

Association between cord blood IgE and genetic polymorphisms of interleukin-4, the β -subunit of the high-affinity receptor for IgE, lymphotoxin- α , and tumor Necrosis factor- α

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High cord blood immunoglobulin E (cbIgE) is known to be associated with increased risks of atopic diseases in childhood. The relationship between genetic polymorphisms and high cbIgE has not been well documented. A cross-sectional study was conducted to assess the association between cbIgE and genetic polymorphisms of interleukin (IL)-4 -590C/T, the β -subunit of the high-affinity receptor for IgE (Fc ϵ RI- β) E237G, lymphotoxin (LT)- α NcoI alleles, and tumor necrosis factor (TNF)- α -308G/A. A total of 320 mother–neonate pairs were recruited from four maternity hospitals from different locations of Taiwan. Cord blood was obtained and assayed for cbIgE. Polymerase chain reaction followed by restriction fragment length polymorphism was used to assess the genotypes. Three hundred pairs of mothers and neonates were included in the final analysis. Infants with IL-4 -590 C allele were found to have higher risk of elevated cbIgE (≥ 0.35 IU/ml, 24.3%) ($p = 0.004$). After adjusting for gender, birth order, maternal age, and history of allergic disease in maternal and paternal families, odds ratios for CC and CT genotypes were 4.41 and 3.16 (95% confidence interval 0.78–22.67, and 1.66–6.13), respectively, using TT genotype as reference. The genotypes of Fc ϵ RI- β , LT- α , and TNF- α were not associated with cbIgE before or after the adjustment. Our finding suggested a significant association of cbIgE with genetic polymorphism of IL-4 -590C/T, but not with the genotypes of Fc ϵ RI- β , LT- α , and TNF- α .

Hui-Ju Wen¹, Ying-Chu Lin^{1,2}, Yung-Ling Lee¹ and Yueliang Leon Guo³

¹Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan, ²College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, ³Department of Environmental and Occupational Medicine, National Taiwan University Medical College and NTU Hospital, Taipei, Taiwan

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Yueliang Leon Guo, Department of Environmental and Occupational Health, College of Medicine, National Taiwan University, 1 Sec 1, Jen-Ai Road, Taipei 100, Taiwan

Tel.: 886 2 3322 8216

Fax: 886 2 3322 8215

E-mail: leonguo@ha.mc.ntu.edu.tw

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The prevalence of atopic diseases, such as asthma, allergic rhinitis, and atopic dermatitis, has increased in the past decades in many countries, including Taiwan (1). Environmental and genetic factors are both important determinants in the development of atopic diseases (2).

Elevated cord blood immunoglobulin E (cbIgE) was found associated with an increased risk of atopic diseases in later childhood (3). Several candidate genes are reported to have influence on the development of atopic disease. Chromosome 5q31–33 comprises of numerous

candidate genes that may affect IgE synthesis, such as cytokine gene cluster (IL-3, IL-4, IL-5, IL-9, and IL-13) and granulocyte-macrophage colony stimulating factor (GM-CSF) gene (4). Interleukin (IL)-4 plays an important role in IgE synthesis by activating pre-T helper cells to become Th₂ cells that in turn trigger isotype switching from IgM/IgG to IgE in B cells (5). The IL-4 promoter polymorphism, a C to T change at position -590, has been associated with atopy and asthma (6). In some studies, IL-4 -590T allele was associated with higher risk of

developing atopy, asthma, and rhinitis in infants (7) as well as with higher total serum IgE (8). However, other studies did not show association between IL-4 -590C/T genotypes and atopic diseases (9). The effect of IL-4 -590C/T on cbIgE is not well documented.

FcεRI-β, a high-affinity IgE receptor, is found on the surface of mast cells and basophiles. The genetic variants of this receptor on chromosome 11q13 were associated with atopy and total IgE levels (10, 11). An amino acid substitution of FcεRI-β E237G alters the hydrophilic nature of the C-terminus of FcεRI-β and affects the intracellular signal transduction. This variant is associated with elevated total IgE and specific IgE and with the onset of childhood asthma (12).

Tumor necrosis factors (TNFs) are potent pro-inflammatory cytokines in asthmatic airway and lavage fluid from asthmatic lung (13). The gene for TNF is located on chromosome 6p21.1–21.3 and forms the TNF cluster with the two-lymphotoxin (LT) genes (LT-α and LT-β) (14). LT-α plays an important role in B cell proliferation and IgE synthesis. The *NcoI* alleles of LT-α was associated with atopy and increasing total IgE levels (15). The promoter polymorphism of TNF-α -308 G to A has influence on severity of asthma and on the development of childhood asthma (16). The LT-α *NcoI* alleles and TNF-α are in linkage disequilibrium. Some studies showed that the LT-α *NcoI**1 and TNF-α -308A haplotype played a potential role in asthma (17).

According to previous studies, the genotypes of IL-4 -590C/T, FcεRI-β E237G, TNF-α -308G/A, and LT-α *NcoI* alleles would affect the synthesis of IgE, which might cause the development of atopic diseases in childhood. However, it is unclear that whether these genetic polymorphisms influence the synthesis of cbIgE. We conducted a hospital-based birth cohort study in general population to investigate the association between cbIgE levels and the genetic polymorphisms.

Materials and methods

Subjects

Four private maternity hospitals were randomly selected at four locations of Taiwan, Annan (Tainan city), Singying (Tainan country), Hsin-Juang (Taipei country), and Taitung (Taitung city). Pregnant women at their third trimester were recruited to participate in this study, and informed consent was obtained. Mother was interviewed for medical and allergic history, and their peripheral blood was collected during the

third trimester, and the neonate's cord blood was collected at delivery after mother's informed consent. Failure of collecting any of this would result in exclusion of the pair in this study. Mothers of foreign nationality were also excluded. Serum and lymphocytes were aliquoted and stored in liquid nitrogen tank till analysis.

Questionnaire

Mothers were asked to complete a questionnaire at 1 month before the calculated due day. Demographic data and histories of atopic diseases (asthma, allergic rhinitis, and atopic dermatitis) in maternal and paternal families were collected by mother's report. Neonatal data of birth weight, height, and head circumference, gender, and gestational age were recorded by nurses.

IgE measurement

The Pharmacia UniCap IgE assay system (CAP system; Pharmacia Diagnostics, Sweden) was applied to determine the levels of cbIgE. The detected range was 0.35–100 IU/ml for CAP system. CbIgE ≥ 0.35 IU/ml was regarded as high (18).

IgA measurement

IgA levels of cord blood were also analyzed by enzyme-linked immunosorbent assay (ELISA) to determine the subjects whose cord blood may be contaminated by maternal blood. Neonates with IgA concentration above the 95th percentile of IgA (10 ng/ml) distribution were considered contaminated by maternal blood and were excluded from the analysis.

Genetic polymorphisms

Genomic DNA was extracted from cord blood by standard genomic DNA extraction methods. Screening for genetic polymorphisms of IL-4 -590C/T, LT-α *NcoI* alleles, and TNF-α -308G/A were performed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) with adequate restriction enzyme, as described in previous studies (17, 19). The PCR amplification of FcεRI-β 237E/G was performed by the following experimental procedure and primers. The upstream primer was 5'-GTCTCTTGAGCGAGACTTCTA-3', and the downstream primer was 5'-CTTATAAATCAATGGGAGGAAAACA-3'. The total volume of 25-μl PCR product contains 2.5 μl of

reaction buffer, 2 μ l of 100- μ M dNTP, 1.5 μ l of Mg^{+2} (1.5 mM), 0.1 μ l of *Taq* DNA polymerase (5 U/ μ l), 0.25 μ l of each primer (20 μ M), and 200 ng of genomic DNA. The conditions of PCR were one cycle of 94°C for 5 min, 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and a cycle of 72°C for 5 min. Then, a total of 15- μ l reagent containing 10 μ l of the PCR product, 1.5 μ l of buffer from New England Biolabs 2, 0.15 μ l of bovine serum albumin (100 μ g/ml), and two units of *Xmn*I enzyme (New England Biolabs, MA, USA) were digested at 37°C for 1 h. Digested products were separated on 2.5% agarose gels. The genotypes of Fc ϵ RI- β 237E/G resulted from a 23-bp band, a 148-bp band for E/E, a 23-bp band, a 148-bp band, and a 171-bp band for E/G, or two 171-bp bands for G/G.

Statistical analysis

Data analysis was done with JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA). The Pearson chi-square test and Fisher's exact test were used to determine the association between categorical data and cbIgE. For continuous data, Mann-Whitney *U*-test was used. As multiple comparisons were done for genotype effects on cbIgE, the α value was adjusted. Therefore, *p* value ≤ 0.0125 (0.05 divided by 4) was considered statistically significant.

To determine the association between genetic polymorphism and cbIgE, multiple logistic regression analysis was applied. Neonatal gender, maternal age, maternal and paternal family histories of atopic diseases were adjusted in the logistic regression model. Adjusted odds ratio (OR) for elevated cbIgE was calculated for each genetic variant.

Results

A total of 320 mother–neonate pairs were recruited from July 2001 to February 2003. Among them, 20 neonates had elevated cord serum IgA and were excluded from further analysis. As a result, 300 pairs of mothers and neonates were included in the final analysis, including 55 pairs from Annan (Tainan city), 81 pairs from Singying (Tainan country), 56 pairs from Hsin-Juang (Taipei country), and 108 pairs from Taitung (Taitung city). The range of cbIgE levels was underdetected (<0.35 IU/ml) to 4.27 IU/ml. The frequencies of cbIgE levels below 0.35, 0.35–0.5, 0.5–1.0, and ≥ 1.0 IU/ml were 75.7%, 6.0%, 12.3%, and 6.0%, respectively. Approximately one-quarter of neonates had elevated cbIgE (≥ 0.35 IU/ml, Table 1). In

Table 1. The characteristics in pregnant women and neonates and neonatal genotypes

Variables	n (%)
Total n	300
Maternal age at delivery (yr)	27.9 (4.83)†
Paternal family history of atopic diseases*	
Yes	37 (12.3)
No	232 (77.3)
Maternal family history of atopic diseases*	
Yes	39 (13.0)
No	227 (75.7)
Neonatal gender	
Boys	162 (54.0)
Girls	134 (44.7)
Cord blood immunoglobulin E	
≥ 0.35 IU/ml	73 (24.3)
<0.35 IU/ml	227 (75.7)
Interleukin-4 -590C/T	
C/C	9 (3.0)
C/T	114 (38.0)
T/T	177 (59.0)
Fc ϵ RI- β 237E/G	
E/E	206 (68.7)
E/G	85 (28.3)
G/G	9 (3.0)
Lymphotoxin- α <i>Nco</i> I alleles	
1/1	63 (31.0)
1/2	162 (54.0)
2/2	75 (25.0)
Tumor necrosis factor- α -308G/A	
G/G	248 (82.7)
G/A	50 (16.7)
A/A	2 (0.7)

*Including parents and grandparents of the neonates.

†Mean (s.d.).

All genes were tested by Hardy-Weinberg test (*p* > 0.05).

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

the four areas, the frequencies of cbIgE ≥ 0.35 IU/ml were not significantly different (*p* = 0.71) (data not shown). The mean age of mothers was not different between infants with elevated cbIgE and those with normal cbIgE. Boys had increasing frequency in cbIgE ≥ 0.35 IU/ml than girls (*p* = 0.04) (Table 2). Neonates with maternal family history of atopic diseases had higher cbIgE levels (*p* < 0.001). Such effect is not seen with paternal family history (*p* = 0.62) (Table 2).

The frequency of genotypes in IL-4 -590C/T, Fc ϵ RI- β 237E/E, LT- α *Nco*I alleles, and TNF- α -308G/A of infants are shown in Table 1. By χ^2 test, the IL-4 -590C/T genotypes were related to cbIgE (*p* = 0.004). The frequency of cbIgE ≥ 0.35 IU/ml was higher in IL-4 -590C/C and C/T groups than in T/T group. The genetic polymorphisms of Fc ϵ RI- β 237E/G, LT- α *Nco*I alleles, and TNF- α -308G/A were not associated with cbIgE levels (*p* = 0.21, 0.31, and 0.19, respectively) (Table 2).

Table 2. Relationship between parental factors, neonates' genetic polymorphisms, and cord blood immunoglobulin E (cbIgE)

Genotypes	cbIgE (IU/ml)		p-value†
	≥0.35	<0.35	
Maternal age at delivery (yr)	27.0 (0.56)*	28.2 (0.32)*	0.09‡
Maternal family history of atopic diseases			
Yes	18 (46.2)	21 (53.8)	<0.001
No	46 (20.3)	181 (79.7)	
Paternal family history of atopic diseases			
Yes	10 (27.0)	27 (73.0)	0.62
No	54 (23.3)	178 (76.7)	
Neonatal gender (%)			
Boys	47 (29.0)	115 (71.0)	0.04
Girls	25 (18.7)	109 (81.3)	
Interleukin-4 -590C/T			
C/C	3 (33.3)	6 (66.7)	0.004
C/T	39 (34.2)	75 (65.8)	
T/T	31 (17.5)	146 (82.5)	
FcεRI-β 237E/G			
E/E	53 (25.7)	153 (74.3)	0.21
E/G	20 (23.5)	65 (76.5)	
G/G	0 (0.0)	9 (100.0)	
Lymphotoxin-α NcoI alleles			
1/1	12 (19.1)	51 (80.9)	0.31
1/2	45 (27.8)	117 (72.2)	
2/2	16 (21.3)	59 (78.7)	
Tumor necrosis factor-α -308G/A			
G/G	9 (17.3)	43 (82.7)	0.19
G/A; A/A	64 (25.8)	184 (74.2)	

All results are shown as n (%).

*Mean (s.d.).

†p-value was calculated by chi-squared test.

‡p-value was calculated by Mann-Whitney U-test.

To be cautious, we try to avoid classifying or dichotomizing the genotypes, and took an approach of using the homozygous genotype with higher percentage as the reference. Therefore, IL-4 promoter -590 CC and CT were individually compared with TT by logistic regression (Table 3). Similar approach was taken for LT-α *NcoI* polymorphism. As for TNF-α -308G/A and FcεRI-β E307G, owing to low prevalence of homozygous variant genotype, this genotype was grouped together with the heterozygous genotype for the logistic regression. To obtain the adjusted OR of these four genotypes, baby gender, birth order, mother's delivery age, maternal and paternal family histories of atopic diseases were included in the multiple logistic regression analysis (Table 3). After adjustment, IL-4 -590CT had higher OR of 3.16 (95% confidence interval, CI = 1.66–6.13) of having elevated cbIgE as compared with TT. Although there is no statistical significance, IL-4 -590CC also had higher OR than TT of elevated cbIgE levels (OR [95% CI] = 4.41 [0.78–22.67]). The genotypes of FcεRI-β 237E/G, TNF-α -308GG/GA, and LT-α *NcoI* alleles were not related to elevated cbIgE.

Table 3. The association between neonatal genotypes and cord blood immunoglobulin E (cbIgE) levels as shown by odds ratio (OR) estimated by multiple logistic regression analysis

Genotypes	cbIgE (IU/ml)		OR (95% CI)*	AOR (95% CI)†‡
	≥0.35	<0.35		
Interleukin-4 -590C/T				
C/C	3 (33.3)	6 (66.7)	2.35 (0.48–9.45)	4.41 (0.78–22.67)
C/T	39 (34.2)	75 (65.8)	2.45 (1.42–4.26)	3.16 (1.66–6.13)
T/T	31 (17.5)	146 (82.5)	1.0	1.0
FcεRI-β 237E/G				
E/G; G/G	20 (21.3)	74 (78.7)	0.78 (0.43–1.38)	0.62 (0.31–1.22)
E/E	53 (25.7)	153 (74.3)	1.0	1.0
Lymphotoxin-α <i>NcoI</i> alleles				
2/2	12 (19.1)	51 (980.9)	1.15 (0.50–2.71)	0.63 (0.24–1.70)
1/2	45 (27.8)	117 (72.2)	1.63 (0.82–3.47)	1.07 (0.48–2.48)
1/1	16 (21.3)	59 (78.7)	1.0	1.0
Tumor necrosis factor-α -308G/A				
G/A; A/A	9 (17.3)	43 (82.7)	0.60 (0.26–1.25)	0.44 (0.16–1.05)
G/G	64 (25.8)	184 (74.2)	1.0	1.0

All results are shown as n (%).

* (95% CI): (95% confidence interval).

†AOR (95%): adjusted odds ratio (95% confidence interval).

‡Adjusted by neonatal gender, birth order, maternal age at delivery, maternal history of atopic diseases, and paternal history of atopic diseases.

Discussion

To the best of our knowledge, this was the first study conducted to investigate the association between genotypes and cbIgE. We found that elevated cbIgE was associated with IL-4 -590C allele but not associated with genotypes of FcεRI-β 237E/G, TNF-α -308G/A, and LT-α *NcoI* alleles.

Using the CAP system for cord IgE measurement, the detection limit is 0.35 IU/ml. We therefore used this value as a cut-off point. Alternatively, this value can be treated as continuous. We examined the effects of genotypes on the value of cbIgE, and substituted a half (0.175 IU/ml) of the detection limit for those under detection. Only IL-4 genotype was statistically related to cbIgE ($p = 0.0062$ by ANOVA comparing those of CC, CT, and TT, data not shown). Such a result is similar to the findings shown in Table 3.

Our data also showed that maternal family history of atopic diseases was related to cbIgE. The prevalence of cbIgE ≥0.35 IU/ml was higher in neonates with maternal atopy. This was consistent with previous studies. A Belgian study on newborn described that maternal atopy was associated with elevated cbIgE (20). Johnson et al. (21) also reported an association between maternal history of atopic disease and cbIgE concentration.

In the present study, the IL-4 -590CC/CT increased the risk of elevated cbIgE. This result

was different from previous studies, which have been reported that the IL-4 -590T was associated with increased risk of allergic diseases, higher total serum IgE levels, and higher IL-4 gene promoter activity as compared with the C allele (7). The IL-5 and -13 genes were also recognized as strongly risk factors for IgE production and located in the same gene cluster (4). A study in Asia reported that genotypes of IL-13 were genetically linked with the genotypes of IL-4 gene promoter (22). A study in China indicated that the IL-13 Arg130Gln genotype was associated with high specific IgE production (23). Although genotypes of IL-13 were not determined in the present study, it was possible that the IL-4 -590C allele was genetically linked with IL-13 Arg130Gln genotype or other candidate genes like IL-5 response for IgE production. The immune mechanism by which IL-4 -590T allele or haplotypes regulate cbIgE production warrants further investigation.

Moreover, the frequencies of genotypes in IL-4 -590C/T are in racial difference (6, 19, 22, 24). Walley and Cookson (25) indicated that the frequencies of IL-4 -590C to T allele were 31% and 27% in Oxon participants and in Busselton families, respectively. In contrast, Kawashima et al. (6) reported that the frequencies of T alleles were 70% and 77% in control and atopic dermatitis offspring of Japanese. In the present study, we observed that the prevalence of T allele was 78.0%. Although the frequency of IL-4 -590T allele was similar to that of Kawashima's study, our finding was inconsistent with his finding that T allele homozygotes were associated with atopic dermatitis. The difference might have been caused by ethnic difference or different study outcomes.

Th₂-skewed immunity response is strongly associated with allergic disease. Environmental allergens could cross the placenta and cause Th₂ immune response in fetus (26). Hagendorens et al. (27) found that prenatal exposure to house dust mite allergen (Der p 1) had influences on cord blood T lymphocytes. Our data showed that mothers sensitized to dust mite, cat dander, dog dander, and cockroach (specific IgE ≥ 0.7 IU/ml) might increase the risk of infants with cbIgE ≥ 0.35 IU/ml (data not shown). Atopic reactions are complex and involve both environmental and genetic components. Our finding that IL-4 -590C/T was related to cbIgE might be because of environment-gene interaction, and requires further investigation.

The associations between the other three genes and IgE production remain inconsistent among studies from different countries (7, 22). Previous studies have identified the genes of Fc ϵ RI- β on

chromosome 11q13 as markers for atopy and elevated IgE response (10). In Australian population, Hill et al. (11) found that the genetic variants of Fc ϵ RI- β were associated with atopy and bronchial hyperresponsiveness. Fc ϵ RI- β E237G was reported to associate with total IgE, specific IgE to allergens, and childhood asthmatic response. However, this result was not found in Japanese children and Canadian infants (8). TNFs are important candidate genes of atopic disease. The linkage between LT- α NcoI 2/2 genotype and an increase of IgE levels had been detected in Italian women (28). Previous studies showed the association between LT- α NcoI*2 allele and asthma in Australian and British population (29). Moffatt et al. (30) indicated that LT- α NcoI alleles and TNF- α -308G/A genotypes were associated with asthma but not with IgE levels. TNF- α -308G/A polymorphism was associated with severe asthma and bronchial hyperreactivity (16). In combination, LT- α NcoI*2/TNF- α -308A was shown as a significant risk factor for asthma and atopy (17, 29). Our study demonstrated that Fc ϵ RI- β 237E/G, LT- α NcoI alleles, and TNF- α -308G/A were not associated with elevated cbIgE concentration. The mechanism of cbIgE production remains unclear. Ethnic variation in genetic background, strategies for participants' collection, and immune response and regulation for cbIgE production by different environmental exposures could partially explain such lack of association. Further follow-up of our population will be needed to determine whether cbIgE predicts occurrence of atopic diseases in this birth cohort.

One strength of this study is the birth cohort design. All participants were recruited from general population and enrolled when pregnant women were at their third trimesters. Besides, genotypes and cbIgE were measured by standard methods, therefore minimizing misclassification of these measurements. Therefore, neither selection bias nor information bias seemed to be a problem in this present study.

In conclusion, this study suggests that the IL-4 -590C allele was associated with elevated cbIgE. Confirmation in a larger population and assessment of the gene-gene and gene-environmental interactions are warranted.

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