

Atopy in cough sensitivity to capsaicin and bronchial responsiveness in young females

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Atopy in cough sensitivity to capsaicin and bronchial responsiveness in young females. M. Fujimura, K. Kasahara, M. Yasui, S. Myou, Y. Ishiura, Y. Kamio, T. Hashimoto, T. Matsuda. ©ERS Journals Ltd 1998.

ABSTRACT: We have shown previously that female sex is a determinant of cough sensitivity to inhaled capsaicin, but the relationship between atopy and the cough sensitivity has not been examined.

The capsaicin cough threshold, defined as the lowest concentration of capsaicin causing five or more coughs, nonspecific bronchial responsiveness, defined as the provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (PC₂₀), total immunoglobulin E (IgE) and specific IgEs to eight common aeroallergens (house dust 1, 2 and 6, *Dermatophagoides pteronyssinus* and *D. farinae*, Japanese cedar, ragweed and orchard grass) in the serum were measured in 71 nonsmoking, healthy young women aged 20.6±0.1 yrs (mean±SEM). A structured interviewer-led questionnaire on allergic diseases revealed that one and six subjects had mild current and past asthma, respectively. These seven subjects were excluded from the data analysis.

PC₂₀ was significantly lower in 42 subjects showing a positive specific IgE than in 22 subjects showing a negative specific IgE to any of the eight allergens (p<0.05), while the capsaicin cough threshold was not significantly different between the subgroups. PC₂₀ was significantly lower in subjects with positive specific IgE to *Dermatophagoides* and house dust, but not to the three kinds of pollen examined.

It was confirmed that atopy indicated by specific immunoglobulin E to mite-related antigens, but not to pollen antigens, is associated with nonspecific bronchial responsiveness, and it is suggested that atopy is not a determinant of airway cough sensitivity in healthy, nonasthmatic subjects.

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It has previously been reported that cough sensitivity to a chemostimulant, tartaric acid, which may produce cough by stimulating irritant receptors, is greater in young females than in young males [1]. In addition, it was shown recently that cough reflexing to capsaicin was more hypersensitive in females than in males, regardless of age, height, weight and pulmonary function [2]. The gender difference in cough sensitivity may explain why females are more susceptible to coughs induced by angiotensin-converting enzyme (ACE) inhibitors [3], and why chronic, persistent nonproductive cough which is resistant to bronchodilator therapy and in which eosinophils are detected in hypertonic saline-induced sputum [4], is more frequent in females [4–6].

It is well recognized that nonspecific bronchial responsiveness is associated with atopy indicated by allergen skin-prick tests [7–9] and serum specific immunoglobulin E (IgE) [10], but the association between airway cough sensitivity and atopy is not known. This study was designed to elucidate whether capsaicin-induced cough, which may depend on C-fibre afferents [11–13], is associated with atopy, expressed as serum-specific IgE to common aeroallergens, serum total IgE level and peripheral blood eosinophil count.

Subjects and methods

Subjects

Seventy-one, randomly selected, nonsmoking, female college students aged 20.6±0.1 yrs (mean±SEM), participated in this study. None of them had experienced upper respiratory tract viral infections for at least 8 weeks before or during the study period. The study was approved by the Ethics Committee of Kanazawa University, and informed consent was obtained from all subjects after the purpose of the test had been explained.

Assessment of atopy

Based on a structured, interviewer-led questionnaire, each subject was asked about respiratory symptoms, complicated allergic diseases and a past and family history of allergic diseases such as bronchial asthma, allergic rhinitis, atopic dermatitis and urticaria.

Total white blood cell and eosinophil counts were performed according to a standard method. The serum total IgE level and specific IgE to eight common aeroallergens were measured by the radioimmunosorbent test (RIST) and the CAP method (Pharmacia, Uppsala, Sweden). A participant was considered as having measurable specific serum IgE when the response level was >0.35 IU·mL⁻¹ to any of the following allergens: house dust 1, 2, or 6, *D. pteronyssinus*, *D. farinae*, ragweed, Japanese cedar or orchard grass.

Assessment of cough sensitivity to capsaicin

Measurement of cough threshold to inhaled capsaicin was carried out using the method reported previously [2, 4, 6]. Capsaicin (30.5 mg) was dissolved in Tween 80 (1 mL) and ethanol (1 mL) and then dissolved in physiological saline (8 mL) to provide a stock solution of 1×10^{-2} M, which was stored at -20°C . This solution was diluted with physiological saline to make solutions of 0.49, 0.98, 1.95, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1,000 μM . Each subject inhaled a control solution of physiological saline, followed by progressively increasing concentrations of the capsaicin solution. Solutions were inhaled for 15 s every 60 s by tidal mouth-breathing wearing a noseclip from a Bennett Twin nebulizer (3012-60 cc; Puritan-Bennett Co., Carlsbad, CA, USA) operated by compressed air at 5 L·min⁻¹. Increasing concentrations were inhaled until five or more coughs were elicited. The output of the nebulizer was 0.21 mL·min⁻¹. It has been reported that aerodynamic mass median diameter (MMD) of the particle is 3.60 μm , with a geometric standard deviation of 3.47 [14]. The number of capsaicin-induced coughs was counted by two medical technicians in the Pulmonary Function Laboratory of Kanazawa University. The cough threshold was defined as the lowest concentration of capsaicin that elicited five or more coughs.

Assessment of nonspecific bronchial responsiveness

Nonspecific bronchial responsiveness was evaluated by methacholine challenge. Methacholine chloride (Sigma Chemical Co., St Louis, MO, USA) was dissolved in physiological saline to make solutions of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg·mL⁻¹. Saline and methacholine were inhaled from a Devilbiss 646 nebulizer (Devilbiss Co, Somerset, PA, USA) operated by compressed air at 5 L·min⁻¹. The output of the nebulizer was 0.28 mL·2 min⁻¹. Firstly, saline was inhaled for 2 min and the forced expiratory volume in one second (FEV₁) was measured on a dry wedge spirometer (Transfer Test; P.K. Morgan, Chatham, Kent, UK). After confirming that the change in FEV₁ from the baseline after inhalation of saline was $\delta 10\%$ in all patients, the inhalation of methacholine was started. Methacholine was inhaled for 2 min by tidal mouth-breathing wearing a nose clip, followed immediately by spirometry. Methacholine of increasing concentrations was successively inhaled until a fall of $\delta 20\%$ in the FEV₁ occurred. The measured values were plotted on semilogarithmic graph paper and the provocative concentration of methacholine producing a 20% fall in the FEV₁ (PC₂₀) was calculated.

Data analysis

One and six subjects were found to have current and past asthma, respectively, based on the questionnaire on allergic diseases and these seven subjects were excluded from the data analysis.

A PC₂₀ $\delta 10$ mg·mL⁻¹ was defined as bronchial hyperresponsiveness [15]. Fourteen of 64 nonasthmatic subjects (21.9%) had bronchial hyperresponsiveness. The number of nonasthmatic subjects with a capsaicin cough threshold of $\delta 1.95$ μM , $\delta 3.9$ μM and $\delta 7.8$ μM was 4, 14 and 31, respectively. Considering the proportion of subjects with bronchial hyperresponsiveness, cough hypersensitivity in this study was defined as a capsaicin cough threshold of $\delta 3.9$ μM .

Values of the capsaicin cough threshold, PC₂₀ and total IgE in the serum were expressed as geometric means with the geometric standard error of the mean (GSEM) expressed as a factor. Logarithmically converted values of the capsaicin cough threshold, PC₂₀ and serum IgE were used in assessing the relationships and differences between these by simple regression analysis and analysis of variance (ANOVA), respectively. Chi-squared analyses of contingency tables were also used to determine the degree of association between variables. A p-value of <0.05 was taken as significant.

The odds ratio (OR) was used as an effect estimator to measure associations in this cross-sectional study [16]. In a situation in which exposure to a given factor is believed to be a cause of a given disease (or other outcome), the population attributable risk is the proportion of the disease (*i.e.* bronchial hyperresponsiveness and cough receptor hypersensitivity) in the total population that can be attributed to exposure to the factor (atopy). This estimator was calculated with regression techniques using the logit link for OR [16].

Results

Cough sensitivity

A capsaicin cough threshold was obtained in all 64 nonasthmatic subjects. The serum total IgE level, specific IgE to any of the allergens, peripheral blood eosinophil count, pulmonary function and PC₂₀ were not significantly different between the 14 subjects with and 50 subjects without cough hypersensitivity. The cough threshold was not correlated with total IgE, peripheral blood eosinophil count or PC₂₀. Furthermore, the cough threshold was not significantly different between subjects with and without positive specific IgE to either of the examined aeroallergens (table 1).

OR and 95% confidence intervals of cough hypersensitivity for specific IgEs are presented in table 2. An approximately twofold excess risk of cough hypersensitivity was observed in subjects with specific IgE to *D. pteronyssinus* (OR=2.00) and ragweed (OR=2.61), but this was not statistically significant.

Bronchial responsiveness

A 20% or greater fall in FEV₁ was not obtained by the final concentration of methacholine (160 mg·mL⁻¹) in 20

Table 1. – Cough sensitivity and bronchial responsiveness in nonasthmatic young females with positive and negative specific IgE in the serum

	Number of subjects		Capsaicin cough threshold μM		PC20 $\text{mg}\cdot\text{mL}^{-1}$	
	Positive	Negative	Positive	Negative	Positive	Negative
<i>Dermatophagoides pteronyssinus</i>	28	36	9.5 (1.19)	12.4 (1.19)	17.8 (1.35)****	86.1 (1.27)
<i>Dermatophagoides farinae</i>	19	45	9.7 (1.25)	11.6 (1.16)	14.0 (1.39)***	69.7 (1.26)
House dust 1	20	40	10.3 (1.22)	11.4 (1.17)	15.7 (1.43)***	68.5 (1.26)
House dust 2	14	50	10.0 (1.29)	11.4 (1.15)	11.4 (1.44)***	62.8 (1.25)
House dust 6	19	45	9.7 (1.25)	11.6 (1.16)	14.0 (1.39)***	69.7 (1.26)
Orchard grass	25	39	10.6 (1.20)	11.3 (1.18)	35.3 (1.39)	49.3 (1.32)
Ragweed	5	59	9.0 (1.66)	11.2 (1.14)	13.0 (2.52)	47.9 (1.24)
Japanese cedar	16	48	13.1 (1.26)	10.4 (1.16)	40.1 (1.53)	44.4 (1.28)
Any of the above allergens	42	22	9.8 (1.15)	13.8 (1.26)	31.0 (1.30)*	82.0 (1.37)

Values are shown as geometric means (SEM). IgE: immunoglobulin E; PC20: provocative concentration causing a 20% fall in the forced expiratory volume in one second. *: $p<0.05$; ***: $p<0.001$; ****: $p<0.0001$ by analysis of variance.

Table 2. – Association of cough sensitivity and bronchial responsiveness with specific IgE in the serum in nonasthmatic young females

	Cough sensitivity			Bronchial responsiveness		
	Positive specific IgE %		Odds ratio (95% CI)	Positive specific IgE %		Odds ratio (95% CI)
	Hyper n=14	Nonhyper n=50		Hyper n=14	Nonhyper n=50	
<i>Dermatophagoides pteronyssinus</i>	57.1	40.0	2.00 (0.6–6.6)	71.4*	36.0	4.44# (1.3–15.3)
<i>Dermatophagoides farinae</i>	35.7	28.0	1.43 (0.4–5.0)	50.0	24.0	3.17 (1.0–10.5)
House dust 1	35.7	30.0	1.30 (0.4–4.5)	57.1*	24.0	4.22# (1.3–13.9)
House dust 2	21.4	22.0	0.97 (0.2–4.2)	35.7	18.0	2.53 (0.7–9.1)
House dust 6	35.7	28.0	1.43 (0.4–5.0)	50.0	24.0	3.17 (1.0–10.5)
Orchard grass	28.6	42.0	0.55 (0.2–2.0)	28.6	42.0	0.55 (0.2–2.0)
Ragweed	14.3	6.0	2.61 (0.4–16.5)	14.3	6.0	2.61 (0.4–16.5)
Japanese cedar	14.3	28.0	0.43 (0.1–2.1)	28.6	24.0	1.27 (0.3–4.8)
Any of the above allergens	71.4	64.0	1.41 (0.4–5.1)	78.6	62.0	2.25 (0.6–8.9)

IgE: immunoglobulin E; CI: confidence interval. A capsaicin cough threshold of $\delta 3.9 \mu\text{M}$ was defined as cough hypersensitivity. Provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (PC20) of $\delta 10 \text{ mg}\cdot\text{mL}^{-1}$ was defined as bronchial hyperresponsiveness. *: $p<0.05$ by Chi-squared analysis. #: CI that do not contain the value 1.0 represent a significant increase in risk.

subjects and their PC20 values were assumed to be $320 \text{ mg}\cdot\text{mL}^{-1}$ for statistical analysis. PC20 was weakly correlated with total IgE level ($r=0.322$, $p<0.01$), but not with the peripheral blood eosinophil count. Subjects with specific IgE to *D. pteronyssinus* ($p<0.0001$), *D. farinae* ($p<0.001$), house dust 1 ($p<0.001$), house dust 2 ($p<0.001$), and house dust 6 ($p<0.001$), but not to any of pollen examined, had significantly lower PC20 than those without the specific IgEs (table 1).

OR and 95% confidence intervals of bronchial hyperresponsiveness for specific IgE are presented in table 2. An approximately twofold excess risk of bronchial hyperresponsiveness was observed in subjects with specific IgE to *D. pteronyssinus* (OR=4.44), *D. farinae* (OR=3.17), house dust 1 (OR=4.22), house dust 2 (OR=2.53), house dust 6 (OR=3.17) and ragweed (OR=2.61). The prevalence of positive specific IgE to *D. pteronyssinus* and house dust 1 was significantly different ($p<0.05$) between subjects with and without bronchial hyperresponsiveness, respectively (table 2).

Discussion

The present study showed that the capsaicin cough threshold was not associated with atopy indicated by specific IgEs to eight common aeroallergens, serum total IgE level and peripheral blood eosinophil count in nonasthma-

tic, healthy young females, and confirmed the association between nonspecific bronchial hyperresponsiveness and atopy, especially with specific IgE to *D. pteronyssinus*, *D. farinae*, house dust 1, 2 and 6, each of which is related to the mite antigen.

Cough reflex testing using capsaicin has been commonly used for studies on the pathophysiology of the cough reflex and antitussive effects of drugs. The reproducibility of sensitivity and of dose-response curve for capsaicin-induced coughs has been established in normal subjects [17]. In this study, the cough threshold to inhaled capsaicin was measured as an index representing airway cough sensitivity, which was defined as the lowest concentration of capsaicin eliciting five or more coughs [2, 4, 6]. The reproducibility of the cough threshold was confirmed in a previous study in which the challenge was repeated at an interval of 30 min to 2 weeks in normal subjects [18, 19].

As some factors, such as sex [1, 2], menopause [2], cigarette smoking [1, 20–22] and ACE genotype [23], have been proposed to influence cough sensitivity, only non-smoking, young females were examined to avoid the factors likely to influence cough sensitivity in the present study, whereas we did not examine the ACE genotype. In the results, *i.e.* atopy, indicated by specific IgEs to eight common aeroallergens, the serum total IgE level and peripheral blood eosinophil count, did not relate to cough sensitivity to inhaled capsaicin in nonasthmatic young females.

It has been well established that there is an association between atopy, as a global feature and nonspecific bronchial hyperresponsiveness [7–10, 24]. In all of these studies, the association between atopy and bronchial hyperresponsiveness was analysed in the entire population including apparent current and past asthmatics. As it is well recognized that bronchial hyperresponsiveness is the fundamental feature of asthma as well as chronic airway inflammation, and there is a high prevalence of positive specific IgEs to aeroallergens in children and young adults with asthma [25], data analysis on bronchial responsiveness and atopy in a population is strongly influenced if subjects with asthma are included. Therefore, this study examined the association between atopy and nonspecific bronchial responsiveness to methacholine in a randomly selected population of young females. Current and past asthmatics were excluded based on a structured interviewer-led questionnaire on respiratory symptoms and allergic diseases.

The results confirmed the association between atopy and nonspecific bronchial hyperresponsiveness even in non-asthmatic subjects and showed that nonspecific bronchial hyperresponsiveness was significantly associated with specific IgEs to mite-related antigens but not to three kinds of pollen. Orchard grass, ragweed and Japanese cedar are the major allergens of pollinosis, but seldom cause asthma in Japan.

In conclusion, airway cough reflexes to inhaled capsaicin were not associated with atopy in nonasthmatic young females. The result is consistent with previous data showing that cough reflexing to inhaled tartaric acid is not heightened in asthma [26] and adds atopic status to the list of factors not directly influencing cough sensitivity, *i.e.* age, height, weight, pulmonary function [2], bronchomotor tone [15] and nonspecific bronchial responsiveness [26]. The independent factors that have been proven to exert an influence are sex, menopause [2], smoking history [1, 20–22] and angiotensin-converting enzyme genotype [23].

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