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Interaction of maternal atopy, CTLA-4 gene polymorphism and gender on antenatal immunoglobulin E production

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Summary

Background Genetic heritability and maternal atopy have been correlated to antenatal IgE production, but very few studies have studied gene–maternal atopy interaction on antenatal IgE production. This study investigated the interaction of CTLA-4 polymorphism with prenatal factors on the elevation of cord blood IgE (CBIgE).

Methods Pregnant women were antenatally recruited for collection of prenatal environmental factors by a questionnaire. Umbilical cord blood samples were collected for CBIgE detection by fluorescence-linked enzyme assay and CTLA-4 polymorphism measurement by restriction fragment length polymorphism.

Results A total of 1104 pregnant women initially participated in this cohort study, and 898 of them completed cord blood collection. 21.4% of the newborns had elevation of CBIgE (≥ 0.5 kU/L). The CTLA-4+49A allele ($P = 0.021$), maternal atopy ($P < 0.001$) and gender ($P = 0.034$), but not the CTLA-4+49G allele, –318C allele, –318T allele, parental smoking or paternal atopy, were significantly correlated with the CBIgE elevation in multivariate analysis. A dichotomous analysis of gene–maternal atopy interactions identified maternal atopy and CTLA-4+49A allele had an additive effect on the CBIgE elevation, especially prominent in male newborns; and in the absence of maternal atopy, CTLA-4+49GG genotype had a protective effect on CBIgE elevation in female newborns.

Conclusions Maternal but not paternal atopy has significant impacts on CBIgE elevation depending on gender and CTLA-4+49A/G polymorphism of newborns. Control of maternal atopy and modulation of CTLA-4 expression in the prenatal stage may be a target for the early prevention of perinatal allergy sensitization.

Keywords cord blood IgE, CTLA-4, maternal atopy, polymorphism

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Introduction

Prevalence of allergy diseases has dramatically increased in the past two decades, especially in children [1]. Allergic mechanism has long been attributed to IgE-mediated reaction, and elevation of serum IgE levels has been correlated to allergic diseases [2]. The fact that systemic administration of humanized anti-IgE antibodies causes a 95–99% decrease in serum IgE, along with anti-inflammatory feature of allergic diseases [3], further supports the IgE-mediated mechanism of allergic diseases. It remains unclear whether the genetic effect on IgE production occurs early in fetal life. In a study with homozygous and heterozygous twins, the estimated heritability for cord blood IgE (CBIgE) levels is estimated around 84–95%,

which is much higher than the heritability for total IgE levels in childhood [4]. Recently, evidence suggests that allergic sensitization might occur *in utero* and might be related to the future development of allergic diseases [5, 6]. Elevation of CBIgE, although not good enough to predict the development of allergic diseases in childhood [7, 8], has been shown to be a risk predictor for the development of aeroallergen sensitization [9] and for the later development of childhood asthma [10]. In a cohort study, we have previously shown that maternal but not paternal atopy has a significant impact on the elevation of CBIgE levels [11]. Whether maternal atopy can interact with *certain genes* to promote IgE production early in the antenatal stage remains to be determined.

Many studies have demonstrated that many genes in different chromosomes are implicated in increase of total IgE levels and allergic disorders in different ethnic populations [12, 13]. A specific blockade of the CTLA-4 expression early in the allergen sensitization stage significantly attenuates allergen-induced airway hyperresponsiveness and IgE production [14]. Recently, the CTLA-4 promoter (−318C/T) and exon 1 (+49A/G) polymorphisms have been correlated to serum autoantibody titres in patients with Graves' disease [15]. A study with Chinese by Liu et al. [16] had also shown an association of these two single nucleotide polymorphisms (SNPs) with systemic lupus erythematosus (SLE). We were therefore interested in whether CTLA-4 polymorphisms at promoter (−318C/T) and exon 1 (+49A/G) were associated with elevation of total IgE levels and allergy diseases in Chinese population. We have previously shown that the CTLA-4+49A allele was associated with elevation of CBIgE levels [17], and associated with total IgE levels and allergic rhinitis (AR) in adults [18]. Extending from those studies, we attempted to explore the interaction of CTLA-4 polymorphism with prenatal environmental factors on antenatal IgE production in this study. Clarification of the antenatal gene-environment interactions on IgE production may provide a prospect for the decrease of antenatal IgE production, resulting in less allergy sensitization in childhood.

Materials and methods

Subjects and cord blood collections

A total of 1104 pregnant families were prenatally recruited for this study after informed consent was obtained. A research nurse was trained to explain the purpose of the study to eligible pregnant parents as they visited our obstetric clinic between September 1999 and August 2004 at Chang Gung Memorial Hospital, Kaoshiung, Taiwan. A questionnaire was applied to collect family allergy history, along with personal history data upon recruitment. Eight hundred and ninety eight newborn infants of the cohort population completed umbilical cord blood collection for CBIgE detection and DNA extraction for determination of CTLA-4+49A/G and −318C/T polymorphisms by restriction fragment length polymorphism (RFLP).

Cord blood immunoglobulin E detection and determination of parental atopic diseases

CBIgE levels were determined by a sensitive low-range IgE detection system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) [10, 17]. Elevation of CBIgE levels was defined by ≥ 0.5 kU/L as previously described [10, 17, 19]. Determination of parents with allergic diseases is defined by phenotypic asthma, rhinitis or atopic dermatitis, to-

gether with detectable serum-specific IgE directed against the common allergens in Taiwan including *Dermatophagoides pteronyssinus*, cockroach, dog dander, seafood or pollen mixture. Maternal atopy was referred to those mothers who had asthma, rhinitis or atopic dermatitis along with detectable specific IgE; and paternal atopy was referred to those fathers having asthma, rhinitis or atopic dermatitis along with detectable specific IgE.

DNA extraction and restriction fragment length polymorphism determination

Cord blood cells were subjected to DNA extraction with 0.5% sodium dodecyl sulphate lysing buffer followed by proteinase K (1 mg/mL) digestion of nuclear protein for 4 h at 60 °C. Total DNA was harvested by standard phenol-chloroform extraction followed by 70% alcohol precipitation. As modified from our previous report [17], we amplified the PCR reactions of the CTLA-4+49A/G polymorphism, using forward primer GCT CTA CTT CCT GAA GAC CT and reverse primer CAA GCC AGA TTG AGT TTT AC for 40 cycles. The PCR products were incubated with the BbvI restriction enzyme (New England Biolabs, Boston, MA, USA) at 37 °C for 6 h before being subjected to a 2% agarose gel electrophoresis with ethidium bromide staining (Sigma Inc., St Louis, MO, USA). Three visible different genotypes could be read on the photos of the agarose gels presenting AA, AG and GG genotypes, respectively. Similarly, the CTLA-4 promoter −318C/T polymorphism was also studied by RFLP. We amplified the PCR reactions with forward primer ATT TAG GAG GAC CCT TGT AC and reverse primer TTA CGA GAA AGG AAG CCG TG in a total of 40 cycles of amplification. The PCR products were incubated with the MseI restriction enzyme (New England Biolabs) at 37 °C for 6 h before being subjected to a 2% agarose gel electrophoresis with ethidium bromide staining. Again, there were three possible band results, reflecting on the agarose gels to present C/C, C/T and T/T genotypes.

Data analysis and statistics

Elevation of CBIgE levels was cut-off ≥ 0.5 kU/L as previously described [10, 17, 19–22]. The rates of elevated CBIgE in subjects with different atopic and smoking history, genders, genotypes or alleles were compared and analysed by univariate analysis. To exclude the possible confounding among variables, multivariate logistic regression was used to explore the variables predictive of elevated CBIgEs. Comparisons of the CBIgE elevation among different haplotypes were carried out by a stepwise analysis of one haplotype vs. others by χ^2 test. A dichotomous gene-environment interaction model (2×4 table) [23] was used to analyse the interaction of CTLA-4 polymorphisms and maternal atopy on CBIgE elevation. A

Table 1. Demographic data between newborns with and without completing cord blood collection

Cord blood collection	Completion (<i>N</i> = 898) (%)	Loss of collection (<i>N</i> = 206) (%)	<i>P</i> -values
Maternal atopy*	23.2	23.8	0.871
Paternal atopy*	21.0	16.4	0.164
Prematurity†	14.4	15.4	0.687
Parental smoking	34.1	33.5	0.875

A total of 1104 women with pregnancy were initially recruited for this study, 898 completed cord blood collection, and 206 lost the cord blood collection.

*Atopy is defined by phenotypic asthma, rhinitis or atopic dermatitis along with detectable serum specific IgE to one or more common allergens.

†Prematurity is defined by gestational age <37 weeks. Demographic data between those with and without cord blood collection were not significantly different (χ^2 -test).

binary decision-making tree analysis with χ^2 Automatic Interaction Detection [24] was used to stratify the rank of a risk to CBIgE elevation. In brief, variables predictive of CBIgE elevation among the 12 independent variables including different CTLA-4 polymorphisms, gender, maternal atopy, paternal atopy and parental smoking were classified into a binary stratification with the highest risk factor predictive of CBIgE elevation in the first stratification, followed by other variables predictive of CBIgE elevation, which are significantly related to the risk factor in the first stratification, in the second stratification and so on. The study power estimation was originally set at 0.9, based on a two-group χ^2 -test with the two-side significance level at $\alpha = 0.05$ and the effect size at 10% of the difference of the elevated CBIgE levels (≥ 0.5 kU/L) between the two groups. The estimated sample size was 600 and we finally included 898 samples. A *P*-value < 0.05 was regarded as statistically significant.

Results

Demographic data of the subjects studied

A total of 1104 pregnant families were antenatally recruited for this study. Eight hundred and ninety eight newborns from the 1104 families completed cord blood collection, and the rest of 206 newborns lost cord blood collection due to stillbirth (12), loss of cord blood collection (130) and drop-off due to delivery in other hospitals (64). Analysis of the demographic data between newborns with and without cord blood collection revealed no significant differences on maternal atopy, paternal atopy or parental smoking history, as shown in Table 1.

Distribution of cord blood immunoglobulin E levels

Using a low-range IgE detection system, we found that male newborns tended to have significantly higher CBIgE levels than females (0.443 ± 0.073 vs. 0.236 ± 0.035 kU/L, *P* = 0.009). The mode of the CBIgE levels was located at 0.18 kU/L, the mean level was 0.34 kU/L and the frequency of newborns with elevated CBIgE (≥ 0.5 kU/L) was 21.4%,

similar to previous studies reported from Western countries [19, 20, 25]. We also tried to transform the CBIgE levels into log scales in order to formulate a normal distribution for parametric analysis, but the log-transformed CBIgE data remain not normal distribution. Thus, we chose to cut-off the CBIgE levels at 0.5 kU/L (>80 percentile), as defined in our and other previous studies [7–11, 17, 19–22], for the non-parametric association analyses below.

Correlation of cord blood immunoglobulin E levels with CTLA-4 polymorphisms and prenatal environmental factors in univariate and multivariate analysis

To study effects of CTLA-4 polymorphism and prenatal environment, including maternal atopy, paternal atopy, gender and parental smoking on elevation of CBIgE levels, we found that maternal atopy (*P* < 0.001) and CTLA-4+49 polymorphism (*P* = 0.032) were associated with elevations of CBIgE in univariate analysis. Maternal asthma (*P* = 0.036), rhinitis (*P* < 0.001), and atopic dermatitis (*P* = 0.019) were also associated with CBIgE elevation. In contrast, newborn babies with paternal atopy or parental smoking were not associated with elevated CBIgE (Table 2). Using multivariate analysis, it was found that maternal atopy [*P* < 0.001; odds ratio (OR) = 4.83] gender (*P* = 0.041; OR = 1.42) and CTLA-4+49A allele (*P* = 0.021; OR = 1.57), but not paternal atopy, parental smoking, CTLA-4+49G allele or CTLA-4 –318C/T alleles were significantly correlated with elevation of CBIgE levels (Table 3).

A dichotomous analysis of the effects of CTLA-4 gene haplotypes and gene-environment interactions on elevation of cord blood immunoglobulin E

To analyse whether certain haplotypes of the CTLA-4 polymorphisms at promoter (–318C/T) and exon 1 (+49A/G) had different risks to CBIgE elevation, we found that there is a significant linkage disequilibrium between these two loci –318C/T and +49A/G (a linkage between –318C and +49G alleles at *D* = 0.448, *r*² = 7.57;

Table 2. Univariate analysis of the correlation of CBIgE elevation (≥ 0.5 kU/L) with CTLA-4 polymorphisms and prenatal environmental factors

Variables	Rates of elevated CBIgE levels (%)	<i>P</i> -values [†]
CTLA-4 polymorphisms		
– 318: CC vs. CT vs. TT	21.5 vs. 20.3 vs. 30.0	0.982
– 318: CC/CT vs. TT	21.3 vs. 30.0	0.453
– 318: TT/CT vs. CC	20.9 vs. 21.5	0.844
+49: AA vs. AG vs. GG	21.3 vs. 24.5 vs. 17.9	0.079
+49: AA/AG vs. GG	23.9 vs. 17.9	0.032
+49: GG/GA vs. AA	21.4 vs. 21.3	0.979
Prenatal factors		
Maternal atopy	36.5 vs. 16.8	< 0.001
Asthma	35.1 vs. 20.7	0.036
Rhinitis	33.5 vs. 18.2	< 0.001
Atopic dermatitis	32.8 vs. 20.3	0.019
Paternal atopy	17.6 vs. 22.3	0.166
Asthma	12.0 vs. 21.6	0.250
Rhinitis	16.8 vs. 22.0	0.147
Atopic dermatitis	17.8 vs. 21.5	0.554
Gender (male vs. female)	23.8 vs. 18.7	0.061
Parental smoking*	22.8 vs. 20.6	0.454

*Thirty-four percent (306/898) of the fathers had smoking habit, but only four out of the 898 mothers had smoking habit.

[†]*P*-values are tested by univariate analysis of χ^2 -test.

CBIgE, cord blood IgE.

Table 3. Multivariate analyses of the correlation of CBIgE elevation (≥ 0.5 kU/L) with CTLA-4 polymorphisms and prenatal environmental factors

Variables	<i>P</i> -values	OR (95% CI)
CTLA-4 polymorphisms		
– 318: CC/CT vs. TT	0.400	0.514 (0.109–2.423)
– 318: TT/CT vs. CC	0.245	0.759 (0.477–1.208)
+49: A A/AG vs. GG	0.021	1.565 (1.070–2.287)
+49: GG/GA vs. AA	0.960	1.016 (0.557–1.852)
Prenatal factors		
Maternal atopy	<0.001	4.830 (2.194–10.636)
Asthma	0.546	0.787 (0.361–1.714)
Rhinitis	0.124	0.546 (0.252–1.181)
Atopic dermatitis	0.774	1.095 (0.591–2.027)
Paternal atopy	0.336	2.405 (0.402–14.392)
Asthma	0.319	0.428 (0.081–2.274)
Rhinitis	0.205	0.328 (0.058–1.839)
Atopic dermatitis	0.272	0.381 (0.068–2.127)
Gender of newborns	0.041	1.423 (1.015–1.995)
Parental smoking	0.322	1.192 (0.842–1.686)

Data are analysed by multivariate logistic regression.

OR, odds ratio; CI, confidence interval; CBIgE, cord blood IgE.

$P < 0.001$). The haplotype –318C/+49G was the major haplotype, and the haplotypes –318C/+49A, –318T/+49A and –318T/+49G were the minor haplotypes in

Table 4. Association between CBIgE elevation and different CTLA-4 haplotypes

Haplotypes (–318C/T; +49A/G)	Rates of CBIgE ≥ 0.5 kU/L	<i>P</i> -values
C-A vs. non-C-A	121/509 (23.8%) vs. 71/389 (18.3%)	0.027
C-G vs. non-C-G	172/804 (21.4%) vs. 20/94 (21.2%)	0.550
T-A vs. non-T-A	39/187 (20.6%) vs. 153/711 (21.5%)	0.311
T-G vs. non-T-G	31/134 (23.1%) vs. 161/764 (21.1%)	0.332

Comparisons of the CBIgE elevation among different haplotypes were done by a step-wise analysis of one haplotype vs. others by χ^2 -test. CBIgE, cord blood IgE.

the study population. In the haplotype analysis, it was found that the haplotype –318C/+49A had a significantly ($P = 0.027$) higher rate of CBIgE elevation than those without –318C/+49A haplotype as shown in Table 4. In consideration of gene-environment interactions, we also analysed CTLA-4 gene-maternal atopy interactions on CBIgE elevation by a dichotomous (2×4 table) model. Results showed that presence of maternal atopy had a significant impact on CBIgE elevation in newborns with CTLA-4 A allele (AA/AG) or GG genotype ($P \geq 0.001$); and maternal atopy and CTLA-4 A allele had an additive effect on CBIgE elevation (Table 5A). The additive effect of CTLA-4 gene-maternal atopy interaction was more prominent in male newborns (OR = 3.86 vs. 2.20, 1.66 departure from expected; Table 5B) but not present in female newborns (OR = 1.36 vs. 1.39; Table 5C). In the absence of maternal atopy, the CTLA-4 GG genotype had a significantly protective effect on CBIgE elevation in female newborns (OR = 0.45, $P = 0.015$; Table 5C) but not significantly in male newborns (OR = 0.66, $P = 0.143$; Table 5B).

A decision-making tree analysis of the rank of a risk to cord blood immunoglobulin E elevation

To prospect for early prevention of CBIgE elevation, we also stratified the predictive variables of CBIgE elevation into a binary decision-making tree by χ^2 Automatic Interaction Detection Analysis. Of the 12 variable predictors, three variables significantly predictive of CBIgE elevation could be stratified into a binary classification as shown in Fig. 1. Newborns from atopic and non-atopic mothers had the most significantly different frequency of elevated CBIgE in the first stratification (36.5% vs. 16.8%; $P = 0.000$). Following the first stratification of atopic and non-atopic mothers, male babies from atopic mothers had the highest frequency of elevated CBIgE (43.8%; $P = 0.028$), and babies from non-atopic mothers with the

Table 5. Gender-dependent interaction of CTLA-4 polymorphisms and maternal atopy on CBIgE elevation

CTLA-4+49	Maternal atopy	CBIgE ≥0.5	CBIgE < 0.5	Odds ratio	95% CI	P-value
(A) CTLA-4 and maternal atopy interaction in the whole population studied						
GG	+	32	54	2.35	1.42–3.88	0.001
GG	–	36	257	0.56	0.36–0.85	0.006
AG or AA	+	44	78	2.24	1.44–3.48	<0.001
AG or AA	–	80	317	1.00	(Reference)	
	Expected A	Departure from expected				
Additive	0.56+2.24 – 1 = 1.8	2.35 – 1.80 = 0.55				
Multiplicative	0.56 × 2.24 = 1.25	2.35/1.25 = 1.88				
(B) CTLA-4 and maternal atopy interaction in male newborns						
GG	+	21	21	3.86	1.94–7.70	<0.001
GG	–	22	129	0.66	0.38–1.15	0.143
AG or AA	+	25	38	2.54	1.39–4.65	0.002
AG or AA	–	44	170	1.00	(Reference)	
	Expected A	Departure from expected				
Additive	0.66+2.54 – 1 = 2.2	3.86 – 2.20 = 1.66				
Multiplicative	0.66 × 2.54 = 1.68	3.86/1.68 = 2.30				
(C) CTLA-4 and maternal atopy interaction in female newborns						
GG	(+)	11	33	1.36	0.63–2.95	0.434
GG	(–)	14	128	0.45	0.23–0.87	0.015
AG or AA	(+)	19	40	1.94	1.01–3.74	0.046
AG or AA	(–)	36	147	1.00	(Reference)	
	Expected A	Departure from expected				
Additive	0.45+1.94 – 1 = 1.39	1.36 – 1.39 = – 0.03				
Multiplicative	0.45 × 1.94 = 0.87	1.36/0.87 = 1.56				

CBIgE, cold blood IgE; CI, confidence interval.

CTLA-4+49GG genotype had the lowest frequency of elevated CBIgE (12.3%; $P = 0.006$) in the second stratification. This binary classification result is compatible with the dichotomous gene-maternal atopy interaction analysis (Table 5) and highlights the sequence of preventive strategies for antenatal prevention of allergy sensitization from control of maternal atopy in male newborns, and modulation of CTLA-4 expression in female newborns.

Discussion

Elevation of serum total IgE levels is well known to be correlated to atopic diseases [2, 3, 10, 14, 18, 19]. Recently, many genes have been linked to the elevation of IgE production. These genetic-association studies however have raised controversial results. For instance, some studies show that the CTLA-4 locus is not associated with asthma in Japanese and Germans [26, 27]. However, Howard *et al.* [28] have shown that the CTLA-4+49A/G polymorphism was linked to elevated IgE levels in Dutch, White, Africa-American and Hispanic populations. We also found that the CTLA-4+49A/G polymorphism was associated with elevation of CBIgE in newborns [17] and AR in adults [18]. In this study, we have further demonstrated a significant interaction among CTLA-4 A allele, gender and maternal atopy on the antenatal IgE produc-

tion reflecting on CBIgE elevations. Maternal atopy alone had the most significant impact on the frequency of elevated CBIgE in male newborns, and female babies with CTLA-4+49GG genotype from non-atopic mothers had a significantly lower rate of CBIgE elevation. Results from this study indicate that maternal inheritance, maternal allergic mediators or placental factors play the most important role on antenatal IgE production, and certain gene(s) such as CTLA-4 can interact with maternal but not paternal atopy to promote antenatal IgE production in a gender-limited fashion. More efforts to explore *in utero* programming of allergy sensitization via epigenetic imprint of maternal inheritance or environment on antenatal allergy sensitization may make perinatal allergy sensitization preventable.

Elevation of CBIgE has been significantly associated with increase of the serum IgE levels at the age of 18 months [25] and at the age of 5 years [29]. Newborns with elevated CBIgE were also associated with aeroallergen sensitization in their childhood [9]. However, the elevation of CBIgE was shown to be not associated with allergy diseases in some studies [7, 8, 30] but associated with the early development of food allergy [31] and with the later development of asthma [10]. Taken together, this suggests that allergy sensitization although may begin early in fetal life, a kinetic interaction between gene and

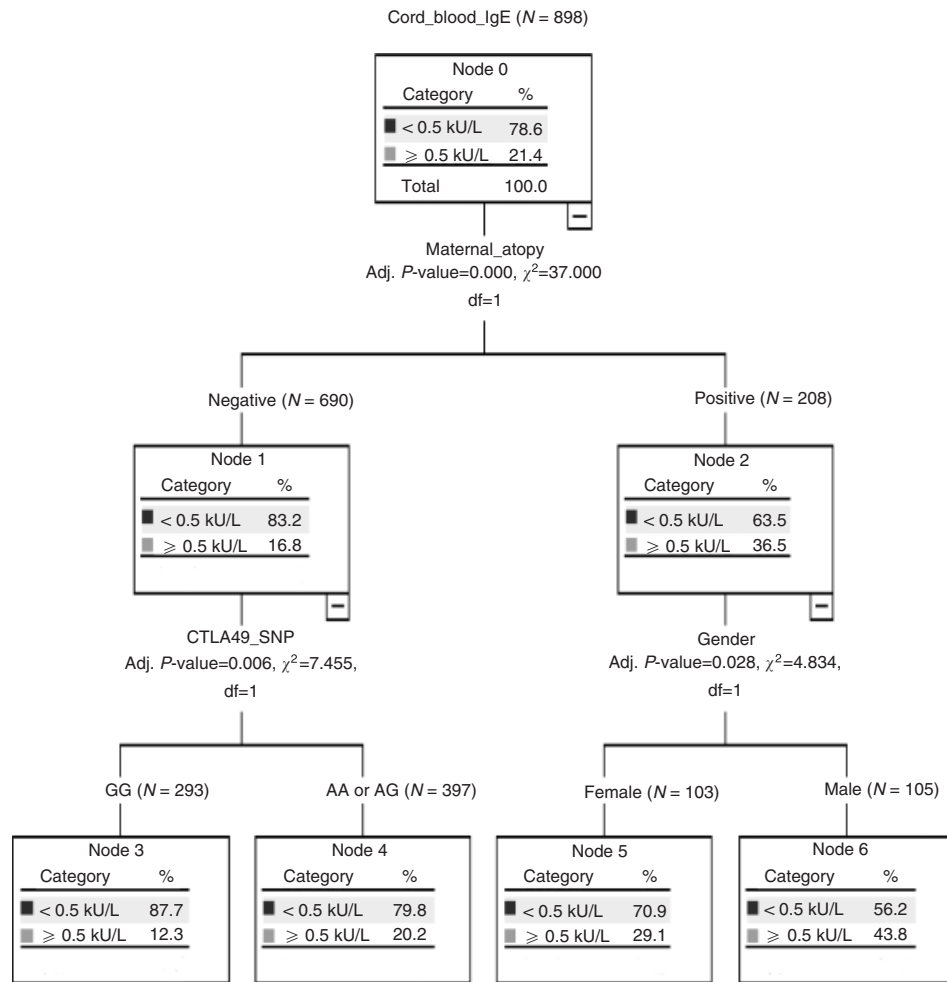


Fig. 1. A binary decision-making tree analysis of variables predictive of cord blood IgE (CBIGe) elevation (≥ 0.5 kU/L). Of the 12 independent variables predictive of CBIGe elevation, the most significant predictor was maternal atopy (first stratification, $P = 0.000$). Following the stratification of maternal atopy, female newborns with CTLA-4+49GG genotype from non-atopic mother had the lowest rate of CBIGe elevation (second stratification, left column; 12.3%, $P = 0.006$) and male newborns from atopic mothers had the highest rate of CBIGe elevation (second stratification, right column; 43.8%, $P = 0.028$).

environment during the perinatal stage determines the outcomes of skin or airway allergy diseases. A larger perinatal cohort study of gene-gene and gene-environment interactions on the development of allergy diseases is required.

It remains controversial whether parental smoking, especially maternal smoking, is associated with CBIGe elevation and childhood allergy sensitization [21, 22, 32]. In this study, we did not find an association of parental smoking with CBIGe elevation. It was, however, found that maternal smoking was not common in Chinese population ($< 0.5\%$; 4/898), as seen in this cohort study. Paternal smoking rate in this study was similar to other studies [21, 22]. The rationale to explain a lower rate of maternal smoking may be because Chinese culture limits female smoking or Chinese mothers concern passive smoking effect on fetal development. Apparently, certain inter-culture situations can influence prenatal environment,

including passive smoking, that can affect antenatal IgE production.

There have been few reports describing functional implications of CTLA-4+49A/G polymorphisms in human allergic diseases. Certain studies have previously shown that lower CTLA-4 expression of T cells tend to drive T-helper type 1 (Th1) polarisation while a high affinity binding of CTLA-4 induces pro-allergy Th2 polarisation in a murine model [33]. Kouki et al. [34] showed that patients with CTLA-4+49GG genotype had lower inhibitory function of CTLA-4. Mäurer et al. [35] also demonstrated that subjects with CTLA-4+49G allele had a lower CTLA-4 expression in T cell activation. In association studies, we [17] and others [36] suggested that CTLA-4 polymorphisms are implicated in a variety of immune diseases in different ethnic populations. In this study, we found that babies with the CTLA-4+49GG genotype from non-atopic mothers had the lowest rate of elevated CBIGe levels.

Taken together, these results suggest that the CTLA-4+49GG genotype may mediate lower CTLA-4 expression, resulting in a higher Th1 polarisation that may limit Th2-derived IgE production early in the antenatal stage.

A gender discrepancy has long been recognized in certain immune diseases including allergy diseases and autoimmune diseases. Males tend to have a higher prevalence of allergic asthma [37] and females are more susceptible to Graves' disease and SLE [38]. The genetic basis for this gender discrepancy has not been identified yet. The CTLA-4+49GG genotype has been associated with higher frequency of Graves' disease and SLE in certain studies [39, 40]. Using a binary decision-making tree analysis, we found that maternal atopy determines elevation of CBIgE the most significantly in male newborns, and CTLA-4 polymorphism determines elevated CBIgE more prominent in female newborns from non-atopic mothers. Based on the binary decision-making tree analysis, further studies to explore whether males are prone to maternal imprint of antenatal IgE production and females with CTLA-4+49GG genotype tend to have lower IgE production but higher autoimmunity are mandatory. In summary, this study demonstrated that antenatal IgE production is largely regulated by maternal atopy, gender, and CTLA-4 gene. Maternal, but not paternal, atopy and CTLA-4+49A allele had an additive effect on CBIgE elevation in male newborns. In the absence of maternal atopy, female newborns with CTLA-4+49GG genotype had a protective effect on CBIgE elevation. Control of maternal atopy in pregnancy and modulation of CTLA-4 expression may be a target for decrease of antenatal IgE production and possibly less perinatal allergy sensitization.

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References

- 1 Global burden of asthma. Global Initiative for Asthma (GINA) Workshop Report 2005 at Website: <http://www.ginasthma.org/index.asp>
- 2 Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989; 320:271-7.
- 3 Holgate ST, Djukanovic R, Casale T, Bousquet J. Anti-immunoglobulin E treatment with omalizumab in allergic diseases: an update on anti-inflammatory activity and clinical efficacy. *Clin Exp Allergy* 2005; 35:408-16.
- 4 Jacobsen HP, Herskind AM, Nielsen BW, Husby S. 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. *J Allergy Clin Immunol* 2001; 107:659-63.
- 5 Devereux G, Barker RN, Seaton A. Antenatal determinants of neonatal immune responses to allergens. *Clin Exp Allergy* 2002; 32:43-50.
- 6 Herz U, Joachim R, Ahrens B, Scheffold A, Radbruch A, Renz H. Allergic sensitization and allergen exposure during pregnancy favor the development of atopy in the neonate. *Internatl Arch Allergy Immunol* 2001; 124:193-6.
- 7 Edenharter G, Bergmann RL, Bergmann KE *et al*. Cord blood-IgE as risk factor and predictor for atopic diseases. *Clin Exp Allergy* 1998; 28:671-8.
- 8 Bergmann RL, Edenharter G, Bergmann KE *et al*. Predictability of early atopy by cord blood-IgE and parental history. *Clin Exp Allergy* 1997; 27:752-60.
- 9 Tariq SM, Arshad SH, Matthews SM, Hakim EA. Elevated cord serum IgE increases the risk of aeroallergen sensitization without increasing respiratory allergic symptoms in early childhood. *Clin Exp Allergy* 1997; 29:1042-8.
- 10 Sadeghnejad A, Karmaus W, Davis S, Kurukulaaratchy RJ, Matthews S, Arshad SH. Raised cord serum immunoglobulin E increases the risk of allergic sensitization at ages 4 and 10 and asthma at age 10. *Thorax* 2004; 59:936-42.
- 11 Liu CA, Wang CL, Chuang H, Ou CY, Hsieh TY, Yang KD. Prenatal prediction of infant atopy by maternal but not paternal total IgE levels. *J Allergy Clin Immunol* 2003; 112:899-904.
- 12 Malerba G, Pignatti PF. A review of asthma genetics: gene expression studies and recent candidates. *J Applied Genet* 2005; 46:93-104.
- 13 Cookson WO, Moffatt MF. Genetics of asthma and allergic disease. *Human Mol Genet* 2000; 9:2359-64.
- 14 Hellings PW, Vandenberghe P, Kasran A *et al*. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. *Eur J Immunol* 2002; 32:585-94.
- 15 Zalete K, Krhin B, Gaberscek S, Hojker S. Thyroid autoantibody production is influenced by exon 1 and promoter CTLA-4 polymorphisms in patients with Hashimoto's thyroiditis. *Int J Immunogenet* 2006; 33:87-91.
- 16 Liu MF, Wang CR, Lin LC, Wu CR. CTLA-4 gene polymorphism in promoter and exon-1 regions in Chinese patients with systemic lupus erythematosus. *Lupus* 2001; 10:647-9.
- 17 Chang JC, Liu CA, Chuang H *et al*. Gender-limited association of cytotoxic T lymphocyte antigen 4 (CTLA-4) polymorphism with cord blood IgE levels. *Pediatr Allergy Immunol* 2004; 15:506-12.
- 18 Yang KD, Liu CA, Wang CL, Ou CY, Hsieh TY. Gender-limited correlation of CTLA-4 polymorphisms with total IgE levels and allergic rhinitis. *Clin Exp Allergy* 2004; 34:32-7.
- 19 Kaan A, Dimich-Ward H, Manfreda J *et al*. Cord blood IgE: its determinants and prediction of development of asthma and other allergic disorders at 12 months. *Ann Allergy Asthma Immunol* 2000; 84:37-42.
- 20 Karmaus W, Arshad H, Mattes J. Does the sibling effect have its origin in utero? Investigating birth order, cord blood immunoglobulin E concentration, and allergic sensitization at age 4 years. *Am J Epidemiol* 2001; 154:909-15.

- 21 Ownby DR, Johnson CC, Peterson EL. Maternal smoking does not influence cord serum IgE or IgD concentrations. *J Allergy Clin Immunol* 1991; **88**:555-60.
- 22 Atici A, Altintas D, Yuksel B *et al.* Do parental smoking and history of allergy influence cord-serum IgE? *Pediatr Allergy Immunol* 1995; **6**:213-5.
- 23 Botto LD, Khoury MJ. Commentary: facing the challenge of gene-environment interaction the two-by-four table and beyond. *Am J Epidemiol* 2001; **153**:1016-20.
- 24 Barton CA, McKenzie DP, Walters EH, Abramson MJ, Victorian Asthma Mortality Study Group. Interactions between psychosocial problems and management of asthma: who is at risk of dying? *J Asthma* 2005; **42**:249-56.
- 25 Hansen LG, Host A, Halken S *et al.* Cord blood IgE. III. Prediction of IgE high-response and allergy. A follow-up at the age of 18 months. *Allergy* 1992; **47**:404-10.
- 26 Heinzmann A, Plesnar C, Kuehr J, Forster J, Deichmann KA. Common polymorphisms in the CTLA-4 and CD28 genes at 2q33 are not associated with asthma or atopy. *Eur J Immunogenet* 2000; **27**:57-61.
- 27 Nakao F, Ihara K, Ahmed S *et al.* Lack of association between CD28/CTLA-4 gene polymorphisms and atopic asthma in the Japanese population. *Exp Clin Immunogenet* 2000; **17**: 179-84.
- 28 Howard TD, Postma DS, Koppelman GA *et al.* Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002; **110**:734-51.
- 29 Hansen LG, Halken S, Host A, Moller K, Osterballe O. Prediction of allergy from family history and cord blood IgE levels. A follow-up at the age of 5 years. Cord blood IgE. IV. *Pediatr Allergy Immunol* 1993; **4**:34-40.
- 30 Croner S, Kjellman NI. Natural history of bronchial asthma in childhood. A prospective study from birth up to 12-14 years of age. *Allergy* 1992; **47**:150-7.
- 31 Kaan A, Dimich-Ward H, Manfreda J *et al.* Cord blood IgE: its determinants and prediction of development of asthma and other allergic disorders at 12 months. *Ann Allergy Asthma Immunol* 2000; **84**:37-42.
- 32 Strachan DP, Cook DG. Health effects of passive smoking: 5 parental smoking and allergic sensitisation in children. *Thorax* 1998; **53**:117-23.
- 33 Padrid PA, Mathur M, Li X *et al.* CTLA4Ig inhibits airway eosinophilia and hyperresponsiveness by regulating the development of Th1/Th2 subsets in a murine model of asthma. *Am J Respir Cell Mol Biol* 1998; **18**:453-62.
- 34 Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol* 2000; **165**:6606-11.
- 35 Mäurer M, Loserth S, Kolb-Mäurer A *et al.* A polymorphism in the human cytotoxic T-lymphocyte antigen 4 (CTLA4) gene (exon 1+49) alters T-cell activation. *Immunogenetics* 2002; **54**:1-8.
- 36 Munthe-Kaas MC, Carlsen KH, Helms PJ *et al.* CTLA-4 polymorphisms in allergy and asthma and the TH1/TH2 paradigm. *J Allergy Clin Immunol* 2004; **114**:280-7.
- 37 Selnes A, Nystad W, Bolle R, Lund E. Diverging prevalence trends of atopic disorders in Norwegian children. Results from three cross-sectional studies. *Allergy* 2005; **60**:894-9.
- 38 Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am J Pathol* 1985; **121**:531-51.
- 39 Vaidya B, Imrie H, Perros P *et al.* The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Hum Mol Genet* 1999; **8**:1195-9.
- 40 Ahmed S, Ihara K, Kanemitsu S *et al.* Association of CTLA-4 but not CD28 gene polymorphisms with systemic lupus erythematosus in the Japanese population. *Rheumatology* 2001; **40**: 662-7.