

IgE in unselected like-sexed monozygotic and dizygotic twins at birth and at 6 to 9 years of age: High but dissimilar genetic influence on IgE levels

Hans Peter Jacobsen, MD,^a Anne Maria Herskind, MD, PhD,^a Bent Windelborg Nielsen, MD, PhD,^b and Steffen Husby, MD, PhD^a Odense and Aarhus, Denmark

Background: IgE is a major determinant of allergic disease. Twin analysis of serum levels of IgE has been carried out previously in children and adults with heritability estimates of 30% to 70% on the basis of ANOVA.

Objective: This study included the analysis of serum IgE in a population of 126 twins, 27 monozygotic pairs and 36 dizygotic pairs, studied at birth (cord blood [CB] IgE) and consecutively at the age of 6 to 9 years of age (serum IgE).

Methods: IgE was determined by means of RIA. ANOVA, correlation analysis, and structural equation modeling by maximal likelihood analysis was used for genetic analysis.

Results: Structural equation modeling by maximal likelihood analysis showed the best-fitting model to be the AE model (A for additive genetic variance and E for environmental variance) both at birth and later in childhood. The estimated heritability was 0.92 (95% CI, 0.84-0.95) for CB IgE and 0.78 (95% CI, 0.60-0.87) for serum IgE. The correlation between CB IgE and serum IgE was 0.04.

Conclusions: The study demonstrated a higher genetic dependency of serum IgE than previously recognized. The low correlation between the IgE levels at birth and later in childhood suggested that different effector mechanisms may be operating at different ages. (*J Allergy Clin Immunol* 2001;107:659-63.)

Key words: Twins, IgE, cord blood, children, heritability, maximum likelihood analysis

IgE has been shown to be a major determinant of allergic disease.¹⁻³ Atopy is a familial syndrome underlying asthma, eczema, and allergic rhinoconjunctivitis and is characterized by the presence of serum IgE antibodies to allergens, as well as elevated total serum IgE concentrations and eventually eosinophilia. Several chromosomes have been suggested to harbor candidate genes for asthma and atopic disease, including chromosome 11q and chromosome 5q.^{4,5} However, it appears that alleles at multiple loci may be involved in the complex cause of asthma and allergies.

Abbreviations used

AIC: Akaike information criterion

CB: Cord blood IgE

DZ: Dizygotic

MZ: Monozygotic

Studies of monozygotic (MZ) and dizygotic (DZ) twins can provide important information on the relative influence of genetic and environmental risk factors on diseases. Several studies in twins, both adult and children, have indicated a significant genetic influence on serum IgE levels with heritability levels of 30% to 70%.⁶⁻⁸ In a previous study of twins, our data on IgE in cord blood (CB) IgE suggested a substantial genetic influence on the levels of CB IgE.⁹ In the present study we investigated consecutive samples from nonselected twins at birth and at 6 to 9 years of age for serum IgE levels, an approach that, to our knowledge, has not been done before. The aim of the study was to explore the nature and magnitude of genetic influences on interindividual differences in serum IgE levels among children to find out whether genetic influences on IgE changes with time and furthermore to evaluate whether CB IgE and serum IgE later in childhood are influenced by common genetic factors.

METHODS

Twin population

The study consisted of twins born in the counties of Funen and Aarhus in Denmark during the period from 1989 to 1992. The twins who participated in our previous study on genetic influences on CB IgE were all born in the county of Funen. At the follow-up, twins from both counties participated. Of a total of 198 subjects with available CB IgE, 126 children agreed to take part in the present follow-up study on serum IgE. Parental informed consent was obtained from the parents of the children. Table I shows sex, distribution, zygosity, and age of the twin population. No sets of twins with a parent in common participated in the study.

Interview

At the time of follow-up, when the twins were 6 to 9 years of age, their parents underwent a structured interview to determine the possible presence of allergic disease. The interview was based on a questionnaire that was a translated version of the ISAAC study questionnaire.¹⁰ The interview included details of atopy, dietary history, housing conditions, pets, smoking, socioeconomic data, respiratory tract infections, wheezing, atopic dermatitis, adverse reactions to

From ^athe Department of Pediatrics, Odense University Hospital, Odense; and ^bthe Department of Pediatrics, Aarhus University Hospital, Aarhus.

Supported by Ebba Celinders Foundation; The Allergy Foundation for Aarhus County, Denmark; The Illum Foundation; The Jacob Madsen and Wife Olga Madsen Foundation; The King Christian X Foundation; and educational grants from Mead-Johnson Inc and from Abbott Inc.

Received for publication August 29, 2000; revised December 14, 2000; accepted for publication December 14, 2000.

Reprint requests: Steffen Husby, MD, PhD, Department of Pediatrics, Odense University Hospital, DK-5000 Odense C, Denmark.

Copyright © 2001 by Mosby, Inc.

0091-6749/2001 \$35.00 + 0 1/83/113565

doi:10.1067/mai.2001.113565

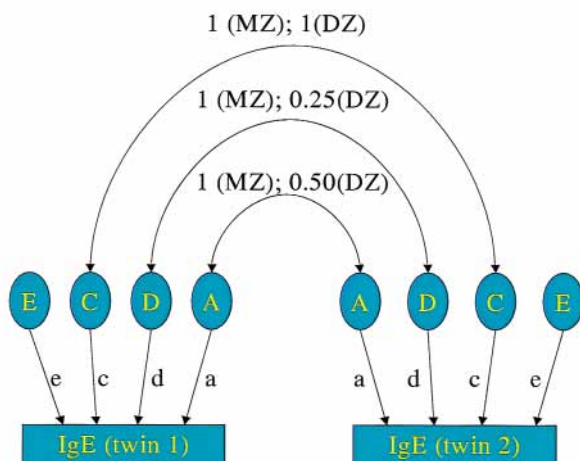


FIG 1. Path model for the influences on serum IgE in twins. The influences are divided into additive factors (A), genetic dominance factors (D), and shared (C) and nonshared (E) environmental factors. Additive genetic factors and genetic dominance factors are both perfectly correlated in MZ twins, whereas in DZ twins the correlation between additive genetic factors equals one half and the correlation between genetic dominance factors equals one fourth.

TABLE I. Study population by zygosity

	Zygosity	N (pairs)	Mean $\pm$ SD	Correlation
CB IgE	MZ	27	0.17 $\pm$ 0.18	0.88
	DZ	36	0.69 $\pm$ 1.8	0.46
Serum IgE	MZ	27	193.22 $\pm$ 316.92	0.8
	DZ	36	104.74 $\pm$ 238.51	0.38

foods, and other possible signs of atopic disease. Current atopic disease defined as manifestations of the disease within the previous 12 months was diagnosed according to the criteria mentioned below.

Asthma was defined as 3 or more episodes with expiratory wheezing diagnosed by a physician and requiring treatment with β_2 -agonist, inhaled steroids, or both.

Recurrent wheezing was recorded when the child had a history of at least 2 episodes with expiratory wheezing. The wheezing was nearly always associated with upper respiratory tract infection and was diagnosed by a physician.

Atopic dermatitis was diagnosed if physical examination showed areas of scaly, erythematous, and itchy eczematous rash primarily of the face and scalp, behind the ears, and at the flexure folds. Only eczema localized to at least 2 typical areas was recorded.

Gastrointestinal symptoms were defined as recurrent colic, vomiting, and/or diarrhea after excluding ordinary eating problems, coincidental infections, and lactose intolerance.

Food allergy/intolerance was diagnosed by means of 2 generally accepted criteria: (1) definite disappearance of symptoms after each of 2 dietary eliminations of the food in question and (2) recurrence of identical symptoms after one controlled challenge.

Physical examinations of the twins were performed by one of the authors (H.P.J., A.M.H., or S.H.), and the children's individual case history and personal data were recorded.

Genetic analysis

The CB IgE and serum IgE data were analyzed for deviation from Gaussian distributions by determination of skewness and kurtosis. Because the model-fitting procedures assume normally dis-

tributed CB IgE and serum IgE, data were transformed by using the natural logarithm. The effect of sex on CB IgE and serum IgE levels, respectively, was analyzed with ANOVA.

For many years, researchers have used the method of twin analysis described by Falconer,^{11,12} which estimates the heritability as the difference between phenotypic correlations for identical and fraternal twins ($H^2 = 2 [r_{MZ} - r_{DZ}]$). This method is simple; however, it assumes that all genetic effects are additive and tends to overestimate heritability in the case of genetic intralocus interaction (dominance) or interlocus interaction (epistasis).

Model-fitting procedures are increasingly used to analyze data from twin studies because these methods allow us to test competing hypotheses about the nature of genetic and environmental influences on the trait (ie, whether twin similarity is a result of additive genetic effects, genetic dominance, or shared environmental effects or whether more than one of these are required to explain the data). In this study proportions of variance attributable to genetic and environmental factors were assessed from variance-covariance matrices by using the structural equation model approach,¹³ with Mx used as statistical software.¹⁴

The path diagram in Fig 1 illustrates the univariate model for decomposing variance in CB IgE or serum IgE. Lower case letters represent genetic and environmental loadings on the trait. The total phenotypic variance can be decomposed into 2 genetic and 2 environmental components. Additive genetic factors (A) are the effects of genes taken singly and added over multiple loci, whereas genetic dominance (D) represents genetic interaction (within loci). Shared environmental effects (C) are the environmental effects that are shared by family members, and nonshared environmental effects (E) are the environmental influences that are unique to each individual and not shared by family members. The diagram indicates how each type of factor contributes to the covariance within an MZ or DZ twin pair. Additive genetic factors and genetic dominance are perfectly correlated in MZ twins, whereas DZ twins, like ordinary siblings, share only half of the additive genetic effects and one quarter of the genetic dominance effects. Shared environmental effects are assumed to be equal in MZ and DZ twins. The model assumes negligible effects of assortative mating, epistasis, genotype-environment interaction, or correlation.

Model fitting was performed by using maximum likelihood analysis, and the selection of the best-fitting model was based on the following 3 criteria: (1) a nonsignificant *P* value in the χ^2 goodness of fit test; (2) minimizing the Akaike information criterion ($AIC = \chi^2 - 2 \times df$); and (3) no parameter could be eliminated from the model without a significant increase in the χ^2 goodness of fit statistic. This approach reflects a balance between goodness of fit and parsimony.

Heritability was computed as genetic variance divided by total phenotypic variance. Genetic and environmental variances were derived from the best fitting model.

Laboratory methods

At birth, CB was obtained by means of cannulation. Blood samples were examined for levels of IgA. If the CB IgA level was above 39 mg/L, the sample was considered to be contaminated with maternal blood, and the twin and the cotwin were excluded from the study.⁹ Serum IgA was determined with Luminometric Immuno Assay (ALK A/S Denmark).

Twin zygosity was determined by 15 to 17 blood serum and enzyme types: ABO, MNS, Rhesus, K, Fya, Hp, *Gc, Tf, PGM-1, Acp, GPT, EsD, GLO, AK, PGD, AFA, and GLT/Gt. Twins were classified as DZ if they were discordant for one or more of those types and MZ when all types were concordant.

IgE levels were determined by using PRIST (Pharmacia, Uppsala, Sweden). The blood samples were examined for IgE antibodies to a mixture of several inhalant and food allergens (Phadiatop, Pharmacia). If positive, sera were further examined for specific antibodies.

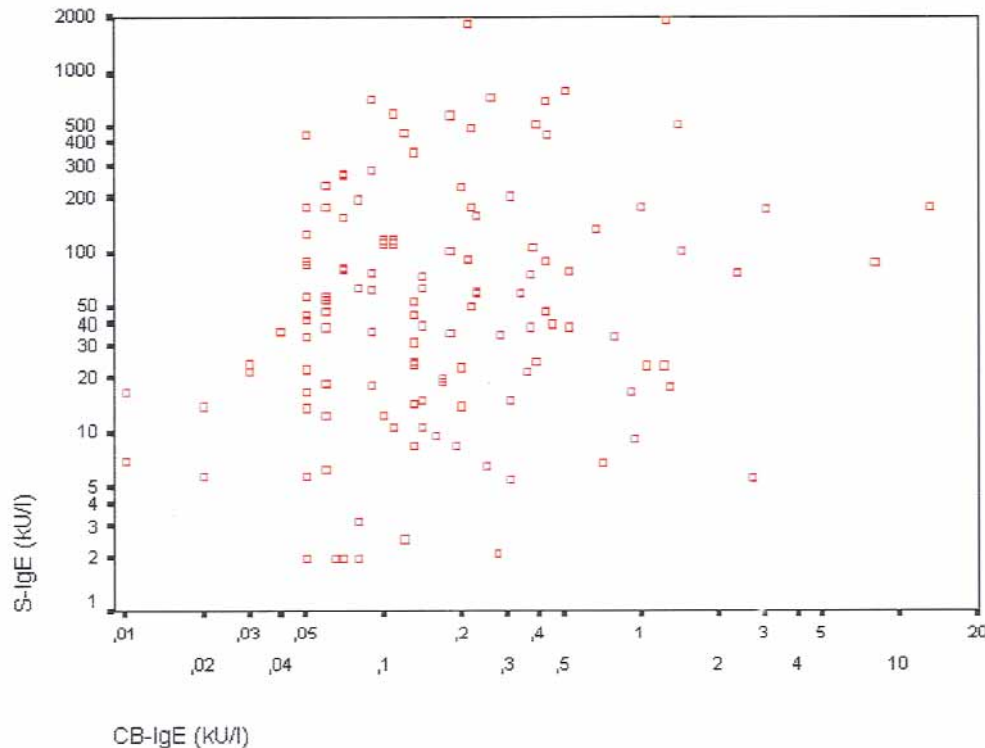


FIG 2. Scatter plot on a logarithmic scale of CB IgE versus serum IgE (*S-IgE*). Correlation between CB IgE and serum IgE is low (0.04).

Ethical considerations

The study was approved by the Regional Ethical Committees for Scientific Research for the County of Aarhus and for the Counties of Funen and Vejle, Denmark.

RESULTS

In the 126 subjects studied, the cumulative incidence of asthma was 11.1%, the cumulative incidence of atopic dermatitis was 13.5%, and the cumulative incidence of rhinoconjunctivitis was 8.7%. Serum IgE levels did not show any statistically significant difference between the atopic and nonatopic subjects.

ANOVA showed no significant effect of sex on CB IgE or IgE. Therefore data were not adjusted for differences in means between male and female subjects. Intraclass correlations for both CB IgE and serum IgE were significantly higher in MZ twins than in DZ twins, indicating a substantial genetic contribution to CB IgE and IgE variation (Table I). Biometric models for CB IgE and serum IgE were applied separately. To find the most parsimonious explanation of the observed pattern of resemblance in twins for CB IgE and IgE, respectively, 5 biometric models were fitted to the normalized data (Table II). In both cases the AE model (A for additive genetic variance and E for environmental variance) gave the best fit by AIC, indicating that additive genetic factors and nonshared environmental factors were important for both CB IgE and IgE levels. Pure environmental models (CE and E) did not fit the data well. The genetic and environ-

mental contributions to variation in CB IgE and IgE for the best-fitting model by AIC is shown in Table III. The estimated heritability was 0.92 (95% CI, 0.84-0.95) for CB IgE and 0.78 (95% CI, 0.60-0.87) for serum IgE. By using the ACE model, shared environmental variance was estimated to be 0.00 (95% CI, 0.00-0.32) for CB IgE and 0.02 (95% CI, 0.00-0.49) for serum IgE. From Table II, it can be seen that D and C can be excluded from the ADE and ACE model, respectively, with only marginal increase in χ^2 , whereas exclusion of A from the model results in a large increase in χ^2 , suggesting a major role for additive genetic factors.

When CB IgE and serum IgE were directly compared (Fig 2), the correlation between CB IgE and serum IgE was 0.04, indicating no substantial influences of common genetic or environmental factors on the 2 traits.

Exclusion of outliers did not change any of the above results.

DISCUSSION

The results of this study show a high genetic dependency of serum IgE estimated to be in the order of 75% to 85%, which is considerably higher than in previously published studies. The methodology for IgE determination showed a technical variability far below the biologic variability. Furthermore, the statistical methodology with biometrical modeling enabled 95% confidence limits to be made that, to our knowledge, were not used in previous studies of serum IgE. The confidence limits for

TABLE II. Biometric models for CB IgE and serum IgE

	Model	Model fit index			
		χ^2	df	P value	AIC
CB IgE	AE*	5.50	4	0.24	-2.5
	ADE	5.35	3	0.15	-0.65
	ACE	5.50	3	0.14	-0.48
	CE	28.20	4	0.00	20.2
	E	52.00	5	0.00	42.8
IgE	AE*	1.87	4	0.76	-6.14
	ADE	1.86	3	0.6	-4.14
	ACE	1.86	3	0.6	-4.14
	CE	9.00	4	0.06	1.00
	E	33.33	5	0.00	23.00

A, Additive genetic factors; D, genetic dominance factors; C, shared environmental factors; E, nonshared environmental factors.

*Best-fitting model according to the AIC ($AIC = \chi^2 - 2 \times df$).

TABLE III. Genetic and environmental contributions to variation in CB IgE and IgE

	Variance* caused by additive genes (heritability)	Variance* caused by the nonshared environment
CB IgE	0.92 (0.84-0.95)	0.08 (0.05-0.16)
Serum IgE	0.78 (0.60-0.87)	0.22 (0.13-0.40)

*Variances are standardized.

the heritability estimates, which depend on the sample size, were 0.92 (95% CI, 0.84-0.95) for CB IgE and 0.78 (95% CI, 0.60-0.87) for serum IgE by using the AE model. The study was done in a population of unselected twins, adding further reliability to the heritability estimate, which may otherwise be biased upward.

Elevated levels of total serum IgE are closely associated with allergic disease, as demonstrated in several studies.^{2,15} However, there is a considerable overlap between the ranges of serum IgE values in healthy and allergic subjects,¹⁶ rendering IgE useless in the individual diagnosis of asthma or other allergies. The population in our study contained both atopic and nonatopic subjects. Atopy was defined according to accepted international guidelines from the ISAAC study.^{10,17} The study did show most of the variation to depend on additive genetic influence (Fig 1), and no discernible common environmental influence was present.

Surprisingly, the data did not indicate any correlation between the serum IgE levels at birth and at a later age (Fig 2). This is at variance with studies where serum IgE was measured at birth and at 6 months of age,¹⁸ as well as up to the age of 3 years.¹⁹ The present study was not designed to evaluate consecutive changes in IgE levels and only included 2 measurements caused by practical and ethical constraints. However, the high heritability estimates in our study at birth and at 6 to 9 years of age combined with the low correlation of the IgE levels (Fig 2) suggest that different genetic factors regulate the serum IgE levels at the 2 time points.

The factors known to regulate IgE levels in particular in experimental animals include T_H2 -type cytokines, such as IL-4^{20,21} and IL-13,²² as well as their receptors, which are closely related and share the IL-4Ra chain.²³ IgE produced by B cells and excreted from plasma cells may further react in a noncognate fashion with effector cells, such as mast cells, basophils, and T cells. To what degree the levels of IL-4, IL-13, and other cytokines may influence the fetus and newborn infant as opposed to later life has been investigated in relation to later development of allergy.²⁴ High IL-4 levels seemed to determine the later development of allergy in one study,²⁵ whereas low levels of IFN- γ seemed to be more indicative of the later development of atopy in another study.²⁶

At the molecular genetic level, studies have investigated IgE antibody levels and IgE levels as markers of atopy in nonselected and asthmatic populations. Specific IgE antibody levels have been related to HLA class II antigens, such as HLA-D on chromosome 6.²⁷⁻²⁹ Two methodologies have been used to dissect the complex genetics of increased levels of total IgE, positional cloning, and candidate gene studies. Positional cloning has identified several regions of interest, including chromosomes 2q, 5q, 6q, 12q, and 13q.³⁰ Linkage between chromosome 11q13 and atopy, increased serum IgE levels, and airway hyperresponsiveness has been reported.³¹ However, other workers have reported no linkage between these phenotypes and chromosome 11q13 in different populations.^{32,33} Chromosome 11q13 contains the genes encoding the Fc ϵ RI high-affinity IgE receptor and the Clara cell secretory protein (CC16). Variations have been identified within the gene for the Fc ϵ RI β chain, and these were associated with atopic dermatitis.³⁴ Furthermore, a polymorphism in the gene for CC16 was associated with an increased risk of asthma,³⁵ but this observation was not confirmed in other populations.³⁶ Linkage between chromosome 5q31 and IgE levels, bronchial hyperresponsiveness,³⁷ and number of blood eosinophils⁴ has been reported. The cytokine gene cluster located on chromosome 5q31 includes the gene encoding IL-4, the *IL4R* gene is located to chromosome 16p12, and these genes or variations within these genes have been associated with IgE levels,³⁸ atopy,^{39,40} and FEV₁ in asthma.⁵

Recently, a mutation in the promoter region of the gene for the leukocyte antigen CD14, which has been mapped to chromosome 5q31-33, has been associated with protection against increased serum IgE levels, as well as asthma.⁴¹ CD14 is a glycoprotein present in tissues in 2 forms: a membrane-bound form (mCD14) present on monocytes and a soluble form (sCD14) present in serum, as well as in human milk.⁴² CD14 functions as a receptor for bacterial LPS.⁴³ The CD14 molecule may have important functions in the maturation of the immune response as a protection mechanism against the development of atopic disease.⁴⁴

Because of the present multitude of genes that may play a role in elevated IgE levels and atopic disease, it seemed justified to reanalyze, in a classical twin setting with novel

techniques, the heritability levels of quantitative serum IgE levels. The even higher heritability estimates obtained in the present study underscores the importance of serum IgE levels as an important avenue for further research. A partial explanation of the difficulties in identifying solitary genes responsible for atopic disease may lie in the fact that different effector mechanisms may be operating at different periods of development and perhaps even mutually in the individual infant and child.

REFERENCES

1. Stenius B, Wide L, Seymour W, et al. Clinical significance of specific IgE to common allergens. *Clin Allergy* 1971;1:37-55.
2. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991;325:454-66.
3. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
4. Martinez FD, Solomon S, Holberg CJ, Graves PE, Baldini M, Erickson RP. Linkage of circulating eosinophils to markers on chromosome 5q. *Am J Respir Crit Care Med* 1998;158:1739-44.
5. Burchard EG, Silverman EK, Rosenwasser LJ, et al. Association between a sequence variant in the IL-4 gene promoter and FEV1 in asthma. *Am J Respir Crit Care Med* 1999;160:919-22.
6. Bazarel M, Orgel HA, Hamburger RN. Genetics of IgE and allergy: serum IgE levels in twins. *J Allergy Clin Immunol* 1974;54:288-304.
7. Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. *J Allergy Clin Immunol* 1984;73:265-70.
8. Hanson B, McGue M, Roitman-Johnson B, Segal NL, Bouchard TJ, Blumenthal MN. Atopic disease and immunoglobulin E in twins reared apart and together. *Am J Hum Genet* 1991;48:873-9.
9. Husby S, Holm NV, Christensen K, Skov R, Morling N, Petersen PH. Cord blood immunoglobulin E in like-sexed monozygotic and dizygotic twins. *Clin Genet* 1996;50:332-8.
10. Pearce N, Weiland S, Keil U, et al. Self-reported prevalence of asthma symptoms in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC protocol. *Eur Respir J* 1993;6:1455-61.
11. Falconer DS. Introduction to quantitative genetics. New York: Ronald Press; 1960.
12. Falconer DS. Introduction to quantitative genetics. 2nd ed. Essex, England: Longman Scientific & Technical; 1961.
13. Neale MC, Cardon LR. Methodology for genetic studies of twins and families. 1st ed. Dordrecht: Kluwer Academic Publishers; 1992.
14. Neale MC. Mx: statistical modeling, 2nd ed. Richmond (VA): University of Virginia, Department of Psychiatry; 1994.
15. Marsh DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. *N Engl J Med* 1981;305:1551-9.
16. Kjellman NIM. Predictive value of high IgE levels in children. *Acta Paediatr Scand* 1976;65:465-71.
17. Mutius VE, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994;149:358-64.
18. Halonen M, Stern D, Lyle S, Wright A, Taussig L, Martinez FD. Relationship of total serum IgE levels in cord and 9-month sera of infants. *Clin Exp Allergy* 1991;21:235-41.
19. Hansen LG, Høst A, Halken S, et al. Cord blood IgE. I. IgE screening in 2814 newborn children. *Allergy* 1992;47:391-6.
20. Mossman TR, Coffmann RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 1989;7:145-73.
21. Del Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988;140:4193-8.
22. Grunig G, Warnock M, Wakil AE, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
23. Izuhara K, Shirakawa T. Signal transduction via the interleukin-4-receptor and its correlation with atopy. *Int J Mol Med* 1999;3:3-10.
24. Holt PG, Sedgewick JD. Suppression of IgE responses following inhalation of antigen. *Immunol Today* 1987;8:14-5.
25. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994;24:423-30.
26. Prescott SL, Macaubas C, Holt BJ, et al. Transplental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730-7.
27. Sparholt SH, Georgsen J, Madsen HO, Svendsen UG, Schou C. Association between HLA-DRB3*0101 and immunoglobulin E responsiveness to Bet v I. *Hum Immunol* 1994;39:76-8.
28. Hizawa H, Freidhoff LR, Chiu YF, et al. Genetic regulation of Dermatophagoides pteronyssinus-specific IgE responsiveness: a genome-wide multipoint linkage analysis in families recruited through 2 asthmatic sibs. Collaborative Study on the Genetics of Asthma (CSGA). *J Allergy Clin Immunol* 1998;102:436-42.
29. Stephan V, Kuehar J, Seibt A, et al. Genetic linkage of HLA-class II locus to mite-specific IgE immune responsiveness. *Clin Exp Allergy* 1999;29:1049-54.
30. Moffatt MF, Cookson WOCM. Genetics of asthma and inflammation: the status. *Curr Opin Immunol* 1999;11:606-9.
31. Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E response and underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989;1:1292-5.
32. Amelung PJ, Panhuysen CIM, Postma DS, et al. Atopy, asthma and bronchial hyperresponsiveness: exclusion of linkage to markers on chromosome 11q and 6p. *Clin Exp Allergy* 1992;22:1077-84.
33. Amelung PJ, Postma DS, Xu J, Meyers DA, Bleecker ER. Exclusion of chromosome 11q and the FcεRI-β gene as aetiological factors in allergy and asthma in a population of Dutch asthmatic families. *Clin Exp Allergy* 1998;28:397-403.
34. Cox HE, Moffatt MF, Faux JA, et al. Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor. *Br J Dermatol* 1998;138:182-7.
35. Laing IA, Goldblatt J, Eber E, et al. A polymorphism of the CC16 gene is associated with an increased risk of asthma. *J Med Genet* 1998;35:463-7.
36. Mao X-Q, Shirakawa T, Kawai M, et al. Association between asthma and an intragenic variant of CC16 on chromosome 11q13. *Clin Genet* 1998;53:54-6.
37. Postma S, Bleecker ER, Amelung PJ, et al. Genetic susceptibility to asthma-bronchial hyperresponsiveness cohered with a major gene for atopy. *N Engl J Med* 1995;333:894-900.
38. Marsh D, Neely JD, Breazeale DR, et al. Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
39. Eichmann KA, Heinzmann A, Forster J, et al. Linkage and allelic association of atopy and markers flanking the IL4-receptor gene. *Clin Exp Allergy* 1998;28:151-5.
40. Kruse S, Japha T, Tedner M, et al. The polymorphisms S503P and Q576R in the interleukin-4 receptor α gene are associated with atopy and influence the signal transduction. *Immunology* 1999;96:365-71.
41. Baldini M, Lohman C, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999;20:976-83.
42. Laluta MO, Vidal K, Nore JE, et al. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. *J Exp Med* 2000;191:1807-12.
43. Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. *J Immunol* 1988;141:547-52.
44. Martinez FD. Maturation of immune responses at the beginning of asthma. *J Allergy Clin Immunol* 1999;103:355-61.