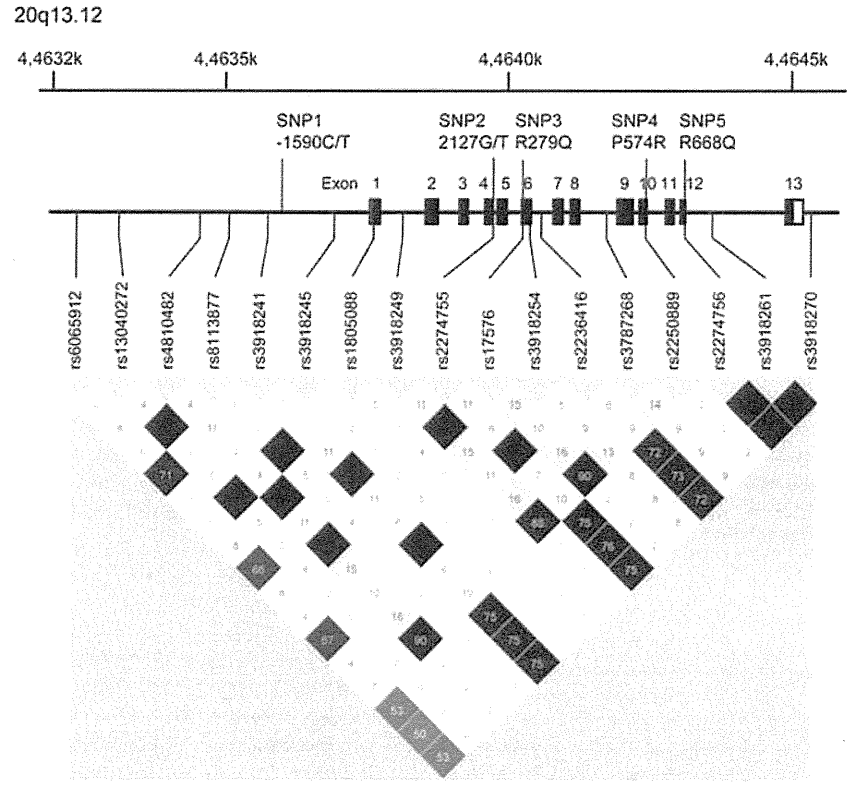

Figure 1 Structure and location of SNPs of the *MMP9* gene. Exons are shown by squares. Filled squares are coding exons. Five SNPs genotyped in this study are shown above the gene map, and HapMap SNPs and their LD status are shown underneath. Data used for LD are based on Japanese data in HapMap data Phase III/Rel3, May 10 on NCBI B36 assembly, dbSNP b126. The nucleotide position is based on February 2009 (GRCh37/hg19) assembly. SNP1 was not found in HapMap database but was known to be in complete LD with rs2918241 and SNP2.[17] Numbers in diamonds in the LD map are $r^2 \times 100$.

Table 2 Association between SNPs of *MMP9* and cedar pollinosis

	Genotype frequency												*Pcor*		
	Cedar pollinosis			*Orchard grass pollinosis*			*Mite-positive perennial AR*			*Control*					
SNP	*11*[b]	*12*[c]	*22*[d]	*11*[b]	*12*[c]	*22*[d]	*11*[b]	*12*[c]	*22*[d]	*11*[b]	*12*[c]	*22*[d]	*Cedar pollinosis vs control*[a]	*Orchard grass pollinosis vs control*	*Mite-positive perennial AR vs control*
−1590C/T (SNP1)	0.783	0.204	0.013	0.870	0.111	0.019	0.765	0.235	0.000	0.611	0.343	0.046	**0.039**	**0.011**	0.844
2127G/T (SNP2)	0.789	0.191	0.020	0.870	0.111	0.019	0.725	0.275	0.000	0.611	0.343	0.046	**0.025**	**0.011**	>1
R279Q (SNP3)	0.421	0.493	0.086	0.463	0.426	0.111	0.480	0.460	0.060	0.509	0.435	0.056	>1	>1	>1
P574R (SNP4)	0.520	0.408	0.072	0.574	0.333	0.093	0.608	0.333	0.059	0.556	0.417	0.028	>1	>1	>1
R668Q (SNP5)	0.737	0.237	0.026	0.833	0.130	0.037	0.725	0.275	0.000	0.546	0.398	0.056	**0.023**	**0.0049**	0.466

Abbreviations: AR, allergic rhinitis; MMP9, matrix metalloproteinase 9; SNP, single-nucleotide polymorphism.
[a]*P*-values for association between an SNP and a phenotype in the dominant model. *Pcor*-values are calculated by multiplying raw *P*-value by 15 (that is, number of SNPs tested×number of diseases).
[b]Homozygous for major allele.
[c]Heterozygous.
[d]Homozygous for minor allele.
Significant *Pcor*-values (<0.05) are in boldface.

Because the numbers of subjects with orchard grass pollinosis and mite-positive perennial AR were similar, the association of SNP1, SNP2 and SNP5 with mite-positive perennial AR may be weaker than that with orchard grass (with or without cedar) pollinosis.

Association between SNPs and serum IgE levels

In the analysis of serum IgE levels, all individuals were included, irrespective of the presence of atopy or disease status. We first examined the distribution of log-transformed total serum IgE [log(-total IgE)] in the 670 school children (data not shown). The rate of values lower than the cutoff was low and the shape of the distribution was almost normal; therefore, we evaluated the association between SNPs and log(total IgE) using analysis of variance. By contrast, because censored values were not negligible, we evaluated the association between SNPs and log-transformed specific IgE values with tobit regression analysis. The effects of SNPs on the IgE values were adjusted for age and sex, and the results of the tests are shown in Table 3. We excluded SNP2 from this analysis because it was thought to be a

Table 3 Association between SNPs and serum IgE levels

			log(total IgE)			*log(cedar IgE)*			*log(orchard grass IgE)*			*log(mite IgE)*		
SNP	*Allele*	*Number of subjects*	*Mean*	*s.d.*	*Pcor*[a]	*Mean*	*s.d.*	*Pcor*[b]	*Mean*	*s.d.*	*Pcor*[b]	*Mean*	*s.d.*	*Pcor*[b]
−1590C/T (SNP1)	C/C	470	2.13	0.68		0.43	0.95		−0.15	0.60		0.48	1.02	
	C/T+T/T	199	2.04	0.67	0.517	0.23	0.90	0.066	−0.25	0.49	0.613	0.34	0.98	>1
R279Q (SNP3)	R/R	308	2.07	0.68		0.42	0.98		−0.19	0.56		0.41	0.99	
	R/Q+Q/Q	359	2.12	0.68	0.737	0.33	0.90	>1	−0.17	0.59	>1	0.46	1.03	>1
P574R (SNP4)	P/P	341	2.07	0.68		0.41	0.96		−0.19	0.56		0.44	1.00	
	P/R+R/R	328	2.13	0.68	>1	0.34	0.91	>1	−0.17	0.59	>1	0.44	1.02	>1
R668Q (SNP5)	R/R	439	2.14	0.68		0.45	0.95		−0.14	0.61		0.48	1.02	
	R/Q+Q/Q	231	2.03	0.67	0.173	0.22	0.90	**0.022**	−0.26	0.48	0.257	0.36	0.99	>1

Abbreviation: SNP, single-nucleotide polymorphism.
[a]Raw *P*-values of coefficients of SNPs for log-transformed total IgE value are calculated as a general linear model with age and sex as covariates. *Pcor*-values are calculated by multiplying raw *P*-values by 16 (that is, number of SNPs tested×number of IgE values).
[b]Raw *P*-values of coefficients of SNPs for log-transformed specific IgE values are calculated using a tobit regression model with age and sex as covariates. *Pcor*-values are calculated by multiplying raw *P* tobit values by 16 (that is, number of SNPs tested×number of IgE values).
Significant *Pcor*-values (<0.05) are in boldface.

Table 4 Association between haplotype and allergic rhinitis (RA)

		Frequency				*Pcor*[a]		
Haplotype	*SNP1–5*	*Cedar pollinosis*	*Orchard grass pollinosis*	*Mite-positive perennial AR*	*Control*	*Cedar pollinosis vs control*	*Orchard grass pollinosis vs control*	*Mite-positive perennial AR vs control*
Haplotype 1	CGGCG	0.566	0.612	0.575	0.510	>1	>1	>1
Haplotype 2	CGAGG	0.254	0.225	0.217	0.214	>1	>1	>1
Haplotype 3	TTGCA	0.107	0.071	0.120	0.230	**0.0012**	**0.0059**	0.201
Haplotype 4	CGACG	0.074	0.092	0.088	0.046	>1	>1	>1

Abbreviations: AR, allergic rhinitis; SNP, single-nucleotide polymorphism.
[a]Raw *P*-values are multiplied by 12 (that is, number of haplotypes×number of diseases).
Significant *Pcor*-values (<0.05) are in boldface.

marker of functional SNP1 and almost the same results were expected. The lowest *P*-value for log(total IgE), 0.043, was observed for SNP5 and was not significant if the value was corrected by the number of SNPs. SNP5 was significantly associated with cedar pollen-specific IgE (*Pcor*=0.022). Although SNP1 had a raw *P*-value of 0.0041, the value was >0.05 after a multiple-test correction. A similar tendency was observed with regard to the association of these SNPs with orchard grass-specific IgE. The raw *P*-values for these SNPs were slightly <0.05 and did not reach the significance level after correction. None of the SNPs showed significant association with mite-specific IgE.

Association of haplotype with AR and serum IgE levels

The estimated frequencies of haplotypes consisting of SNP1 to SNP5 are shown in Table 4. The frequency of the TTCGA haplotype [haplotype 3 (H3)] between cedar (10.7%) and orchard grass (7.1%) of childhood AR was significantly lower than that in child controls (23.0%) (*Pcor*=0.0012 and *Pcor*=0.0059, respectively). This haplotype was not significantly associated with mite-positive perennial AR (*Pcor*=0.201). H3 consists of minor alleles of SNP1, SNP2 and SNP5, whereas other haplotypes [haplotype 1 (H1), haplotype 2 (H2) and haplotype 4 (H4)] correspond to the major allele of SNP1, SNP2 and SNP5 (CGXXG, X: any allele of SNP3/SNP4). Because the test of association was performed with H3 vs H1+H2+H4, the result is equivalent to the association between single SNP of SNP1, SNP2 or SNP5 and disease. The frequency of H1, H2 and H4 was higher in all three types of AR patient groups than in the control group. Association test of these haplotypes with the diseases, however, did not reach the level of significance. CGXXG-type haplotypes were equally divided into H1, H2 and H4 in all AR groups and in the control group by SNP3–SNP4 alleles that were not in LD with SNP1–SNP2–SNP5 and not associated with any diseases. This reduced the power to detect an association between each haplotype (H1, H2 or H4) and the disease type.

Next, we determined whether H3 has an impact on serum total and specific IgE levels (Table 5). Individuals with at least one H3 allele showed significantly lower cedar-specific IgE levels (*Pcor*=0.0041). The *Pcor*-value for the association with orchard grass-specific IgE was slightly >0.05. This suggests that individuals with H3 are less prone to pollen sensitization. No effect of H3 on total IgE level and mite-specific IgE level was evident.

Effect of amino-acid changes on MMP9 activity

Different promoter activities of alleles of SNP1 were reported previously.[17] However, it was unknown whether the amino-acid change of SNP5 has any effect on MMP9 activity or function. To evaluate the effect of SNP5, we constructed four different MMP9 proteins composed of different combination of SNP3, SNP4 and SNP5 alleles because SNP3 and SNP4 may influence the effect of SNP5 on the enzyme activity.

As shown in Figure 2, type 1 enzyme showed significantly higher proteolytic activity than any of the other types of enzyme tested. Because H1 corresponds to type 1 enzyme and did not show significant association with pollinosis, a difference in enzyme activity

Table 5 Association between haplotype 3 and serum IgE levels

		log(total IgE)			*log(cedar IgE)*			*log(orchard grass IgE)*			*log(mite IgE)*		
	Number of subjects	*Mean*	*s.d.*	*Pcor*[a]	*Mean*	*s.d.*	*Pcor*[b]	*Mean*	*s.d.*	*Pcor*[b]	*Mean*	*s.d.*	*Pcor*[b]
Haplotype 3													
H3/H3+H3/others	194	2.03	0.67		0.21	0.89		−0.26	0.49		0.33	0.97	
(H3)													
Others/others	474	2.13	0.69	0.237	0.44	0.95	**0.0041**	−0.15	0.60	0.064	0.48	1.02	0.262

[a]*P*-values of coefficients of haplotype 3 (H3) for log-transformed total IgE value are calculated as a general linear model with age and sex as covariates. *Pcor*-values are calculated by multiplying raw *P*-values by 4 (that is, number of IgE values).
[b]*P*-values of coefficients of H3 for log-transformed specific IgE values are calculated using a tobit regression model with age and sex as covariates. *Pcor*-values are calculated by multiplying raw *P*-values by 4 (that is, number of IgE values).
Significant *Pcor*-values (<0.05) are in boldface.

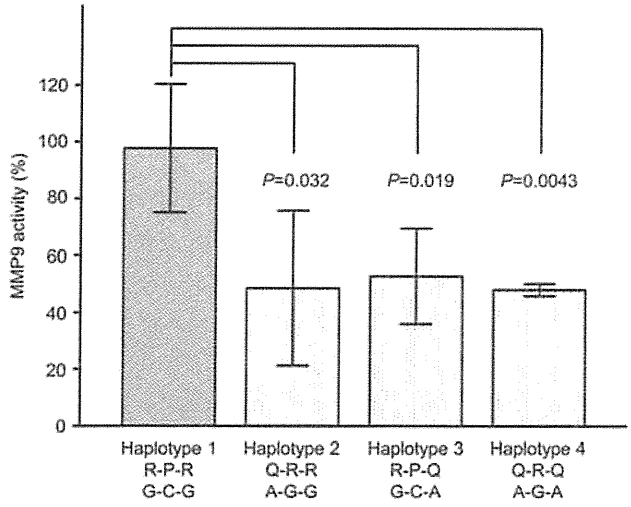

Figure 2 Comparison of peptide cleavage activity of different sequences of MMP9. MMP9 activity for peptide cleavage was monitored by 5-carboxyfluorescein fluorescence. In each experiment, duplicate samples of cell culture medium were assayed. The activity of wild-type enzyme was set to 100% in each experiment. Values are expressed as mean and standard deviation of three independent experiments. Significance was evaluated using analysis of variance test.

was not associated with risk for pollinosis development in a straightforward manner. Compared with the type 1 (wild-type) enzyme, the amino-acid changes from R to Q at SNP5 (type 3) decreased enzyme activity by half (P=0.019). The amino-acid change of 279R (SNP3) + 574R (SNP4) (type 2) also decreased the activity (P=0.032) to a similar extent. The activity of type 4 was similar to that of type 2 and type 3, suggesting no additive effect by type 2 and type 3 amino-acid changes. SNP5 showed an association with cedar pollen sensitization and pollinosis and had an impact on MMP9 enzyme activity. However, a reduction in enzyme activity was also observed with SNP3 and SNP4, neither of which showed an association with disease. Thus, reduced activity was not enough to show an association with clinical phenotypes.

DISCUSSION

In this study, we investigated the association between (potentially) functional sequence variations of the *MMP9* gene and AR in Japanese children. The SNPs −1590C/T (SNP1) and R668Q (SNP5) were in strong LD and were significantly associated with cedar and orchard grass pollinosis. SNP5 was also significantly associated with cedar pollen sensitization. To evaluate the pathological importance of SNP5, we measured the proteolytic activity of different types of MMP9 due to SNP3, SNP4 and SNP5. To our knowledge, the present study is the first to experimentally evaluate the effects of amino-acid changes in MMP9 on its proteolytic activity. Compared with the wild-type enzyme, enzymes with any tested combination of amino-acid substitution showed lower enzyme activity. However, lower enzyme activity and disease risk were not exactly correlated. As seen in Table 3, frequencies of H1 that correspond to the wild-type enzyme in the disease groups were higher than in the control group. It is possible that H1 is associated with higher risk for pollinosis, but this was not statistically significant. H3 was significantly associated with lower risk for pollinosis and is the only haplotype that contains the T allele of SNP1, which was shown previously to have higher promoter activity.[17,24] From these observations, we speculate that different promoter activity associated with SNP1 may be more necessary or important for change in pollinosis risk than different enzyme activity associated with SNP5.

Although we observed significant associations between orchard grass pollinosis and both SNP1 and SNP5, this finding should be interpreted with caution because 89% (48/54) of orchard grass pollinosis patients also had cedar pollinosis. We could not test an association between the SNPs and genuine orchard grass pollinosis because only six children showed orchard grass-only pollinosis. In this test, we found an association between SNPs and pollinosis in patients with sensitization for two different pollens and longer duration of symptoms. Lower OR values and more significant *P*-values found in these patients compared with cedar pollinosis-only patients suggest that children with MMP9 susceptible allele tend to show a more severe phenotype: sensitization for more pollen types and longer duration of symptoms.

Patients with perennial AR with positive mite IgE did not show significant association with *MMP9* SNPs. ORs of SNP1 and SNP5 of those children who also had pollinosis were a little lower than in the entire group, but were higher than in patients with both cedar and orchard grass pollinosis. Further, ORs in mite-positive perennial AR-only patients were similar to those in control children. The relationship between cedar pollinosis and mite-positive perennial AR was not significant. These results suggest a different pathogenesis for pollinosis and mite-positive perennial AR; in addition, MMP9 may have a more important role in the pathogenesis of cedar sensitization and pollinosis than in that of mite sensitization and mite-positive perennial AR.

A strong association between *MMP9* gene variation and serum pollen-specific IgE levels suggests that MMP9 may be involved in the

sensitization process for pollen in the upper airways. Several studies have investigated the role of MMP9 in the immune system. Ichiyasu *et al.*[25] reported that DCs of the bone marrow from MMP9-deficient mice may have impaired migration through the tight junctions. Yen *et al.*[26] reported that DCs matured within inflammatory sites require both chemokine receptor type 7 and prostaglandin E2-induced MMP9 for directional migration to draining lymph nodes. Hintzen *et al.*[27] reported that continuous DC-mediated transport of inhaled antigen to the bronchial lymph node is critical for the induction of tolerance to innocuous antigens. The roles of MMP9 are not restricted to DC migration. MMP9 was also reported to be involved in transmigration of lymphocyte,[28] neutrophils[28] and eosinophils.[29] Recent results demonstrated that tissue-type plasminogen activator promoted several types of bone marrow cells to move to tissue remodeling sites and that MMP9 had a key role on this process by promoting Kit-ligand secretion and vascular endothelial growth factor A (VEGF-A) tissue store release.[30] Furthermore, MMP9 is also known to be expressed in airway epithelial and subepithelial cells.[31,32] These results suggest multipotent effects of MMP9 in the immune system and on tissue remodeling. The results of our association studies, and of many other studies, suggest that MMP9 is involved in the sensitization processes, in particular through migration of DCs and other cell types.

The T allele of SNP1 has been shown to have higher promoter activity,[17] whereas enzyme activity associated with H3 was lower than H1 enzyme. Thus, the association between MMP9 expression/activity and risk for disease development appears to be complex. Because SNP1 and SNP5 are in strong LD and associated with cedar and orchard grass pollinosis to a similar extent, we cannot determine conclusively from the present results which of the SNPs is more important for disease development. As noted above, MMP9 is essential for the action of a variety of cell types, and some of these actions may antagonize *in vivo* biologic an immunologic processes such as IgE production, inflammation and tissue remodeling. Even in the standard allergic asthma model of *Mmp9* knockout mice, totally discrepant results have been reported.[4,5] It is therefore possible that subtle differences in environmental or genetic backgrounds are responsible for the changes in MMP9 expression/activity. Dissection of the mechanistic link between *MMP9* variations and predisposition to sensitization or disease risk is very difficult at present.

A recent large cohort study in Germany investigating *MMP9* SNPs and asthma development showed an association between Q279R and "non-atopic" asthma.[33] This result is somewhat different from our observation that *MMP9* SNPs were involved in pollen sensitization, suggesting that *MMP9* may be involved in chronic airway inflammation processes through a mechanism other than sensitization. The different results may be due to the fact that cedar pollen is not a prevalent allergen in Germany and the larger statistical power of the study may reveal different roles of *MMP9*. Different patterns of association between SNPs and phenotypes may be reflected by the multifaceted nature of *MMP9* and its complex interactions with other genes and factors.[34] Genotype–phonotype association studies under different environmental conditions may shed light on different functions of the *MMP9* gene.

Currently, data on the relevance of MMP9 to nasal tissue pathogenesis in humans are limited. Lee *et al.*[35] examined nasal polyp tissues from asthma patients and showed that the MMP9 level was correlated with the level of inflammatory cell markers such as eosinophilic cationic protein and tryptase. The expression of MMP9 mRNA was higher in nasal polyps when compared with inferior turbinate mucosa in patients with chronic rhinosinusitis.[36] Wang *et al.*[37] reported that –1590C/T and R668Q were associated with chronic rhinosinusitis with nasal polyposis. The haplotype corresponding to H3 in our study was also associated with the disease (P=0.0045). However, negative results have also been reported.[38] Biopsy specimens of nasal mucosa were taken from patients with perennial AR and non-rhinitic control subjects. MMP1, 2, 3 and 9 mRNA were measured and no upregulation of MMPs was found in the tissue from patients. MMP9 in nasal tissue in animals has also been studied. Lim *et al.*[12] investigated upper- and lower-airway remodeling in murine model. OVA (ovalbumin)-sensitized mice were repeatedly exposed to inhalation of OVA for 1 month or 3 months. Repetitive OVA challenge for 3 months induced circumferential peribronchial fibrosis in the lung. Subepithelial fibrosis, increased MMP9 and Timp-1 expression, goblet cell hyperplasia and submucosal grand hypertrophy were observed in the nose. These findings are important because nasal mucosa may show similar pathologic changes to lung tissue and the *MMP9* gene may have a significant role in the pathogenesis of nasal mucosa.

The present results support an important role of the *MMP9* gene in pollen sensitization and pollinosis in Japanese children. Identifying the role of the *MMP9* gene in the sensitization process in upper-airway tissues is of importance to understand the development of pollinosis. In addition, whether stimulation or inhibition of MMP9 activity may benefit treatment of AR is of interest.

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ORIGINAL ARTICLE

The onset of allergic rhinitis in Japanese atopic children: A preliminary prospective study

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Abstract

Conclusion: This preliminary prospective study suggests that background factors may differ among allergic diseases. The beneficial interventions for reducing development of allergic rhinitis (AR) are also effective for the prevention of subsequent onset of bronchial asthma (BA). *Objective:* To determine the risk factors associated with onset of AR in atopic children in a prospective study. *Methods:* All patients with atopic dermatitis (AD) or food allergy with or without BA who visited the Pediatric Unit of Chiba University Hospital from 2005 to 2006 were enrolled in the study and received allergy examinations every 3–6 months. *Results:* A total of 100 patients were followed up for more than 2 years. Among the 60 patients without BA at entry to the study, 12 developed BA during the follow-up period. Development of AR preceded BA in 10 of the 12 patients (83.3%). In the background factors at the entry, positive sensitization to house dust mite (HDM) was significantly related to development of BA. Among the 48 patients without AR, 20 developed AR. High titers of serum HDM-specific IgE and high eosinophil counts in blood, and detection of eosinophils in nasal smears at the entry were significantly related to development of AR.

Keywords: *Bronchial asthma, atopic dermatitis, food allergy, house dust mite specific IgE*

Introduction

The concept of chronological development of food allergy (FA), atopic dermatitis (AD), bronchial asthma (BA), and allergic rhinitis (AR) in children with atopic risk factors is referred to as 'allergic march.' However, the detailed interactions and relationships among these allergic diseases have yet to be clarified. Settipane et al. [1] found that college students with AR developed BA at a rate three times higher than those without AR in a 23-year follow-up study. Similar studies in Finland [2] and the United States [3] also concluded that AR was an independent risk factor for development of BA. However, the asthma type (atopic or non-atopic) and development of AR in the follow-up period were not examined in these retrospective studies. Furthermore, the underlying mechanisms of development of allergic diseases are unclear and might differ with age, gender, and ethnicity.

Early intervention is currently thought to be important for prevention of progression of allergic diseases to more serious conditions. Therefore, an understanding of the interactions among allergic diseases in the development phase is required for establishment of an optimal strategy for early intervention, and particularly for secondary intervention. In this study, we prospectively examined the risk factors associated with onset of AR in atopic children who were closely followed at our institute for more than 2 years (mean 3.5 years), although the number of examined subjects was limited. Onset of AR preceded development of

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BA in many patients, and high serum titers of house dust mite (HDM)-specific IgE and increased eosinophil counts in blood and nasal smears were identified as possible predictive factors for development of AR.

Material and methods

Subjects

All AD or FA patients with or without asthma who visited the Pediatric Unit of Chiba University Hospital from 2005 to 2006 were enrolled in the study. Prior approval for the study was obtained from the Ethics Committee of Chiba University. Written, witnessed, informed consent was obtained from the parents of all patients.

Study protocol

All patients were examined by the same otorhinolaryngologist (S.Y.) and pediatrician (N.S.), who specialize in allergology. The examination included a nasal inspection, a respiratory test including spirometry and peak flow monitoring, and allergy tests for total IgE, HDM, Japanese cedar pollen, orchard grass, egg, wheat-specific IgE, ratio of eosinophils in leukocytes in peripheral blood, and cytology of a nasal smear. The respiratory test was conducted to determine the extent and change in lower airway obstruction. Positive sensitization to inhalants and food allergens was a necessary condition for diagnosis of AR and FA, respectively. The allergy tests were performed every 3–6 months.

Diagnosis of allergic diseases

Diagnosis of AR was based on the following criteria: representative symptoms of AR (e.g. paroxysmal sneezing, runny nose, nasal congestion, nasal itching, and eye itching) that persisted for more than 2 weeks, positive sensitization to typical allergens, and positive identification of eosinophils in a nasal smear. Nasal cytology has been shown to be useful in diagnosis of AR in children [4]. Positive sensitization was defined as a serum CAP-radioallergosorbent test (CAP-RAST, SRL Inc., Tokyo, Japan) score ≥ 2. The specific IgE level was ≥ 0.7 UA/ml.

Diagnosis of FA was based on symptoms after a meal (e.g. eczema, cough, vomiting, and diarrhea), positive sensitization to foods, and food challenge tests. Diagnosis of AD was based on symptoms of eczema with pruritus that repeatedly aggravated and palliated. A personal or parental history of allergic diseases and a high IgE titer were also considered in the AD diagnosis [5]. Diagnosis of BA was based on a history of symptoms and reversible bronchoconstriction. Patients or their parents completed a daily diary of respiratory symptoms (e.g. recurrent wheeze, dyspnea, exercise-induced symptoms, and symptoms causing waking at night) and these were checked by a doctor.

Treatment of the diseases

Allergic diseases were treated using the Japanese practical guidelines [6]. Local application of steroid ointment and oral antihistamines were used for AD. FA was treated by removal of the causal foods from the diet. Inhalation of steroids or leukotriene receptor antagonists was used for control of BA, with inhalation of β-blockers when needed based on the severity of symptoms. Oral antihistamines were mainly used for AR. Nasal steroids were only used in a few patients because these drugs are not approved for patients under 6 years old in Japan.

Statistical analysis

Data analysis was performed with two-tailed tests at a significance level of 5%. A Mann-Whitney U test was used for comparison of age and background factors at the first examination and in the follow-up period. A chi-squared test was used for other factors. All analyses were performed using SAS v. 8.02 (Cary, NC, USA).

Results

Background of subjects

A total of 114 pediatric patients were enrolled in the study and 100 patients were followed for more than 2 years. Fourteen patients dropped out of the study for personal reasons. The mean follow-up period was 3.5 ± 1.9 years. The background of the 100 patients at entry, including diagnosis, age, gender, birth order (first child or not), breast feeding, parental history of allergic disease (AD, BA, and HDM AR), parental smoking, pets, ratio of eosinophils in leukocytes in peripheral blood, and HDM-specific IgE are shown in Table I.

Follow-up of 60 patients without BA at the first examination

Among the 60 patients who did not have BA at the time of entry, 12 developed BA in the follow-up period. A comparison of the background factors at the time of entry between these 12 patients and the 48 patients who did not develop BA is shown in Table II. The HDM sensitization rate was higher ($p < 0.05$) in

Table I. Backgrounds of the enrolled patients at the time of entry to the study ($n = 100$).

Parameter	%
Boys	76.0
First child	67.0
Breast feeding	76.0
Parental medical history of allergic diseases*	37.0
Parental smoking	28.0
Pet	14.0
Ratio of blood eosinophils	
<5%	51.0
≥5%	49.0
HDM-specific IgE (UA/ml)	
<0.70	24.0
0.70–17.5	20.0
>17.5	56.0
Diseases present	
BA only	9.0
BA + AR	27.0
BA + AD (± FA)	4.0
AD (± FA)	60.0

Age at time of entry was 4.0 ± 2.7 years (mean ± SD) and the follow-up period was 3.5 ± 1.9 years. AD, atopic dermatitis; BA, bronchial asthma; FA, food allergy; HDM, house dust mite.
*Parental history of allergic disease included at least one of three diseases (AD, BA, and HDM AR).

patients who developed BA. Age, gender, birth order, breast feeding, parental clinical history, parental smoking, pets, diagnosis of HDM AR, detection of nasal eosinophils, and ratio of blood eosinophils did not differ significantly between the groups. Titers of HDM-specific IgE were not correlated with development of BA. Of the 12 patients with newly developed BA, 5 had AR at the time of entry and 7 developed AR in follow-up (Figure 1). In five of these seven patients, onset of AR occurred more than 3 months earlier than onset of BA. In the other two patients, the onset times for AR and BA were within 3 months of each other. Therefore, development of AR preceded BA in 10 of 12 patients (83.3%) with new onset BA.

Follow-up of 48 patients without AR at the first examination

Among 48 patients who did not have AR at the time of entry, 20 developed AR in the follow-up period. A comparison of the background factors at entry between these 20 patients and the 28 patients who did not develop AR is shown in Table III. Patients with new onset AR had significantly higher levels of eosinophils in nasal smears ($p < 0.01$), ratio of blood eosinophils ($p < 0.01$), and HDM-specific IgE ($p < 0.01$). HDM-specific IgE titers were correlated with development of AR. Age, gender, birth order, breast feeding, parental clinical history, parental smoking, pets, and diagnosis of BA did not differ significantly between the groups. Among the 48 patients initially without AR, eosinophils in the nasal smear were detected in 8 at the time of entry and in another 15 during follow-up. Of these 23 patients, 20 (86.4%) developed AR. Among the 20 patients with new onset AR, 14 (70.0%) were not complicated with BA (Figure 1). Of the six patients with AR and BA, four (20.0%) had BA at the time of entry and two (10.0%) developed BA at the same time as onset of AR.

Influence of treatment on development of new allergic diseases

The association of development of BA and AR with the severity of eczema during the follow-up period is shown in Table IV. The severity of eczema was significantly associated with development of AR ($p < 0.05$), but not with development of BA.

Discussion

Epidemiological studies have shown that BA and AR often co-exist, and the Allergic Rhinitis Impact on Asthma (ARIA) guidelines [7] recommend treating these conditions as a series of airway diseases, rather than as separate diseases. However, the interactions and relationships in the development of these allergic diseases have not been clarified. In the current prospective study, in which pediatric patients with AD or FA were followed up for a mean period of 3.5 years, we found that development of AR preceded that of BA in atopic children with AD or FA. This result is in agreement with several retrospective studies [1–3] and suggests that AR is an independent risk factor for development of BA.

In a recent birth cohort study of the sequential progression of multiple allergic diagnoses using the General Practice Research Database in the UK [8], diagnosis of eczema followed by asthma, which was in turn followed by rhinitis, was found to be the most common trajectory. However, as pointed out by the authors, rhinitis may have been under-diagnosed in the study. Diagnosis of AR has the risk of inclusion of a high rate of false-positive or false-negative cases because of the different concerns of patients, parents, and doctors. AR is sometimes difficult to distinguish from acute infectious rhinitis and even healthy people may exhibit mild, nonspecific nasal symptoms such as sneezing and nasal secretion. Thus, an allergic

Table II. Association between background factors and the development of bronchial asthma (BA).

Parameter	Patients without BA on entry to study (n = 60)		p value
	12 patients with newly developed BA	48 patients without BA	
Age at time of entry (years) (mean ± SD)	2.5 ± 0.7	3.3 ± 2.5	0.47
Follow-up period (years) (mean ± SD)	2.8 ± 1.9	3.6 ± 1.4	0.28
Background factors at time of entry (%)			
Boys	91.7	72.9	0.32
First child	83.3	64.6	0.21
Breast feeding	91.7	85.4	0.92
Parental medical history of allergic diseases*	58.3	37.5	0.33
Parental smoking	25.0	18.8	0.94
Pet	20.0	8.3	0.75
Diagnosis of HDM AR	41.7	41.7	0.74
Positive for eosinophils in nasal smear	50.0	52.1	0.85
Ratio of blood eosinophils in nasal smear			0.061
<5%	25.0	60.4	
≥5%	75.0	39.6	
HDM-specific IgE (UA/ml)			< 0.05
<0.70	0	33.3	
>0.70	100	66.7	

AD, atopic dermatitis; AR, allergic rhinitis; FA, food allergy; HDM, house dust mite.
*Parental history of allergic disease included at least one of three diseases (AD, BA, and HDM AR).

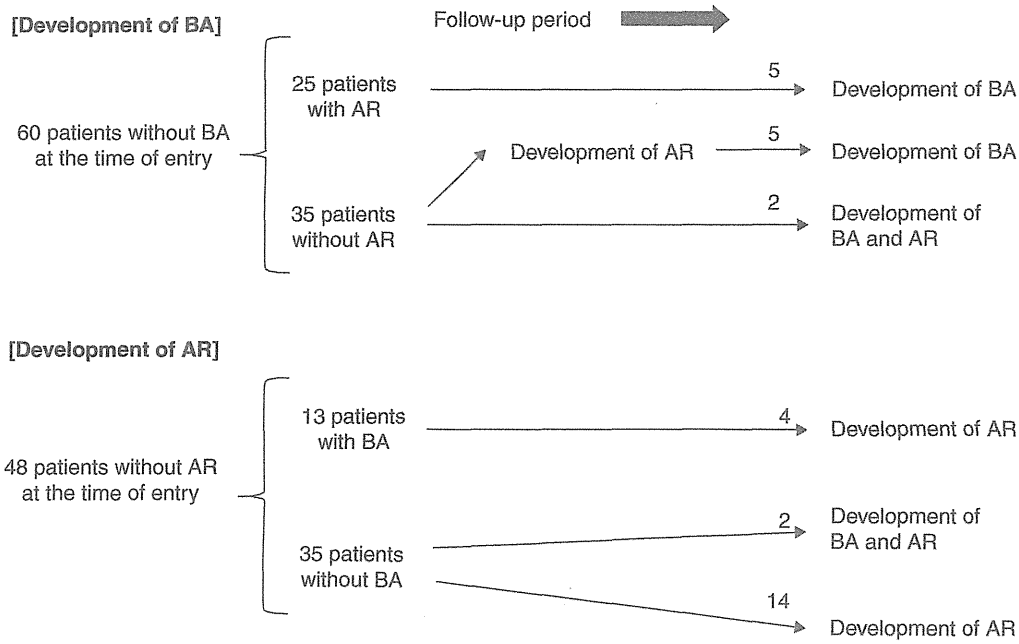

Figure 1. Development of allergic rhinitis (AR) preceded bronchial asthma (BA) in 10 (83.3%) of 12 patients with new onset BA. In contrast, development of BA preceded AR in only 4 (20.0%) of 20 patients with new onset AR.

Table III. Association between background factors and the development of allergic rhinitis (AR).

Parameter	Patients without AR on entry to study (n = 48)		p value
	20 patients with newly developed AR	28 patients without AR	
Age at time of entry (years) (mean ± SD)	2.9 ± 1.8	3.5 ± 3.2	0.66
Follow-up period (years) (mean ± SD)	4.1 ± 1.2	2.7 ± 2.0	0.10
Background factors at time of entry (%)			
Boys	80.0	82.1	0.85
First child	85.0	71.4	0.45
Breast feeding	75.0	92.9	0.19
Parental medical history of allergic diseases*	20.0	35.7	.23
Parental smoking	20.0	35.7	0.39
Pet	5.0	7.1	0.76
Diagnosis of BA	20.0	32.1	0.54
Positive for eosinophils in nasal smear	40.0	0	< 0.01
Ratio of blood eosinophils			< 0.01
<5%	40.0	85.7	
≥5%	60.0	14.3	
HDM-specific IgE (UA/ml)			< 0.01
<0.70	20.0	60.7	
0.7–17.5	20.0	25.0	
>17.5	60.0	14.3	

AD, atopic dermatitis; HDM, house dust mite.
*Parental history of allergic disease included at least one of three diseases (AD, BA, and HDM AR).

examination is necessary to avoid a high incidence of false positives and false negatives.

In the current study, detection of eosinophils in nasal smears, increased peripheral blood eosinophils, and high HDM-specific IgE in serum were identified as risk factors for onset of AR in atopic children with AD or FA. However, only 20% of the patients with new onset AR had preceding BA, while 70% of patients developed AR without BA. Many factors are likely to be involved in development of BA and a subgroup of younger children may develop BA before AR. However, the mean ages at entry of patients without BA and with BA were 3.2 and 5.5 years, respectively, and rates of AR in these subjects were 41.6 and 67.5%, respectively. Thus, AR can develop at a younger age and we were unable to identify BA as an independent risk factor for AR.

Table IV. Association between the control of eczema and the development of bronchial asthma (BA) or allergic rhinitis (AR).

Patient group	Status	Control of eczema (%)			p value
		n	Good*	Poor†	
Subjects without BA at entry to study		60			0.75
	BA developed	12	50.0	50.0	
	BA undeveloped	48	50.0	50.0	
Subjects without AR at entry to study		39			< 0.05
	AR developed	18	38.8	61.1	
	AR undeveloped	21	76.2	23.8	

*Control of eczema was defined as 'Good' when the skin was dry and eczema was not observed clearly.
†Control of eczema was defined as 'Poor' when eczema was observed clearly.

In recent years, many countries have experienced an increase in the prevalence of AR. Natural resolution of AR is not commonly observed, particularly in childhood, and most pediatric patients with AR grow to adulthood without natural improvement. Genetic and environmental factors appear to be involved in onset of AR, as seen with many other diseases [9–12]. The relevance of genetic factors cannot be ignored, but it appears that environmental factors have played a major role in the recent increase of AR. Factors that change the predisposition to produce IgE are thought to have contributed to this increase. The findings in the current study are consistent with this suggestion, since development of AR was associated with an increased titer of HDM-specific IgE, but was not clearly linked to parental history of allergy.

Early intervention strategies to inhibit the increase of specific IgE are important in the management of AR. Allergen avoidance is clearly important for prevention of AR, but may not achieve effective results [13]. Allergen-specific immunotherapy is the only current treatment with the potential for long-term cure of AR. Randomized controlled trials (RCTs) have shown that antigen-specific immunotherapy is also effective for changing the natural course of allergic diseases [14], decreasing antigen-specific IgE [15], and reducing new allergic sensitization [16]. However, administration by a subcutaneous route as an early intervention is difficult in children, in particular, because it is associated with a risk, albeit low, of anaphylactic shock and the inconvenience of frequent visits to a physician [17]. A recent review of RCTs of sublingual immunotherapy (SLIT) for AR suggested that this approach is safe and easy for children [18]. Thus, SLIT may be effective as an alternative route of administration and as an early intervention strategy.

Poor control of AD is associated with a risk of development of AR, since damage to the skin barrier by AD itself and by resultant scratching might aggravate skin sensitization to allergens [19]. A clinical RCT of the efficacy of antihistamines for prevention of BA in children with AD or allergen sensitization showed some positive results [20], but this treatment is not accepted widely as a standard because of the risk of adverse events, particularly when taken over a long period. Effective treatment with safe medication to improve scratching and skin sensitization, even if not treating the underlying disease, may be useful for preventing onset of AR in patients with AD.

The results of our prospective study indicate that preventing an increase of HDM-specific IgE and sensitization to HDM are likely to be beneficial interventions for prevention of AR and BA, respectively, and effective control of AD would be prevention of AR. The beneficial interventions for reducing development of AR are also effective for the prevention of subsequent onset of BA. We note that these results are based on a relatively small number of patients, and further large-scale studies are needed to clarify these important issues.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Effects of Aging on the Natural History of Seasonal Allergic Rhinitis in Middle-Aged Subjects in South Chiba, Japan

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Key Words
Seasonal allergic rhinitis · Japanese cedar pollen · Remission · Development · Specific IgE

Abstract
Background: The natural history of allergic rhinitis has been examined in a few longitudinal studies. The purpose of the study was to investigate the course, development and remission of seasonal allergic rhinitis (SAR) over 10 successive years in middle-aged subjects. ***Methods:*** An annual questionnaire survey on allergic rhinitis symptoms combined with an examination of specific IgE has been undertaken in a rural town in south Chiba since 1995. The analyzed subjects were 703 residents who underwent every examination in 1995, 2004 and 2005. In the last 15 years, the annual pollen count in Chiba was highest in 2005. ***Results:*** The sensitization rates to cedar pollen decreased with age in the same subject groups over 10 years, but the prevalence of SAR was higher in 2005 compared with 1995. Of the 52 subjects with SAR in 1995, the symptoms had disappeared in 10 subjects in 2005. Specific IgE had converted to negative or borderline in 4 of these patients, had decreased but was still positive in 4 and was increased or unchanged in 2. During the 10-year period, 22 subjects developed SAR, of whom 12 had increased specific IgE and 10 had similar or decreased specific IgE in 2005. ***Conclusion:*** SAR induced by cedar pollen takes a chronic course in the majority of middle-aged patients in south Chiba, Japan. The prevalence of SAR increased over 10 years due to a high level of pollen exposure. Changes in specific IgE were not directly associated with the development or remission of SAR.

Introduction

Recent observations have suggested a significant worldwide increase in the prevalence of allergic rhinitis [1]. In Japan, Japanese cedar *(Cryptomeria japonica)* pollens are the major allergens. Since cedar forests cover nearly 18% of the total forest area of Japan [2], Japanese cedar produces enormous amounts of pollen that are dispersed over many kilometers and causes widespread sea-

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sonal allergic rhinitis (SAR) induced by cedar pollen in major cities, including Tokyo [3]. The seasonal dispersion of cedar pollen normally starts in early February in the south Kanto area (Chiba and Tokyo), peaks from late February to March and ends in April [4].

The prevalence of SAR induced by cedar pollen has increased by more than 10% in the last 10 years based on questionnaire surveys [5–7]. However, findings based on only questionnaire data carry the risk of including many false-positive cases, as allergic rhinitis can be difficult to distinguish from acute upper respiratory infection and even healthy individuals may exhibit mild, nonspecific nasal symptoms such as sneezing and nasal secretion [8]. In addition, the cedar pollen season coincides with the winter season when upper respiratory infections are common, and an allergen-specific IgE test is necessary to avoid a high incidence of false positives. Moreover, sensitization to cedar pollen and the incidence of SAR induced by cedar pollen are influenced by variable seasonal pollen counts, especially for tree pollens. For example, dispersal of tree pollens such as Japanese cedar pollen has annual differences of more than 15-fold, in contrast to grass pollens [9]. The pollen-specific IgE titer also shows a seasonal change, and the sensitization and incidence rates of SAR induced by cedar pollen change every year [9, 10]. Thus, an accurate evaluation of the development, remission and natural course of SAR induced by cedar pollen is difficult.

Allergic rhinitis is a typical type 1 allergic disease, but the mechanisms of development and remission are complicated and have not been clarified yet. These are important fundamental phenomena that require understanding for the development of new treatment strategies for cure of the disease. In this study, we followed a population of middle-aged Japanese subjects in a typical small town in Japan for 10 years, including the years with the highest and lowest pollen counts during this period, to examine the course of allergic rhinitis.

Subjects and Methods

Subjects and Study Design

Maruyama-cho is a small rural town in the south of Chiba where movement of residents into the town is uncommon, and the town has been losing its population of children and young adults. We have determined the incidence of allergic rhinitis among the residents of Maruyama-cho annually since 1995. In 1995, we sent a letter to all residents of Maruyama-cho over 39 years of age (n = 3,771) to ask them to undergo a medical examination, and 1,560 subjects agreed to this request. The examination has subsequently been performed annually in the middle of June 2 months after the cedar pollen season. At the time of the examination the physicians checked the current nasal symptoms, history of allergic rhinitis and other allergic disease, and family history of allergic disease. Serum CAP-radioallergosorbent test (CAP-RAST; SRL Inc., Tokyo, Japan) scores for Japanese cedar pollen and mite *(Dermatophagoides pteronyssinus)* were also examined. Serum samples were frozen and all were analyzed within 2 days. We examined the changes of sensitization rates for Japanese cedar pollen and mite, and prevalence of SAR induced by cedar pollen and mite perennial allergic rhinitis over a 10-year period in the residents who had undergone every examination in 1995, 2004 and 2005.

The study was performed in compliance with the Ethical Guidelines for Clinical Studies and Good Clinical Practice and the Declaration of Helsinki (1989 and 2000 revisions). The protocol was approved by the Ethics Committee of Chiba University Hospital, and written informed consent was obtained from each subject prior to their participation in the study.

Pollen Counts

Pollen counts were determined with a Durham sampler (Nishiseiki Co., Funabashi, Japan) using the gravimetric method. In Japan, pollen counts are typically measured using the gravimetric method with a Durham sampler, in contrast to the typical use of a Burkard sampler in Western countries.

Diagnosis of Allergic Rhinitis

Mite perennial allergic rhinitis was defined as the presence of typical nasal symptoms almost all year around and a serum CAP-RAST score for mite of ≥ 2. SAR induced by cedar pollen was defined as the presence of nasal symptoms in at least three successive pollen seasons since 1993, based on differences in annual pollen dispersal, and a serum CAP-RAST score for Japanese cedar pollen of ≥ 2 in 1995. The specific IgE level was classified using the following scores: 6, $\geq$100 UA/ml; 5, 50.0–99.9 UA/ml; 4, 17.5–49.9 UA/ml; 3, 3.50–17.4 UA/ml; 2, 0.70–3.49 UA/ml; 1, 0.35–0.69 UA/ml; 0, $\leq$0.34 UA/ml. A CAP-RAST score of 2–6 was regarded as positive, a score of 1 as borderline and a score of 0 as negative. The sensitization rates were calculated based on the number of subjects with a score of 2–6.

Remission and Development of SAR Induced by Cedar Pollen

Subjects who had SAR induced by cedar pollen in 1995 but did not have nasal symptoms in the cedar pollen season in 2005 (during which extremely high amounts of cedar pollen were scattered) were defined as in remission. In contrast, those who did not have nasal symptoms in 1995 but had both typical nasal symptoms and positive cedar-specific IgE in 2005 were defined as patients with newly developed SAR induced by cedar pollen.

Statistical Analysis

Comparison of the profiles of subjects was performed with two-tailed tests at a significance level of 5% using the χ^2 test in SAS v.8.02 (SAS Inc., Cary, N.C., USA). Sensitization rates, prevalence rates as well as the association between the background factors and the disappearance/development of symptoms were also analyzed by the χ^2 test.

Results

Profile of Subjects

The number of residents who underwent examinations in 1995, 2004 and 2005 was 703. The other 857 residents who dropped out of the study were older and had significantly lower rates for sensitization to Japanese cedar pollen and SAR induced by cedar pollen in 1995, compared to the subjects who were followed. There was no difference in gender between the two groups (table 1).

Table 1. Profile of subjects in 1995

	Followed in 2005 (n = 703)	Dropped out in 2005 (n = 857)
Age in 1995, %		
40s	24.3	14.9
50s	31.0	15.1
60s	36.7	35.0
70s	8.0	35.0
p value	<0.001 (70s vs. total of others)	
Gender, %		
Female	59.5	57.8
Male	40.5	42.2
p value	0.50	
Sensitization to cedar pollen, %		
Positive	22.0	16.5
Negative or borderline	78.0	73.5
p value	<0.01	
Diagnosis of SAR induced by cedar pollen, %		
Yes	7.4	4.3
No	92.6	95.7
p value	<0.01	
Sensitization to mite, %		
Positive	18.3	14.6
Negative or borderline	81.7	85.4
p value	<0.05	
Diagnosis of mite perennial allergic rhinitis, %		
Yes	1.8	1.3
No	98.2	98.7
p value	0.37	

Pollen Counts

Dispersal of Japanese cedar pollen generally exhibits a bell-shaped pattern with time: cedar pollen dispersal starts in early February and reaches a peak between late February and early March, with some variation due to changes in the climate each year. The pollen dispersal season lasts for more than 8 weeks in the Chiba area.

The annual counts of cedar pollen (the total of daily counts from February to April) were 4,600, 500 and 7,850 grains/cm^2/season in 1995, 2004 and 2005, respectively. The average annual pollen count was 3,000 grains/cm^2/season over the 15 years since the start of the pollen count survey in Chiba, and was lowest in 2004 and highest in 2005.

Sensitization Rates for Japanese Cedar Pollen and Prevalence of SAR Induced by Cedar Pollen

Sensitization rates for Japanese cedar pollen in males are shown in figure 1a. Changes of sensitization rate in the same subjects from 1995 to 2005 are shown by arrows in the figure: these rates decreased over a 10-year period by 1.7% (1/59), 5.1% (4/79) and 12.7% (15/118) in their 40s, 50s and 60s, respectively, in 1995. The decrease was significantly greater in the 60s age group compared to the 40s or 50s age groups. Sensitization rates for Japanese cedar pollen in females are shown in figure 1b. These rates decreased by 1.8% (2/112) and 6.5% (9/139) from 1995 to 2005 in their 40s and 50s, respectively, in 1995, with a significantly greater decrease in the 50s age group.

The prevalence of SAR induced by cedar pollen in 2004 slightly decreased compared with 1995 in males in their 40s and 60s in 1995 (fig. 2a). However, the prevalence increased in 2005 compared with 1995, except for subjects in their 60s in 1995. The changes in rates from 1995 to 2005 were 5.1% (3/59) and 6.3% (5/79) in their 40s and 50s, respectively, in 1995, with no significant difference between these changes. The prevalence in females is shown in figure 2b, and an increased prevalence over a 10-year period was found in their 40s and 60s in 1995. The prevalence was similar in 1995 and 2004. The changes in rates from 1995 to 2005 were 4.5% (5/112) and 1.4% (2/140) in their 40s and 60s, respectively, in 1995, with no significant difference between these changes.

Table 2 shows changes in age-specific sensitization rates for Japanese cedar pollen and prevalence rates of SAR in all subjects over a 10-year period. The sensitization rate among the 50s age group in 2005 was significantly greater than that among the 50s age group in 1995. The prevalence rates of SAR among the 50s and 60s age groups in 2005 were significantly greater than those among the 50s and 60s age groups in 1995.

Sensitization Rates for Mite and Prevalence of Mite Perennial Allergic Rhinitis

Sensitization rates for mite in males decreased with age, and the changes in sensitization from 1995 to 2005 were 6.8% (4/59), 10.1% (8/79) and 11.9% (14/118) in males in their 40s, 50s and 60s, respectively, in 1995, with no

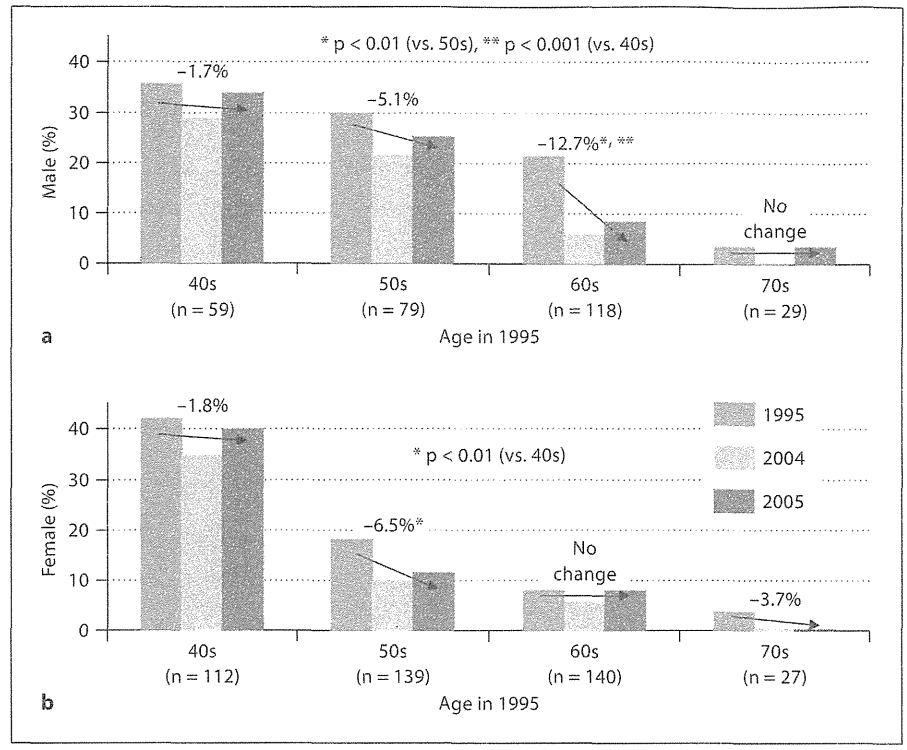

Fig. 1. Sensitization rates for Japanese cedar pollen. The changes in sensitization in the same subjects from 1995 to 2005 are shown by arrows. The rates decreased over a 10-year period in most groups except for males in their 70s (**a**) and in females in their 60s (**b**) in 1995.

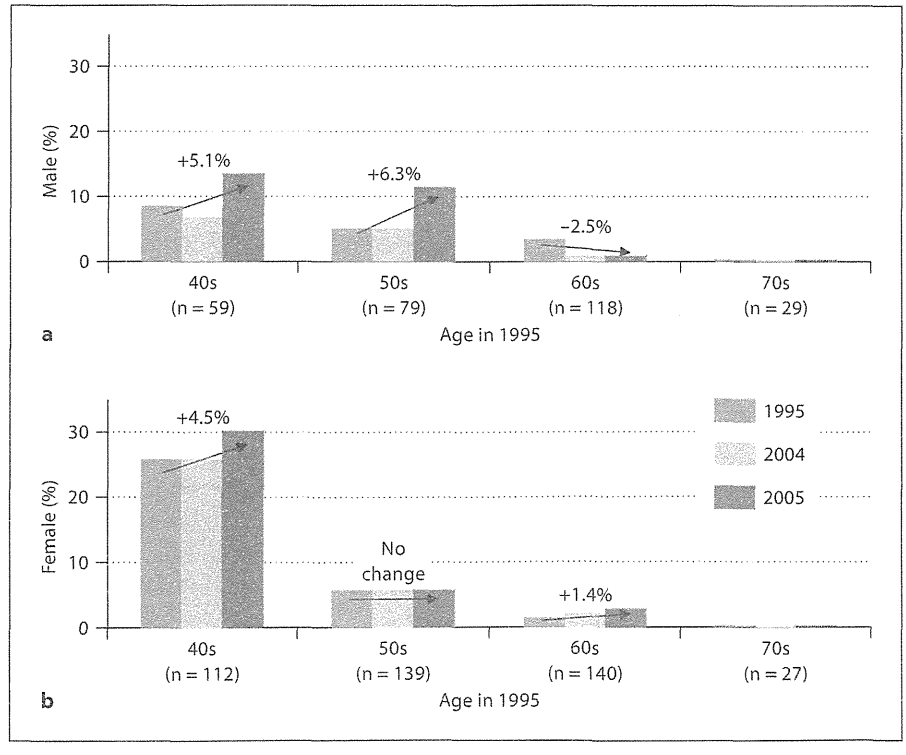

Fig. 2. Prevalence of SAR induced by Japanese cedar pollen. The changes in prevalence in the same subjects from 1995 to 2005 are shown by arrows. The prevalence increased over a 10-year period in most groups except for males in their 60s (**a**) and females in their 50s (**b**) in 1995.

Table 2. Changes in age-specific sensitization rates for Japanese cedar pollen and prevalence rates of SAR in all subjects over a 10-year period

Age	Sensitization rates for Japanese cedar pollen, %			Prevalence rates of SAR, %		
	1995	2005	p value	1995	2005	p value
40s	39.8	–	–	19.9	–	–
50s	22.5	38.0	$<1.0\times10^{-3}$	5.5	24.6	$<1.0\times10^{-7}$
60s	14.0	16.5	0.44	2.3	7.8	<0.01
70s	3.6	8.1	0.36	0	1.9	0.64
80s	–	1.8	–	–	0	–

significant differences among the data. Sensitization rates for mite in females also decreased with age, and the decreases from 1995 to 2005 were 8.9% (10/112) and 11.5% (16/139) in females in their 40s and 50s, respectively, in 1995, with a significantly greater decrease in the 50s age group (data not shown).

The prevalence of mite perennial allergic rhinitis was low in males and females (<7%). The prevalence decreased with age in all groups and was unaffected by the annual pollen count (data not shown).

Remission of Symptoms of SAR Induced by Cedar Pollen

Among the 703 subjects, 52 had been diagnosed with SAR induced by cedar pollen in 1995. The disease had not resolved in 42 (80.8%) of these patients, but 10 (19.2%) did not have symptoms of SAR induced by cedar pollen in 2005, a year in which there was an extremely high pollen count (table 3). These 10 subjects continued to have no nasal symptoms in pollen seasons 2006 and 2007 (data not shown). Older and male patients showed a tendency for more chance of remission, but there was no correlation with the age at onset of SAR induced by cedar pollen, other allergic diseases or family history of SAR induced by cedar pollen. Cedar-specific IgE levels were higher in patients without remission than in those with remission. However, the cedar-specific IgE of each subject changed to negative or borderline in 4, decreased but was still positive in 4 and increased or was unchanged in 2 of the 10 subjects with remission (fig. 3).

Development of SAR Induced by Cedar Pollen

Among the 703 subjects, 651 did not have symptoms of SAR induced by cedar pollen in 1995. Of these subjects, 629 had not developed SAR induced by cedar pollen in 2005, but 22 (3.4%) had developed the disease over 10 years (table 4). Younger subjects showed a tendency for more chance of development. There was no difference between genders. Cedar-specific IgE levels in 1995 were higher in patients who developed the disease compared to those without disease development. Among the 703 subjects, 103 were sensitive to Japanese cedar pollen, but did not have any symptoms of SAR induced by cedar pollen in 1995. Of these 103 subjects, 17 (16.5%) had symptoms of SAR induced by cedar pollen in 2005. The cedar pollen-specific IgE increased in 7, decreased in 8 and was unchanged in 2 of the 17 subjects with development of the

Table 3. Association between the background factors and the disappearance of symptoms in subjects with SAR induced by cedar pollen in 1995

	Symptoms disappeared in 2005 % (n = 10)		Symptoms continued in 2005 % (n = 42)
Age in 1995			
40s	30.0		73.8
50s	40.0		19.0
60s	30.0		7.2
p value		<0.05	
Gender			
Female	40.0		83.3
Male	60.0		16.7
p value		<0.05	
Cedar-specific IgE			
CAP-RAST 2–3	80.0		33.3
CAP-RAST 4–6	20.0		66.7
p value		<0.05	
Mite-specific IgE			
CAP-RAST<2	50.0		61.9
CAP-RAST≥2	50.0		38.1
p value		0.74	
Age at the onset, years			
<40	40.0		57.1
≥40	60.0		42.9
p value		0.53	
Other allergic disorders[1]			
With disorders	20.0		11.9
Without disorders	80.0		88.1
p value		0.87	
Family histories of SAR induced by cedar pollen[2]			
With family histories	10.0		11.9
Without family histories	90.0		88.1
p value		0.70	

[1] Other allergic disorders: mite perennial allergic rhinitis, bronchial asthma and atopic dermatitis.

[2] Histories of parents, brothers, and sisters.

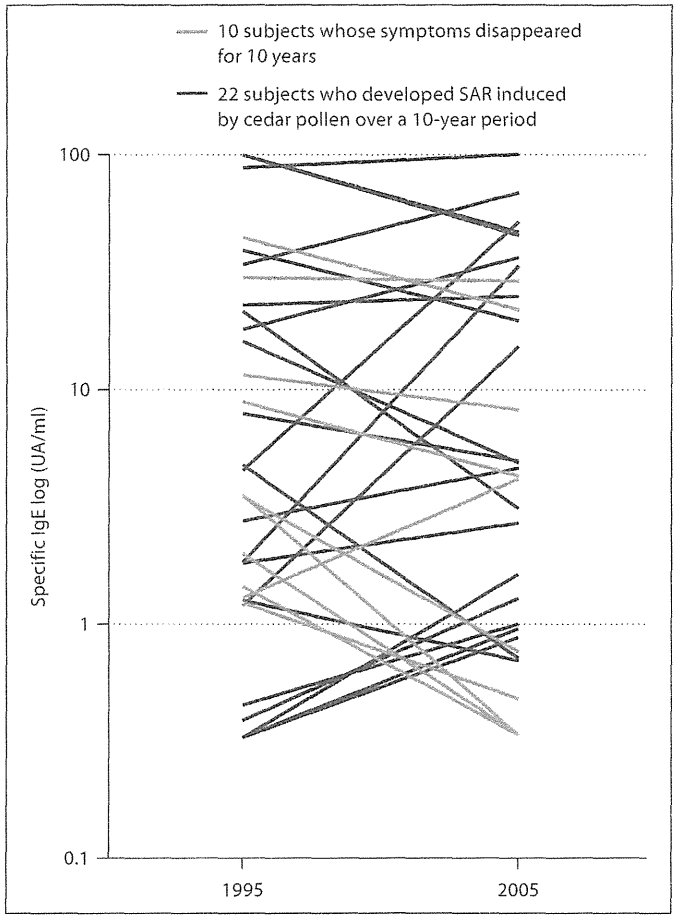

Fig. 3. Changes of specific IgE titer to Japanese cedar pollen. The changes of cedar pollen-specific IgE did not have a constant tendency. Changes in specific IgE were not directly associated with development or remission of SAR.

Table 4. Association between the background factors and the development of symptoms in subjects without SAR induced by cedar pollen in 1995

	With SAR in 2005, % (n = 22)		Without SAR in 2005, % (n = 629)
Age in 1995			
40s	50.0		20.0
50s	40.9		31.3
60s	9.1		39.8
70s	0		8.9
p value		<0.001	
Gender			
Female	54.5		58.3
Male	45.5		41.7
p value		0.72	
Cedar-specific IgE			
CAP-RAST<2	22.7		86.3
CAP-RAST 2–3	40.9		13.5
CAP-RAST 4–6	36.4		0.2
p value		<0.001	
Mite-specific IgE			
CAP-RAST<2	50.0		84.6
CAP-RAST≥2	50.0		15.4
p value		<0.001	
Other allergic disorders[1]			
With disorders	0		4.0
Without disorders	100		96.0
p value		0.69	
Family histories of SAR induced by cedar pollen[2]			
With family histories	4.5		3.5
Without family histories	95.5		96.5
p value		0.74	

[1] Other allergic disorders: mite perennial allergic rhinitis, bronchial asthma, and atopic dermatitis.
[2] Histories of parents, brothers, and sisters.

disease (fig. 3). Five of the 548 (0.9%) subjects who were not sensitive to cedar pollen in 1995 were sensitized and had symptoms of SAR induced by cedar pollen in the 2005 pollen season. Eighteen of the 22 patients with newly developed SAR induced by cedar pollen continued to have symptoms during the pollen seasons in 2006 and 2007, when the pollen counts were low.

Discussion

This study provides a longitudinal analysis of the same middle-aged subjects for 10 years, including comparisons among seasons with large differences in cedar pollen counts: the highest in 2005 and lowest in 2004 over the last 15 years. The number of subjects in the study was limited and the gender and age distributions indicate that the population is not representative of the national middle-aged Japanese population. However, the subjects who dropped out of the study included more nonsensitized subjects without allergic rhinitis who were older than 70 years of age in 1995. Therefore, we believe that the longitudinal epidemiological survey of allergic rhinitis in the same middle-aged subjects combined with an examination of specific IgE and annual pollen counts provides new information on the current status of allergic rhinitis, since very few such surveys have been performed.

A decreased sensitization and prevalence to cedar pollen and mite were observed in the middle-aged subjects

in this study, as also reported in other countries [11–13]. There also seemed to be a difference between genders, with reduced sensitization occurring about 10 years earlier in females. However, with exposure to a high pollen level, sensitization to cedar pollen and the prevalence of SAR induced by cedar pollen increased in subjects in their 50s to 70s regardless of gender. The annual pollen count in 2005 (7,850 grains/cm^2/season) was about 2.5 times higher than the mean over the last 15 years. Subjects with low cedar pollen-specific IgE levels (CAP-RAST sores of 2–3) tended to contribute to the decrease in sensitization, whereas subjects with high specific IgE levels tended to have symptoms under exposure to a high level of cedar pollen.

Genetic and environmental factors are involved in the onset of allergic rhinitis, as with many other diseases [14, 15]. The relevance of genetic factors cannot be ignored, but environmental factors may also have played a major role in the recent increase in allergic diseases. In Japan, after World War II, an extensive afforestation program was initiated using Japanese cedar because of its fast growth and its value as a building material. Consequently, however, the amount of airborne Japanese cedar pollen has increased dramatically after the 1970s [16]. Pollen dispersal varies from year to year, but annual pollen counts have shown a tendency to increase with time and are a major environmental factor in allergic rhinitis [17]. There are other environmental factors which can change the predisposition to produce IgE and may also have contributed to the increase in allergic rhinitis. However, increased pollen dispersal probably has a major role in the increased prevalence of SAR induced by cedar pollen in middle-aged subjects, since sensitization to mite and the prevalence of mite perennial allergic rhinitis decreased with age in the current study.

Remission or development of allergic rhinitis has only been examined in a few longitudinal studies [9, 18–20] and evaluation of these processes is particularly difficult in SAR induced by tree pollens, as described above. During the 10 years of the present study, remission and development of SAR induced by cedar pollen occurred in 19.2% (10/52) and 3.4% (22/651) of the middle-aged subjects, respectively. Mite-allergic rhinitis remitted in 8 of 13 subjects (61.5%) and did not newly develop in any subjects (data not shown). SAR took a chronic course in the majority of middle-aged patients in the current study. Younger subjects showed a tendency for more risk of development of SAR induced by cedar pollen and less chance of remission, but the change in the pollen-specific IgE level in each individual was not directly associated with remission or development. These data showed no differences between genders.

There was a high rate of asymptomatic sensitization in this study. 103 subjects were sensitized without symptoms at baseline, and of these only 17 developed symptoms during the follow-up period. One of the reasons of this high rate of asymptomatic sensitization is low CAP-RAST scores of the 103 subjects. Among the 103 subjects, 94 (91.3%) showed scores of 2–3. The sensitization rates were calculated based on the number of subjects with a CAP-RAST score of 2–6. We did not perform skin tests. Skin prick test might be more sensitive and show a higher correlation with allergic symptoms; however, CAP-RAST test allowed a quantitative analysis. In this study, high CAP-RAST scores could be a predictive factor for the new development of SAR (table 4). The cross-reactivity to carbohydrate determinants may cause false-positive specific IgE determinations as reported in some pollens in vitro [21–24]; however, the cross-reactivity of specific IgE to carbohydrate determinants in cedar pollen has not been clarified yet.

Overall, this study suggests that SAR induced by cedar pollen does not resolve in the majority of middle-aged patients over a 10-year period and takes a chronic course. The prevalence of mite-allergic rhinitis has remained low and decreased since 1995, but that of SAR induced by cedar pollen has increased in middle-aged subjects in south Chiba, Japan. This increase is associated with the rise of cedar pollen dispersal. Remission and development of SAR induced by cedar pollen were both observed in middle-aged subjects over a 10-year period, and were not directly associated with the change in the specific IgE level in each subject. Further studies are required to clarify the basis of these observations.

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