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ORIGINAL ARTICLE

Symptoms of Allergic Rhinitis in Women during Early Pregnancy Are Associated with Higher Prevalence of Allergic Rhinitis in Their Offspring

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ABSTRACT

Background: Epigenetic control of gene expression profiles is a ubiquitous mechanism during cell differentiation, organogenesis and chronic inflammatory reactions. Recent studies have shown that allergen exposure during very early pregnancy increases bronchial hypersensitivity in offspring in a murine model of bronchial asthma. However, no such phenomena were reported in humans. In the present study, the role of epigenetic control in the onset of allergic diseases was investigated.

Methods: A total of 400 pairs of mothers with physician-diagnosed allergic rhinitis (AR) and their offspring (age 7–18 months) who participated in a large-scale medical check-up were enrolled in this retrospective cohort study. Family history of allergic diseases and the presence or absence of AR symptoms during pregnancy were inquired about using a self-answered questionnaire. A logistic regression model adjusted for age, gender, birth month and father's history of allergic diseases was statistically analyzed.

Results: Offspring whose mothers had any AR symptoms during early pregnancy showed a significantly higher adjusted odds ratio for the onset of AR in offspring than those whose mothers had no symptoms during pregnancy (adjusted Odds Ratio: 6.26, $p = 0.036$). However, the symptoms of AR during late pregnancy showed no effects on the odds ratio. In contrast, the presence or absence of AR symptoms during early or late pregnancy showed no association with the prevalence of food allergy, atopic dermatitis or asthma in offspring.

Conclusions: Our results suggest the presence of possible epigenetic mechanisms regulating the onset of AR in humans presumably through increased organ-specific hypersensitivity.

KEY WORDS

allergic rhinitis, epigenetics, offspring, pregnancy, symptoms

INTRODUCTION

During the ontogenesis of multicellular organisms, a single cell proliferates and differentiates into many different cell types each with a unique function and gene expression pattern. This fact clearly indicates that additional information beyond that generated by the genetic sequence must be present in the generation of the diversity of genomic expression, because all somatic cells in a single organism possess an identical set of chromosomes with identical sequences.

Epigenetics is the term used to describe such meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself.[1] The molecular mechanisms by which gene expression is epigenetically regulated are explained by DNA methylation and chromatin modifications, including histone acetylation, methylation, ubiquitination, sumoylation and phosphorylation.[2] Epigenetic regulation is not only critical for generating diversity of cell types during mammalian development, but is also important for maintaining the stability and integrity of the expression profiles of different cell types.[1] In this respect, disruption of epigenetic control leads

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to several major pathologies, including cancer and syndromes involving chromosomal instabilities.[3] It is also known that several mechanical stresses, such as radiation, malnutrition, and exposure to certain drugs and smoking can induce epigenetic changes in humans.[4] Thus, epigenetic changes are potential targets for therapeutic interventions.[2]

In chronic inflammatory diseases such as bronchial asthma and allergic rhinitis (AR),[5] chronic exposure to certain cytokines or chronic inflammation itself can also induce epigenetic changes in cells in target tissues.[6,7] In most cases, such changes support the perpetuation of chronic inflammatory reactions and might lead to resistance against therapeutic agents such as corticosteroids.[8]

On the other hand, intrauterine events can also affect offspring development through epigenetic mechanisms. For instance, maternal nutrition during pregnancy is reported to be associated with the onset of metabolic syndromes in adult offspring.[9] Recently, Hamada *et al*. reported that maternal exposure to allergens during very early pregnancy in a mouse model of bronchial asthma significantly increased bronchial hypersensitivity and allergic inflammation in offspring.[10] However to date, no report has presented clear evidence that allergic symptoms of mothers during pregnancy affect the onset of allergic diseases in human offspring. In the present study, we attempted to clarify whether or not such an epigenetic control mechanism is present and involved in the onset of AR in the offspring.

METHODS

SUBJECTS

A total of 400 pairs of mothers with physician-diagnosed AR and their offspring (187 boys and 211 girls, age 1.7–18.7 months) who participated in a large-scale medical check-up was enrolled in this retrospective cohort study. Mothers were enrolled only if the guardian's answer to the question, "Has the mother of the child ever received a diagnosis of allergic rhinitis by a doctor?" was "Yes". Along with the age and the gender of the offspring, paternal history of allergic diseases and the presence or absence of the symptoms of AR in parents during pregnancy were inquired about using a self-answered questionnaire. The presence of AR symptoms during pregnancy was based on the guardian's response to the question, "Has the mother of the child showed any symptoms of allergic rhinitis during the pregnancy?" In this study, the first and the second half of the gestation period was considered to be early and late pregnancy, respectively. The primary outcome measure was the presence of physician-diagnosed allergic diseases in the offspring. The diagnosis of AR in the offspring was made only if the guardian's answer to the question, "Has your child ever received a diagnosis of allergic rhinitis by a doctor?" was "Yes". The prevalence of other allergic disease was also determined similarly. This study was approved by the Ethics Review Board of Kochi Medical School.

STATISTICS

A logistic regression model adjusted for age, gender, birth month of the offspring and paternal history of allergic diseases was analyzed using STATA software (StataCorp LP, College Station, TX, USA) and considered to be significant if $p < 0.05$.

RESULTS

DESCRIPTIVE CHARACTERISTICS OF THE OFFSPRING

Initially, the offspring enrolled in this study were divided into three groups in the context of the presence or absence of symptoms of AR in their mothers during pregnancy. The descriptive characteristics of the three groups were compared (Table 1). The male/female proportion did not differ among these three groups ($p = 0.681$). However, age and the prevalence of a paternal history of allergic diseases were not the same in these three groups ($p = 0.010$ and 0.005, respectively). In addition, the month of birth differed significantly among these three groups ($\chi^2 = 18.95$, $p = 0.0001$). In particular, the month of birth of offspring whose mothers had symptoms of AR during early pregnancy was not unimodal throughout the year, the frequency being higher in the September to November period.

ASSOCIATION BETWEEN THE SYMPTOMS OF AR IN MOTHERS DURING PREGNANCY AND THE PREVALENCE OF AR IN THEIR OFFSPRING

In order to determine the association between symptoms of AR in mothers during pregnancy and the prevalence of AR in their offspring, detailed information was obtained from all subjects. According to the differences in background shown in Table 1, a logistic regression model adjusted for age, gender, birth month of the offspring and paternal history of allergic diseases was analyzed (Table 2). The presence of symptoms of AR in mothers during early pregnancy was associated with a significantly higher prevalence of AR in their offspring (adjusted odds ratio: 6.332, 95% CI: 1.134–35.360, $p = 0.035$). In contrast, no such association was found between symptoms of AR in mothers during late pregnancy and the prevalence of AR in their offspring (adjusted odds ratio: 0.476, 95% CI: 0.115–1.976, $p = 0.307$). Some mothers had AR symptoms during both early and late pregnancy. In the offspring of these mothers, the prevalence of AR was not high enough to reach statistical significance (adjusted OR: 1.472, 95% CI: 0.398–5.448). This result is reasonable because only symptoms during early pregnancy, and not late pregnancy, were significantly correlated with a higher prevalence of AR in the offspring.

Table 1 Descriptive characteristics of offspring enrolled in this study

Confounding Factors	Symptoms of Allergic Rhinitis during Pregnancy in Mothers				p value
	Total	None	Early	Late	
	Data † (*n* = 400)	Data † (*n* = 150)	Data † (*n* = 219)	Data † (*n* = 173)	
Age (mo) ‡	9.9 (1.7－18.7)	10.7 (2.7－18.7)	9.0 (1.7－18.4)	9.7 (2.6－18.0)	0.010*
Gender §					
Male	187 (47.0)	67 (44.7)	105 (48.4)	76 (44.4)	0.681
Female	211 (53.0)	83 (55.3)	112 (51.6)	95 (55.6)	
Month of Birth §					
January	36 (9.1%)	19 (12.8%)	16 (7.3%)	13 (7.6%)	0.0001*
February	24 (6.0%)	14 (9.4%)	7 (3.2%)	8 (4.7%)	
March	24 (6.0%)	7 (4.7%)	12 (5.5%)	15 (8.7%)	
April	24 (6.0%)	8 (5.4%)	13 (6.0%)	15 (8.7%)	
May	32 (8.0%)	11 (7.4%)	11 (5.0%)	20 (11.6%)	
June	29 (7.3%)	14 (9.4%)	11 (5.0%)	12 (7.0%)	
July	30 (7.5%)	11 (7.4%)	18 (8.3%)	13 (7.6%)	
August	29 (7.3%)	8 (5.4%)	18 (8.3%)	14 (8.1%)	
September	37 (9.3%)	10 (6.7%)	27 (12.4%)	14 (8.1%)	
October	44 (11.1%)	15 (10.1%)	29 (13.3%)	14 (8.1%)	
November	47 (11.8%)	15 (10.1%)	32 (14.7%)	16 (9.3%)	
December	42 (10.6%)	17 (11.4%)	24 (11.0%)	18 (10.5%)	
Paternal History of Allergic Diseases §					
No	187 (46.8)	56 (37.3)	112 (51.1)	94 (54.3)	0.005*
Yes	213 (53.3)	94 (62.7)	107 (48.9)	79 (45.7)	

† Mean value or number of offspring, and percent, range or SD.

‡ Kruskal-Wallis test.

§ χ^2 test. * $p < 0.05$.

Table 2 Association of the prevalence of allergic rhinitis in offspring with allergic rhinitis symptoms in mothers during pregnancy

Allergic Rhinitis (*n* = 10) Total (*n* = 400)	Allergic Rhinitis Yes/No	(%)	OR	(95% CI)	aOR †	(95% CI)	p value
Age			1.004	(0.999－1.009)	1.005	(1.000－1.010)	0.051
Gender							
Male	7/180	(3.7)	1.000		1.000		
Female	3/208	(1.4)	0.371	(0.095－1.455)	0.416	(0.103－1.672)	0.226
Month of Birth			0.985	(0.826－1.174)	0.992	(0.832－1.184)	0.932
Paternal History of Allergic Diseases							
No	4/183	(2.1)	1.000		1.000		
Yes	6/207	(2.8)	1.326	(0.368－4.772)	3.027	(0.611－15.010)	0.175
Symptoms of Allergic Rhinitis in Mothers							
During Pregnancy							
None	2/148	(1.3)	1.000		1.000		
Anytime	8/242	(3.2)	2.446	(0.513－11.675)	3.204	(0.638－16.101)	0.157
Early Pregnancy							
No	2/179	(1.1)	1.000		1.000		
Yes	8/211	(3.7)	3.393	(0.711－16.185)	6.332	(1.134－35.360)	0.035 *
Late Pregnancy							
No	6/221	(2.6)	1.000		1.000		
Yes	4/169	(2.3)	0.872	(0.242－3.138)	0.476	(0.115－1.976)	0.307

† Adjusted odds ratio and 95% confidence intervals for allergic rhinitis in offspring were calculated by logistic regression analysis after adjustment for age, gender, paternal history of allergic diseases and month of birth.

* $p < 0.05$.

Table 3 Associations of the prevalence of other allergic diseases in offspring with the symptoms of allergic rhinitis in mothers during pregnancy

Allergic Disease of Offspring Total (n = 400)	Allergic Diseases Yes/No	(%)	OR	(95% CI)	aOR †	(95% CI)	p value
Bronchial Asthma							
Symptoms of Allergic Rhinitis in Mothers							
During Pregnancy							
None	3/147	(2.0)	1.000		1.000		
Anytime	3/247	(1.2)	0.595	(0.119 – 2.987)	1.023	(0.190 – 5.496)	0.979
Early Pregnancy							
No	3/178	(1.7)	1.000		1.000		
Yes	3/216	(1.4)	0.824	(0.164 – 4.133)	2.399	(0.283 – 20.317)	0.422
Late Pregnancy							
No	4/223	(1.8)	1.000		1.000		
Yes	2/171	(1.2)	0.652	(0.118 – 3.602)	0.419	(0.042 – 4.189)	0.459
Food Allergy							
Symptoms of Allergic Rhinitis in Mothers							
During Pregnancy							
None	7/143	(4.7)	1.000		1.000		
Anytime	15/235	(6.0)	1.304	(0.519 – 3.275)	1.788	(0.720 – 4.442)	0.211
Early Pregnancy							
No	9/172	(5.0)	1.000		1.000		
Yes	13/206	(5.9)	1.206	(0.503 – 2.889)	1.617	(0.555 – 4.714)	0.379
Late Pregnancy							
No	11/216	(4.8)	1.000		1.000		
Yes	11/162	(6.4)	1.333	(0.564 – 3.151)	1.104	(0.383 – 3.180)	0.854
Atopic Dermatitis							
Symptoms of Allergic Rhinitis in Mothers							
During Pregnancy							
None	7/143	(4.7)	1.000		1.000		
Anytime	20/230	(8.0)	1.776	(0.733 – 4.307)	0.559	(0.225 – 1.390)	0.211
Early Pregnancy							
No	8/173	(4.4)	1.000		1.000		
Yes	19/200	(8.7)	2.054	(0.877 – 4.810)	2.099	(0.776 – 5.681)	0.144
Late Pregnancy							
No	14/213	(6.2)	1.000		1.000		
Yes	13/160	(7.5)	1.236	(0.565 – 2.702)	0.921	(0.374 – 2.270)	0.858

† Adjusted odds ratio and 95% confidence intervals for other allergic diseases in offspring were calculated by logistic regression analysis after adjustment for age, gender, paternal history of allergic diseases and month of birth.

To confirm the validity of the logistic regression model, we determined the post-estimation goodness-of-fit parameter for this logistic regression model using the Hosmer-Lemeshow goodness-of-fit test and found the model to be valid (p = 0.5027). Thus, we are convinced of the statistical significance of this study, even though the number of offspring with AR was relatively small.

In addition, the effect of maternal smoking and the exposure of the mothers to passive smoking during pregnancy was included in the present logistic regression model. Maternal smoking and the exposure of the mothers to passive smoking during pregnancy is known to be a strong confounding factor for the onset of AR[11]; however, even after these factors were considered, the adjusted odds ratio was virtually unchanged (data not shown).

ASSOCIATION BETWEEN THE SYMPTOMS OF AR IN MOTHERS DURING PREGNANCY AND THE PREVALENCE OF OTHER ALLERGIC DISEASES IN THEIR OFFSPRING

Just as in the aforementioned analysis, the association between symptoms of AR in mothers during pregnancy and the prevalence of other allergic diseases in their offspring was determined (Table 3). No significant association was observed between symptoms of AR in mothers during either early or late

Table 4 Association of the prevalence of allergic rhinitis in offspring with the symptoms of allergic rhinitis in fathers during pregnancy

Allergic Rhinitis (n = 10) Total (n = 400)	Allergic Rhinitis Yes/No	(%)	OR	(95% CI)	aOR †	(95% CI)	p value
Age			1.004	(0.999 – 1.009)	1.004	(0.999 – 1.001)	0.114
Gender							
Male	7/180	(3.7)	1.000		1.000		
Female	3/208	(1.4)	0.371	(0.095 – 1.455)	0.429	(0.108 – 1.715)	0.232
Birth Months			0.985	(0.826 – 1.174)	0.987	(0.832 – 1.171)	0.879
Symptoms of Allergic Rhinitis in Fathers							
During Pregnancy							
None	8/286	(2.7)	1.000		1.000		
Anytime	2/104	(1.9)	0.688	(0.144 – 3.290)	0.563	(0.100 – 3.151)	0.513
Early Pregnancy							
No	8/300	(2.6)	1.000		1.000		
Yes	2/90	(2.2)	0.833	(0.174 – 3.995)	2.609	(0.426 – 15.989)	0.300
Late Pregnancy							
No	10/309	(3.1)	1.000		1.000		
Yes	0/81	(0.0)	/	/	/	/	/

† Adjusted odds ratio and 95% confidence intervals for allergic rhinitis in offspring were calculated by logistic regression analysis after adjustment for age, gender and month of birth.

pregnancy and the prevalence of either physician-diagnosed bronchial asthma, food allergy or atopic dermatitis in their offspring.

ASSOCIATION BETWEEN THE SYMPTOMS OF AR IN FATHERS DURING PREGNANCY AND THE PREVALENCE OF AR IN THEIR OFFSPRING

Just as in the aforementioned two analyses, the association between symptoms of AR in fathers during pregnancy and the prevalence of AR in their offspring was determined (Table 4). No significant association was observed between symptoms of AR in fathers during either early or late pregnancy and the prevalence of AR in their offspring. In addition, there was no significant association between symptoms of AR in fathers during either early or late pregnancy and the prevalence of physician-diagnosed bronchial asthma, food allergy or atopic dermatitis in their offspring (data not shown).

DISCUSSION

Epigenetic control of gene expression profiles is a ubiquitous mechanism during cell differentiation, organogenesis and chronic inflammatory reactions.[1] A recent study showed that allergen exposure during very early pregnancy increased bronchial hypersensitivity and allergic inflammation in offspring in a murine model of bronchial asthma,[10] but no such phenomena have been reported in humans. In the present study, mothers with physician-diagnosed AR, from whom detailed information about their pregnancy and offspring could be obtained, were enrolled.

Before starting the statistical analysis, we carefully considered the descriptive characteristics of the offspring who were divided into three groups in the context of the presence or absence of symptoms of AR in their mothers during pregnancy (Table 1). These characteristics are known to be common confounding factors for the onset of allergic diseases.[12,13] The gender ratio of offspring did not differ among these three groups. However, age and the prevalence of the paternal history of allergic diseases were not the same in these three groups. In addition, the month of birth also differed among them, presumably because the season for the major allergen of rhinitis, Japanese Cedar pollen, is exclusively March to May.[14,15] In contrast, the frequency of offspring whose mothers had symptoms during late pregnancy was slightly higher in March to May, as expected. In order to eliminate the effect of the month of birth and so on, these factors were considered and adjusted for in our logistic regression model. In other words, our statistical analysis allowed us to determine the effect of symptoms of the mothers free of the influence of the difference in birth month profile.

After consideration of these confounding factors, we found that the presence of symptoms of AR in mothers during early but not late pregnancy was significantly associated with a higher prevalence of AR in their offspring (Table 2). The fact that the positive association was found only in the offspring with symptoms during early pregnancy, *i.e.* not in those with symptoms during late pregnancy, strongly suggests that this association is not due simply to the severity of the mothers' rhinitis or genetic predisposition but rather to some mechanisms operating spe-

cifically during early pregnancy, which is the time when organogenesis is being undertaken. It was previously reported that allergen exposure just before mating was critical to producing increased bronchial hyperreactivity of offspring in a mouse model of asthma, while exposure during late pregnancy showed no effect.[10] Our findings are highly compatible with this previous report and emphasize that the maternal allergic reactions during very early pregnancy are critical.

Note that symptoms of AR in mothers correlated only with a higher prevalence of AR, not with those of other allergic diseases such as bronchial asthma, food allergy or atopic dermatitis in their offspring (Table 3). It is now well-understood that both atopy (hyper secretion of antigen-specific IgE) and organ-specific hypersensitivity regulate the onset and phenotype of allergic diseases.[16] Together with this observation, our results suggest that the symptoms of AR in mothers during early pregnancy do not influence IgE production in their offspring but rather their organ-specific (nasal) hypersensitivity. In fact, T cells from fetuses acquire the ability to mount a proliferative response to a common allergic trigger (β-lactoglobulin, house dust mite, etc) only after 22 weeks of pregnancy.[17] Thus, T cells may not be the target of this effect or T cells may not even exist during this period in the fetus.

In order to confirm that the positive association we found is mother-specific, we also tested the effect of paternal symptoms. The fathers' symptoms of AR during either early or late pregnancy showed no correlation with the prevalence of AR in their offspring (Table 4). Thus, this effect does not reflect simply the transfer of genetic predisposition from the parents or the dose of allergen exposure during that period, but rather is maternal symptom-specific.

Taken together, our findings imply the presence of possible mechanisms that can transfer susceptibility to AR or organ-specific hypersensitivity from pregnant women to their offspring when AR symptoms occur during early, but not late, pregnancy. The data suggest that this phenomenon cannot simply be explained by genetic transfer of the allergic predisposition from the parent to the offspring. Some intrauterine events have actually been reported to be associated with the onset of allergic diseases in offspring.[18] However, the time of exposure and the target of the effect clearly differ from the observations made in this study. Our results taken together thus support the presence of epigenetic control of gene expression profiles in susceptibility to AR in offspring rather than a simple transfer of genetic predispositions.

Recent studies of the molecular basis of epigenetics have shown that DNA methylation and chromatin modifications, including histone acetylation, methylation, ubiquitination, sumoylation and phosphorylation are the main mechanisms underlying such phenomena.[2] However, which gene loci are selectively modulated or how such loci are selected is essentially unknown. Hamada *et al*. have suggested that premating treatment with neutralizing anti-IL-4 antibody abrogated the maternal effect.[10] However, it is as yet unknown whether IL-4 critically regulates allergic symptoms directly and thus epigenetic transfer was inhibited or epigenetic modulation of the IL-4 gene locus is critical. On the other hand, it was reported that administration of IFN-γ to the pregnant female mouse during middle pregnancy (gestation day 6.5) diminished the Th2 immune responses in their offspring.[19] In that case, IFN-γ administration is likely to affect immune cell generation in the offspring directly or indirectly, and thus the time of exposure and the target of modification appear to be critically different from those in our study. In the present study, genes or loci responsible for the transfer of the higher prevalence of AR or nasal hypersensitivity are very likely to be involved. However, identification of the genes or loci requires further investigations.

It has been widely reported that the prevalence of several allergic diseases differs depending on the birth month of the subjects in several countries.[20-22] Such differences in allergic disease prevalence or sensitization to seasonal allergens were explained by the immature immune responses of infants with high perinatal exposure to allergens. However, recent intervention studies have suggested that the dose of allergen exposure prenatally or during early infancy has only marginal effects on the allergy sensitization of the offspring.[23,24] Together with our results, these findings suggest that maternal allergic symptoms during early pregnancy, rather than early-life exposure to the allergen, might be a more important confounding factor. In addition, in Japan the prevalence of AR is reportedly higher in subjects born in autumn to winter.[14,25] Our results confirmed this tendency (Table 1) and in addition, imply the involvement of an epigenetic effect of maternal exposure to the allergen in this tendency.

In the present study, only young children, less than two years of age, were enrolled. It is unknown whether this influence might be enhanced or diminished by the interaction with environmental factors later in life. Though the number is not large, we found for the first time in humans a statistically significant correlation between the symptoms of mothers and a higher prevalence of AR in their offspring.

This study has some limitations. First, the diagnosis of AR was questionnaire-based, and no laboratory data, including the total IgE or allergen-specific IgE titers, were measured. Second, the pregnancy period was divided into only two groups because the number of offspring given a diagnosis of AR was relatively small. Further detailed analysis will be necessary to specify the critical period during pregnancy. Finally, as this is a retrospective cohort study, a controlled in-

tervention study should also be performed in the near future.

Our results suggest not only the presence of epigenetic control in the onset of allergic diseases in humans but also suggest the clinical importance of aggressive control of AR symptoms in women during early pregnancy; the best strategy being allergen avoidance.[26,27]

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REFERENCES

1. Cheung P, Lau P. Epigenetic regulation by histone methylation and histone variants. *Mol. Endocrinol.* 2005;**19**: 563-573.
2. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;**429**:457-463.
3. Adcock IM, Ford P, Barnes PJ, Ito K. Epigenetics and airways disease. *Respir. Res.* 2006;**7**:21.
4. Moodie FM, Marwick JA, Anderson CS *et al*. Oxidative stress and cigarette smoke alter chromatin remodeling but differentially regulate NF-kappaB activation and proinflammatory cytokine release in alveolar epithelial cells. *FASEB J.* 2004;**18**:1897-1899.
5. Nagai H. Immunopharmacological approach to elucidating the mechanism of allergic inflammation. *Allergol. Int.* 2005;**54**:251-261.
6. Ito K, Ito M, Elliott WM *et al*. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N. Engl. J. Med.* 2005;**352**:1967-1976.
7. Barnes PJ, Adcock IM, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur. Respir. J.* 2005;**25**:552-563.
8. Roth M, Johnson PR, Borger P *et al*. Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N. Engl. J. Med.* 2004;**351**:560-574.
9. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol. Rev.* 2005;**85**:571-633.
10. Hamada K, Suzaki Y, Goldman A *et al*. Allergen-independent maternal transmission of asthma susceptibility. *J. Immunol.* 2003;**170**:1683-1689.
11. Miyake Y, Miyamoto S, Ohya Y *et al*. Association of active and passive smoking with allergic disorders in pregnant Japanese women: baseline data from the Osaka Maternal and Child Health Study. *Ann. Allergy Asthma Immunol.* 2005;**94**:644-651.
12. Peroni DG, Piacentini GL, Alfonsi L *et al*. Rhinitis in preschool children: prevalence, association with allergic diseases and risk factors. *Clin. Exp. Allergy* 2003;**33**:1349-1354.
13. Platts-Mills TA, Erwin E, Heymann P, Woodfolk J. Is the hygiene hypothesis still a viable explanation for the increased prevalence of asthma? *Allergy* 2005;**60**:s25-31.
14. Saitoh Y, Dake Y, Shimazu S *et al*. Month of birth, atopic disease, and atopic sensitization. *J. Investig. Allergol. Clin. Immunol.* 2001;**11**:183-187.
15. Enomoto T, Onishi S, Sogo H *et al*. Japanese cedar pollen in floating indoor house dust after a pollinating season. *Allergol. Int.* 2004;**53**:279-285.
16. Kurukulaaratchy RJ, Matthews S, Arshad SH. Defining childhood atopic phenotypes to investigate the association of atopic sensitization with allergic disease. *Allergy* 2005;**60**:1280-1286.
17. Warner JA. Primary sensitization in infants. *Ann. Allergy Asthma Immunol.* 1999;**83**:426-430.
18. Jones CA, Holloway JA, Popplewell EJ *et al*. Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J. Allergy Clin. Immunol.* 2002;**109**:858-866.
19. Lima C, Souza VM, Faquim-Mauro EL *et al*. Modulation of the induction of lung and airway allergy in the offspring of IFN-gamma-treated mother mice. *J. Immunol.* 2005; **175**:3554-3559.
20. Yoo Y, Yu J, Kang H, Kim DK, Koh YY, Kim CK. Birth month and sensitization to house dust mites in asthmatic children. *Allergy* 2005;**60**:1327-1330.
21. Chew FT, Goh DY, Teo J, Quak SH, Lee BW. Month of birth and childhood atopic diseases in the tropics. *Allergy* 1998;**53**:962-968.
22. Kusunoki T, Asai K, Harazaki M, Korematsu S, Hosoi S. Month of birth and prevalence of atopic dermatitis in schoolchildren: dry skin in early infancy as a possible etiologic factor. *J. Allergy Clin. Immunol.* 1999;**103**:1148-1152.
23. Horak F, Jr., Matthews S, Ihorst G *et al*. Effect of mite-impermeable mattress encasings and an educational package on the development of allergies in a multinational randomized, controlled birth-cohort study—24 months results of the Study of Prevention of Allergy in Children in Europe. *Clin. Exp. Allergy* 2004;**34**:1220-1225.
24. Woodcock A, Lowe LA, Murray CS *et al*. Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. *Am. J. Respir. Crit. Care Med.* 2004;**170**:433-439.
25. Kusunoki T, Korematsu S, Nakahata T, Hosoi S. Cedar pollinosis in Japanese schoolchildren: results from a large questionnaire-based survey. *Arerugi* 2002;**51**:15-19.
26. Arshad SH. Primary prevention of asthma and allergy. *J. Allergy Clin. Immunol.* 2005;**116**:3-14.
27. Liccardi G, Cazzola M, Walter Canonica G, Passalacqua G, D'Amato G. New insights in allergen avoidance measures for mite and pet sensitized patients. A critical appraisal. *Respir. Med.* 2005;**99**:1363-1376.