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Risk Factors for Infantile Atopic Dermatitis and Recurrent Wheezing

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Abstract

Background: The pathogenic mechanisms of atopic dermatitis (AD) and recurrent wheezing (RW) during infancy are not fully understood.
Objective: We evaluated immunological markers associated with AD and RW during infancy.
Methods: We followed a cohort (n=314) from birth to 14 months of age. Some of the participants underwent a physical examination and blood test at 6 and 14 months of age. Univariate and multivariate logistic regression analysis and receiver operating characteristic curve analysis were performed to find which immunological markers could be risk factors for AD and RW.
Results: Of 16 immunological markers found in cord blood, only immunoglobulin (Ig) E was associated with AD at 6 months of age (adjusted OR [aOR], 1.607). None of the markers was associated with AD or RW at 14 months of age. Of 23 immunological markers at 6 months of age, total IgE (aOR, 1.018) and sensitization to egg white (aOR, 23.246) were associated with AD at 14 months of age. Phytohemagglutinin (PHA)–induced production of interleukin (IL) 4 from peripheral blood mononuclear cells (PBMCs) (aOR, 1.043) was associated with RW at 14 months of age.
Conclusion: Cord blood IgE was a risk factor for AD at 6 months of age. Total IgE and sensitization to egg white at 6 months of age were risk factors for AD at 14 months of age. PHA-induced IL-4 production in PBMCs at 6 months of age was a risk factor for RW at 14 months of age.

Key words: Birth cohort. Infants. Cord blood. Atopic dermatitis. Recurrent wheezing.

Resumen

Antecedentes: Los mecanismos patógenos de la dermatitis atópica (DA) y las sibilancias recurrentes (SR) durante el primer año de vida no se conocen completamente.
Objetivo: Se evaluaron los factores inmunológicos asociados a DA y SR durante el primer año de vida.
Métodos: Se realizó el seguimiento de una cohorte (n = 314) desde el nacimiento hasta los 14 meses de edad. Algunos de los participantes se sometieron a una exploración física y a un análisis de sangre a los 6 y a los 14 meses de edad. Se realizaron análisis de regresión logística unifactoriales y multifactoriales y análisis de la curva de eficacia diagnóstica para determinar qué factores inmunológicos podrían ser factores de riesgo para la DA y las SR.
Resultados: De 16 factores inmunológicos hallados en la sangre del cordón umbilical, solo la inmunoglobulina E (IgE) se asoció a DA a los 6 meses de edad (oportunidad relativa ajustada [ORa], 1,607). Ninguno de los factores se asoció a DA o SR a los 14 meses de edad. De 23 factores inmunológicos a los 6 meses de edad, el total de IgE (ORa, 1,018) y la sensibilización a la clara de huevo (ORa, 23,246) se asociaron a DA a los 14 meses de edad. La producción de interleucina (IL) 4 inducida por fitohemaglutinina (FHA) de las células mononucleares de sangre periférica (CMSP) (ORa, 1,043) se asoció a SR a los 14 meses de edad.
Conclusión: La presencia de IgE en la sangre del cordón umbilical fue un factor de riesgo de DA a los 6 meses de edad. El total de IgE y la sensibilización a la clara de huevo a los 6 meses de edad fueron factores de riesgo de DA a los 14 meses de edad. La producción de IL-4 inducida por FHA en las CMSP a los 6 meses de edad fue un factor de riesgo de SR a los 14 meses de edad.

Palabras clave: Cohorte de nacimiento. Lactantes. Sangre del cordón umbilical. Dermatitis atópica. Sibilancias recurrentes.

Introduction

Allergic diseases such as atopic dermatitis (AD), asthma, and food allergy are common diseases in children. AD is the most common allergic disease during infancy, and onset occurs during the first 6 months of life in 45% of those affected [1]. Recurrent wheezing (RW) is also an important problem during the first few years of life, because some children with RW develop bronchial asthma [2]. Genetic factors [3] and environmental factors [4] affect the development of allergic diseases. The role of genetic factors has been reported by our group [5,6] and by other authors [7]. We also reported on the role of cord blood factors associated with allergic diseases [8-10]. However, the pathogenic mechanisms of these diseases are still not fully understood.

Immunoglobulin (Ig) E and eosinophils are considered markers of allergic disease in early childhood [11,12]. Today, several new immunological markers have been investigated. Helper T cell (T_H) imbalance is considered to be associated with allergic diseases [13]. Type 2 T_H cells (T_H2), which produce cytokines such as interleukin (IL) 4, IL-5, and IL-13 promote secretion of IgE and differentiation of eosinophils and dominate T-cell responses in allergic diseases [14]. T_H1 cells, which produce IL-2, IL-12, and interferon (IFN) γ, confer immunity against infection and suppress T_H2 cells [14]. Several studies have reported the importance of regulatory pathways, such as regulatory T cells (Treg), IL-10, and transforming growth factor ß [14,15]. However, the question as to which immunological markers really affect allergic disease in early childhood remains unclear.

We have been performing a cohort study (the Gifu Allergy and Immunology Cohort Study [GAICS]) since 2004. To our knowledge, few birth cohort studies have examined immunological changes during infancy. We describe some of the immunological markers of cord blood and peripheral blood at 6 and 14 months of age and study their role in AD and RW.

Methods

Study Participants and Design

We performed this analysis as a part of the GAICS, which included 314 infants born in a maternity hospital from October 2004 to July 2005. The details of the study were explained to the parent(s) before birth. Informed consent was obtained from all the parent(s). Cord blood samples were taken at birth. All participants were followed up using questionnaires at birth and at 6 and 14 months of age.

In order to analyze the immunological markers of allergic diseases, more than half of the participants were recruited to a group in which the participants underwent further tests, including a physical examination by a pediatric allergist and blood sampling (previous written agreement from the parent[s]). All the questionnaires were completed. The remaining participants were followed up by questionnaire only and will be analyzed in future papers. All parts of this cohort study were approved by the Ethics Committee of the Graduate School of Medicine, Gifu University, Gifu, Japan.

Diagnosis

AD was diagnosed by a pediatric allergist according to Hanifin and Rajka's minor criteria [11]; RW was diagnosed by a pediatric allergist based on a history of more than 2 episodes of physician-diagnosed wheezing.

Laboratory Determinations

Total white blood cell (WBC) counts were performed using an automated instrument. Blood smears from peripheral blood samples were stained (Wright-Giemsa), and a white blood cell differential test was performed. Absolute eosinophil and basophil counts were calculated from total WBC numbers.

Cord blood and peripheral blood serum IgE were examined using LUMIPULSE IgE (Fujirebio Inc). Specific IgE was measured using the radioallergosorbent test, and sensitization was defined as a specific IgE concentration >0.34 U/mL for certain allergens (egg white, cow's milk, wheat, soy bean, house dust [h1], *Dermatophagoides farinae*, dog dander, and cat dander). Cord blood serum IgA was analyzed to evaluate the contamination of maternal blood, and samples of that IgA ≥1 mg/dL were excluded [16].

Flow Cytometry of Lymphocyte Subpopulations

Flow cytometry was performed on a Cytomics FC500 device (Beckman Coulter). Whole blood samples were stained with CD3, CD19, CD4, CD8, CD56/CD16, and CD4/CD25 antibodies (Beckman Coulter). An appropriate amount of fluorescence antibody and 100 μL of whole blood were mixed and incubated for 20 minutes. The whole blood samples were treated with ammonium chloride to lyse the erythrocytes, and the cells were washed twice with phosphate-buffered saline (PBS). The cells were then resuspended in 0.5 mL of PBS and analyzed immediately. The percentages of $CD3^+$, $CD19^+$, and $CD4^+$ cells in the lymphocytes were named, respectively, CD3% (T cell), CD19% (B cell), and CD4% (helper T cell). The $CD8^+$ cells were divided into $CD8^{bright}$ and $CD8^{dull}$. Most of the $CD8^{dull}$ cells expressed CD56 (data not shown) and were considered to be natural killer cells. On the other hand, most of the $CD8^{bright}$ cells expressed CD3, but not CD56 (data not shown). Therefore, the percentages of $CD8^+$ cells and $CD8^{bright}$ and $CD8^{dull}$ in the lymphocytes were named CD8%, $CD8^{bright}$% (cytotoxic T cell), and $CD8^{dull}$% (some of the natural killer cells). The percentages of double-positive CD56 and CD16 cells in the lymphocytes were named $CD56^+CD16^+$%. The percentage of CD56-positive and CD16-negative cells were named $CD56^+CD16^-$%. The $CD25^+$ cells in the $CD4^+$ lymphocytes were named $CD4^+CD25^+$%.

Intracellular Cytokine Analysis

Intracellular cytokine staining was performed as reported elsewhere [17]. A total of 0.5 mL of heparinized whole blood was added to 0.5 mL of an RPMI-1640 medium, incubated with a combination of 25 ng/mL of phorbol myristate acetate (PMA) and 1 μg/mL of ionomycin in the presence of 10 μg/mL of brefeldin A (Sigma), and cultured for 4 hours at 37ºC in a humidified atmosphere containing 5% CO_2. Surface staining (CD4, CD8) was carried out at room temperature for 20 minutes in the dark. The blood samples were then

fixed and permeabilized with an IntraPrep Permeabilization Reagent (Beckman Coulter). Finally, the cells were incubated with monoclonal antibodies of anti–IFN-γ fluorescein isothiocyanate (BD Biosciences) or anti–IL-4 phycoerythrin (BD Biosciences) for 40 minutes in the dark and then analyzed using flow cytometry. The percentage of IFN–γ-producing cells in a $CD4^+$ and a $CD8^{bright}$ cell was considered to be T_H1% and cytotoxic T cells (T_C)1%, respectively. The percentage of IL-4–producing cells in a $CD4^+$ and a $CD8^{bright}$ cell was considered to be T_H2% and T_C2%, respectively.

Cell Preparation and Culture

Cord blood mononuclear cells (CBMCs) or peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on Ficoll/Paque (Pharmacia AB) and washed 3 times. The isolated CBMCs/PBMCs were suspended at a final concentration of 1×10^6 cells/mL in an RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2mM L-glutamine, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin. Cell suspensions were cultured in the presence or absence of 10 μg/mL of phytohemagglutinin (PHA) (Gibco BRL) for 24 hours at 37ºC in a humidified atmosphere containing 5% CO_2. The culture supernatants were measured using a human IFN-γ enzyme-linked immunosorbent assay (ELISA) kit (Ohtsuka) and an IL-4 US ELISA kit (BioSource). The detection limits of the ELISAs were 15.6-1000 pg/mL (IFN-γ) and 0.39-25.0 pg/mL (IL-4).

Statistics

Multivariate logistic analyses were performed to determine whether there were any cord blood immunological markers that could predict AD at 6 months of age, whether there were any cord or peripheral blood immunological markers at birth or at 6 months of age that could predict AD at 14 months of age, and whether there were any cord or peripheral blood immunological markers at birth or at 6 months of age that could predict RW at 14 months of age.

SPSS version 17.0 (SPSS, Inc) was used for univariate and multivariate logistic regression analysis. Values below the detection limit were allotted half the detection limit. A separate univariate risk factor analysis was conducted for AD at 6 and 14 months of age. To predict AD and RW, we fitted a multiple regression model that included all potential predictors with a univariate P value <.05. A backward stepwise (likelihood ratio) procedure was used to select a final model.

Receiver operating characteristic (ROC) curve analysis was also performed with SPSS version 17.0 to evaluate the predictability of the factors selected in the logistic regression analysis.

Results

Study Participants and Baseline Characteristics

A flowchart of the GAICS is shown in the Figure. Of the 314 participants, 171 (54.5%) were female, mean (SD) gestational age was 278.0 (7.5) days (39 weeks and 5.0 days), and mean birth weight was 3120.5 (388.2) g.

We recruited participants who had undergone physical examination at 6 and 14 months of age (Figure). First, we compared data for participants who had undergone the physical examination and for the remaining participants. No significant differences were observed for gestational age, birth weight, sex, and family history of the parent(s) of patients from both groups (Table 1). The final sample comprised participants who had undergone a physical examination.

Prevalence of Atopic Dermatitis and Recurrent Wheezing

Of the 174 participants who had a physical examination at 6 months of age, 43 (24.7%) and 0 (0.0%) suffered from AD and RW, and were referred to as 6MAD(+) or 6MRW(+), respectively. Those who did not have AD or RW at 6 months of age were referred to as 6MAD(–) and 6MRW(–), respectively. Of the 157 participants who had a physical examination at 14 months of age, 30 (19.1%) and 12 (7.6%) suffered from AD and RW, and were referred to as 14MAD(+) or 14MRW(+), respectively. Those who did not have AD or RW at 14 months of age were named 14MAD(–) and 14MRW(–), respectively. Among the 30 14MAD(+) participants, only 4 also suffered from RW.

Risk Factors for Atopic Dermatitis at 6 Months of Age

We analyzed whether immunological markers in cord blood could be risk factors for 6MAD(+) (Table 2). Univariate logistic regression analysis showed that increases in cord blood

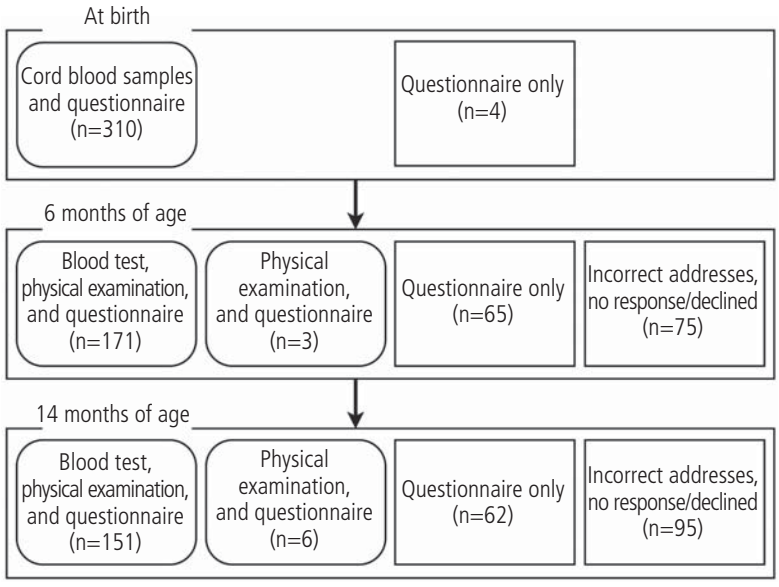

Figure. Flowchart of study participants in the Gifu Allergy and Immunology Cohort Study. For this analysis of immunological markers and physician-diagnosed allergic disease, we used participants who had undergone a physical examination at 6 and 14 months of age (shown as a round-cornered box).

Table 1. Comparison Between Participants Who Underwent a Physical Examination and the Remaining Participants

Physical Examination at 6 Months of Age	Yes	No	*P* Value
Gestational age	278.15 (6.89) d (n=169) 39 wk 5.15 d	277.75 (8.88) d (n=59) 39 wk 4.75 d	NS[a]
Weight at birth	3118.48 (380.28) g (n=173)	3125.95 (411.67) g (n=65)	NS[a]
Male/male+female	81/174 (46.55%)	62/140 (44.29%)	NS[b]
Allergic history			
Father	55/162 (33.95%)	48/123 (39.02%)	NS[b]
Mother	70/164 (42.68%)	58/125 (46.40%)	NS[b]
At least 1 parent	98/162 (60.50%)	78/124 (62.90%)	NS[b]
Both parents	27/162 (16.67%)	28/124 (22.58%)	NS[b]
Physical Examination at 14 Months of Age	**Yes**	**No**	***P* Value**
Gestational age	278.46 (7.10) d (n=148) 39 wk 5.46 d	277.29 (8.01) d (n=80) 39 wk 4.29 d	NS[a]
Weight at birth	3100.68 (404.22) g (n=151)	3154.97 (358.46) g (n=87)	NS[a]
Male/male+female	68/157 (43.31%)	75/157 (47.77%)	NS[b]
Allergic history			
Father	51/144 (35.42%)	52/141 (36.88%)	NS[b]
Mother	66/146 (45.21%)	62/143 (43.36%)	NS[b]
At least 1 parent	91/144 (63.20%)	84/142 (59.86%)	NS[b]
Both parents	26/144 (18.06%)	29/142 (20.42%)	NS[b]

Abbreviation: NS, nonsignificant.
[a]Mann-Whitney test.
[b]Fisher exact test.

Table 2. Odds Ratio (OR) for Having Atopic Dermatitis (AD) at 6 Months of Age According to the Univariate Logistic Regression Analysis of Markers in Cord Blood

	AD 6M (–)		AD 6M (+)		Total				95% CI for OR	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	*P* Value	OR	Lower	Upper
Cord blood										
WBC	12.51	(3.31)	12.21	(4.17)	12.44	(3.53)	.676	0.976	0.872	1.093
Eosinophils	332.4	(186.2)	348.3	(171.3)	336.4	(182.0)	.664	1.000	0.998	1.003
Basophils	78.89	(57.06)	94.03	(76.85)	82.67	(62.63)	.233	1.004	0.998	1.010
IgE	0.54	(0.75)	0.95	(1.42)	0.64	(0.97)	.034	1.462	1.029	2.078
T_H1%	0.25	(0.26)	0.27	(0.21)	0.25	(0.24)	.629	1.439	0.328	6.303
T_H2%	0.17	(0.21)	0.20	(0.23)	0.17	(0.21)	.475	1.833	0.348	9.669
T_C1%	0.60	(0.72)	0.59	(0.62)	0.60	(0.69)	.981	0.994	0.579	1.706
T_C2%	0.03	(0.08)	0.02	(0.07)	0.03	(0.08)	.535	0.148	0.000	61.836
CD3%	51.22	(14.93)	54.75	(16.06)	52.10	(15.24)	.223	1.016	0.991	1.041
CD19%	9.68	(5.57)	8.33	(4.58)	9.35	(5.36)	.184	0.948	0.876	1.026
CD4%	39.92	(11.83)	41.83	(12.22)	40.40	(11.92)	.406	1.014	0.982	1.047
$CD8^{bright}$%	11.62	(4.79)	13.32	(6.36)	12.03	(5.24)	.107	1.060	0.988	1.138
$CD56^+CD16^+$%	9.09	(5.06)	10.05	(6.03)	9.33	(5.31)	.348	1.034	0.964	1.108
$CD56^+CD16^-$%	1.65	(1.21)	1.50	(0.87)	1.61	(1.13)	.504	0.882	0.610	1.275
$CD4^+CD25^+$%	11.62	(2.79)	12.50	(3.07)	11.84	(2.88)	.114	1.108	0.976	1.260
IFN-γ (PHA)	556.6	(961.3)	1111.6	(1883.3)	687.4	(1255.9)	.043	1.000	1.000	1.001
IL-4 (PHA)	5.74	(4.61)	6.13	(4.98)	5.84	(4.69)	.668	1.017	0.941	1.099

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; PHA, phytohemagglutinin; WBC, white blood cells.

IgE levels and PHA-induced IFN-γ production by CBMCs were associated with 6MAD(+).

After stepwise multivariate logistic regression, only cord blood IgE (adjusted OR [aOR]), 1.607; 95% CI, 1.082-2.387; *P*=.019) remained in the model (Table 3).

A ROC curve analysis to evaluate the predictability of cord blood IgE for distinguishing 6MAD(+) from 6MAD(–) revealed the area under the curve (AUC) of cord blood IgE to be 0.564 (95% CI, 0.456-0.672; *P*=.230]. Hence, cord blood IgE is a risk factor but not a predictive factor for AD at 6 months of age.

Table 3. Adjusted Odds Ratio (aOR) for Having Atopic Dermatitis at 6 Months of Age According to Multivariate Logistic Regression Analysis of Markers in Cord Blood

				95% CI for aOR	
		P Value	aOR	Lower	Upper
Cord blood					
Step 1	IgE	.039	1.552	1.022	2.359
	IFN-γ (PHA)	.144	1.000	1.000	1.001
Step 2	IgE	.019	1.607	1.082	2.387

Abbreviations: IFN, interferon; Ig, immunoglobulins; PHA, phytohemagglutinin.

Table 4. Odds Ratio (OR) for Having Atopic Dermatitis (AD) at 14 Months of Age According to Univariate Logistic Regression Analysis of Markers in Cord Blood and at 6 Months

	AD 14M (–)		AD 14M (+)		Total				95% CI for OR	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	*P* Value	OR	Lower	Upper
Cord blood										
WBC	12.22	(3.40)	12.58	(4.18)	12.30	(3.58)	.646	1.028	0.912	1.160
Eosinophils	314.8	(180.8)	342.2	(182.4)	321.2	(180.8)	.489	1.001	0.998	1.003
Basophils	72.15	(52.99)	92.81	(74.04)	76.87	(58.77)	.118	1.005	0.999	1.012
IgE	0.54	(0.81)	0.60	(0.56)	0.55	(0.76)	.684	1.107	0.679	1.805
T_H1%	0.25	(0.26)	0.31	(0.29)	0.26	(0.27)	.284	2.205	0.519	9.370
T_H2%	0.20	(0.24)	0.16	(0.15)	0.19	(0.23)	.394	0.404	0.050	3.249
T_C1%	0.58	(0.71)	0.58	(0.62)	0.58	(0.69)	.991	1.003	0.552	1.825
T_C2%	0.03	(0.04)	0.04	(0.14)	0.03	(0.07)	.525	4.816	0.038	613.400
CD3%	51.57	(15.72)	55.07	(12.88)	52.33	(15.17)	.273	1.016	0.988	1.044
CD19%	9.42	(5.77)	9.28	(4.77)	9.39	(5.55)	.906	0.995	0.923	1.073
CD4%	40.09	(12.38)	43.15	(10.73)	40.74	(12.08)	.235	1.021	0.986	1.058
$CD8^{bright}$%	11.60	(4.82)	12.96	(5.60)	11.89	(5.00)	.213	1.053	0.971	1.143
$CD56^+CD16^+$%	9.31	(5.21)	11.18	(6.48)	9.72	(5.54)	.118	1.060	0.985	1.141
$CD56^+CD16^-$%	1.65	(1.26)	1.57	(0.83)	1.63	(1.17)	.748	0.940	0.645	1.371
$CD4^+CD25^+$%	11.56	(2.79)	12.20	(3.15)	11.70	(2.88)	.291	1.078	0.938	1.239
IFN-γ (PHA)	519.6	(771.7)	939.0	(1493.2)	609.5	(980.1)	.064	1.000	1.000	1.001
IL-4 (PHA)	5.65	(4.37)	6.55	(5.99)	5.84	(4.76)	.263	1.448	0.758	2.768
Six months of age										
WBC	10.78	(3.11)	11.23	(3.16)	10.86	(3.11)	.527	1.045	0.911	1.199
Eosinophils	371.1	(553.0)	567.5	(454.8)	404.7	(541.1)	.183	1.000	1.000	1.001
Basophils	55.86	(29.58)	61.31	(31.89)	56.79	(29.94)	.420	1.006	0.992	1.019
IgE	14.36	(23.46)	49.72	(61.12)	20.43	(35.39)	.001	1.022	1.010	1.035
T_H1%	2.07	(1.13)	2.11	(0.92)	2.08	(1.09)	.888	1.030	0.685	1.550
T_H2%	0.33	(0.19)	0.39	(0.28)	0.34	(0.21)	.247	3.325	0.434	25.467
T_C1%	7.15	(8.57)	6.76	(5.56)	7.08	(8.12)	.835	0.994	0.939	1.052
T_C2%	0.05	(0.17)	0.04	(0.07)	0.05	(0.16)	.654	0.414	0.009	19.459
CD3%	62.38	(12.50)	58.66	(14.56)	61.74	(12.89)	.204	0.979	0.949	1.011
CD19%	14.43	(9.19)	15.76	(9.52)	14.65	(9.22)	.520	1.015	0.969	1.064
CD4%	44.47	(10.44)	41.82	(10.53)	44.02	(10.47)	.260	0.976	0.935	1.018
$CD8^{bright}$%	13.76	(5.27)	12.68	(3.51)	13.58	(5.02)	.336	0.954	0.867	1.050
$CD56^+CD16^+$%	6.86	(4.46)	6.35	(4.95)	6.77	(4.53)	.614	0.974	0.878	1.080
$CD56^+CD16^-$%	0.98	(1.14)	0.86	(0.56)	0.96	(1.06)	.647	0.839	0.397	1.777
$CD4^+CD25^+$%	10.26	(2.22)	10.98	(2.18)	10.39	(2.22)	.154	1.155	0.947	1.408
IFN-γ (PHA)	3148.0	(3480.5)	1935.2	(2552.4)	2951.8	(3369.5)	.124	1.000	1.000	1.000
IL-4 (PHA)	13.20	(11.90)	13.70	(9.25)	13.28	(11.48)	.056	0.472	0.218	1.020

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; PHA, phytohemagglutinin; WBC, white blood cells.

Risk Factors for Atopic Dermatitis at 14 Months of Age

We analyzed whether immunological markers in cord blood could be risk factors for 14MAD(+). However, no significant differences were found in any of the immunological markers of cord blood between 14MAD(+) and 14MAD(–) (Table 4).

We also analyzed whether immunological markers at 6 months of age could be a risk factor for 14MAD(+). According to univariate logistic regression analysis, an increase in total IgE levels was associated with 14MAD(+) (Table 4). Risk also significantly increased among participants who were sensitized to egg white, milk, wheat, or dog dander at 6 months of age (Table 5).

After stepwise multivariate logistic regression, total IgE at 6 months of age (aOR, 1.018; 95% CI, 1.002-1.034; *P*=.030) and egg white–specific IgE (aOR, 23.246; 95% CI, 2.801-192.905; *P*=.004) remained in the model (Table 6).

A ROC curve analysis performed to evaluate the predictability of total IgE level at 6 months of age for distinguishing 6MAD(+) from 6MAD(–) revealed the AUC of the total IgE levels to be 0.726 (95% CI, 0.607-0.845; *P*=.001). Since sensitization to egg white is not a continuous value, a ROC curve analysis was not performed. Hence, total IgE levels at 6 months of age were not only risk factors, but also predictors, for AD at 14 months of age.

Risk Factors for Recurrent Wheezing at 14 Months of Age

We analyzed whether immunological markers in cord blood could be risk factors for 14MRW(+). However, no significant differences were observed between 14MRW(+) and 14MRW(–) in any markers (Table 7).

We also analyzed whether immunological markers at 6 months of age could be risk factors for 14MRW(+). Using

Table 5. Odds Ratio (OR) for Having Atopic Dermatitis (AD) at 14 Months of Age According to Univariate Logistic Regression Analysis of Markers at 6 Months

		AD 14M (–)		AD 14M (+)				95% CI for OR	
			(n/total)		(n/total)	*P* Value	OR	Lower	Upper
Six months of age									
Egg white	≥ class 1	31.90%	(37/116)	87.50%	(21/24)	<.001	14.946	4.192	53.282
Cow's milk	≥ class 1	6.90%	(8/116)	37.50%	(9/24)	<.001	8.100	2.710	24.209
Wheat	≥ class 1	4.65%	(4/86)	33.33%	(5/15)	.002	10.250	2.358	44.561
Soy	≥ class 1	0%	(0/116)	4.17%	(1/24)	1.000	8.148×10^9	–	–
Dog dander	≥ class 1	6.03%	(7/116)	25.00%	(6/24)	.007	5.190	1.565	17.216
Cat dander	≥ class 1	1.72%	(2/116)	4.17%	(1/24)	.466	2.478	0.216	28.487
Df	≥ class 1	0.86%	(1/116)	8.33%	(2/24)	.060	10.455	0.908	120.354

Abbreviations: Df, *Dermatophagoides farinae*.

Table 6. Adjusted Odds Ratio (aOR) for Having Atopic Dermatitis at 14 Months of Age According to Multivariate Logistic Regression Analysis of Markers at 6 Months

				95% CI for aOR	
		P Value	aOR	Lower	Upper
Six months of age					
Step 1	IgE	.125	1.020	0.995	1.045
	Egg white ≥ class 1	.005	21.706	2.489	189.321
	Cow's milk ≥ class 1	.636	0.539	0.042	6.964
	Wheat ≥ class 1	.933	1.094	0.135	8.888
	Dog dander ≥ class 1	.215	3.316	0.499	22.038
Step 2	IgE	.105	1.020	0.996	1.044
	Egg white ≥ class 1	.004	22.072	2.617	186.170
	Cow's milk ≥ class 1	.639	0.555	0.047	6.527
	Dog dander ≥ class 1	.205	3.358	0.517	21.828
Step 3	IgE	.057	1.015	1.000	1.032
	Egg white ≥ class 1	.005	20.921	2.522	173.568
	Dog dander ≥ class 1	.222	3.100	0.503	19.088
Step 4	IgE	.030	1.018	1.002	1.034
	Egg white class ≥1	.004	23.246	2.801	192.905

Abbreviations: Ig, immunoglobulin.

Table 7. Odds Ratio (OR) for Having Recurrent Wheezing (RW) at 14 Months of Age According to in Univariate Logistic Regression Analysis of Markers in Cord Blood and at 6 Months

	RW 14M (–)		RW 14M (+)		Total				95% CI for OR	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	*P* Value	OR	Lower	Upper
Cord blood										
WBC	12.31	(3.51)	12.11	(4.90)	12.30	(3.58)	.884	0.984	0.793	1.221
Eosinophils	323.6	(183.8)	282.8	(129.0)	321.2	(180.8)	.561	0.999	0.994	1.003
Basophils	75.00	(57.36)	106.5	(77.06)	76.87	(58.77)	.183	1.007	0.997	1.016
IgE	0.57	(0.78)	0.23	(0.12)	0.55	(0.76)	.122	0.082	0.003	1.953
T_H1%	0.25	(0.26)	0.34	(0.38)	0.26	(0.27)	.366	2.752	0.307	24.698
T_H2%	0.19	(0.23)	0.15	(0.20)	0.19	(0.23)	.617	0.376	0.008	17.435
T_C1%	0.58	(0.70)	0.47	(0.61)	0.58	(0.69)	.649	0.751	0.218	2.584
T_C2%	0.02	(0.04)	0.11	(0.25)	0.03	(0.07)	.061	516.318	0.742	359 457
CD3%	52.24	(15.30)	53.55	(14.12)	52.33	(15.17)	.801	1.006	0.961	1.052
CD19%	9.33	(5.59)	10.26	(5.15)	9.39	(5.55)	.627	1.028	0.921	1.147
CD4%	40.88	(12.26)	38.84	(9.49)	40.74	(12.08)	.625	0.986	0.931	1.044
$CD8^{bright}$%	11.97	(5.08)	10.74	(4.00)	11.89	(5.00)	.476	0.946	0.813	1.102
$CD56^+CD16^+$%	9.67	(5.54)	10.27	(5.80)	9.72	(5.54)	.755	1.019	0.905	1.148
$CD56^+CD16^-$%	1.66	(1.20)	1.28	(0.59)	1.63	(1.17)	.344	0.662	0.282	1.557
$CD4^+CD25^+$%	11.65	(2.87)	12.39	(3.07)	11.70	(2.88)	.461	1.086	0.872	1.351
IFN-γ (PHA)	579.2	(875.6)	1056.7	(2023.3)	609.5	(980.1)	.204	1.000	1.000	1.001
IL-4 (PHA)	5.62	(4.49)	8.96	(7.19)	5.84	(4.76)	.053	1.117	0.999	1.249
Six months of age										
WBC	10.89	(3.16)	10.47	(2.47)	10.86	(3.11)	.696	0.955	0.759	1.202
Eosinophils	407.9	(554.0)	358.7	(309.8)	404.7	(541.1)	.793	1.000	0.998	1.002
Basophils	57.50	(30.43)	46.51	(20.20)	56.79	(29.94)	.270	0.982	0.951	1.014
IgE	21.07	(36.39)	11.11	(11.79)	20.43	(35.39)	.439	0.984	0.945	1.025
T_H1%	2.06	(1.07)	2.31	(1.42)	2.08	(1.09)	.505	1.215	0.685	2.157
T_H2%	0.34	(0.21)	0.40	(0.18)	0.34	(0.21)	.434	3.192	0.175	58.318
T_C1%	7.06	(8.19)	7.42	(7.58)	7.08	(8.12)	.897	1.005	0.927	1.090
T_C2%	0.05	(0.17)	0.02	(0.03)	0.05	(0.16)	.614	0.033	0.000	19 784.957
CD3%	61.60	(13.08)	63.76	(10.09)	61.74	(12.89)	.625	1.014	0.958	1.074
CD19%	14.42	(9.18)	18.13	(9.66)	14.65	(9.22)	.247	1.041	0.972	1.115
CD4%	44.02	(10.53)	43.98	(10.09)	44.02	(10.47)	.992	1.000	0.937	1.067
$CD8^{bright}$%	13.49	(4.93)	14.85	(6.41)	13.58	(5.02)	.433	1.050	0.930	1.186
$CD56^+CD16^+$%	6.95	(4.55)	4.17	(3.45)	6.77	(4.53)	.075	0.779	0.592	1.025
$CD56^+CD16^-$%	0.95	(1.08)	1.05	(0.78)	0.96	(1.06)	.797	1.067	0.651	1.747
$CD4^+CD25^+$%	10.41	(2.21)	10.12	(2.52)	10.39	(2.22)	.712	0.943	0.691	1.287
IFN-γ (PHA)	2981.3	(3381.0)	2536.0	(3368.9)	2951.8	(3369.5)	.701	1.000	1.000	1.000
IL-4 (PHA)	12.73	(10.61)	21.33	(19.46)	13.28	(11.48)	.045	1.043	1.001	1.086

Abbreviations: AD, atopic dermatitis; IFN, interferon; Ig, immunoglobulin; IL, interleukin; PHA, phytohemagglutinin; WBC, white blood cells.

Table 8. Odds Ratio (OR) for Having Recurrent Wheezing (RW) at 14 Months of Age According to Univariate Logistic Regression Analysis of Markers at 6 Months

		RW 14M (–)		RW 14M (+)				95% CI for OR	
			(n/total)		(n/total)	*P* Value	OR	Lower	Upper
Six months of age									
Egg white	≥ class 1	40.46%	(53/131)	55.56%	(5/9)	.380	1.840	0.472	7.170
Cow's milk	≥ class 1	12.98%	(17/131)	0%	(0/9)	.998	0.000	–	–
Wheat	≥ class 1	8.60%	(8/93)	12.50%	(1/8)	.712	1.518	0.165	13.935
Soy	≥ class 1	0.76%	(1/131)	0%	(0/9)	1.000	0.000	–	–
Dog dander	≥ class 1	9.92%	(13/131)	0%	(0/9)	.999	0.000	–	–
Cat dander	≥ class 1	2.29%	(3/131)	0%	(0/9)	.999	0.000	–	–
Df	≥ class 1	2.29%	(3/131)	0%	(0/9)	.999	0.000	–	–

Abbreviations: Df, *Dermatophagoides farinae*.

the 14MRW(–) group as a reference, the risk of becoming 14MRW(+) increased significantly in PHA-induced IL-4 production from PBMCs at 6 months of age (Table 7). Specific IgE analyses at 6 months of age revealed no factor to be significant (Table 8).

Since only 1 factor was significant in the univariate logistic regression, stepwise multivariate logistic regression analysis was not performed.

A ROC curve analysis performed to evaluate the predictability of the PHA-induced IL-4 production from PBMCs at 6 months of age for distinguishing 14MRW(+) from 14MRW(–) revealed the AUC of PHA-induced IL-4 production from PBMCs at 6 months of age to be 0.629 (95% CI, 0.419-0.839; *P*=.196). Hence, higher PHA-induced IL-4 production from PBMCs at 6 months of age is a risk factor—but not a predictive factor—for RW at 14 months of age.

Discussion

We are conducting the GAICS to understand the genetic and environmental factors that affect sensitization to allergens and the development of allergic disorders. We are also investigating which laboratory data are useful for predicting the development of allergic diseases. Although several birth cohort studies have been performed in Europe and United States, the number of birth cohort studies in Asia remains low. Our cohort study is one of the few such studies in an Asian community.

We focused on the relationship between infantile AD/RW and laboratory data (values for IgE, WBC, eosinophils, basophils, and lymphocyte subpopulations, as well as cytokine production in CBMCs or PBMCs). Several new findings in allergic disease have been recorded. The T_H1/T_H2 paradigm provides a useful model for understanding the pathogenesis of allergic diseases, and the immunosuppressive function of the Treg subset is of interest. We also analyzed subpopulations of helper T cells (T_H1/T_H2 or Tregs) in cord blood and peripheral blood at 6 and 14 months of age.

Several lines of research suggest that the prevalence of AD in infancy is about 15% to 20%, although this value varies according to the study. We showed that the prevalence of AD at 6 months and 14 months of age was 24.7% and 19.1%, respectively. Moore et al [18] found the prevalence of AD in first 6 months in the United States to be 17.1%. Dunlop et al [19] reported that the prevalence of AD in 14-year-old Slovak children was 15.6%. Benn et al [20] reported that the prevalence of AD in children aged 18 months in Denmark was 11.5%. Each study was performed in children of different ages and different races in different areas using different diagnostic methods. Our diagnosis was based on physical examination by well-trained pediatric allergists, while other research was based on questionnaires [18,19] or data obtained by a trained interviewer [20]. Our research results are highly representative of the prevalence of AD in Asian infants.

We also investigated the prevalence of RW in infancy. Our study showed that the respective prevalence of RW at 6 months and 14 months of age was 0% and 7.6%. Other reports showed higher values. Henderson et al [21] reported that the prevalence of RW at 6 months and 7-18 months of age in the UK was 8.8% and 15.2%, respectively. Visser et al [22] reported that the prevalence of RW in the first year of life among Dutch infants was 14.5%. However, Chulada et al [23] reported that the prevalence of RW in the USA was 7.6%. Our results were similar to those of Chulada et al and provide important information on the prevalence of RW in Asian infants.

We showed that higher cord blood IgE is significantly associated with AD at 6 months of age (aOR, 1.607). However, ROC curve analysis revealed that AD is not a good predictor of AD at 6 months of age. We previously reported that cord blood IgE level is not a good predictor of infant AD [9]. Other authors [24,25] reported that cord blood IgE cannot be recommended as a screening instrument for primary prevention. The results of our study are consistent with these findings. The predictability of allergic diseases using cord blood IgE has received much attention. However, our latest finding is that cord blood IgE is the only risk factor out of 16 cord blood immunological markers, including relatively new markers such as T_H1, T_H2, T_C1, T_C2, and $CD4^+CD25^+$ cells. These data indicate that cord blood IgE is the best marker of 16 cord blood immunological markers but not an ideal predictor for AD at 6 months of age.

Our study also showed that high total IgE and egg white–specific IgE at 6 months of age were significantly associated with AD at 14 months of age and that the aOR was 1.018 and 23.246, respectively. Moreover, ROC curve analysis showed AD to be a good predictor of AD at 14 months of age. Other authors did not find similar results at this age. Perkin et al [26] reported that total IgE at 12 months was a predictor of eczema at age 5; this finding is similar to ours for total IgE, except for the difference in age. As for sensitization to hen egg, Nickel et al [27] reported that hen egg–specific IgE at 12 months is a valuable marker for subsequent allergic sensitization. Although the outcome of these authors was sensitization to common indoor and outdoor allergens at the age of 3 years, which is different from the outcome we observed, the results are nonetheless similar to ours. We found that high total IgE and sensitization to hen egg are risk factors and predictive factors of AD at 14 months of age. These findings are useful for clinical practice.

In contrast to the importance of IgE in AD, total IgE and specific IgE were not risk factors for RW at 14 months of age. Our results show that high IL-4 production was a risk factor for RW at 14 months of age. IL-4 is a T_H2 cytokine that induces isotype class switching to IgE in human B cells [28,29]. Whether participants with RW at 14 months of age show a subsequent increase in IgE or not will be analyzed in future studies. Although PHA-induced production of IL-4 from PBMCs was a risk factor for RW in our study, the percentage of T_H2 was not, indicating that, although cell count does not change drastically, the ability to produce IL-4 does change in children with RW.

Our cohort is relatively smaller than other birth cohorts. While most studies were based on questionnaires or interviews by nurses, our participants underwent a physical examination by well-trained pediatric allergists, as well as blood tests at 6 and 14 months of age. This makes our study design quite unique. Moreover, we tested new markers of the T_H1/T_H2 paradigm, which includes the T_H1/T_H2 subset, Treg, and $T_H1/$

T_H2 cytokine production. These new markers have not been analyzed in other cohort studies. Our findings provide useful information on allergic disease in infants and on the use of markers in clinical practice.

Total and specific IgE are important markers for AD in infants. In contrast, total and specific IgE are not important markers for RW.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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