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Prevalence of inhaled antigen sensitization and nasal eosinophils in Japanese children under two years old

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ABSTRACT

Objective: The increasingly younger age of onset of allergic rhinitis (AR) has recently become a problem. This study examined the prevalence of inhaled antigen sensitization and nasal eosinophils in children younger than two years old, with measurement of the serum concentrations of aeroallergen-specific IgE antibodies to house dust mites, cat fur, and Japanese cedar pollen, measurement of nasal eosinophil counts, and a questionnaire administered to the children's parents.

Methods: The subjects were a group of healthy children undergoing 18-month infant health checks provided by the local government, and sick children younger than two years old at the pediatric hospital.

Results: Among 408 healthy infants, 44 (10.7%) had antigen-specific IgE antibodies, 29 (7.1%) had nasal eosinophils, and eight (2.0%) had both specific IgE antibodies and nasal eosinophils. Nasal assessment revealed that 125 children had rhinorrhea. Of the infants who showed both sensitization to antigens and nasal eosinophils, six (1.5%) had confirmed rhinorrhea. Among 186 sick children younger than two years old at the pediatric hospital, aeroallergen-specific IgE antibodies were detected in five (2.6%). The presence of nasal eosinophils was confirmed in six children (3.2%), which percentage was smaller than that of the healthy group. No infant had either sensitization to antigens or nasal eosinophils.

Conclusion: The findings described above indicate that the minimum prevalence of AR might be 1.5% in 18-month-old children and that around 10% of affected children have aeroallergen-specific IgE antibodies in Japan. The incidence of AR in young children might increase further.

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1. Introduction

Allergic rhinitis (AR) is an important global public health problem because of its markedly increased prevalence worldwide. Our recent study revealed that over 35% of the Japanese population aged 20–50 years old suffers from allergies during the Japanese cedar pollen (JCP) season [1]. Infantile allergic disease has been described as the 'allergic march' and is believed to begin with atopic dermatitis accompanied by infantile asthma, with subsequent progression to house dust mite-induced perennial AR. Otitis media with effusion is also included in allergic march among children 2–6 years. From

around elementary school age onwards, seasonal allergic rhinitis (SAR) occurs [2]. Other authors have suggested that AR can occur earlier and can progress to bronchial asthma [3,4]. Despite the lack of definite evidence, a younger age of onset for AR has recently become a problem in Japan. However, because infants (unlike adults) develop AR with diverse symptoms, it is often difficult for pediatricians to make a diagnosis of AR. Therefore, even when symptoms of rhinitis are present, the contribution of AR is not strongly suspected in many cases. These points might explain why the incidence of AR and the sensitization rate have not been determined in children younger than two years old. In a clinical setting, however, it is important to have exact data for these parameters.

The accuracy of most studies performed to date is open to question because they have relied on parents' completed questionnaires to assess the incidence of AR. More recently, some studies have combined the results of surveys with measurement of antigen-specific IgE [5,6]. Nevertheless, other diagnostic procedures are also required, such as identification of symptoms with

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duration of at least two weeks and the skin prick test. According to Allergic Rhinitis and its Impact on Asthma (ARIA), for the diagnosis of AR, information about the existence of symptoms and identification of inhalant antigens must be obtained [7]. Furthermore, the Japanese Guidelines for Allergic Rhinitis (JG-AR) specify as requirements for the diagnosis the presence of nasal eosinophils or positive result of nasal provocation test, in addition to the above-mentioned ARIA's requirements [8]. Consequently, we combined a conventional questionnaire survey of AR completed by parents with direct observation by an ear, nose and throat specialist, measurement of the nasal eosinophil count, and qualitative measurements of aeroallergen-specific IgE antibodies. The subjects were a control group of healthy 18-month-old children and a group of children younger than two years for whom hospital care had been sought.

2. Methods

2.1. Subjects

In Japan, 18-month-old children undergo a free health check by a pediatrician and vaccination status. In this study, children undergoing this infant health check in Fukui City during September–November 2008 were enrolled as group 1. Of 539 children underwent the infant health check, 408 (220 boys, 188 girls, mean age of 18.4 months) participated in this study.

Group 2 comprised 186 infants younger than two years old (109 boys, 77 girls, with mean age of 10.9 months) who received care at the Department of Pediatrics at Fukui Aiku Hospital during September–October, 2008. They were grouped by age: 0–5 months, $n = 53$; 6–11 months, $n = 49$; 12–17 months, $n = 46$; and 18–23 months, $n = 38$. Informed consent was obtained from the parents of all participating children. The Ethics Review Board of the University of Fukui approved this study.

2.2. Questionnaire survey

Each child's parents were asked about siblings, the past history of allergic disease, exposure to environmental tobacco smoke, family history of allergy, and pets (Table 1). To exclude 'diagnosis' of the allergic state by the parents, the item 'cured' was included in the questionnaire to exclude any temporary condition that differed from constitutional allergic disease.

2.3. Assessment of nasal eosinophils

Nasal scraping from both inferior nasal turbinates was performed with a disposable swab and sampled onto a glass slide. Each sample was stained with Wright–Giemsa stain to confirm the presence or absence of eosinophils. Each child was classified as negative or positive. Positive means that more than 10 eosinophils were found under 200 $\times$ magnification.

Table 1
Questionnaire.

Q1: About your baby

The baby has _____ siblings, and the baby's birth order is _____th.

Has the baby ever been diagnosed as having an allergic disease at a hospital or clinic?

Please check the baby's disease(s) and mark the condition with a circle if "Yes".

No	Yes
() Atopic dermatitis	(From _____month, treated cured wait and see)
() Bronchial asthma	(From _____month, treated cured wait and see)
() Allergic rhinitis	(From _____month, treated cured wait and see)
() Allergic conjunctivitis	(From _____month, treated cured wait and see)
() Food allergy	(From _____month, treated cured wait and see)

Q2: About the baby's family

Please check the disease(s) and mark the person(s) with a circle if the baby has a family member(s) with an allergy diagnosed at a hospital or clinic.

() Atopic dermatitis	(Brothers, Sisters, Father, Mother, Others)
() Bronchial asthma	(Brothers, Sisters, Father, Mother, Others)
() Allergic rhinitis	(Brothers, Sisters, Father, Mother, Others)
() Allergic conjunctivitis	(Brothers, Sisters, Father, Mother, Others)
() Food Allergy	(Brothers, Sisters, Father, Mother, Others)

Is there a smoker at home?

No	Yes (Smoker: Father, Mother, Other(s))
()	()

Q3: About pets

Are there any pets at home?

No	Yes (Kind: _____)
()	()

2.4. Identification of inhaled antigens

After finger prick with a needle, blood was collected into a pipette, and specific IgE antibodies for house dust mites, cat fur, and JCP were measured (IMMFAST Check J1; Mitsubishi Chemical Medience Corp., Tokyo, Japan). This test contains reagents to detect allergen-specific IgE antibodies using immunochromatography and corresponded to a Class 2 level or higher result according to the CAP-RAST method [9].

2.5. Intranasal observation

To check the existence of nasal discharge and observed inferior turbinate, each child was examined by an otorhinolaryngology and allergy specialist (Y. Osawa) using an anterior rhinoscope before the blood collection. Intranasal observation was defined as positive when rhinorrhea and inferior conchal swelling were noted. This criterion enabled us to detect more patients than when the diagnoses were based on AR symptoms, and led to identification of numerous children.

After that, if a thick deposit of mucus was obtained on the disposable cotton swab at the time of nasal eosinophil measurement, then intranasal observation was regarded as positive.

2.6. Statistical analysis

Statistical analysis was conducted using Fisher's exact test. Differences were considered statistically significant for $p < 0.05$.

3. Results

3.1. Group 1

The results are presented in Table 2. Of the children who showed positivity for antigen-specific IgE antibodies, 31 (7.6%) were positive for house dust mites, 12 (2.9%) for cat fur, and five (1.2%) for JCP. Some children had antibodies for more than one antigen. Therefore, a total of 44 (10.7%) had one or more positive antibodies for these antigens. Among the 29 children (7.1%) who

Table 2

Details of children undergoing an 18-month infant health check (Group 1) who had antigen-specific IgE antibody or nasal eosinophils.

	Antigen-specific IgE				Total
	Mite	Cat	JCP	Negative	
<i>Group 1</i>					
Eosinophils in nasal smear					
Positive	7	1	0	21	29
Negative	24	11	5	343	379
Total	31	12	5	364	408

had nasal eosinophils, eight (2.0%) also had antigen-specific IgE antibody for house dust mite (seven children) or cat fur (one child).

Demographic and social data are presented in Table 3. In 30 children, a pet cat was significantly associated with positivity for specific IgE antibodies to house dust mite, cat fur, and JCP. The same trend was apparent for the presence of any pet (68 children), irrespective of the species. The other hand, a significant correlation was found between the presence of nasal eosinophils and a 'positive paternal history of allergic disease', as well as having three or more siblings.

Intranasal observation revealed rhinorrhea in 125 of the 408 children (30.6%). Among the children who were positive for antigen-specific IgE antibodies, an intranasal observation revealed rhinorrhea in 12 where the antigen was house dust mites, six where it was cat fur, and one where it was JCP.

Table 4 presents details of eight children who had evidence of both sensitization to antigens and positivity for nasal eosinophils. Of these eight children, six (1.5%) were positive for intranasal rhinorrhea, who had definite AR according to the criteria of JG-AR [8]. Of these eight children, four (50%) had close contact with pets. A diagnosis of AR had been made for only one (Case 3) of the eight children described above. The simultaneous questionnaire survey results revealed that 11 children (2.7%) had been diagnosed with AR. However only one of 11 children had sensitization to antigens or none had nasal eosinophils.

Table 3

Demographic and social data—group 1 (*/**/****: there are overlapping cases).

	Number	Antigen-specific IgE positive cases						Eosinophils in nasal smear	
		Mite	P value	Cat	P value	JCP	P value	Positive	P value
Past history of allergic diseases									
No	334	23		9		4		22	
Yes—any allergic diseases*	74	8	0.2340	3	0.4624	1	>.9999	7	0.4509
Yes—atopic dermatitis*	29	1	0.2547	1	0.5698	1	0.3241	3	0.4371
Yes—asthma*	12	2	0.2120	0	>.9999	0	>.9999	3	0.0475
Yes—food allergy *	29	2	0.2616	2	0.2164	0	>.9999	3	0.4371
Family history of allergic diseases									
No	207	13		7		3		12	
Yes—maternal**	136	12	0.4009	4	>.9999	2	>.9999	13	0.2073
Yes—paternal**	125	12	0.2879	3	0.2667	1	>.9999	17	0.0257
Number of brothers (sisters)									
Only one	219	14		8		4		12	
Two	131	13	0.3004	3	0.5467	1	0.6543	9	0.6449
Three or more	58	4	>.9999	1	0.69	0	0.5828	8	0.0429
Passive smoking									
No	181	14		6		3		11	
Yes—by mother	29	2	>.9999	1	>.9999	0	>.9999	3	0.4173
Yes—by other(s)	198	15	>.9999	5	0.7668	2	0.6792	15	0.7668
Pet(s) keeping									
No	340	21		5		2		23	
Yes—any pets***	68	10	0.023	7	0.0011	3	0.0345	6	0.6036
Yes—a cat***	30	6	0.015	7	<.0001	2	0.0345	2	>.9999
Yes—a dog***	40	5	0.1741	0	>.9999	1	0.2844	4	0.5093

Table 4

Details of eight children with antigen sensitization/nasal eosinophils in group 1.

Case	Age (months)	Sex	Antigen specific IgE	Eosinophils in nasal smear	Intranasal observation	Complications and past history of allergic disease	Family history of allergic disease	Siblings (order)	Pet(s)	Passive smoking
1	18	M	Mite	++	+	–	Father, mother	1 (1)	Cat, dog, bird	+
2	18	F	Mite	+++	+	–	Father, mother	1 (1)	Dog	+
3	19	M	Cat	+++	+	Asthma	–	2 (2)	Cat	–
4	19	F	Mite	+	–	Food allergy	–	1 (1)	–	–
5	20	M	Mite	+	+	–	–	2 (2)	Cat	–
6	18	M	Mite	+	+	–	–	2 (2)	–	+
7	18	F	Mite	+	+	–	Mother, sister	3 (3)	–	+
8	19	M	Mite	+	–	–	Father	2 (2)	–	–

3.2. Group 2

Of group 2, 150 (80.6%) had upper respiratory tract infections; 36 (19.4%) had viral infections of the digestive tract. No other severe illness was found or reported. Five children showed positivity for aeroallergen-specific IgE antibodies (2.6%) (Table 5). Among them, three (1.6%) had antibodies against house dust mites; two (1.1%) had antibodies against cat fur. They were classified according to age as follows: 6–11 months—one (0.5%, IgE antibody specific to cat fur), 12–17 months—one (0.5%, IgE antibody specific to house dust mite), and 18–23 months—three (two with IgE antibodies specific to house dust mites [5.3%], and one with IgE antibodies specific to cat fur [2.6%]). The youngest child, nine months old, resided with a cat. Although the presence of cat fur-specific IgE antibodies was confirmed, this infant was negative on intranasal observation and had no personal or family history of allergic disease.

The presence of nasal eosinophils was confirmed in six children (3.2%) (Table 5). These children were classified according to age: 0–5 months—two, 6–11 months—one, 12–17 months—one, and 18–23 months—two. The youngest child, for whom respiratory syncytial virus infection had been diagnosed, was only one month old (data not shown). In group 2, many children had infections, and the percentage of children in whom intranasal observation had detected rhinorrhea was high (63.4%, 118). Intranasal observation for rhinorrhea was positive in all six children with nasal eosinophils. These children were characterized by their 'positive family history of allergic disease' and having 'two or more siblings'; each was the youngest child in the family.

4. Discussion

This study showed that the prevalence of AR in health 18-month-old Japanese children might be at least 1.5% according to the criteria in the JG-AR [8]. Because this survey was performed outside the JCP season, the prevalence could be as high as 6.1% when the following children are included: five who had specific IgE antibodies for JCP, two who were negative for intranasal observation of rhinorrhea despite sensitization to antigens and positivity for nasal eosinophils, and 12 who had a combination of

sensitization to antigens, negative nasal eosinophil test, and positive intranasal rhinorrhea. The incidence of specific IgE antibodies to three antigens (house dust mites, cat fur, and JCP) was as high as 10.7% at the age of 18 months. These findings suggest that the actual prevalence of AR at the age of 18 months might be higher than 1.5%.

Results for group 2 show that no child younger than two years old had either antigen-specific IgE antibodies or nasal eosinophils. Furthermore, the positivity rate for specific IgE antibodies to the three antigens in group 2 was lower than in group 1. Classification of group 2 according to age showed that 38 were 18–23 months old. Because the overall positivity rate for antigen sensitization among group 1 was estimated as 10.7%, the corresponding incidence in group 2 would be approximately 10% of 38: four children. In fact, three were positive in the age group of 18–23 months (7.9%). Consequently, the positivity rates for sensitization to antigens are equivalent for group 1 and group 2.

Regarding previously reported epidemiological studies of AR in children younger than three years of age, the GINA study in Germany revealed that the positivity rate for sensitization to common inhaled antigens among 1290 infants aged 12 months was 2.3% [6]. In the Netherlands, 5.9% of 751 infants aged 12–24 months were sensitized [5]. According to a survey conducted on 562 infants aged 18 months in Denmark, persistent sensitization was noted in 10%, and 3% had a positive skin prick test [10]. Taken together with the results of the present study, these results indicate no difference in the positivity rate for sensitization to antigens or the prevalence of AR between Europe and Japan, as judged by the skin prick test.

Many previous investigations of infantile AR have been based solely on questionnaire surveys for the parents. Their reliability is open to question. According to this questionnaire survey, 11 children (2.7%) had a history of AR that had been diagnosed by a medical practitioner. Although this prevalence is intermediate between the percentages of 1.5% and 6.1% found in this study, sensitization to antigens was confirmed in only one of the 11 children. Furthermore, none of the 11 children had nasal eosinophils. These findings indicate that the diagnoses of AR in infants younger than two years old by medical practitioners or based on reports of AR from the parents are not accurate. Under the age of two years, reports of upper respiratory tract infections might be excessive in many cases because it is impossible for infants to describe their symptoms reliably.

In this study, nasal eosinophils were counted. Nasal eosinophil measurement has a long history as an important parameter for diagnosis of AR: its use has been reported since the 1970s. Malmberg reported that eosinophilia in nasal smears was correlated significantly with a history of AR, nasal mucosal swelling, and nasal discharge [11]. Thereafter, much work has been done to assess the usefulness of assessing nasal eosinophils in the diagnosis of AR. For example, Zeiger and Heller performed a prospective study of infants with a strong family history of allergic disease and showed that eosinophils were present at higher levels

Table 5

Details of children to hospital (group 2) who had antigen-specific IgE antibody or nasal eosinophils.

	Antigen-specific IgE				Total
	Mite	Cat	JCP	Negative	
Group 2					
Eosinophils in nasal smear					
Positive	0	0	0	6	6
Negative	3	2	0	175	180
Total	3	2	0	181	186

in the nasal scrapings of allergic children between birth and four years of age, but were uncommon in children without allergic disease [12]. Crobach et al. reported that nasal smear eosinophilia were significantly associated with the diagnosis of AR, but the contribution of this parameter was small and clinically irrelevant [13]. Because of such difficulty in interpreting the clinical significance of nasal smear eosinophils, this test is not often performed for the diagnosis of AR. However, reviews of the importance of nasal eosinophilia in AR have confirmed that the eosinophil count is correlated with various symptoms and with an immunological marker. Therefore, nasal eosinophil measurement is useful [14–16].

In this study, 29 children of group 1 nasal eosinophils were positive (7.1%). Nasal eosinophilia was found in two of 46 children (12–17 months in group 2, 4.3%) and two of 38 children (18–23 months in group 2, 5.3%). We expected that positive rate of nasal eosinophils in group 2 would be higher than that of group 1 because viral and bacterial infection in the respiratory tract will induce eosinophils in nasal mucosa. The association between nasal eosinophils and the respiratory tract infection is now under investigation. Furthermore, for respiratory syncytial virus (RSV) infection, eosinophils appear in infantile rhinorrhea [16]. However, recently reports suggested local allergic rhinitis; allergic rhinitis with skin test and serum specific IgE negative [17,18]. Rondón et al. revealed local allergic rhinitis patients had local production of specific IgE and eosinophilic cationic protein induced by allergens [18]. These findings suggest that detecting the combination of specific IgE antibodies to inhaled antigens and nasal eosinophils supports a diagnosis of AR.

Demographic and social analyses of this study revealed that sensitization to inhaled antigens is correlated with the presence of pets, especially cats, which agrees with reports from western countries [19–21]. In contrast nasal eosinophilia was correlated in the present study with a family history (paternal) of AR and with the number of siblings. No comparisons can be made because of the paucity of literature describing nasal eosinophilia in infants, the influence of a family history of allergic disease, or the number of siblings [22]. It has been reported that nasal eosinophils even appear in infants with food allergy [22]. However, there was no significant correlation between the presence of specific IgE/nasal eosinophilia and a positive history of food allergy (Table 3).

Our aim in this study is to estimate the minimum prevalence rate of AR in Japanese children under two years old. The minimum prevalence of AR is 1.5% in Japanese healthy 18-month-old children, although the maximum prevalence of AR might be 6–10%. Results show that AR can be assumed to occur from the age of 18 months onwards. Therefore, it is necessary to establish diagnostic criteria for AR in children younger than two years old to assist with prevention by early intervention and early diagnosis.

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References

- [1] M. Sakashita, T. Hirota, M. Harada, R. Nakamichi, T. Tsunoda, Y. Osawa, et al., Prevalence of allergic rhinitis and sensitization to common aeroallergens in a Japanese population, *Int Arch Allergy Immunol* 151 (2009) 255–261.
- [2] S. Illi, E. von Mutius, S. Lau, R. Nickel, C. Grüber, B. Niggemann, et al., The natural course of atopic dermatitis from birth to age 7 years and association with asthma, *J Allergy Clin Immunol* 113 (2004) 925–931.
- [3] P.A. Pedersen, E.R. Weeke, Asthma and allergic rhinitis in the same patients, *Allergy* 38 (1983) 25–29.
- [4] R.J. Settipane, G.W. Hagy, G.A. Settipane, Long-term risk factors for developing asthma and allergic rhinitis: a 23-year follow-up study of college students, *Allergy Proc* 15 (1994) 21–25.
- [5] A. Baatenburg de Jong, L.D. Dikkeschei, P.L.P. Brand, High prevalence of sensitization to aeroallergens in children 4 yrs of age or younger with symptoms of allergic disease, *Pediatr Allergy Immunol* 20 (2009) 735–740.
- [6] I. Brockow, A. Zutavern, U. Hoffmann, A. Gröbl, A. von Berg, S. Koletzko, et al., Early allergic sensitizations and their relevance to atopic diseases in children aged 6 years: results of GINI study, *J Investig Allergol Clin Immunol* 19 (2009) 180–187.
- [7] J. Bousquet, N. Khaltaev, A.A. Cruz, J. Denburg, W.J. Fokkens, A. Togias, et al., Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen), *Allergy* 63 (Suppl. 86) (2008) 8–160.
- [8] K. Okubo, Y. Kuroki, S. Fujieda, S. Ogino, E. Uchio, H. Odajima, et al., Japanese guideline for allergic rhinitis, *Allergol Int* 60 (2) (2011) 171–189.
- [9] T. Ono, M. Kawamura, S. Arai, H. Nariuchi, A highly sensitive quantitative immunochromatography assay for antigen-specific IgE, *J Immunol Methods* 272 (2003) 211–218.
- [10] H. Jøhnke, L.A. Norberg, W. Vach, A. Høst, K.E. Andersen, Patterns of sensitization in infants and its relation to atopic dermatitis, *Pediatr Allergy Immunol* 17 (2006) 591–600.
- [11] H. Malmberg, Symptoms of chronic and allergic rhinitis and occurrence of nasal secretion granulocytes in university students, school children and infants, *Allergy* 34 (1979) 389–394.
- [12] R.S. Zeiger, S. Heller, Development of nasal basophilic cells and nasal eosinophils from age 4 months through 4 years in children of atopic parents, *J Allergy Clin Immunol* 91 (1993) 723–734.
- [13] M.J. Crobach, J. Hermans, A.A. Kaptein, J. Ridderikhoff, H. Petri, J.D. Mulder, The diagnosis of allergic rhinitis: how to combine the medical history with the results of radioallergosorbent tests and skin prick tests, *Scand J Prim Health Care* 16 (1998) 30–36.
- [14] G. Ciprandi, A. Vizzaccaro, I. Cirillo, M. Tosca, A. Massolo, G. Passalacqua, Nasal eosinophils display the best correlation with symptoms, pulmonary function and inflammation in allergic rhinitis, *Int Arch Allergy Immunol* 136 (2005) 266–272.
- [15] S.T. Chen, H.L. Sun, K.H. Lu, K.H. Lue, M.C. Chou, Correlation of immunoglobulin E, eosinophil cationic protein, and eosinophil count with the severity of childhood perennial allergic rhinitis, *J Microbiol Immunol Infect* 39 (2006) 212–218.
- [16] M. Shinohara, H. Wakiguchi, H. Saito, K. Matsumoto, Presence of eosinophils in nasal secretion during acute respiratory tract infection in young children predicts subsequent wheezing within two months, *Allergol Int* 57 (2008) 359–365.
- [17] C. Rondón, J.J. Romero, S. López, C. Antúnez, E. Martín-Casañes, M.J. Torres, et al., Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis, *J Allergy Clin Immunol* 119 (4) (2007) 899–905.
- [18] C. Rondón, J. Fernández, S. López, P. Campo, I. Doña, M.J. Torres, et al., Nasal inflammatory mediators and specific IgE production after nasal challenge with grass pollen in local allergic rhinitis, *J Allergy Clin Immunol* 124 (5) (2009) 1005–1011.
- [19] G. Anyo, B. Brunekreef, G. de Meer, F. Aarts, N.A. Janssen, P. van Vliet, Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children, *Clin Exp Allergy* 32 (2002) 361–366.
- [20] J.C. Celedon, A.A. Litonjua, L. Ryan, T. Platts-Mills, S.T. Weiss, D.R. Gold, Exposure to cat allergen, maternal history of asthma, and wheezing in first 5 years of life, *Lancet* 360 (2002) 781–782.
- [21] B. Holscher, C. Frye, H.E. Wichman, J. Heinrich, Exposure to pet and allergies in children, *Pediatr Allergy Immunol* 13 (2002) 334–341.
- [22] H. Shioda, T. Mishima, The significance of mast cells in nasal smears from patients with food allergy, *Allergy* 37 (1966) 321–328.