

Gender-limited association of cytotoxic T-lymphocyte antigen-4 (CTLA-4) polymorphism with cord blood IgE levels

Chang J-C, Liu C-A, Chuang H, Ou C-Y, Hsu T-Y, Huang E-Y, Yang KD. Gender-limited association of cytotoxic T-lymphocyte antigen-4 (CTLA-4) polymorphism with cord blood IgE levels. *Pediatr Allergy Immunol* 2004; 15: 506–512. © 2004 Blackwell Munksgaard

Allergic mechanism has long been attributed to IgE-mediated reaction. The relationship between gene polymorphism and cord blood IgE (CB IgE) is unclear. We investigated whether elevation of CB IgE levels was associated with polymorphisms of cytotoxic T-lymphocyte antigen 4 (CTLA-4) at (–318) CT and (+49) AG positions in a gender-limited fashion. CB IgE levels were determined by Pharmacia CAP system and the CTLA-4 polymorphisms at (–318) and (+49) were determined by restriction fragment length polymorphism (RFLP). A total of 644 consecutive umbilical cord bloods were collected for this study. 32.9% of newborn infants had detectable IgE levels (≥ 0.35 kU/l). 25.6% of the male newborns had elevated CB IgE levels (≥ 0.5 kU/l) similar to those of the female newborns (22.7%). The CTLA-4 polymorphism at (+49) but not (–318) was significantly associated with elevated CB IgE levels ($p = 0.004$). The association of CTLA-4 (+49) A allele with elevated CB IgE levels was found only in females. Both male and female infants with different CTLA-4 (–318) genotypes had no difference in the rates of elevated CB IgE levels. A linkage disequilibrium between CTLA-4 (+49) G and (–318) C allele was found in this Chinese population. Subjects with the (+49, GG and –318, CC) genotype had a significantly lower rate of elevated CB IgE levels. Association of the CTLA-4 (+49) polymorphism with elevated CB IgE levels was found only in female infants. Newborn infants with the (+49, GG and –318, CC) genotype tended to have a low rate of elevated CB IgE.

Jen-Chieh Chang¹, Chieh-An Liu¹, Hau Chuang¹, Chia-Yu Ou², Te-Yao Hsu², Eng-Yen Huang³ and Kuender D. Yang¹

¹Departments of Pediatrics and ²Obstetrics, Chang Gung Memorial Hospital, ³Graduate Institute of Clinical Medical Sciences, Chang Gung University, Kaohsiung, Taiwan

Key words: polymorphism; gender; cord blood immunoglobulin E; cytotoxic T-lymphocyte antigen-4

Kuender D. Yang, Department of Medical Research (12F 12R), Chang Gung Memorial Hospital, No. 123, Ta-Pei Road, Niau-Sung Hsiang, Kaohsiung 833, Taiwan.

Tel.: 886-7-7317123 ext. 8848

Fax: 886-7-7312867

E-mail: yangkd@adm.cgmh.org.tw

Accepted 29 January 2004

Prevalence of allergic disorders has increased significantly in the past two decades (1–3). Results from epidemiological studies suggest that allergic diseases are hereditary as monozygotic twins are more frequently affected than heterozygotic twins (4), and offspring of affected parents also have a higher risk for developing the disease (5). Many studies worldwide and in different ethnic populations demonstrated that there are many loci in different chromosomes that are implicated in allergic disorders (6–9). Allergic mechanism has long been attributed to IgE-mediated reaction and elevation of serum IgE levels has been implicated in allergic diseases (10, 11). Whether certain genetic polymorphisms are related to elevated serum total IgE levels

remains controversial. The rationale for the controversy may be because IgE production could be influenced by environmental exposure of specific antigens or non-specific stimulants.

The estimated heritability for cord blood (CB) IgE levels is much higher than that in childhood serum IgE levels (0.92; 95% CI, 0.84–0.95 vs. 0.78; 95% CI, 0.60–0.87) (12). This suggests that IgE production in a fetus may be modulated mostly by inherited but less by environmental factors. To study the correlation of CB IgE levels with certain gene polymorphisms may be a better model to explore the relationship between genes and IgE production. Thus, we proposed in this study that certain genetic polymorphisms might have impacts on fetal IgE production reflecting

on elevated CB IgE levels and certain genetic allele(s) may have a gender-limited influence on the early IgE production.

A genetic study has provided evidence for the linkage of a candidate atopy gene to chromosome 2q33, where CTLA-4 and CD28 genes are colocalized (13). Lack of association between CD28/CTLA-4 gene polymorphisms and atopic asthma has been reported in the Japanese population (14). However, Hizawa et al. (15) showed that elevation of total serum IgE levels in patients with asthma was correlated to the polymorphisms of CTLA-4. In a functional study, subjects with CTLA-4 (+49) G allele were found to have higher autoantibody levels (16). A treatment with CTLA-4-Ig significantly reduced the generation of IL-4, IL-5, and IgE, and reduced eosinophilic inflammation of the lungs in an animal asthma model (17). There is no functional discrepancy of the CTLA-4 gene polymorphisms between males and females described yet. We investigated male/female discrepancies in the relationship between polymorphisms of the CTLA-4 at (+49) and (-318) positions and umbilical CB IgE levels.

Methods

Subjects and IgE detection

A total of 644 consecutive cord bloods from 304 female and 340 male newborns were collected for this study after informed consent was obtained from parents. Samples were collected between September 2001 and September 2002 at Chang Gung Memorial Hospital, Kaoshiung, Taiwan. A research nurse was trained to explain the purpose of the study to eligible pregnant parents as they visited our obstetric clinic. A questionnaire was applied to collect family allergy history, parental smoking and gestational age after cord blood was collected. Serum samples from the cord blood were separated by a centrifuge at 800 *g* for 15 min and stored at -70°C until analysis. The blood cells after serum collection were subject to DNA extraction for determination of CTLA-4 (+49) and (-318) polymorphisms as described below.

Cord blood total IgE levels were determined by a low range IgE detection system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) (18). The sensitive low range IgE detection system is used to detect cord blood total IgE levels. Elevation of CB IgE levels was defined by ≥ 0.5 kU/l as previously described (19).

DNA extraction and RFLP protocol

Blood cells were subjected to DNA extraction with 0.5% SDS lysing buffer followed by proteinase K (1 mg/ml) digestion of nuclear protein for 4 h at 60°C (20). Total DNA was harvested by standard phenol-chloroform extraction followed by 70% alcohol precipitation. As modified from our previous report (20), we amplified the PCR reactions of the CTLA-4 (+49) AG 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 *Bbv*I 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). The bands of AG genotypes showed 1/3 possible results. There may have been a non-digested higher molecular weight single band, or two heterozygous bands representing digestion of target sequence of enzyme in one allele only, or a complete digestion with a low molecular weight single band. These represent the genotypes AA, AG and GG, respectively.

Similarly, the CTLA-4 promoter (-318) CT polymorphism was also studied by restriction fragment length polymorphism (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 40 cycles of amplification. The PCR products were incubated with the *Mse*I 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. Again, there were three possible band results. CC genotype samples showed up as a higher non-digested single band, CT genotype samples revealed digestion of target sequence of enzyme in one allele only and showed as two heterozygous bands, and TT samples were completely digested, appearing as a low molecular weight single band.

Statistics

Elevation of cord blood total IgE levels was cut off at ≥ 0.5 kU/l as previously described (19). Differences of the rates of elevated CB IgE in subjects with different genotypes or alleles were analyzed by Chi-square or Fisher's exact test depending on the expected sample sizes of each subcategories. The power estimation was originally set at 0.9, based on a two group χ^2 test with a

one-side significance level at $\alpha = 0.05$ and the effective size at 10% difference of the elevated CB IgE levels (≥ 0.5 kU/l) between two groups. The estimated sample size was 600 and we finally included 644 samples. p -value < 0.05 was regarded as statistical significance. SPSS (version 10.0, Chicago, IL, USA) was our statistical analysis software.

Results

Gestational age and maternal smoking are two important factors other than genetic inheritance that can significantly influence the CB IgE levels. We first sought to make sure the gestational age, family allergy history and rate of parental smoking among subjects with different genotypes were not different. As shown in Table 1, gestational age and family allergy history were not significant difference among subjects with different CTLA-4 (+49) AG genotypes or (-318) CT genotypes. The proportion of smoking mothers and fathers was also not significantly different among subjects with different CTLA-4 (+49) AG genotypes or (-318) CT genotypes (Table 1). There were very few mothers having smoking habit in this Chinese population although prevalence of paternal smoking was similar to the studies with Caucasians (21–23).

There were 32.9% newborns revealing detectable CB IgE (≥ 0.35 kU/l). 24.2% of the newborns had elevated CB IgE at ≥ 0.5 kU/l. Male newborns had higher rate of the elevated CB IgE ≥ 0.5 kU/l than female newborns (25.6 vs. 22.7%), but there was no significant difference between males and females ($p = 0.393$).

Analysis of the association of elevated CB IgE with different CTLA-4 (+49) and (-318) polymorphisms showed that the (+49) AG but not

(-318) CT polymorphism was significantly associated with elevation of CB IgE levels (Table 2). Further analysis showed that the genotypes with (+49) AG or AA had higher rate of elevated CB IgE levels indicating the association of CTLA-4 (+49) A allele with elevation of CB IgE levels. As different genetic polymorphisms may have different impacts on the IgE production in males and females. We have further analyzed the associations of CTLA-4 polymorphisms with CB IgE levels in males and females. Interestingly, we found that the association of CTLA-4 (+49) A allele with elevated CB IgE was only significantly found in female but not male newborns (Table 3). The CTLA-4 (+49) GG genotype in females appeared to have a protective effect on limiting the elevation of CB IgE levels. The CTLA-4 (-318) polymorphism had no significant effect on elevation of CB IgE levels in males or females.

Studies were also pursued to study effects of the linkage disequilibrium between (+49) AG and (-318) CT on CB IgE levels. According to Mendelian law, inheritance of two alleles in one chromosome should follow the frequency of $1/2 \times 1/2 = 0.25$. In our series, the frequency of CTLA-4 (+49) G allele and (-318) C allele was $574/644 = 0.89$. Apparently, a linkage disequilibrium between (+49) G and (-318) C alleles (expected 0.25 vs. observed 0.89; $p < 0.001$) was found in the Chinese population. Certain combined genotypes such as (+49) GG and (-318) CT as well as (+49) GG and (-318) TT never appeared in the

Table 1. Demographic data in participants with different cytotoxic T-lymphocyte antigen-4 (CTLA-4) polymorphisms

Polymorphisms	Family Hx +	Parental smoking	Preterm
+49 genotypes			
AA	50/69 (72.5%)	15/70 (21.4%)	1/70 (1.4%)
AG	214/301 (71.1%)	69/302 (22.8%)	12/301 (4.0%)
GG	194/270 (71.9%)	58/272 (21.3%)	13/271 (4.8%)
p-values	0.965*	0.900*	0.443+
-318 genotypes			
CC	359/500 (71.8%)	106/504 (21.0%)	20/502 (4.0%)
CT	96/135 (71.1%)	35/135 (25.9%)	6/135 (4.4%)
TT	3/5 (60%)	1/5 (20.0%)	0/5 (0.0%)
p-values	0.837+	0.473+	0.873+

p -values were analyzed by Chi-square test (*) or Fisher's exact test (+) depending on whether the expected sample sizes of each subcategories were greater than 5 or not.

Table 2. Association of cytotoxic T-lymphocyte antigen-4 (CTAL-4) (+49), but not (-318), polymorphism with elevated cord blood IgE levels (kU/l)

Polymorphisms	IgE < 0.5	IgE ≥ 0.5
+49 genotypes		
AA	56 (80.0%)	14 (20.0%)
AG	211 (69.9%)	91 (30.1%)
GG	221 (81.3%)	51 (18.8%)
p-values	0.004*	
+49 A allele		
AA or AG	267 (71.8%)	105 (28.2%)
GG	221 (81.3%)	51 (18.8%)
p-values	0.006*	
-318 genotypes		
CC	378 (75.0%)	126 (25.0%)
CT	106 (78.5%)	29 (21.5%)
TT	4 (80.0%)	1 (20.0%)
p-values	0.681+	
-318 T allele		
CT or TT	110 (78.6%)	30 (21.4%)
CC	378 (75.0%)	126 (25.0%)
p-values	0.383*	

p -values were analyzed by Chi-square test (*) or Fisher's exact test (+) depending on whether the expected sample sizes of each subcategories were greater than 5 or not.

Table 3. Gender discrepancy of the association of cytotoxic T-lymphocyte antigen-4 (CTAL-4) (+49) polymorphisms with elevated cord blood IgE level (kU/l)

Polymorphisms	Female subjects		Male subjects	
	IgE < 0.5	IgE ≥ 0.5	IgE < 0.5	IgE ≥ 0.5
+49 genotypes				
AA	27 (81.8%)	6 (18.2%)	29 (78.4%)	8 (21.6%)
AG	97 (70.3%)	41 (29.7%)	114 (69.5%)	50 (30.5%)
GG	114 (85.7%)	19 (14.3%)	107 (77.0%)	32 (23.0%)
p-values	0.008*		0.265*	
+49 A allele				
AA or AG	124 (72.5%)	47 (27.5%)	143 (71.1%)	58 (28.9%)
GG	114 (85.7%)	19 (14.3%)	107 (77.0%)	32 (23.0%)
p-values	0.006*		0.231*	
−318 genotypes				
CC	192 (78.4%)	53 (21.6%)	186 (71.8%)	73 (28.2%)
CT	43 (78.2%)	12 (21.8%)	63 (78.8%)	17 (21.3%)
TT	3 (75%)	1 (25%)	1 (100%)	0 (0%)
p-values	0.987+		0.392+	
−318 T allele				
TT or CT	46 (78.0%)	13 (22.0%)	64 (79.0%)	17 (21.0%)
CC	192 (78.4%)	53 (21.6%)	186 (71.8%)	73 (28.2%)
p-values	0.946*		0.200*	

p-values were analyzed by Chi-square test (*) or Fisher's exact test (+) depending on whether the expected sample sizes of each subcategories were greater than 5 or not.

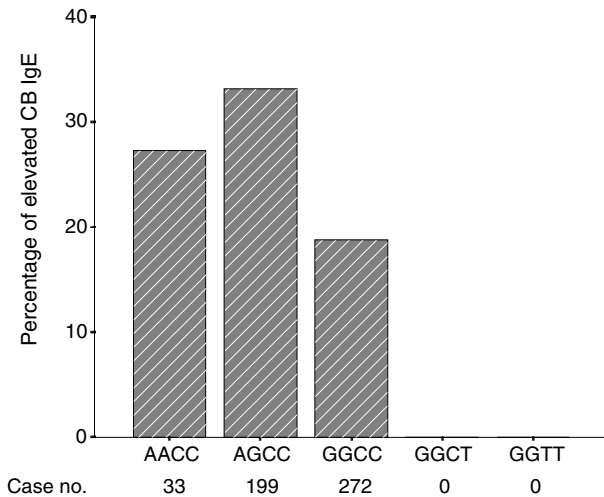


Fig. 1. Association of different combined cytotoxic T-lymphocyte antigen-4 (+49) and (-318) genotypes with the rate of elevated cord blood IgE (CB IgE) level. There is a significant difference ($p = 0.002$) of the elevation of CB IgE among different genotypes. The genotypes: (+49) GG/(-318) CT and (+49) GG/(-318) TT never appeared in the studied population, whereas subjects with (+49) GG/(-318) CC genotypes tended to have a lower rate of elevated CB IgE.

Chinese population studied. More interestingly, it was found that the combined (+49) GG and (-318) CC genotypes appeared to be protective from elevation of CB IgE (Fig. 1). The rates of elevated CB IgE were not different among

Table 4. The relationship of the parental cytotoxic T-lymphocyte antigen-4 (CTLA-4) (+49) genotypes to elevated cord blood IgE levels (kU/l)

CTLA4 +49 genotypes	CB IgE (kU/l)	
	IgE < 0.5	IgE ≥ 0.5
Genotypes of fathers		
GG	187 (76.3%)	58 (23.7%)
AA or AG	284 (75.9%)	90 (24.1%)
p-values	0.911*	
Genotypes of mothers		
GG	199 (76.5%)	61 (23.5%)
AA or AG	269 (75.6%)	87 (24.4%)
p-values	0.779*	
Genotypes of both parents		
GG	81 (81.8%)	18 (18.2%)
AA or AG	188 (70.9%)	77 (29.1%)
p-values	0.036*	

p-values were analyzed by Chi-square test (*) or Fisher's exact test (+) depending on whether the expected sample sizes of each subcategories were greater than 5 or not.

Table 5. The relationship of the elevation of cord blood IgE levels (kU/l) to the cytotoxic T-lymphocyte antigen-4 (CTLA-4) (+49) polymorphism of newborns from parents with and without smoking

+49 genotypes	Non-smoking parents (IgE ≥ 0.5)	Smoking parents (IgE ≥ 0.5)	p-values
AA ($n = 70$)	9/55 (16.4%)	5/15 (33.3%)	0.161†
AG ($n = 302$)	70/233 (30.0%)	21/69 (30.4%)	0.950*
GG ($n = 272$)	41/214 (19.2%)	10/58 (17.2%)	0.740*

One hundred and forty-two of the 644 infants studied had smoking parents. They were almost all paternal smoking (139), but few cases with maternal smoking (3). p-values were analyzed by Chi-square test (*) or Fisher's exact test (†) depending on whether the expected sample sizes of each subcategories were greater than 5 or not.

newborns from mothers or fathers with different CTLA-4 (+49) genotypes (Table 4). We did, however, found that babies from both parents with CTLA-4 (+49) GG genotype did have a lower frequency of elevated CB IgE than infants from parents with CTLA-4 (+49) A allele (AA or AG genotypes) (Table 4). This has further supported that CTLA-4 (+49) GG can limit the fetal IgE production. Further analysis showed that the frequency of elevated CB IgE was not different in newborns with different CTLA-4 genotypes from parents with and without smoking although newborns with (+49) AA genotype from non-smoking parents tended to have a lower rate of elevated CB IgE (Table 5).

Discussion

Serum total IgE levels have been associated with asthma, and related to airway hyperresponsiveness (10, 11). Recently, many genes have been

linked to regulation of IgE production (24, 25). There are at least 23 loci reported to be responsible for IgE production or allergic disorders. These genetic association studies however have raised controversial results (6–9). For instance, some studies showed that the CTLA-4 locus was not associated with asthma in Japanese and Germans (14, 26). However, Howard et al. (27) have shown that the (+49) AG polymorphism was linked to elevated IgE levels in Dutch, White, Africa-American and Hispanic populations. The reasons for these discrepant results are not well known. Different ethnic populations in different studies may partly explain the variability. Another reason is that IgE production is regulated by not only genes but also environmental factors. In a previous study, we demonstrated that maternal but not paternal atopy history was correlated to frequency of elevated CB IgE level ($p = 0.001$) (28). Using the CB IgE model that reveals a heritability more than 90% (12), we found that an association of the (+49) A allele with elevated CB IgE levels was significantly found in females. The higher rate of elevated CB IgE was also found in the males with (+49) A allele although it did not reach a significant level. The mechanism for the gender-linked genetic relationship with CB IgE levels deserves further studies.

There were few reports describing functional implications of CTLA-4 (+49) A/G polymorphisms. It was shown that CTLA-4 (+49) GG genotype had a lower inhibitory function of CTLA-4 (29). Mäurer et al. (30) demonstrated that subjects with G allele had a lower CTLA-4 expression in T-cell activation. We found that CTLA-4 (+49) GG genotype in females appeared to have a protective effect on limiting the elevation of CB IgE levels. The underlying reason for the association of CTLA-4 genotypes with elevated CB IgE is not known. A linkage disequilibrium between (+49) G and (–318) C allele was found in the study, and this linkage disequilibrium appeared to limit the elevation of CB IgE. This suggests that an actual susceptibility or regulation locus for IgE production may be located in the nearby of the CTLA-4 gene. A previous study showed that patients with CTLA-4 G allele tended to have higher autoantibody levels (16). Autoantibodies usually belong to IgM or IgG isotype antibody (31). In this study, we found that babies with CTLA-4 (+49) GG genotype had low IgE levels. Taken together, this suggests that different genotypes of CTLA-4 may be involved in the T-dependent B cell switch of isotype antibody production.

Although a gender-association has long been recognized in certain autoimmune diseases such as Graves' disease and systemic lupus erythematosus (SLE), no genetic basis for this gender discrepancy has been demonstrated. Similarly, it has not been described whether any genetic polymorphism is involved in the gender discrepancy of total IgE levels and atopic diseases. Certain autoimmune diseases had previously been linked with the CTLA-4 polymorphism at position 49 (codon 17) of exon 1 (32–36). The GG genotype at this position has been associated with higher frequency of insulin-dependent diabetes mellitus (IDDM), Graves' disease, thyroid associated orbitopathy (TAO) and primary biliary cirrhosis (32–35). In contrast, the AA genotype at this position has been associated with susceptibility to celiac disease (36). It is interesting that Asians, who have higher frequency of GG genotype (14, 37, 38), have higher incidence of SLE and type 1 diabetes (37, 38). In contrast, Caucasians, who possess higher frequency of the AA genotype at this position (32–35), have lower incidence of SLE (39) but higher incidence of celiac disease (36). Thus, the CTLA-4 gene polymorphism apparently acts an important role on regulating certain inflammatory disorders in different populations.

We are the first time in the literature to demonstrate that the association of CTLA-4 (+49) A allele with elevation of CB IgE levels was found significantly in females but not in males. This suggests that the GG genotype in the CTLA-4 position 49 may play an immune braking effect to suppress certain allergic reactions whereas the A allele may augment allergic reaction in a gender limited fashion. Certain polymorphic alleles in the CTLA-4 gene had different linkage disequilibrium in different ethnic populations (32, 40). Masterman et al. (41) have shown that CTLA-4 exon 1, (+49) G allele almost always occurs together on the same chromosome with the promoter (–318) C allele. They found that the presence of homozygosity for a haplotype (+49) G and (–318) C allele conferred to a more than twofold risk for primary progressive multiple sclerosis. We also found a linkage disequilibrium between (+49) G and (–318) C allele, which may provide an inhibition of the CB IgE production. The genotypes: (+49) GG/(–318) CT and (+49) GG/(–318) TT never appeared in the studied population, whereas subjects with (+49) GG/(–318) CC genotypes tended to have a lower rate of elevated CB IgE.

In conclusion, there is no doubt that CTLA-4 is an important gene to regulate CB IgE levels, especially in females. Further studies are however

needed to explore which gene can regulate the CB IgE production in males and which genes directly affect fetal IgE production in both females and males.

Acknowledgements

The authors would like to thank the staff of delivery room for their collection of cord blood samples. This study was in part supported by a grant NSC91-2314-B182A-024 from National Science Council, and a grant CMRPG8016 from Chang Gung Memorial Hospital, Taiwan.

References

1. THE INTERNATIONAL STUDY OF ASTHMA AND ALLERGIES IN CHILDHOOD. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: The international study of asthma and allergies in childhood (ISAAC) steering committee. *Lancet* 1998; 351: 1225–32.
2. Global Initiative for Asthma (GINA) Workshop Report 2002 at Website: <http://www.ginasthma.com>
3. YANG KD. Aspects of global environment, genetics and management. *Chang Gung Medical J* 2000; 23: 641–61.
4. LUBS ML. Empiric risks for genetic counseling in families with allergy. *J Pediatr* 1972; 80: 26–31.
5. OWNBY DR. Environmental factors versus genetic determinants of childhood inhalant allergies. *J Allergy Clin Immunol* 1990; 86: 279–87.
6. COOKSON WO, YOUNG RP, SANDFORD AJ, et al. Maternal inheritance of atopic IgE responsiveness on chromosome 11q. *Lancet* 1992; 340: 381–4.
7. COOKSON WO, MOATT MF. Genetics of asthma and allergic disease. *Hum Mol Genet* 2000; 9: 2359–64.
8. OBER C, COX NJ, ABNEY M, et al. Genome-wide search for asthma susceptibility loci in a founder population. The Collaborative Study on the Genetics of Asthma. *Hum Mol Genet* 1998; 7: 1393–8.
9. LEE YA, WAHN U, KEHRT R, et al. A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. *Nat Genet* 2000; 26: 470–3.
10. 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.
11. SEARS MR, BURROWS B, FLANNERY EM, HERBISON GP, HEWITT CJ, HOLDAWAY MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991; 325: 1067–71.
12. 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.
13. ANONYMOUS. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). *Nat Genet* 1997; 15: 389–92.
14. 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.
15. HIZAWA N, YAMAGUCHI E, JINUSHI E, KONNO S, KAWAKAMI Y, NISHIMURA M. Increased total serum IgE levels in patients with asthma and promoter polymorphisms at CTLA4 and FCER1B. *J Allergy Clin Immunol* 2001; 108: 74–9.
16. TOMER Y, GREENBERG DA, BARBESINO G, CONCEPCION E, DAVIES TF. CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. *J Clin Endocrinol Metab* 2001; 86: 1687–93.
17. 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.
18. KAM KL, HSIEH KH. Comparison of three *in vitro* assays for serum IgE with skin testing in asthmatic children. *Ann Allergy* 1994; 73: 329–36.
19. 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.
20. YANG KD, YANG MY, LI CH, WANG CL, CHEN RF, LIN TY. Altered cellular but not humoral responses in patients with enterovirus 71 infections in Taiwan. *J Infect Dis* 2001; 183: 850–6.
21. MAGNUSSON CGM. Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allergy. *J Allergy Clin Immunol* 1986; 78: 898–904.
22. 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.
23. 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.
24. KIMURA M, TSURUTA S, YOSHIDA T. IL-4 production by PBMCs on stimulation with mite allergen is correlated with the level of serum IgE antibody against mite in children with bronchial asthma. *J Allergy Clin Immunol* 2000; 105: 327–32.
25. GRAVES PE, KABESCH M, HALONEN M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000; 105: 506–13.
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. 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.
28. LIU CA, WANG CL, CHUANG H, OU CY, HSU TY, YANG KD. Prenatal prediction of infant atopy by maternal but not paternal total IgE levels. *J Allergy Clin Immunol* 2003; 112: 899–904.
29. 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.
30. MÄURER M, LOSERTH S, KOLB-MÄURER A, et al. (CTLA4) gene (exon 1 + 49) alters T-cell activation. *Immunogenetics* 2002; 54: 1–8.
31. KOHLER H, BAYRY J, NICOLETTI A, KAVERI SV. Natural autoantibodies as tools to predict the

- outcome of immune response? *Scand J Immunol* 2003; 58: 285–9.
32. MARRON MP, RAEL LJ, GARCHON HJ, et al. Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphism in multiple ethnic. *Hum Mol Genet* 1997; 6: 1275–82.
33. 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.
34. VAIDYA B, IMRIE H, PETROS P, DICKINSON J. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene polymorphism confers susceptibility to thyroid associated orbitopathy. *Lancet* 1999; 354: 743–4.
35. AGARWAL K, JONES DE, DALY AK, et al. CTLA-4 gene polymorphism confers susceptibility to primary biliary cirrhosis. *J Hepatol* 2000; 32: 538–41.
36. NALUAI AT, NILSSON S, SAMUELSSON L, et al. The CTLA4/CD28 gene region on chromosome 2q33 confers susceptibility to celiac disease in a way possibly distinct from that of type 1 diabetes and other chronic inflammatory disorders. *Tissue Antigens* 2000; 56: 350–5.
37. 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.
38. LEE YJ, HUANG FY, LO FS, et al. Association of CTLA4 gene A-G polymorphism with type 1 diabetes in Chinese children. *Clin Endocrinol* 2000; 52: 153–7.
39. HEWARD J, GORDON C, ALLAHABADIA A, BARNETT AH, FRANKLYN JA, GOUGH S-CL. THE A-G polymorphism in exon 1 of the CTLA-4 gene is not associated with systemic lupus erythematosus. *Ann Rheum Dis* 1999; 58: 193–5.
40. HOLOPAINEN PM, PARTANEN JA. Technical note: linkage disequilibrium and disease-associated CTLA-4 gene polymorphisms. *J Immunol* 2001; 167: 2457–8.
41. MASTERMAN T, LIGERS A, ZHANG Z, et al. CTLA4 dimorphisms and the multiple sclerosis phenotype. *J Neuroimmunol* 2002; 131: 208–12.