

ORIGINAL ARTICLE

Prediction of atopic dermatitis in 2-yr-old children by cord blood IgE, genetic polymorphisms in cytokine genes, and maternal mentality during pregnancy

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Keywords

atopic dermatitis; birth cohort; cord blood IgE; genetic polymorphisms; prediction; stress.

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Abstract

Atopic dermatitis (AD) is the most common skin disease in childhood and the first step of atopic march. This study aimed to investigate whether AD in children could be better predicted by biologic markers (cord blood IgE [cbIgE], LT- α *NcoI* alleles, and Fc ϵ RI- β E237G genotypes) and maternal mentality during pregnancy, taking into account gender, socio-demographic factors, and parental atopy. From 2001 to 2005, 1264 mother–infant pairs were recruited to participate in a birth cohort study. Prenatal questionnaire was used to collect family history, maternal gestational conditions and mentality, and environmental exposures. Cord blood was collected and assayed for genotypes and IgE levels. Phone interviews at 6 months and 2 yrs of age were conducted to inquire children's health status, including AD occurrence. In addition to the known risk factors such as gender, maternal education, and parental atopy, biomarkers and maternal mentality during pregnancy were screened by logistic regression as candidate predictors of AD. Area-under-curve (AUC) statistic from receiver-operating characteristic (ROC) curve analysis was used to compare two predicting models with and without biomarkers and maternal mentality. A total of 730 pairs completed the prenatal questionnaire and phone interview and were included in final analysis. The prevalence of ever having physician-diagnosed AD by 2-yr-olds was 5.9%. Elevated cbIgE levels (≥ 0.5 kU/l), LT- α *NcoI* alleles, Fc ϵ RI- β E237G genotype, and maternal psychologic stress during pregnancy were significantly associated with AD. Comparison with AUCs of the classic model (including gender, maternal education, and parental atopy), the model adding cbIgE levels, genotypes in cytokine genes, and maternal stress (model 2) showed higher ability to discriminate between children with and without AD (AUC statistics: 0.63 [95% CI = 0.60–0.67] vs. 0.73 [95% CI = 0.70–0.76], respectively; model comparison, $p = 0.027$). We conclude that elevated cbIgE, LT- α and Fc ϵ RI- β genotypes, and maternal stress during pregnancy were associated with ever having physician-diagnosed AD in 2-yr-old children and increased the predictive ability for AD after taking into account gender, maternal education, and parental atopic history.

Atopic dermatitis (AD) is the most common skin disease in childhood. AD is often the first manifestation of atopic disease. The prevalence of AD has increased in many countries worldwide over the past two decades, including Taiwan (1).

More than 80% of AD in childhood develops before 5 yrs of age and especially during the first 6 months of life (2). The quality of life of children with AD and their families is obviously affected, especially in those children with moderate and

severe symptoms (3). Identification of the early risk factors of AD provides potential for preventing the development of atopic diseases in early childhood.

Atopic disease is likely attributed to skewed T help 2 (Th2) immune response with higher immunoglobulin E (IgE) production by Th2 cytokine stimulation (interleukin [IL]-4, IL-5, IL-13) (4). Umbilical cord blood IgE (cbIgE) is a known biomarker that can predict allergic sensitization in early childhood (5). Elevated cbIgE level is also a risk factor for asthma in school-age children (5) and AD in early childhood (6).

Many candidate genes are reported to contribute to the development of atopic diseases (7). Chromosome 5q31–33 contains numerous genes that affect IgE production, such as cytokine gene cluster (*IL-4*, *IL-5*, and *IL-13*) and granulocyte-macrophage colony-stimulating factor (8). *IL-4* plays an important role in IgE synthesis by stimulating pre-T helper cells to transform into Th2 cells. The *IL-4* promoter polymorphism, a C-to-T change at position –590, has been reported to be associated with the development of atopy, asthma, and rhinitis in children at 12 months of age (9). Tumor necrosis factors (TNFs) are potent pro-inflammatory cytokines involved in the development of atopic disease (10). The TNF cluster located on chromosome 6q21.1–21.3 contains both the *TNF* gene and two lymphotoxin (*LT*) genes (*LT- α* and *LT- β*). *LT- α* is related to B-cell proliferation and IgE synthesis. The *LT- α* *NcoI* alleles are associated with atopy and total IgE levels (11). Higher IgE levels were observed in female with *LT- α* *NcoI* 2/2 genotype (11). The high-affinity receptor for IgE (Fc ϵ RI) found on the surface of mast cells and basophils plays an important role in the IgE-mediated hypersensitivity reaction after binding with allergen-specific IgE antibodies (12). Fc ϵ RI contains three subunits: an α chain, a β chain, and a γ chain. The β subunit of Fc ϵ RI (Fc ϵ RI- β) can amplify the strength of signal transduction and help transport receptors to the cell surface (13). Single-nucleotide polymorphisms (SNPs) of Fc ϵ RI- β located in chromosome 11q13 are also susceptibility factor for atopic diseases and atopy (14).

Psychosocial stress is also reported with a factor for the development of allergic diseases and atopy. A German study in patients with AD showed that stress might increase specific IgE levels and eosinophil counts (15). Parental stress was found to be associated with infantile wheezing (16). It is known that the *in utero* environment can affect fetal immune system development. Maternal stress during pregnancy was reported a risk factor for elevated cbIgE levels (17). Prenatal stress was also found associated with altered adaptive immune responses in cord blood mononuclear cells through increases in IL-13 levels and decreases in interferon (IFN)- γ levels (18).

Because both hereditary and environmental factors contribute to the development of AD, we developed a predictive model for 6-month-old AD from Taiwan birth cohort study (19). Predictive factors of AD included parental atopic diseases, environmental exposure to fungus and renovation/painting at home, and maternal educational level. However, the above-mentioned model used questionnaire data only,

but no biologic markers. This study aimed to investigate the effect of biologic markers (cbIgE and susceptible genetic polymorphisms) and maternal mentality during pregnancy on the development of childhood AD and to examine whether these biologic factors could enhance the prediction of AD after taking into account gender, socio-demographic factors, and parental atopy.

Materials and methods

Subject recruitment

Eight private maternity hospitals were selected at seven locations in Taiwan, including Hsin-Juang (Taipei County), Jiayi City, Huwei Town (Yulin County), Singying (Tainan County), Tainan City, Kaohsiung City, and Taitung (Taitung City). Pregnant women who had undergone prenatal examinations at these hospitals and their neonates born after July 2001 were consecutively recruited in this study (17). In their third trimester of gestation, written informed consent was obtained and the women were asked to complete a structured questionnaire. Venous blood was obtained by a nurse. At birth of the child, umbilical cord blood was collected. Our protocols were approved by the National Cheng Kung University Hospital Institutional Review Board. Failure to collect any of these components resulted in exclusion of the pair from this study. Excluding mothers of foreign nationality, a total of 1264 mother–infant pairs were recruited between July 2001 and July 2005. Preterm births were ($n = 66$, 5.2%) not excluded from this study. These infants were followed up by phone interview at the age of 6 and 24 months. Phone interviews were conducted by well-trained interviewers after each mother's permission.

Data collection

Questionnaires were completed 1 month before the estimated due date. Pregnant women were asked about their demographic data, family history of atopic diseases (asthma, allergic rhinitis, and AD), environmental factors at home (incense burning, pets, carpet, water damage, fungi on wall, pesticide use), and the neonate's birth order. Maternal self-reported mental status (vitality, vigor, happiness, anxiety, discouragement, nervousness, tiredness, exhaustion, and working stress) during pregnancy was also included in the prenatal questionnaire. The questions pertaining to maternal mental status were extracted from the 'modified Chinese version of Short Form 36 Health Survey' (IQOLA SF-36 Taiwan standard version 1.0) after adequate authorization. Each neonate's birth data (gender, weight, height, head circumference, and gestational week) were collected from hospital records.

Telephone interviews were conducted when the children were 6 months and 2 yrs of age. Mothers were asked about their children's health, diet, growing situation, and environmental exposure. Identification of children with or without AD was based on the response to the question 'Did your child ever have physician-diagnosed AD?' and according to the symptoms of itching and scratching the skin and a

characteristic rash in locations typical of the disease (such as arm folds and behind the knees). An answer of 'Yes' to both questions in any follow-up interview was considered to indicate that the child had AD by 2 yrs of age.

IgE measurement

The levels of cbIgE were determined using the Pharmacia UniCap IgE assay system (CAP System; Pharmacia Diagnostics, Uppsala, Sweden). Infants with cbIgE ≥ 0.5 kU/l were defined as having elevated cbIgE levels. The pairs of mother and infants whose cbIgE data were suspected to be contaminated by maternal blood (tIgE/cbIgE ≤ 50) were excluded from the final analysis (20).

Genotype determination

Genomic DNA was extracted from umbilical cord blood cells using standard genomic DNA extraction methods. The genotypes of IL-4-590C/T, TNF- α -308G/A, LT- α *NcoI* 1/2 alleles, and Fc ϵ RI- β E237G were determined using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) as previously described (20).

Statistical analysis

JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA) was used for data analysis. Differences between infants with and without AD were assessed by Student's *t*-test or non-parametric tests for continuous variables and chi-square test or Fisher's exact test for categorical variables. Odds ratio (OR) and 95% confidence interval (95% CI) were used to examine the effects of potential risk factors for AD. *p* Values ≤ 0.05 were considered statistically significant.

Principle component analysis followed by varimax rotation was used to identify the major responses concerning maternal mental status during pregnancy. The number of rotated factors was obtained by examining eigenvalues of the principle components, and factors with eigenvalues > 1.0 were used to analyze the association with AD. We tried to avoid dichotomizing or classifying the genotypes and instead adopted the approach of using the homozygous genotype with lower frequency of ever having AD as the reference. Moreover, the rare homozygous genotype was combined with the heterozygous genotype to avoid the lower sample size as shown in the previous study (20).

Backward stepwise regression was applied to select the predictive factors in multiple regression analysis. Those factors with *p* values < 0.10 in multiple regression analysis would be included in the predictive model for AD. The effects of classic factors (e.g., gender, maternal education, and paternal atopy; model 1) and further added biologic markers and maternal mental status during pregnancy (model 2) for AD were evaluated by multiple regression analysis. The ability of the models to discriminate between children with and without AD was evaluated by receiver–operator characteristic (ROC) curves. Models with area-under-the-curve (AUC) statistics equal to 0.5 were considered not better than chance alone,

whereas models with higher AUC statistics were considered better than chance. We then compared AUC in different models by the Wilcoxon–Mann–Whitney *U* test, which was performed using MedCalc for Windows version 9.2.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Among the 1264 mother–child pairs in our study, 91 that had umbilical cord blood suspectedly contaminated by maternal blood, 13 with multiparity, and one deceased child were excluded from the study. Therefore, 1159 pairs (91.7%) were recruited for follow-up interviews. Among them, 895 mothers (77.2%) completed the telephone interview and data collection. After excluding mother–infant pairs with missing data of potential risk factors, a total of 730 pairs (63%) were entered for the final regression model analysis. The characteristics of parents and children in included pairs and excluded pairs were shown in Table 1. The included and excluded pairs did not differ significantly regarding maternal age during delivery, parent-education levels, gender distribution, gestational weeks, birth weight, and birth order. The lifetime prevalence of AD was 5.9% in 2-yr-old children.

Maternal self-reported occasional anxiety during pregnancy was a risk factor for AD onset before 2 yrs of age (*p* = 0.022). A significant association was also found between childhood AD and other self-reported maternal mental statuses, such as vitality (*p* = 0.0015), depression

Table 1 Characteristics of parents and children in included pairs (*n* = 730) and excluded pairs (*n* = 429)

Characteristic	Included pairs <i>n</i> (%)	Excluded pairs <i>n</i> (%)
Parents		
Maternal age at childbirth (yr)	27.8 $\pm$ 4.7*	27.6 $\pm$ 4.9*
Maternal education		
>12 yrs	241 (33.0)	114 (26.6)
10–12 yrs	415 (56.8)	244 (56.9)
≤ 9 yrs	74 (10.2)	58 (13.5)
Paternal education		
>12 yrs	235 (32.5)	123 (28.7)
10–12 yrs	383 (53.1)	219 (51.0)
≤ 9 yrs	104 (14.4)	63 (14.7)
Children		
Birth weight (g)	3097.4 $\pm$ 447.2*	3059.8 $\pm$ 427.6*
Gestational length (wks)	38.9 $\pm$ 1.2*	38.8 $\pm$ 1.3*
Gender		
Male	361 (49.5)	227 (52.9)
Female	369 (50.5)	194 (45.2)
Birth order		
First	319 (43.7)	149 (34.7)
Second	251 (34.4)	148 (34.5)
$\geq$ Third	114 (15.6)	59 (13.8)

Some numbers do not add up to total *n* because of missing values.

*Mean $\pm$ s.d.

Table 2 Rotated factors of maternal mentality during pregnancy by principle component analysis

	Factor 1	Factor 2
Eigenvalue (% variance explained)	3.61 (30.4%)	1.50 (26.3%)
Self-reported vitality	-0.06	0.83
Self-reported nervousness	0.71	-0.02
Self-reported depression	0.63	-0.37
Self-reported vigor	-0.08	0.80
Self-reported anxiety	0.67	-0.40
Self-reported exhaustion	0.70	-0.28
Self-reported happiness	-0.22	0.79
Self-reported tiredness	0.68	-0.17
Self-reported work stress	0.61	0.13
Conceived indicators	Stress	Energy and happiness

Factors 1 and 2 account for 56.7% of variance.

Factor 1 was conceived to be stress indicator. Factor 2 was conceived to be energetic and happy indicator.

($p = 0.038$), vigor ($p = 0.0087$), exhaustion ($p = 0.0009$), tiredness ($p = 0.015$), and working stress ($p = 0.0056$). Because mental factors were mutually related, principle component analysis was used to summarize these mental factors. Two rotated factors had eigenvalues of >1.0 and were

selected. Factor 1 (stress indicator) was strongly and positively related to nervousness, depression, anxiety, exhaustion, tiredness, and working stress. Factor 2 (energy and happiness indicator) was positively associated with vitality, vigor, and happiness (Table 2). We then divided these two factor scores into low, medium, and high groups. After adjustment for gender, maternal educational levels, parental atopy, cbIgE levels, and susceptible genotype, high maternal emotional stress during pregnancy was significantly associated with early childhood AD at 2 yrs of age (OR [95% CI] = 2.3 [1.1–5.3]; Table 3).

The effects of potential risk factors for childhood AD are presented in Table 3. Mothers ever having AD (OR [95% CI] = 4.4 [0.9–17.0]) and fathers ever having respiratory allergic diseases (RAD) (OR [95% CI] = 2.2 [1.1–4.4]) increased the risk of the children developing AD. Children with elevated cbIgE levels were at a higher risk of developing AD (OR [95% CI] = 2.2 [1.0–4.6]). Children with the LT- α 2/2 genotype might be at a higher risk of AD than those with the 1/1 or 1/2 genotype (OR [95% CI] = 2.6 [1.0–7.7]). A positive effect was found between the Fc ϵ RI- β EG/GG genotype and AD in children (OR [95% CI] = 2.0 [1.1–3.9]). None of the environmental exposure factors (e.g., a pet at home, carpet at home, cockroach appearance at home, and fungus on wall) had a significant effect on the development of AD.

Table 3 Odds ratios and adjusted odds ratios of potential risk factors for ever having AD in 2-yr-old children ($n = 730$)

Variables	N	Percentage of children	No. of AD (%)	OR (95% CI) [†]	AOR (95% CI) [‡]
Gender					
Male	361	49.5	25 (6.9)	1.5 (0.8–2.7)	1.4 (0.7–2.6)
Female	369	50.5	18 (4.9)	Reference	Reference
Birth season					
Spring	164	22.5	8 (4.9)	0.9 (0.3–2.2)	–§
Summer	169	23.2	10 (5.9)	1.1 (0.4–2.6)	
Autumn	200	27.4	14 (7.0)	1.3 (0.6–2.9)	
Winter	197	27.0	11 (5.6)	Reference	
Birth order					
First	333	45.6	23 (6.9)	1.8 (0.7–5.5)	–§
Second	261	35.8	15 (5.8)	1.5 (0.6–4.7)	
≥Third	128	17.5	5 (3.9)	Reference	
Maternal education					
>12 yrs	241	33.0	16 (6.6)	1.7 (0.5–7.4)	1.6 (0.5–7.7)
10–12 yrs	415	56.9	24 (5.9)	1.5 (0.5–6.2)	1.6 (0.5–7.2)
≤9 yrs	74	10.1	3 (4.1)	Reference	Reference
Maternal history of atopic diseases					
AD	16	2.2	3 (18.8)	4.5 (1.0–14.9)*	4.4 (0.9–17.0)*
RAD	141	19.3	12 (8.5)	1.8 (0.9–3.6)	1.4 (0.6–2.9)
No	573	78.5	28 (4.9)	Reference	Reference
Paternal history of atopic diseases					
AD	15	2.0	2 (13.3)	3.2 (0.5–12.5)	3.1 (0.4–13.1)
RAD	145	19.9	15 (10.3)	2.4 (1.2–4.6)**	2.2 (1.1–4.4)*
No	570	78.1	26 (4.6)	Reference	Reference
Cord blood IgE (kU/l)					
≥0.5	106	14.5	12 (11.3)	2.4 (1.2–4.8)*	2.2 (1.0–4.6)*
<0.5	624	85.5	31 (5.0)	Reference	Reference

Table 3 (Continued)

Variables	N	Percentage of children	No. of AD (%)	OR (95% CI)†	AOR (95% CI)‡
IL-4 -590C/T					
CC/CT	244	33.4	16 (6.6)	1.2 (0.6–2.2)	–§
TT	486	66.6	27 (5.6)	Reference	
TNF- α -308G/A					
GA/AA	143	19.6	6 (4.2)	0.6 (0.2–1.5)	–§
GG	585	80.4	37 (6.3)	Reference	
LT- α <i>NcoI</i> allele					
22	186	25.5	17 (9.1)	2.7 (1.1–7.5)*	2.6 (1.0–7.7)*¶
12	379	51.9	20 (5.3)	1.5 (0.6–4.1)	1.5 (0.6–4.2)
11	165	22.6	6 (3.6)	Reference	Reference
Fc ϵ RI- β E237G					
EG/GG	230	31.5	20 (8.7)	2.0 (1.1–3.7)*	2.0(1.1–3.9)*
EE	500	68.5	23 (4.6)	Reference	Reference
Rotated factors of maternal mentality					
Factor 1 (stress indicator)					
High	244	33.4	22 (9.0)	2.3 (1.1–5.2)*	2.3 (1.1–5.3)*
Median	244	33.4	11 (4.5)	1.1 (0.5–2.7)	1.1 (0.4–2.8)
Low	242	33.2	10 (4.1)	Reference	Reference
Factor 2 (energy and happiness indicator)					
High	243	33.3	9 (3.7)	0.5 (0.2–1.1)	–§
Median	244	33.4	16 (6.6)	0.9 (0.4–1.8)	
Low	243	33.3	18 (7.4)	Reference	
Pet at home					
Yes	230	31.5	13 (5.7)	0.9 (0.5–1.8)	–§
No	489	67.0	30 (6.1)	Reference	
Fungus on wall					
Yes	123	16.8	10 (8.1)	1.5 (0.7–3.1)	–§
No	601	82.3	33 (5.5)	Reference	
Carpet at home					
Yes	61	8.4	3 (4.9)	0.8 (0.2–2.3)	–§
No	664	91.0	40 (6.0)	Reference	

Some numbers do not add up to total n because of missing values.

AD, atopic dermatitis; RAD, respiratory allergic disease (asthma and allergic rhinitis).

* $p < 0.05$, ** $p < 0.01$.

†OR (95% CI), Odds ratio (95% confidence interval).

‡AOR, adjusted OR. The variables in multiple regression analysis were selected by backward stepwise regression.

§None in the multiple regression analysis.

¶p Value = 0.055.

For predicting AD, we set up model 1, which included gender, maternal education, and parental atopy, each of which was found to significantly associate with AD in children in a national birth cohort in Taiwan (19). According to backward stepwise regression processes that excluded unimportant factors one at a time from the model, we then added cbIgE levels and susceptible genetic factors (LT- α *NcoI* alleles and Fc ϵ RI- β E237G genotypes) and rotated factor 1 of maternal mental status during pregnancy to develop model 2. Among parental atopy, the strongest factor for AD in children was found to be parents with AD followed by parents with RAD. Therefore, we grouped paternal atopy into the following categories: either parent with AD; either parent with RAD but without AD; and both parents without any

atopic diseases. The ability of models to discriminate between children with and without AD is shown in Fig. 1. Compared with an AUC = 0.5, AUC statistics were significantly different from 0.5 in both model 1 (AUC = 0.63 [95% CI = 0.60–0.67], $p = 0.0049$) and model 2 (AUC = 0.73 [95% CI = 0.70–0.76], $p = 0.0001$). The AUC statistics were significantly better for model 2 as compared with model 1 ($p = 0.027$), indicating better capability of discrimination between children with or without AD by model 2 as compared with model 1. The factors that affect AD in model 2 are shown in Table 4. Elevated cbIgE levels, LT- α 2/2 genotype, Fc ϵ RI- β EG/GG genotype, and rotated factor 1 of maternal mental status (higher stress) during pregnancy were positively associated with early childhood AD.

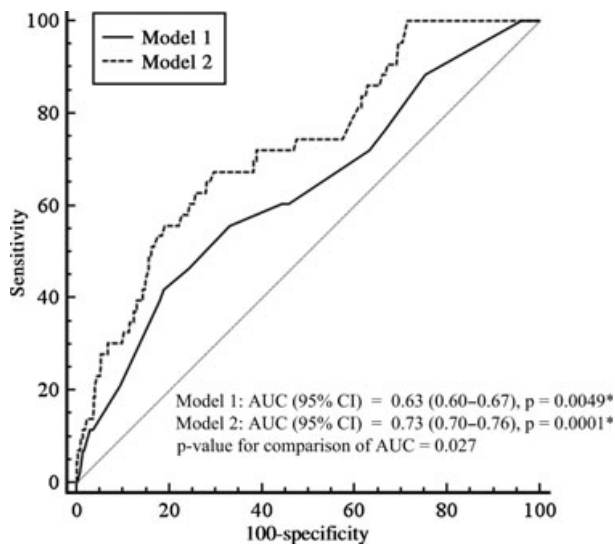


Figure 1 Receiver-operating characteristic curves for the prediction of atopic dermatitis in 2-yr-old children by model 1 and model 2. Factors of model 1 include gender, maternal education, and parental atopy. Model 2 includes model 1 variables, cord blood IgE levels, genetic polymorphisms of LT- α *NcoI* alleles and Fc ϵ RI- β E237G, and maternal stress indicator during pregnancy. The diagonal line indicates a reference area under curve (AUC) = 0.5 (no better than chance alone). * p -value for AUC of models compared with AUC = 0.5.

Table 4 The adjusted odds ratios of factors in predictive model 2 for ever having AD in 2-yr-old children ($n = 730$)

Factors	n	No. of AD (%)	AOR (95% CI)*	p Value
Cord blood IgE (kU/l)				
≥0.5	103	12 (11.7)	2.1 (1.0–4.4)	0.044
<0.5	599	31 (5.2)	Reference	
LT- α <i>NcoI</i> allele				
22	181	17 (9.4)	2.8 (1.1–8.2)	0.04
12	362	20 (5.5)	1.5 (0.6–4.3)	0.4
11	159	6 (3.8)	Reference	
Fc ϵ RI- β E237G				
EG/GG	223	20 (9.0)	2.1 (1.1–4.0)	0.024
EE	479	23 (4.8)	Reference	
Maternal stress during pregnancy				
High	234	22 (9.4)	2.3 (1.1–5.3)	0.036
Median	235	10 (4.3)	1.1 (0.5–2.8)	0.82
Low	233	11 (4.7)	Reference	

*AOR (95% CI), adjusted odds ratio (95% confidence interval). AOR was adjusted by gender, maternal education, and parental history of atopic diseases. AD, Atopic dermatitis.

Discussion

We found that cbIgE levels, infantile LT- α and Fc ϵ RI- β genotypes, and maternal mental stress during pregnancy were associated with AD in children. Comparison with the classic

model (including gender, maternal education, and parental atopy), the model that included cbIgE levels, genotypes in cytokine genes, and maternal stress, could better predict AD in children in our study.

Elevated cbIgE concentration was first reported as a biomarker for predicting atopic diseases in the 1970s (21). In the past few decades, the effect of cbIgE on childhood atopic disease has remained controversial, but there is general agreement that elevated cbIgE level is related to later atopic diseases. In longitudinal studies, cbIgE ≥ 0.3 kU/l was reported to increase the risk of AD development at the age of 18 months (6), and cbIgE ≥ 0.5 kU/l was associated with AD in 5-yr-old children and might predict subsequent atopy up to 20 yrs of age (22). Elevated CbIgE was also associated with AD in 1-month-old, 3-month-old, and 6-month-old children in a birth cohort study in Taichung, Taiwan (23). We also found a significant effect of elevated cbIgE levels on AD in children in the present study. Children with high cbIgE levels may be more sensitive to allergens and have a subsequently increased risk of developing atopic diseases.

Infantile LT- α *NcoI* alleles and Fc ϵ RI- β E237 G genotypes were found associated with children's AD in the present study. LT- α , also known as TNF- β , is produced by lymphocytes and plays an important role in B-cell proliferation and IgE synthesis (24). The *NcoI* variant within the first intron of the LT- α gene has been associated with increased IgE levels (11) and atopy (11) and affects the rate of gene transcription and protein production (25). This finding was inconsistent with that of the other study. Izakovicova Holla et al. (26) found no association between polymorphisms in the LT- α alone and atopic diseases in a Czech population, but there was an interaction between the TAP333 and LT- α polymorphisms. Atopic diseases are complex and involve both environmental and genetic components. To our knowledge, our study might be the first to find that LT- α *NcoI* alleles are risk factors for AD in children. However, our finding might be attributable to a gene–gene or environment–gene interaction. Another genetic polymorphism, an amino acid substitution in exon 7 of the Fc ϵ RI- β gene (E237G), was also associated with the development of AD on children in present study. Fc ϵ RI expression on the surface of basophils and mast cells plays an important role in the IgE-mediated hypersensitivity reaction after binding with allergen-specific IgE antibodies (12). Among the three Fc ϵ RI subunits (α , β , and γ chain), Fc ϵ RI- β plays a central role in signal transduction and helps transport the Fc ϵ RI protein to the surface of mast cells and basophils (13). Polymorphism of Fc ϵ RI- β E237G can alter the hydrophilic nature of the C-terminus of Fc ϵ RI- β and can affect intracellular signal transduction. Kim et al. (27) indicated that E237G substitution in the Fc ϵ RI- β gene may enhance Fc ϵ RI signaling and induce the release of pro-inflammatory mediators and cytokines in response to receptor cross-linking. However, the effect of Fc ϵ RI- β E237G may be attributable to linkage disequilibrium with other variants on chromosome 11q13 (28).

Psychologic stress of the mother during pregnancy was found to increase the risk of AD development in the present study. High emotional stress during pregnancy not only leads

to pregnancy complications but also increases children's risk of developing disease in later life (29). Psychologic stress related to atopic disease is widely reported; however, the mechanism remains unclear. The stress response is initiated in the central nervous system and activates the autonomic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the HPA axis by psychologic stress can lead to the elevated release of corticosteroids/glucocorticoids. Glucocorticoids can drive Th1/Th2 balance toward a Th2 immune response by upregulating IL-4, IL-13, and IL-10 production (30). An animal study showed that maternal stress-induced corticosterone could cross the placenta, affect fetal intrauterine immune modulation, and perhaps lead to prolonged changes in HPA axis activity of the offspring (31). Moreover, the nervous system can affect the course of AD through emotional stress (32), abnormal expression of neuropeptides and neurotrophin in skin lesions, and altered patterns of cutaneous innervations (33, 34). Nerve growth factor (NGF) can regulate neuropeptide synthesis and the allergic response through interactions with immune inflammatory cells (33, 34). Wang et al. (35) indicated that NGF was a good predictor of AD development in children and also found a positive correlation between maternal stress during pregnancy and NGF levels. Higher cord blood neuro-mediated levels were measured in patients with AD than in controls (35). Thus, stress-induced perinatal immunomodulation may affect the expression of atopic diseases in children.

Receiver-operating characteristic curve has been used broadly in assessing predictive models (36, 37). The curve is a plot of sensitivity (the true positive rate) vs. 1-specificity (the false positive rate) evaluated at consecutive cutoff points of the predicted probability. A quantitative summary measure of the ROC curve can be evaluated by AUC (38). In the present study, ROC curves were compared and the AUCs indicated that model 2 better discriminated AD than model 1 (AUC = 0.73 vs. 0.63). The factors chosen in model 1 were based on a national birth cohort study of 25,000 participants in Taiwan (19). We show in this study that adding cbIgE, LT- α and Fc ϵ RI- β genotypes, and maternal stress during pregnancy significantly improve the performance of predictive model.

The fetus is extremely susceptible to environmental challenges, and the *in utero* condition may affect fetal health both before and after birth. Moreover, atopic disease is complex and involves genetic and environmental factors. Genes can modify the impact of environmental exposure and affect the development of atopic diseases. Our results suggested that in addition to heredity and demographic data, biologic markers (cbIgE levels and susceptible genetic polymorphisms) and prenatal exposure including maternal mental status during pregnancy should also be considered when predicting AD development.

The present study used a birth cohort design and enrolled a general population of pregnant women in their third trimester. Levels of cbIgE and genotypes of the LT- α *NcoI* alleles and Fc ϵ RI- β E237G were determined by standard methods. Moreover, information on maternal psychosocial

stress was obtained before childbirth, thus unlikely biased by the children's atopic outcomes.

There are limitations in this study. Firstly, infantile AD was based on maternal reports of physician-diagnosed AD, which might be subject to misclassification. We did not inquire whether this diagnosis was made by a dermatologist, a pediatrician, or a general practice physician. However, this diagnosis is most likely made by a member of one of these specialties. Physician-diagnosed AD reported by mothers was validated by clinical examination in another study (39). Moreover, characteristic symptoms of AD were described in the questionnaire, which might reduce the misclassification of children's AD. Secondly, all questionnaires were completed by the mothers. However, atopic history was obtained for both parents. We obtained atopic history before childbirth, but physician-diagnosed AD at the age of 2 yrs. The interviewers and mothers were unaware of the study hypothesis. Therefore, information bias owing to parental atopy status was not likely. Thirdly, 429 (37%) mother–infant pairs were excluded from the final analysis. Among them, 264 pairs were owing to incorrect contact information, moving abroad, or the mother's refusal to be interviewed. Additional 165 pairs were excluded owing to missing of any information in selected predictive factors. Therefore, selection bias might be a concern. The characteristics of the infants (e.g., gender, birth order, and birth weight) and parents (e.g., maternal age and parental education levels) did not differ significantly between those included and those excluded. Thus, selection bias owing to loss to analysis might not be severe in the present study. Fourthly, our result only showed moderate predictive capacity of model 2 (AUC = 0.73). Lower boundaries of 95% C.I. of the ORs were close to 1.0. Atopic diseases are complex and involve both environmental and genetic components (7). Many genetic and environmental factors affect the development of AD. However, owing to the sample size of our study, genes with low percentage in minor allele were not included. Environmental factors with moderate effects tended to be excluded from the backward stepwise modeling also. It is possible that if more variables were included in model 2, the predictive capacity would be enhanced. Also, broad confidence ranges for the predictive factors were seen (Table 4), despite the adjusted OR of larger than 2.0. Although the predictive capacity of predictive model was moderate, we believe that this method is worth further testing as a tool for predicting development of AD. Finally, caution about the generalizability of our findings is warranted because infants recruited from eight private maternity hospitals at seven locations may not be representative of all infants in Taiwan. External validation study in more representative population is warranted.

In conclusion, cbIgE levels, LT- α and Fc ϵ RI- β genotypes, and maternal stress during pregnancy were associated with ever having AD in 2-yr-old children. Compared with the model including gender, maternal education, and parental atopic history, adding biologic marker and maternal emotional stress during pregnancy increased the predictive ability for AD.

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