented T-helper cell type 2 responses,¹⁰ and reconstitution of CC16 in CC16-deficient mice can reverse this altered phenotype.¹¹

In humans, the *CC16* gene is located on chromosome 11q12-13, a region that was originally reported for its associations with asthma, high serum total IgE levels, and AHR in several genome-wide linkage studies.^{12–14} There have been several reports of an association between pulmonary diseases and a guanine–adenine substitution at position 38 (A38G) downstream from the transcription initiation site within the noncoding region of this gene's exon 1. The A allele is associated with the decreased transcriptional activity of this gene.^{15,16} Numerous studies have shown significant associations of this polymorphism with asthma and the presence of wheeze and AHR, particularly in infants and children.^{17,18} However, the possible association of this polymorphism with adult asthma, particularly adult late-onset asthma, has not been well studied. In addition, the association of this polymorphism with asymptomatic AHR has not been reported.

As part of the authors' research efforts on risk factors for asymptomatic AHR and adult late-onset asthma,^{4,5,19} the present study focused on CC16. First, the possible association between the CC16 A38G polymorphism and nonspecific AHR was evaluated in asymptomatic healthy subjects. Plasma CC16 levels also were measured, and their association with AHR was evaluated. Second, a case–control study was conducted to evaluate the possible association of this polymorphism with the development of late-onset asthma.

Methods

Study Populations

Asymptomatic healthy subjects for nonspecific airway responsiveness determinations

As previously described,¹⁹ 154 asymptomatic healthy subjects (110 men and 44 women; median age, 24 years; range, 18–35 years) were recruited. These subjects' characteristics are listed in Table 1. These subjects were medical students from Hokkaido University (Sapporo, Japan) or young doctors in the authors' hospital who lived in Sapporo. All subjects provided their written informed consent for enrollment in this study and all associated procedures. The ethics committee of the Hokkaido University

Table 1

Characteristics of subjects for measurement of airway hyper-responsiveness

Characteristics	Data
Subjects, n	154
Age (y), median (range)	24 (18–35)
Men, n (%)	110 (71.4)
Atopy, n (%)	118 (76.6)
Smoking status: current/former/never	17/8/129
Dmin (log, unit), mean $\pm$ SD	1.12 $\pm$ 0.62
IgE (log, IU/mL), mean $\pm$ SD	1.89 $\pm$ 0.62
CC16 38GA genotype AA/AG/GG	29/67/58

Abbreviations: CC16, Clara cell secretory protein; Dmin, cumulative dose of inhaled methacholine measured at inflection point at which respiratory conductance starts to decrease; SD, standard deviation.

School of Medicine approved this study. None of these subjects had ever experienced wheezing, shortness of breath, or any other respiratory disease. None of the participants reported any recent respiratory infections. They were examined for total and specific IgE antibodies. Atopic status was defined as a positive response to at least 1 of the 10 common inhaled allergens, as previously described.^{4,5,20} An increase in specific IgE antibody levels (IgE multiple antigen simultaneous test score, lumicount >1.0) was considered a positive response. Spirometry also was performed for all subjects (Autospiro AS-300; Minato, Osaka, Japan).

Case–control study

A total of 1,086 unrelated Japanese subjects were recruited, including 504 patients with asthma and 582 healthy subjects (Table 2 and eTable 1). All patients with asthma were recruited from the pulmonary clinic of the First Department of Medicine, Hokkaido University Hospital. Patients were considered asthmatic based on the presence of recurrent episodes of at least 2 of 3 symptoms (coughing, wheezing, or dyspnea) associated with demonstrable reversible airflow obstruction and/or increased airway responsiveness to methacholine, as previously described.^{4,5,20} As healthy controls, individuals who visited the clinic for annual routine physical examinations and had no history of asthma or any other chronic pulmonary diseases were recruited. All these subjects were unrelated and of Japanese descent. Written informed consent was obtained from all subjects for enrollment in this study. The ethics committee of the Hokkaido University School of Medicine also approved this study. The definition of atopic status was the same as that for the study measuring nonspecific AHR.

Nonspecific Airway Responsiveness

Nonspecific airway responsiveness, as judged by the degree of methacholine-induced bronchoconstriction, was determined by continuous methacholine inhalation with simultaneous measurement of respiratory resistance (Astograph; Chest, Tokyo, Japan), as

Table 2

Characteristics of subjects for case–control study

	Healthy control subjects	Patients with asthma			P value ^a	P value ^b
		Early onset	Middle onset	Late onset		
Subjects, n	582	141	150	213		
Age (y), median (range)	47 (18–79)	26 (16–73)	37 (20–71)	59 (41–81)	<.001	<.001
Men, n (%)	299 (51.4)	82 (58.2)	58 (38.7)	90 (42.3)	<.001	.007
Atopy, n (%)	294 (50.5)	138 (92.0)	112 (79.4)	115 (54.0)	<.001	<.001
Never smoker, n (%)	325 (56.5)	84 (59.6)	78 (52.0)	108 (50.7)	.011	.12
Age of onset (y), median (range)	—	5 (0–19)	30 (20–40)	55 (41–80)	<.001	<.001
IgE (log IU/mL), mean $\pm$ SD	1.84 $\pm$ 0.63	2.60 $\pm$ 0.63	2.38 $\pm$ 0.67	2.25 $\pm$ 0.62	<.001	<.001

Abbreviation: SD, standard deviation.

^aThe χ^2 test was used for proportions and Kruskal–Wallis analysis was used for continuous variables among the 4 groups.

^bThe Cochran linear trend was used for proportions and the Jonckheere–Terpstra test was used for continuous variables among the 3 asthma groups.

described previously.^{19,21} After recording baseline respiratory resistance, beginning with the lowest concentration, increasing doses of methacholine were inhaled at 1-minute intervals. As an index of AHR, the cumulative dose values of inhaled methacholine measured at the inflection point at which respiratory conductance started to decrease (Dmin) were used. One Dmin unit represented a 1-minute inhalation of 1 mg/mL of methacholine.²² Logarithmic Dmin values as an index of AHR were used because previous studies have shown that AHR assessed by Dmin correlates well with degree of airway inflammation, including exhaled nitric oxide concentrations and sputum eosinophil counts, in patients with asthma.^{22,23}

Genotyping

The CC16 A38G polymorphism (rs3741240) was identified using the TaqMan system (assay ID, C_25473445_10; Applied Biosystems, Foster City, California).

Plasma CC16 Concentrations

Plasma samples were available for 137 of the 154 asymptomatic healthy subjects and used for measurements of CC16 levels; 7 mL of whole blood was collected in tubes containing ethylenediaminetetraacetic acid and centrifuged for 10 minutes at 1,000g. The supernatant was separated and stored at -80°C until analysis. Plasma CC16 levels were measured using a commercial enzyme-linked immunosorbent assay kit (Bio Vendor Laboratory Medicine, Inc, Brno, Czech Republic), as in several previous studies.^{17,24} The sensitivity of this assay was 20 pg/mL.

Statistical Analysis

To compare differences among different subject groups, the Kruskal-Wallis test and χ^2 test were used for nonparametric, continuous, and categorical variables. In addition, the Cochran linear trend test or Jonckheere-Terpstra test was used to evaluate the trends among groups with asthma. Furthermore, to compare the results of the standardized continuous variables (log Dmin values and plasma CC16 levels) between the 2 groups (genotype AA + AG vs GG), unpaired *t* test or 1-way analysis of variance was used to adjust for the independent variables of age, sex, smoking status (never, former, and current), and atopic status.

Pearson correlation coefficients were used to analyze the association between log Dmin and plasma CC16 levels. Patients with asthma were divided into 3 subgroups based on age of onset: early onset, 0 to 19 years; middle onset, 20 to 40 years; and late onset, at least 41 years.⁴ Logistic regression analysis was used to estimate odds ratios (ORs) with corresponding 95% confidence intervals (CIs) as estimates of relative risk for the development of asthma (early, middle, and late onset). The ORs were adjusted for sex, age, smoking status, and atopic status. The Hardy-Weinberg equilibrium (HWE) program was used to compare the observed numbers of genotypes with the expected numbers of genotypes under the HWE.⁵ Statistical analyses were performed using SYSTAT 11 for Windows (Systat Software, Inc, Chicago, Illinois).

Results

CC16 A38G Polymorphism Is Associated With Plasma CC16 Levels and Dmin Values

Of the 154 healthy subjects, 118 (76.6%) had specific IgE antibodies to at least 1 of 10 inhaled antigens. A total of 129 subjects (83.8%) had never smoked. Log Dmin values were normally distributed among these 154 asymptomatic subjects (mean $\pm$ standard deviation [SD] for log Dmin, 1.12 ± 0.62). The genotype frequencies of CC16 were 18.8% for AA, 43.5% for AG, and 37.8% for GG. In this study population, the frequency of the CC16 A38G polymorphism did not deviate significantly from the HWE ($P > .05$).

When possible associations between CC16 A38G genotypes and Dmin values were evaluated, the log Dmin values were significantly different among these genotypes: log Dmin was lower for 38A carriers (38AA + AG; mean $\pm$ SD, 1.03 ± 0.61) than for noncarriers (38GG; mean $\pm$ SD, 1.28 ± 0.61 ; $P = .012$; adjusted $P = .020$ by analysis of variance; Fig 1A). Plasma CC16 levels were significantly lower in 38A carriers (mean $\pm$ SD, 6.36 ± 2.74) than in noncarriers (mean $\pm$ SD, 7.57 ± 3.21 ; $P = .020$; adjusted $P = .012$ by analysis of variance; Fig 1B). As shown in Figure 2A, there was a significant positive correlation between log Dmin values and plasma CC16 levels ($P = .012$, $r = 0.214$). Smoking status has been shown to have

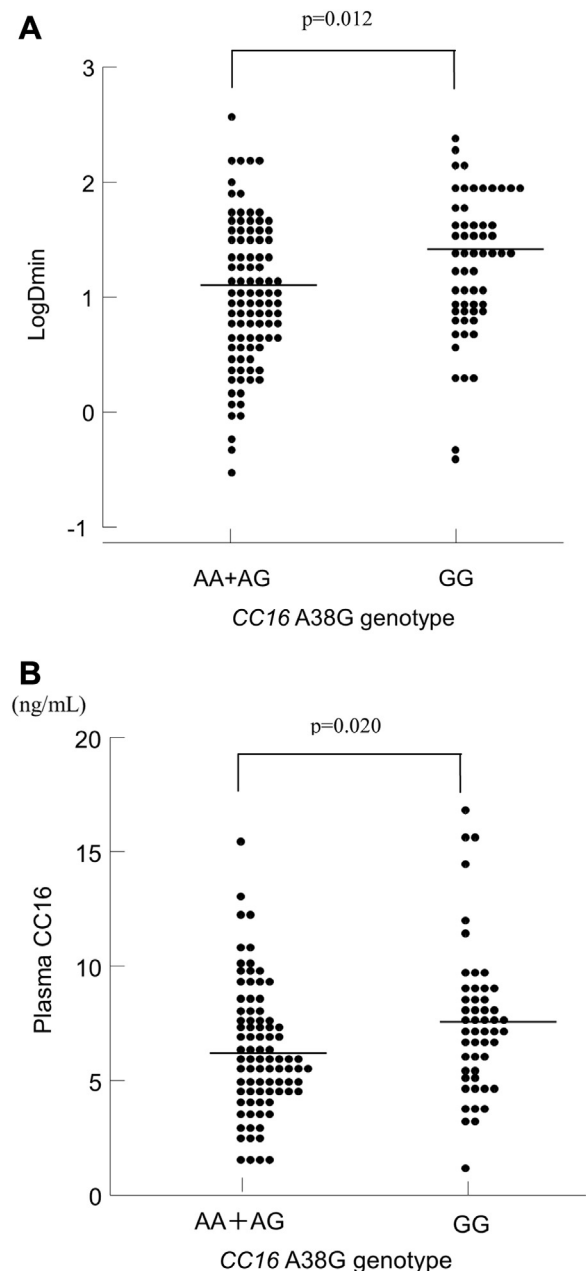


Figure 1. Values of the logarithmic cumulative dose of inhaled methacholine measured at the inflection point at which respiratory conductance starts to decrease (log Dmin) and plasma Clara cell secretory protein (CC16) stratified by CC16 G38A genotype. (A) Airway hyper-responsiveness (log Dmin) in young healthy subjects. A significant difference is seen in log Dmin between 38A allele carriers and noncarriers. (B) CC16 concentration in plasma in young healthy subjects. A significant difference is seen in plasma CC16 levels between 38A allele carriers and noncarriers.

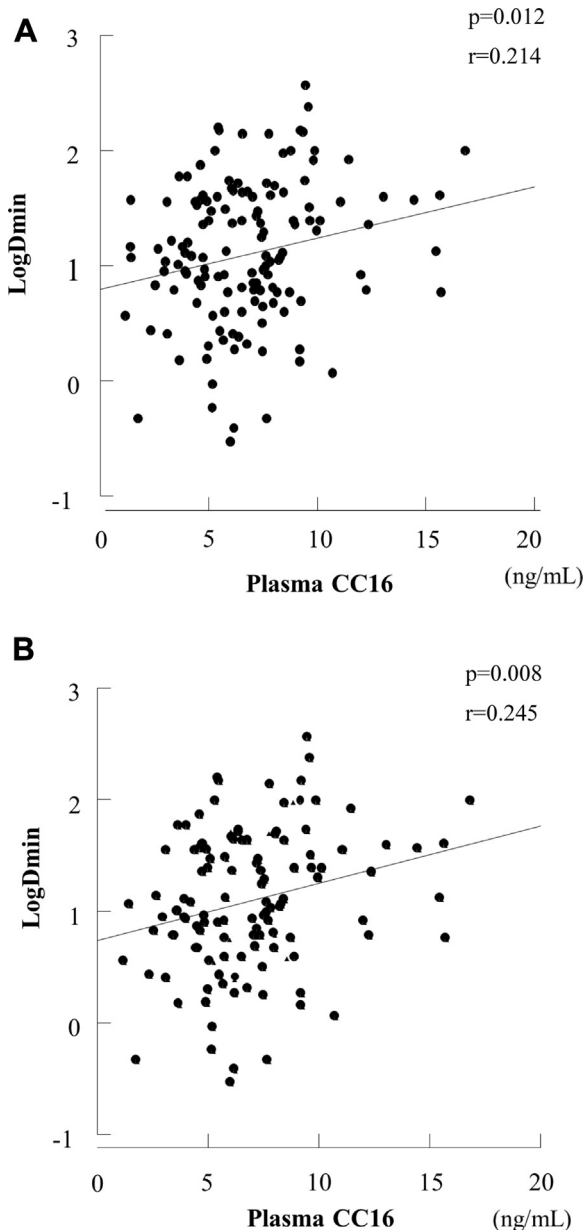


Figure 2. Association between plasma Clara cell secretory protein (CC16) and the logarithmic cumulative dose of inhaled methacholine measured at the inflection point at which respiratory conductance starts to decrease (log Dmin). (A) There is a significant positive correlation between log Dmin values and plasma CC16 levels. (B) There is a significant positive correlation between log Dmin values and plasma CC16 levels in never smokers.

a significant effect on serum CC16 levels^{25–27}; therefore, this association also was evaluated in never smokers ($n = 115$). As shown in Figure 2B, a significant association was observed in never smokers ($P = .008$, $r = 0.245$).

CC16 A38G Polymorphism Is Associated With Late-Onset Asthma

In this study population, patients with early-onset asthma were more frequently atopic than those with late-onset asthma and healthy controls (Table 2). Of the 3 asthma subgroups, patients with late-onset asthma had the lowest mean total serum IgE level and the lowest frequency of atopic status. In addition, the frequency of former smokers was highest in the late-onset asthma group (Table 2). The A38G polymorphism did not deviate significantly from the HWE in the control subjects ($P > .05$). The A allele (AA + AG)

was significantly associated with late-onset asthma (adjusted OR, 1.62; 95% CI, 1.09–2.40; $P = .016$). In contrast, there were no significant differences in the genotype distributions of the A38G polymorphism between healthy control subjects and patients with early- or middle-onset asthma (Table 3). When healthy controls were restricted to those older than 40 years, a similar association was observed; the A38G polymorphism was significantly associated with late-onset asthma (adjusted OR, 1.61; 95% CI, 1.09–2.37; $P = .017$; eTables 2,3,4).

Discussion

In the present study, there was an association between the A allele of the CC16 A38G polymorphism and lower Dmin values, an indicator of nonspecific airway responsiveness assessed by methacholine-induced bronchoconstriction, in asymptomatic healthy subjects. A significant association between the AA + AG genotype and low plasma CC16 levels indicated that decreased expression of CC16 in the airway determined by the A38G polymorphism might contribute to the development of asymptomatic AHR. In addition, the case–control study provided evidence that the CC16 A38G polymorphism was associated with susceptibility to the development of late-onset asthma.

Although AHR is often accompanied by respiratory symptoms, it can occur without any clinical respiratory symptoms (ie, asymptomatic AHR). Several prospective studies have shown that asymptomatic AHR might be a risk factor for future asthma.^{6–8} The significance of asymptomatic AHR as a risk factor for future asthma could have 2 possible explanations. Studies that have examined bronchial biopsies have reported that mild inflammatory and structural changes in the airway mucosa occur in patients with asymptomatic AHR,²⁸ and it was speculated that these changes could progress by the time respiratory symptoms appeared. In contrast, other reports have shown that airway inflammation does not necessarily play a determining role in the development of AHR in patients with asymptomatic AHR, as assessed by the inflammatory cell profiles in sputum.²⁹ This result indicates that asymptomatic AHR is due to a direct increase in airway smooth muscle cell reactivity that is independent of inflammatory processes. Although a significant association of CC16 A38G polymorphism and plasma CC16 levels with AHR was observed in the present study, the precise mechanisms of how CC16 is involved in the pathogenesis of asymptomatic AHR are unclear. Considering the anti-inflammatory role of CC16 in T-helper cell type 2–related airway inflammation, it is plausible to speculate that airway inflammation due to decreased CC16 levels in the airway might contribute to asymptomatic AHR. Pathologic examination by bronchial biopsies or sputum analysis will clarify how CC16 is involved in the development of asymptomatic AHR.

In addition to the association between the A38G polymorphism and asymptomatic AHR, there was a significant association between plasma CC16 levels and Dmin values. Previous reports have suggested that circulating levels of CC16 might be a marker of respiratory disease, which could be influenced by epithelial cell dysfunction. Plasma CC16 levels are low in patients with asthma and those with chronic obstructive pulmonary disease and are correlated with disease activity.^{16,30,31} Therefore, mild airway inflammation, which may develop in patients with asymptomatic AHR, contributes to lower plasma CC16 levels, as shown in this study. Alternatively, intrinsically lower CC16 expression determined by the A38G polymorphism might contribute to the development of asymptomatic AHR and eventually lead to late-onset asthma. In the clinical setting, measuring plasma CC16 levels in young adults may be useful for predicting the presence of nonspecific hyper-responsiveness and the possibility of developing asthma in the future. However, it should be noted that the correlation between

Table 3

Association of CC16 A38G polymorphism with asthma

	Subjects, n	CC16 A38G genotype, n (%)			OR ^a	95% CI ^a	P value ^a
		AA	AG	GG			
Healthy controls	582	103 (17.7)	273 (46.9)	206 (35.4)			
Asthma (all onset ages)	504	83 (16.5)	243 (48.2)	178 (35.3)	0.99	0.77–1.29	.96
Early-onset asthma	141	20 (14.2)	67 (47.5)	54 (38.3)	0.73	0.47–1.14	.16
Middle-onset asthma	150	26 (17.3)	65 (43.3)	59 (39.3)	0.75	0.51–1.11	.15
Late-onset asthma	213	37 (17.4)	111 (52.1)	65 (30.5)	1.62	1.09–2.40	.016

Abbreviations: CC16, Clara cell secretory protein; CI, confidence interval; OR, odds ratio.

^aAdjusted for age, sex, atopy, and smoking status. Odds ratios were calculated for the presence of the 38A allele using multivariate logistic regression models.

plasma CC16 levels and Dmin values was not strong ($r = 0.214$; Fig 2A), which suggests that several environmental and other genetic factors might be involved in asymptomatic AHR.^{19,32}

Although several studies have shown significant associations of the A38G polymorphism and asthma with the presence of wheeze and AHR in infants and children,^{17,18} there have been few reports showing an association between this polymorphism and adult asthma. When the effect of the A38G polymorphism was evaluated for the entire asthma population in the present study, no significant association was observed, which is consistent with a previous report conducted in a Japanese population.³³

Considering the biological function of CC16 in T-helper cell type 2–related inflammation, the prolonged anti-inflammatory effect of CC16 might eventually contribute to the development of late-onset asthma. This may partly explain why an association with this polymorphism was found only with adult-onset asthma, but not with childhood-onset asthma. Alternatively, a significant association of this polymorphism with asymptomatic AHR and with late-onset asthma might support the proposition that asymptomatic AHR is a risk factor for the future development of asthma. However, it is not certain how many subjects in the present study will eventually develop asthmatic symptoms during their lifetimes. Prospective studies will be needed to determine the significance of asymptomatic AHR as a risk factor for future asthma onset in this population.

Recently, asthma has been recognized as a heterogeneous and genetically complex disease that cannot be explained by a single mechanism. A precise definition of asthma phenotypes is becoming more relevant, not only to improve the understanding of their pathophysiologic mechanisms, but also to ascertain the specific genes associated with these phenotypes. Several recent approaches for clustering asthma have suggested that age of onset is one determinant to identify certain asthma phenotypes and their underlying specific pathogenesis.² The age of asthma onset encompasses a wide range, and differentiating asthma based on age at onset of disease can enhance the understanding of asthma pathogenesis. Patients with childhood-onset asthma have been shown to have a significantly greater likelihood of allergic sensitization and clinical responses to triggers than patients with adult-onset asthma. Genetic factors related to atopy are involved in childhood-onset asthma. In contrast, asthma that develops during adulthood is more heterogeneous, there is usually no family history of asthma, and more complex environmental factors appear to be involved in its pathogenesis. Although the role of genetic predisposition in adult-onset asthma is less clear than in atopic childhood-onset asthma, a specific host genetic factor also might be important in the development of adult-onset asthma. In addition to the authors' previous results showing an association of CCL5/RANTES and tissue factor gene variants with adult-/late-onset asthma, the present study may have identified a new genetic factor involved in the development of adult late-onset asthma.^{4,5}

Two major limitations need to be considered in interpreting the present results. First, an oscillation method (Astograph) was used to evaluate AHR in asymptomatic healthy subjects.^{19,21} With this

method, the change in respiratory resistance is monitored instead of forced expiratory volume in 1 second (FEV₁), which is recommended as a primary outcome by the American Thoracic Society guidelines for evaluating AHR.³⁴ However, these guidelines also indicate that the changes in respiratory resistance might be more sensitive than the changes in FEV₁ for detecting bronchoconstriction. Therefore, to evaluate the factors associated with AHR in asymptomatic healthy subjects, as in the present study, it may be better to use respiratory conductance instead of FEV₁. Further studies using changes in FEV₁ as an outcome will be needed to confirm the present results.

Second, case–control studies based on questionnaires are susceptible to recall bias. Definitions of physician-diagnosed asthma have been shown to be specific but are somewhat lacking in sensitivity and probably exclude milder cases of asthma. In the present study, to judge the age of onset of asthma as accurately as possible, patients were asked about episodes of dyspnea, wheezing, or coughing during childhood and puberty. When uncertain, the time of earliest respiratory symptoms was designated as the age of onset of asthma symptoms. A recent study on the age at onset of asthma in 2,524 Japanese adult patients with asthma found that 23.7% had developed the disease before 20 years of age, and 58.5% had developed the disease at older than 20 years. This variation in age at onset of adult asthma is similar to what was found in the present study and provides evidence that the self-reported data on age at onset of asthma obtained in this study are reliable.³⁵

In summary, the results of the present study suggest that the CC16 A38G polymorphism may play a role in asymptomatic AHR and contribute to the development of late-onset asthma. This newly described association between the CC16 A38G polymorphism and asymptomatic AHR and late-onset asthma provides additional evidence suggesting that asymptomatic AHR is a risk factor for the future development of asthma. Further prospective studies will be needed to clarify the significance of asymptomatic AHR, which is determined by the CC16 A38G polymorphism, in the later development of symptomatic asthma.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2013.08.005>.

References

- [1] Lötvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol*. 2011;127:355–360.
- [2] Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med*. 2010;181:315–323.
- [3] Thomsen SF, Duffy DL, Kyvik KO, Backer V. Genetic influence on the age at onset of asthma: a twin study. *J Allergy Clin Immunol*. 2010;126:626–630.
- [4] Hizawa N, Yamaguchi E, Konno S, Tanino Y, Jinushi E, Nishimura M. A functional polymorphism in the RANTES gene promoter is associated with the development of late-onset asthma. *Am J Respir Crit Care Med*. 2002;166:686–690.

- [5] Isada A, Konno S, Hizawa N, et al. A functional polymorphism (–603A → G) in the tissue factor gene promoter is associated with adult-onset asthma. *J Hum Genet.* 2010;55:167–174.
- [6] Laprise C, Boulet LP. Asymptomatic airway hyperresponsiveness: a three-year follow-up. *Am J Respir Crit Care Med.* 1997;156:403–409.
- [7] Xu X, Rijcken B, Schouten JP, Weiss ST. Airways responsiveness and development and remission of chronic respiratory symptoms in adults. *Lancet.* 1997;350:1431–1434.
- [8] Antó JM, Sunyer J, Basagaña X, et al. Risk factors of new-onset asthma in adults: a population-based international cohort study. *Allergy.* 2010;65:1021–1030.
- [9] Broeckaert F, Bernard A. Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. *Clin Exp Allergy.* 2000;30:469–475.
- [10] Chen LC, Zhang Z, Myers AC, Huang SK. Cutting edge: altered pulmonary eosinophilic inflammation in mice deficient for Clara cell secretory 10-kDa protein. *J Immunol.* 2001;167:3025–3028.
- [11] Hung CH, Chen LC, Zhang Z, et al. Regulation of TH2 responses by the pulmonary Clara cell secretory 10-kd protein. *J Allergy Clin Immunol.* 2004;114:664–670.
- [12] Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet.* 1989;1:1292–1295.
- [13] Daniels SE, Bhattacharya S, James A, et al. A genome-wide search for quantitative trait loci underlying asthma. *Nature.* 1996;383:247–250.
- [14] Huang SK, Mathias RA, Ehrlich E, et al. Comparative study on the genetics of asthma. Evidence for asthma susceptibility genes on chromosome 11 in an African-American population. *Hum Genet.* 2003;113:71–75.
- [15] Ohchi T, Shijubo N, Kawabata I, et al. Polymorphism of Clara cell 10-kD protein gene of sarcoidosis. *Am J Respir Crit Care Med.* 2004;169:180–186.
- [16] Laing IA, Hermans C, Bernard A, Burton PR, Goldblatt J, Le Souëf PN. Association between plasma CC16 levels, the A38G polymorphism, and asthma. *Am J Respir Crit Care Med.* 2000;161:124–127.
- [17] Yang KD, Ou CY, Chang JC, et al. Infant frequent wheezing correlated to Clara cell protein 10 (CC10) polymorphism and concentration, but not allergy sensitization, in a perinatal cohort study. *J Allergy Clin Immunol.* 2007;120:842–848.
- [18] Laing IA, Goldblatt J, Eber E, et al. A polymorphism of the CC16 gene is associated with an increased risk of asthma. *J Med Genet.* 1998;35:463–467.
- [19] Fukui Y, Hizawa N, Takahashi D, et al. Association between nonspecific airway hyperresponsiveness and Arg16Gly beta2-adrenergic receptor gene polymorphism in asymptomatic healthy Japanese subjects. *Chest.* 2006;130:449–454.
- [20] Hattori T, Konno S, Hizawa N, et al. Genetic variants in the mannose receptor gene (MRC1) are associated with asthma in two independent populations. *Immunogenetics.* 2009;61:731–738.
- [21] Fukui Y, Yamaguchi E, Hizawa N, et al. [Studies on airway hyperresponsiveness by the Astograph(r) method in asthmatics and young adult non-asthmatic asymptomatics]. *Arerugi.* 2004;53:565–574 (in Japanese).
- [22] Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am J Respir Crit Care Med.* 2003;168:983–988.
- [23] Nogami H, Shoji S, Nishima S. Exhaled nitric oxide as a simple assessment of airway hyperresponsiveness in bronchial asthma and chronic cough patients. *J Asthma.* 2003;40:653–659.
- [24] Ku MS, Sun HL, Lu KH, et al. The CC16 A38G polymorphism is associated with the development of asthma in children with allergic rhinitis. *Clin Exp Allergy.* 2011;41:794–800.
- [25] Shijubo N, Itoh Y, Yamaguchi T, et al. Serum and BAL Clara cell 10 kDa protein (CC10) levels and CC10-positive bronchiolar cells are decreased in smokers. *Eur Respir J.* 1997;10:1108–1114.
- [26] Bernard AM, Roels HA, Buchet JP, Lauwerys RR. Serum Clara cell protein: an indicator of bronchial cell dysfunction caused by tobacco smoking. *Environ Res.* 1994;66:96–104.
- [27] Robin M, Dong P, Hermans C, Bernard A, Bersten AD, Doyle IR. Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. *Eur Respir J.* 2002;20:1152–1161.
- [28] Laprise C, Laviolette M, Boutet M, Boulet LP. Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. *Eur Respir J.* 1999;14:63–73.
- [29] Nomura N, Yoshikawa T, Kamoi H, Kanazawa H, Hirata K, Fujimoto S. Induced sputum analysis in asymptomatic young adults with bronchial hyperresponsiveness to methacholine. *Respirology.* 2007;12:516–522.
- [30] Shijubo N, Itoh Y, Yamaguchi T, et al. Serum levels of Clara cell 10-kDa protein are decreased in patients with asthma. *Lung.* 1999;177:45–52.
- [31] Bernard A, Marchandise FX, Depelchin S, Lauwerys R, Sibille Y. Clara cell protein in serum and bronchoalveolar lavage. *Eur Respir J.* 1992;5:1231–1238.
- [32] Yoshikawa T, Kanazawa H, Tanaka J, et al. Gene polymorphism of epidermal growth factor receptor and airway hyperresponsiveness in young allergic subjects without respiratory symptoms. *Med Sci Monit.* 2010;16:163–171.
- [33] Gao PS, Mao XQ, Kawai M, et al. Negative association between asthma and variants of CC16(CC10) on chromosome 11q13 in British and Japanese populations. *Hum Genet.* 1998;103:57–59.
- [34] Guidelines for methacholine and exercise challenge testing—1999. *Am J Respir Crit Care Med.* 2000;161:309–329.
- [35] Fukutomi Y, Taniguchi M, Tsuburai T, et al. [Survey of asthma control and anti-asthma medication use among Japanese adult patients]. *Arerugi.* 2010;59:37–46 (in Japanese).

eTable 1

Characteristics of healthy control subjects and those with asthma in the case–control study

	Healthy control subjects	Subjects with asthma	P value
Subjects, n	582	504	
Age (y), median (range)	47 (18–79)	49 (16–81)	<.001 ^b
Men, n (%)	299 (51.4)	230 (45.6)	.001 ^a
Atopy, n (%)	294 (50.5)	365 (72.4)	<.001 ^a
Never smoker, n (%)	325 (56.5)	270 (53.6)	.004 ^a
Age of onset (y), median (range)	—	35 (0–80)	
IgE (log, IU/mL), mean ± SD	1.84 ± 0.63	2.39 ± 0.65	<.001 ^b

Abbreviation: SD, standard deviation.

^aBy χ^2 test.

^bBy Kruskal-Wallis analysis.

eTable 2

Characteristics of healthy control subjects in case–control study

	Healthy control subjects		P value
	Age ≤40 y	Age >40 y	
Subjects, n	177	405	
Age (y), median (range)	30 (18–40)	52 (41–79)	<.001 ^b
Men, n (%)	89 (50.3)	210 (51.9)	.73 ^a
Atopy, n (%)	109 (61.6)	179 (44.2)	<.001 ^a
Never smoker, n (%)	108 (61.0)	217 (53.6)	.097 ^a
IgE (log, IU/mL), mean ± SD	1.82 ± 0.66	1.85 ± 0.61	.81 ^b

Abbreviation: SD, standard deviation.

^aBy χ^2 test.

^bBy Kruskal-Wallis analysis.

eTable 3

Characteristics of healthy control subjects and those with asthma older than 40 years in case–control study

	Healthy control subjects	Subjects with asthma	P value
	Age >40 y	Onset age >40 y	
Subjects, n	405	213	
Age (y), median (range)	52 (41–79)	59 (41–81)	<.001 ^b
Men, n (%)	210 (51.9)	90 (42.3)	.023 ^a
Atopy, n (%)	179 (44.2)	115 (54.0)	.021 ^a
Never smoker, n (%)	217 (53.6)	108 (50.7)	.496 ^a
Age of onset (y), median (range)	—	55 (41–80)	—
IgE (log, IU/mL), mean ± SD	1.85 ± 0.61	2.25 ± 0.62	<.001 ^b

Abbreviation: SD, standard deviation.

^aBy χ^2 test.

^bBy Kruskal-Wallis analysis.

eTable 4

Association of CC16 polymorphism with asthma in subjects older than 40 years

	Subjects, n	CC16 A38G genotype, n (%)			OR ^a	95% CI ^a	P value ^a
		AA	AG	GG			
Healthy controls (>40 y old)	405	69 (17.0)	179 (44.2)	157 (38.8)			
Late-onset asthma	213	37 (17.4)	111 (52.1)	65 (30.5)	1.61	1.09–2.37	.017

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age, sex, atopy, and smoking status. Odds ratios were calculated for the presence of the 38A allele using multivariate logistic regression models.