

Associations between total serum IgE levels and the 6 potentially functional variants within the genes *IL4*, *IL13*, and *IL4RA* in German children: The German Multicenter Atopy Study

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Background: Increased total serum IgE levels are a common characteristic of atopic disorders. Six potentially functional variants, including C-590T in the *IL4* gene, C-1055T and Arg130Gln in the *IL13* gene, and Ile50Val, Ser478Pro, and Gln551Arg in the *IL4RA* gene, have been evaluated for their involvement in the control of total serum IgE levels and related atopic disorders, but the results of these studies have been inconsistent.

Objective: We examined whether these 6 variants had genotypic effects on total serum IgE levels in 823 unrelated German children from a large infant cohort, the German Multicenter Atopy Study.

Methods: Marginal effect models were used for the analyses of the repeated IgE measurements. Weighted linear regression and family-based tests of association were performed to minimize the possibility of spurious effects caused by selection bias or confounding on the basis of ethnic background.

Results: There are significant associations between increased total serum IgE levels and 2 variants in the *IL13* gene ($P < .005$ and $.0002$ for Arg130Gln and C-1055T, respectively). These genetic effects are unlikely to be due to solely linkage disequilibrium between 2 polymorphisms, population stratification, or nonrepresentative samples. In addition, exposure to maternal smoking appears to modify the above effects on total serum IgE levels. However, no statistical association was observed between this quantitative phenotype and the other 4 variants examined.

Conclusion: These findings suggest that variants C-1055T and Arg130Gln of the *IL13* gene might play an important role on total serum IgE production in this study population. (*J Allergy Clin Immunol* 2003;112:382-8.)

Key words: Total serum IgE levels, *IL4*, *IL13*, *IL4RA*, single nucleotide polymorphism, association

Increased total serum IgE levels are commonly observed in several atopic disorders that afflict increasing numbers of persons worldwide both seasonally and annually. Total serum IgE levels might be under stronger genetic control than atopic disease.¹ Additionally, the phenotypic definition of total serum IgE is more stable across studies, and therefore studying IgE levels should help to understand the genetic basis of common atopic diseases.

Several studies have repeatedly reported linkage between 5q31-33 and total serum IgE levels or its related atopic disorders²⁻¹⁴; therefore this region might well contain genes controlling IgE. The 2 most promising candidate genes in this region are the genes encoding IL-4 (*IL4*) and IL-13 (*IL13*). Their gene products are the only known cytokines with the ability to induce IgE synthesis in vitro^{15,16} and in vivo.^{17,18} The signal transduction pathway leading to IgE synthesis requires interaction between IL-4 and IL-13 and their receptors.¹⁹ IL-4 receptor α (IL-4R α), the common subunit of IL-4R and IL-13R, is located on chromosome 16p12, which has been shown to be linked with atopy in a German population and with a positive skin prick test response in Hutterites.^{8,20}

As strong candidate genes for IgE synthesis, only C-590T in the *IL4* gene, C-1055T and Arg130Gln in the *IL13* gene, and Ile50Val, Ser478Pro, and Gln551Arg in the gene encoding IL-4R α (*IL4RA*) have been shown to be potentially functional variants.²¹⁻²⁶ Many association studies have been performed to test the effects of these 6 variants on total serum IgE levels and related atopic disorders in various populations.^{21-25,27-46} Results of these studies are conflicting, however, for several reasons. First, these variants might influence total serum IgE levels in some populations but not in others. Second, most studies of total serum IgE levels used individuals ascertained through atopic diseases and nonatopic control subjects, and thus the genetic effects on IgE levels might not be representative of the general population. Third, causal genes and perhaps even modifier genes for the development of atopic diseases might not be identical to those involved in controlling total serum IgE levels, although it

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Abbreviations used

FRET: Fluorescence resonance energy transfer
LD: Linkage disequilibrium
MAS: Multicenter Atopy Study
SNP: Single nucleotide polymorphism

is likely that some genes contribute to both. Fourth, environmental factors and interaction effects between these 2 cytokines and their shared receptor subunit have rarely been considered, so that weak genetic effects might have been overlooked. Finally, small sample sizes in various studies and possible genotyping errors could contribute to conflicting published results.

To fully evaluate the genetic effects of *IL4*, *IL13*, and *IL4RA* on total serum IgE levels, we performed an association study of 6 relevant variants in these 3 genes using 823 German children. Both the mean difference and rate of change in total serum IgE levels over age were estimated across genotypes. Haplotype analyses were performed for genes with more than 2 variants. Additionally, we tested for gene-gene and gene-environment interactions. To eliminate the possibility of a spurious association caused by hidden population stratification, we also performed a family-based test of association by using 382 complete parent-offspring trios.

METHODS

Study subjects

The German Multicenter Atopy Study (MAS) recruited 1314 of 7609 newborns from 6 hospitals in 5 German cities (Berlin, Düsseldorf, Freiburg, Mainz, and Munich) throughout the year 1990. Informed consent was obtained from all parents, and the study was approved by both the local Ethics Committee in Germany and the Committee on Human Research in the Johns Hopkins Bloomberg School of Public Health. The detailed sampling strategy has been described previously.^{47,48} In brief, 1314 children comprised the original MAS study cohort (499 potential high-risk infants with cord blood IgE levels of ≥ 0.9 kU/L and/or ≥ 2 atopic family members and 815 randomly selected control subjects). All cohort infants were brought in for follow-up visits at 1, 3, 6, 12, 18, and 24 months, with annual visits thereafter. The participation rate at the seventh birthday was 71.5%. A total of 823 children whose DNA samples were available and who had at least one German parent were selected for a population-based association study. A total of 382 complete trios were used in the subsequent family-based association study.

To assess possible selection bias, we compared the baseline characteristics of the studied subcohort ($n = 823$) with those of the original cohort children with at least one German parent ($n = 5569$). Consistent with the MAS sampling strategy, there is a substantially higher proportion of children with more than 2 atopic family members and increased cord blood IgE levels, reflecting selection of children with increased serum IgE levels for this longitudinal study. The weighted linear regression analyses described below were used to address this problem.

Total and specific serum IgE measurement

Total serum IgE levels and specific IgE antibodies to hen's egg, cow's milk, soy, wheat, *Dermatophagoides pteronyssinus*, cat dander, mixed grasses, and birch pollen were determined by means of CAP-RAST-FEIA (Pharmacia, Germany) at ages 1 through 7

(except for the age of 4 years) in these children.⁴⁹ IgE concentrations at these 6 time points were log10 transformed before data analyses.

Coding of parental smoking

Exposure to tobacco smoke is a potential environmental risk factor for increased total serum IgE levels on the basis of epidemiologic and animal studies.⁵⁰⁻⁵³ Prenatal exposure to maternal smoking was defined as maternal smoking during pregnancy, and postnatal exposure to maternal smoking was defined when the mother reported that she smoked at home at 2 or more of the first 3 visits or 4 or more time points during the 7-year period of follow-up. Among the remaining subjects, "unexposed" was defined as having a nonsmoking mother for at least 4 of 6 visits. Ambiguous situations were defined as missing.

Genotype analysis

DNA samples were genotyped by using melting-curve analysis. After PCR amplification for each single nucleotide polymorphism (SNP), a pair of fluorescence resonance energy transfer (FRET) probes was applied to the PCR product and analyzed by using the LightCycler system (Roche Diagnostics, Mannheim, Germany). PCR conditions, primers, and FRET probe sequences are available on request (renate.nickel@charite.de). The donor probes comprise 5,6-carboxyfluorescein attached to 3'-*o*-ribose, and the acceptor probes were labeled with LightCycler Red 705 or 640 attached to the 5' terminus. Probes were designed by Olfert Landt (Tib Molbiol, Berlin, Germany). The donor probes are complementary to the mutant sequence. During melting-curve analysis, hybridization of the probe was more stable with the mutant compared with the wild-type allele, resulting in different melting temperatures for each allele.

Statistical analysis

Population-based association study. Allele frequencies for each SNP were calculated. A χ^2 goodness of fit test was used to assess deviation from Hardy-Weinberg equilibrium for genotype frequencies at each locus. The expectation maximization algorithm was used to estimate haplotype frequencies for each pair of SNPs by using the program Arlequin,⁵⁴ and the commonly used linkage disequilibrium (LD) statistic D' was calculated on the basis of these frequencies.⁵⁵ A likelihood ratio test was performed to test significance of departures from linkage equilibrium.⁵⁴

A marginal effect model was fitted to assess the association between genotype and longitudinal measurements of IgE, while accounting for the correlation between repeated IgE measurements. Interaction terms between age, age², age³, and genotypes were added into the model to test the variation in rate of change over the 7-year time period for the different genotypes. For each SNP, both a codominant model and the most commonly used genetic model from previous studies were implemented. A test of gene-gene and gene-environment interaction was performed by adding interaction terms to the regression model. Two-tailed P values of less than .05 were considered statistically significant; however, no corrections for multiple comparisons were made.

To assess the potential influence of selection bias on our results, we applied a weighted linear model to weight the observed data according to the original cohort. Similarly estimated coefficients between a weighted linear model and a marginal effect model suggest that the results might not be biased by selection. The weight for each child was assigned on the basis of the ratio of frequency of a specific combination of increased cord blood IgE levels, atopic family history, and maternal smoking status during pregnancy among 3852 of 5569 children with all the information available in the baseline population and 767 of 823 such children in the studied subcohort.

For the genes with more than 2 variants, haplotype analyses were performed. Three-locus haplotypes in the gene *IL4RA* were recon-

TABLE I. Allele frequencies and genotype distribution of gene variants, as well as LD measurements, in 823 German children

	C-590T	C-1055T	Arg130Gln	Ile50Val	Ser478Pro	Gln551Arg
Genotype distribution						
Mutant/mutant*	28	43	43	249	17	26
Wild-type/mutant	221	301	266	395	222	256
Wild-type/wild-type	537	467	499	160	572	530
Allele frequency (mutant)	17.6%	23.9%	21.8%	55.5%	15.8%	19.0%
LD statistic (<i>D'</i>)						
C-590T		0.19†	0.33†	0.03	0.17	0.16
C-1055T			0.58†	0.02	0.03	0.05
Arg130Gln				0.02	0.01	0.09
Ile50Val					0.37†	0.28†
Ser478Pro						0.99†
Gln551Arg						

*The mutant alleles here are T-590, T1055, Gln130, Ile50, Pro478, and Arg551.

†Pairwise LD measures with *P* values of less than .001.**TABLE II.** Genetic effects of 6 SNPs on mean total serum IgE levels in 823 German children after adjustment for age, age², age³, and sex by using a marginal effect model

	β	95% CI	<i>P</i> value
C-590T			
CC	Reference		
TT and CT	.02	-.06 to .11	.61
C-1055T			
CC and CT	Reference		
TT	.29*	.14 to .45	.0002
Arg130Gln			
Arg/Arg	Reference		
Arg/Gln	.13†	.04 to .21	.0043
Gln/Gln	.29†	.16 to .43	<.0001
Val50Ile			
Val/Val and Val/Ile	Reference		
Ile/Ile	-.01	-.1 to .07	.74
Ser478Pro			
Ser/Ser	Reference		
Pro/Pro and Ser/Pro	.06	-.03 to .15	.20
Gln551Arg			
Gln/Gln	Reference		
Arg/Arg and Gln/Arg	.04	-.05 to .12	.40

*The β estimate from the weighted linear regression is .33. Among nonatopic subjects, the β estimate is .41 (*P* < .0001) on the basis of 3 children with the TT genotype.†The β estimates from the weighted linear regression are .08 and .28, respectively. Among nonatopic subjects, these estimates are .15 (*P* = .05) and .32 (*P* = .03) on the basis of 6 children with the Gln/Gln genotype and 47 children with the Gln/Arg genotype.

structured with PHASE software (version 1.0),⁵⁶ and then ambiguous diplotypes were assigned by using weights calculated with the conditional probability given all the compatible diplotypes matching the observed genotypes. Weighted linear regression was then used for the IgE measurements of each time point separately.

Family-based association study. We used the marginal effect models as in the population-based study above but this time with a different covariate, reflecting transmission of the mutant allele from both parents⁵⁷ rather than the children's genotype.

All univariate analyses were performed by using the STATA (version 7; Stata statistical software, College Station, Tex) statisti-

cal package, and the analyses of longitudinal data and family-based association analyses were performed with the SAS (version 8.2; SAS Institute, Cary, NC) statistical package.

RESULTS

Allele and genotype frequency

Allele frequencies and genotype distributions for the 6 SNPs are shown in Table I. None of these markers deviated significantly from the Hardy-Weinberg expectation. Statistically significant levels of LD were seen both for the variants within the *IL13* gene and for those within the *IL4RA* gene.

Phenotype and genotype association

Nongenetic factors, including sex, maternal smoking, study center, birth season, parents' education levels, number of pregnancies of the mother, and duration of breast-feeding, were not associated with both total serum IgE levels and the genotypic distribution of these 6 SNPs (data not shown) and therefore were not considered as confounders in this study. Analyses of the genetic effects of the 6 variants on total serum IgE levels (Table II) were adjusted for age, age², age³, and sex. Homozygous and heterozygous carriers of the Gln130 allele of the *IL13* gene had 0.29 log numbers of kilounits per liter (kU/L) and 0.13 log (kU/L) higher IgE levels (*P* < .0001 and *P* = .0043, respectively) compared with homozygous carriers of the Arg130 allele under the codominant model. Genotype TT at the *IL13* promoter polymorphism was substantially associated with increased IgE levels by 0.29 log (kU/L), with a *P* value of .0002 under the recessive model. Fig 1 clearly shows the above effects year by year instead of the overall estimates from the marginal effect model. Estimates from the weighted linear model revealed similar genetic effects for these 2 SNPs, which provided evidence that the association between variants in the *IL13* gene and total serum IgE levels does not appear to be due to selection bias. There were no statistical associations between total serum IgE levels and the other 4 SNPs under either the codominant (data not

shown) or the most commonly used model. Neither 2-locus (Ser478Pro-Gln551Arg) nor 3-locus (Ile50Val-Ser478Pro-Gln551Arg) haplotype analyses provide any evidence of association for the *IL4RA* gene with IgE levels (data not shown). Although a substantial difference in total serum IgE levels by *IL13* genotypes was observed across ages, there was no significant effect for any variants on the shape or rate of IgE changes (data not shown).

Studies by Marsh et al² and Graves et al³¹ suggest that genetic variants within the cytokine cluster on chromosome 5q31-q33 are associated with increased total serum IgE levels in nonatopic patients. Genetic effects in 331 atopic children and 165 nonatopic children were reanalyzed separately to address this finding. Atopy was defined as either high specific IgE levels to any common allergens (≥ 0.7 kU/L) or high total IgE levels (defined as previously reported³⁶). Nonatopic children were those not sensitized to any common allergens (ie, children who have IgE measurements available for at least 4 of the 6 time points, although none of these IgE measurements showed detectable specific IgE antibody to the tested allergen) and were not given a diagnosis of atopic dermatitis. Despite a small sample size, we confirmed the findings of Marsh et al² and Graves et al³¹ for variants Arg130Gln and C-1055T in the *IL13* gene (see footnotes in Table II).

C-1055T and Arg130Gln in the *IL13* gene were in strong LD, and therefore the effect on total serum IgE levels of one variant might be due to the substantial lack of independence between the 2 variants. For example, an association between the Gln130 carriers of Arg130Gln and higher total serum IgE levels could result from apparent genetic effects of the TT genotype of C-1055T on IgE. However, stratified analyses suggest a separate effect for each locus not simply caused by LD (Table III). Among the children with reference genotypes (CC and CT) at the *IL13* promoter polymorphism, genotypes Arg/Gln and Gln/Gln of the *IL13* coding region polymorphism showed total serum IgE levels 0.12 log (kU/L) and 0.27 log (kU/L) higher than those with Arg/Arg genotypes. It is important to note that the same trend of genetic effect for a Gln130 carrier was not seen among the children with a TT genotype at the *IL13* promoter polymorphism (Table III) on the basis of the observation of constant genotypic effects for Arg/Arg, Arg/Gln, and Gln/Gln. These different effects of the variant Arg130Gln between strata of the *IL13* promoter variants suggest a possible interaction between these 2 variants. Unfortunately, the tight LD makes tests of interaction unstable given the sparse data.

Test of interaction (gene-gene and gene-environment)

We observed a possible modifier effect of postnatal exposure to maternal smoking on 2 functional SNPs in the *IL13* gene. For children who were not exposed to maternal smoking, genotype TT and Gln/Gln had significantly higher total serum IgE levels by 0.27 log (kU/L) and 0.25 log (kU/L), respectively, compared with their reference genotypes, CC/CT and Arg/Arg. However,

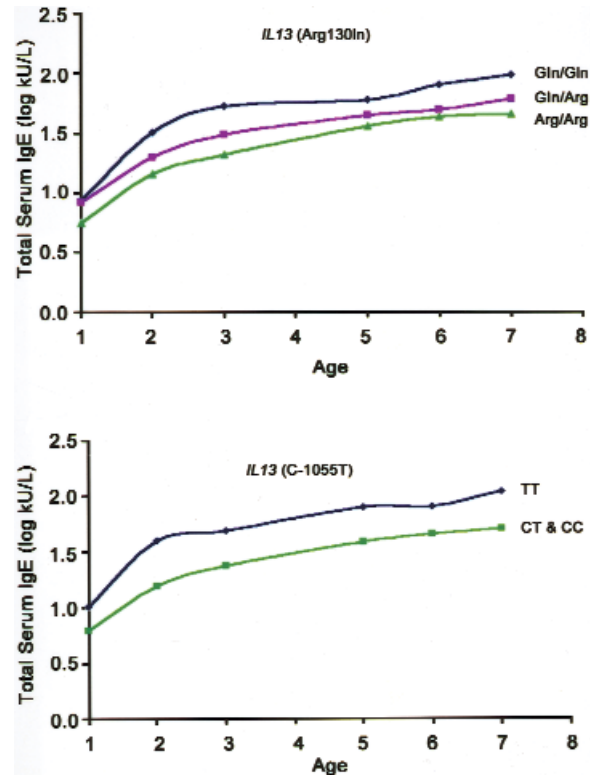


FIG 1. Mean difference of total serum IgE levels across genotypes for the *IL13* variants Arg130Gln and C-1055T, respectively.

TABLE III. Individual and combined genetic effects of C-1055T and Arg130Gln in the *IL13* gene after adjustment for age, age², age³, and sex

Arg130Gln (<i>IL13</i> fourth exon)			
<i>IL13</i> (C-1055T)	Arg/Arg	Gln/Arg	Gln/Gln
CC and CT	Reference (n = 482)	0.12 (n = 251, P = .01)	0.27 (n = 25, P = P = .0002)
TT	0.37 (n = 10, P = .0055)	0.33 (n = 15, P = .02)	0.33 (n = 18, P = .0067)

Note that n indicates the number of children in each cell and P indicates the P value for estimates of each combined genetic effect versus the common reference.

among those who were exposed to maternal smoking, the above genotypic effects become even higher (0.60 log [kU/L] and 0.65 log [kU/L]). These differences between strata of exposure to maternal smoking are statistically significant, with P values of less than .02. Prenatal exposure to maternal smoking showed similar effects; however, it was not possible to distinguish between the effects of prenatal and postnatal exposure because mothers who smoked during pregnancy were less likely to quit afterward. No statistically significant evidence for interaction effects between *IL13* (C-1055T and Arg130Gln), *IL4* (C-590T), and the 3 SNPs in the *IL4RA* gene was seen in these data (data not shown).

Transmission disequilibrium test

Finally, family-based analyses were performed to minimize the possibility of false-positive results. The transmission of allele Gln130 and genotype TT were associated with higher total serum IgE levels, with estimated effects of 0.18 log (kU/L) ($P = .02$) and 0.28 log (kU/L) ($P = .05$), respectively.

DISCUSSION

This study analyzed the genetic effects of 6 potentially functional variants within 3 genes (*IL4* [C-590T], *IL13* [C-1055T and Arg130Gln], and *IL4RA* [Ile50Val, Ser478Pro, and Gln551Arg]) by using a German birth cohort. The 2 variants in the *IL13* gene showed a statistically significant effect on total serum IgE levels that did not appear to be due to LD between them, selection bias, or population stratification. In addition, we observed interaction effects between exposure to maternal smoking and these 2 functional SNPs in the *IL13* gene on total serum IgE levels. There was no effect of the *IL4* promoter polymorphism and 3 functional polymorphisms within the *IL4RA* gene on IgE. The present study also examined variation in rates of change over age across genotypes for these 6 SNPs, although no genotype showed significant results. No significant interactions were observed between the *IL4* promoter variant or variants of the *IL13* gene with the 3 variants in the *IL4RA* gene.

Recently, Graves et al³¹ showed that polymorphisms in the *IL13* promoter and the coding region play an important role in the development of a high IgE phenotype by using data from 2 samples of German children and one sample of US children. The genetic effect was strongest in nonatopic subjects. Our studies confirm their results in a sample comprised of German children. Moreover, we provide evidence that each of these 2 variants might have separate effects on total serum IgE concentration not solely a result of LD between them.

Our results are not likely to be due to selection bias or population stratification. Weighted linear regression was used to reconstruct a cohort that represented the German children from all 6 study centers, and we found very similar estimated genetic effects compared with those obtained from the marginal effect model without this weighting variable. Therefore these genetic effects on total serum IgE levels seem unlikely to be attributable to merely biased sampling. We also addressed potential hidden-population stratification in our data. Nonsignificant D' for SNPs (Table I) in the genes located on different chromosomes (5q31 and 16p12) suggested that population stratification might not be important in this German cohort. In addition, the family-based study with repeated IgE measurements as the dependent variable showed associations for both *IL13* SNPs, suggesting that these 2 variants might either directly affect serum IgE levels or might be in tight LD with a causal gene.

However, a significant association between *IL13* variants and total serum IgE levels was not found in British,

Japanese, and Dutch populations.^{22,35,45} In addition to the relatively small sample sizes and ascertainment bias caused by sampling through asthmatic patients, another possible reason for their negative findings is that environmental factors were not included in their analyses. In the present study we found that children who inherited *IL13* variants and were exposed to maternal smoking had higher total serum IgE levels than similar children who were not exposed to maternal smoking. This finding suggested that genetic susceptibility to high IgE production might be facilitated by increased IgE-positive cells in the nasal mucosa,⁵⁸ lungs,⁵⁹ or both of children exposed to environmental tobacco smoke.

The *IL4* promoter polymorphism did not influence total serum IgE levels in the MAS children. This promoter polymorphism might be in LD with a true causal variant (eg, C-1055T or Arg130Gln of the *IL13* gene or other SNPs nearby),⁶⁰ it might influence the development of atopic disorders rather than influence total serum IgE levels,²⁷⁻³⁰ or it might be a modifier gene that affects disease severity.^{30,32}

Kruse et al²⁵ found Pro478 and Arg551 of the *IL4RA* gene to be associated with lower total serum IgE levels in 181 German children, of whom 91% were atopic. This finding has been confirmed in both Finnish and Dutch populations.^{44,46} We observed similar allele frequencies for these SNPs in the MAS cohort; however, we could not confirm an association with total IgE levels in (1) the 138 children from the same city (Freiburg) as those in the Kruse et al²⁵ study (Pro478: -0.04 log [kU/L]; Arg551: -0.06 log [kU/L]) or (2) 331 atopic German children drawn from our 823 samples (data not shown). Thus different environmental factors or a possible sampling difference between the 2 studies cannot explain the discrepancy. Moreover, haplotype analyses provided no evidence of either synergic effects of the variants Ser478Pro and Gln551Arg or any haplotype effect on total serum IgE levels in the present study.

The effects of the *IL4RA* extracellular variant Ile50Val on total serum IgE levels were inconsistent in 2 Japanese studies.^{23,37} Possibly different environmental factors might explain this difference, or one could be a false-positive result caused by chance alone. In our study the genotype Ile/Ile was not associated with total serum IgE levels in the MAS cohort children, which is consistent with the results of Noguchi et al³⁷ and Heinzmann et al.³⁵

Although no associations for *IL4* and *IL4RA* were observed in our data, the possibility for undetected small genetic effects remains. Our sample of 823 German children is still not large enough to detect small genetic effects. Given the observed marker allele frequencies in this study, the smallest mean difference that could be detected between mutant and wild-type subgroups is about 0.11 log (kU/L) (when type I and type II errors were fixed at 0.05 and 0.2, respectively). Additionally, the present study tested relatively few of the possible gene-gene and gene-environment interactions that could contribute to regulation of IgE levels, and higher-order interactions are possible.

In summary, we used a candidate gene approach on a

cohort of German infants with longitudinal IgE data. Individual and interaction effects for 6 potentially functional variants within the *IL4*, *IL13*, and *IL4RA* genes were tested. Two polymorphisms in *IL13* showed statistically significant association with increased total serum IgE levels. These genetic associations do not appear to be due to solely LD between these polymorphisms of the same gene. Moreover, these associations are modified by exposure to maternal smoking. The important role of these 2 functional variants on total serum IgE production in German children might provide a new focus for future therapeutic and preventive strategies.

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