

Associations between specific serum IgE response and 6 variants within the genes *IL4*, *IL13*, and *IL4RA* in German children: The German Multicenter Atopy Study

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Background: Among many published studies of specific IgE response or atopy, only a few showed positive marginal effects for 6 potentially functional single nucleotide polymorphisms (SNPs; C-590T in the *IL4* gene, C-1055T and Arg130Gln in the *IL13* gene, and Ile50Val, Ser478Pro, and Gln551Arg in the *IL4RA* gene). SNPs were commonly considered individually, and therefore the true effect could be masked by other genes or environmental factors.

Objective: We tested the relationship between these 6 SNPs and sensitization to food, mite, cat, and outdoor allergens in unrelated German children drawn from the Multicenter Atopy Study. Gene-gene and gene-environment interactions were also evaluated.

Methods: Multiple logistic regression models were used for the analyses of 4 sensitization outcomes.

Results: The variant C-1055T was significantly associated with increased risk of sensitization to food and outdoor allergens, with odds ratios of 3.49 (95% CI, 1.52-8.02) and 2.27 (95% CI, 1.04-4.94), respectively. The effects of the TT genotype on food sensitization appear to depend on variants in the *IL4RA* gene, in which marginally significant interaction terms were observed. Significant evidence supported an interaction between exposure to maternal smoking and variant Gln551Arg on risk of cat sensitization. In addition, we found that the effect of variant C-590T on sensitization to mite depended on Der p 1 allergen levels in carpet dust samples.

Conclusions: These findings not only suggested that variants in the *IL4*, *IL13*, and *IL4RA* genes play an important role in controlling specific IgE response but also strengthened our understanding of gene-gene and gene-environment interaction on the development of specific sensitization in this study population. (J Allergy Clin Immunol 2004;113:489-95.)

Key words: Sensitization, *IL4*, *IL13*, *IL4RA*, single nucleotide polymorphism

Atopy is clinically expressed as atopic asthma, allergic rhinitis, and atopic dermatitis and can be defined by at least one positive specific IgE antibody response to food or inhalant allergens, an increased level of total serum IgE, or both. Total serum IgE levels are more likely to be under genetic control, whereas specific IgE response might be determined predominantly by environmental factors, primarily exposure to the antigen in question.¹ Biologic synthesis of specific IgE antibody in a cognate pathway requires the corresponding antigen to be first internalized by B cells and subsequently presented to the T cells by the MHC II molecule.^{2,3} Therefore allergen exposure is always necessary for a positive specific IgE response. There is a dose-response relationship between mite allergen exposure and its corresponding specific IgE response.⁴⁻¹² However, a consistent pattern of association has not been seen for domestic cat allergen exposure levels and cat-specific IgE response.^{8,13-17} In addition to exposure to specific allergens, other environmental factors might also increase the risk of sensitization. Evidence increasingly supports passive smoking as a risk factor for atopy,^{5,18-20} especially for sensitization to food allergens in early childhood.⁵

Although environmental factors are important in the development of atopy, many studies have shown that there is a strong genetic predisposition as well. By using phenotypes related to specific IgE responses (positive skin prick test response, atopy, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and birch-specific IgE antibodies), linkage analyses have identified several chromosomal regions that might contain genes controlling the allergic response, including 5q31²¹⁻²⁹ and 16p12,^{23,28,30} in which *IL4*, *IL13*, and *IL4RA* are mapped. The 6 potentially functional single nucleotide polymorphisms (SNPs) in these 3 genes, 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 widely tested for possible effects on serum IgE levels and related atopic phenotypes in different populations.³¹⁻⁵¹ In most cases the study of specific IgE response did not consider possible modifier effects of corresponding

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Abbreviations used

LRT: Likelihood ratio test

OR: Odds ratio

SNP: Single nucleotide polymorphism

environmental factors, such as levels of exposure to indoor allergens and active or passive smoking. Therefore the true effect of either genotype or environmental factors might be poorly represented in these studies. In addition, most of these previous studies used atopy or positive skin prick test responses as outcomes, which are composite phenotypes for IgE response to any one allergen. This makes it difficult to separate the effects of the genes and exposure to specific allergens from their interaction effects.

In the present study we test whether these 6 potentially functional variants in the *IL4*, *IL13*, and *IL4RA* genes themselves increased the risk of sensitization to food, mite, cat, and outdoor allergens (grass and birch) in German children by using a case-control study design. In addition, we test for modifying effects of passive smoking on the risk of each specific sensitization, for variability with exposure levels to indoor allergens on the genetic effects of sensitization to mite and cat, and for evidence of gene-gene interaction effects on risk of sensitization. To eliminate the possibility of a spurious association caused by hidden population stratification, we also performed a family-based test of association by using a limited number of complete parent-offspring trios. Haplotype analyses with the omnibus likelihood ratio test (LRT) to examine heterogeneity in haplotype frequencies were used to evaluate the effects of these 2 candidate loci on the risk of each sensitization.

METHODS**Study subjects**

The present study included 823 German children who participated in the German Multicenter Allergy Study, as described in a previous study of total serum IgE levels.⁵¹ These children were recruited in 1990 and brought in for follow-up visits at the ages of 1, 3, 6, 12, 18, and 24 months, plus annual follow-up visits thereafter. Total serum IgE levels and specific IgE antibodies to hen's egg, cow's milk, soy, wheat, *D pteronyssinus*, cat dander, mixed grasses, and birch pollen were determined by means of CAP-RAST FEIA (Pharmacia, Germany) at annual visits at the age of 1 through 7 years (except at the age of 4 years). Sensitization to these allergens was defined as serum IgE antibody to the specific allergen equal to or greater than 0.7 kU/L at least once during the 7-year follow-up period. Control subjects were defined as children who had IgE measurements obtained at a minimum of 4 of 6 visits but without IgE measurements showing detectable specific IgE antibodies to the tested allergen (<0.35 kU/L). Four case-control analyses were performed to examine the relationship between the 6 genetic variants and the following phenotypes: sensitization to food allergens, sensitization to mite, sensitization to cat, and sensitization to outdoor allergens.

A total of 159 complete trios, in which children had at least one of the 4 sensitization phenotypes, was available for family-based association tests (the number of trios with children sensitized to food, mite, cat, or outdoor allergens were 93, 56, 36, and 108, respectively).

Exposure assessment

Coding of parental smoking. Maternal and paternal smoking information was obtained by questionnaire at the child's birth and at the ages of 18 months and 3, 4, 5, and 7 years of age. Prenatal exposure was defined as maternal smoking during pregnancy, and postnatal exposure was defined by whether the mother smoked at home for at least 2 of the first 3 reports or for more than 3 reports during the entire 7 years of follow-up. Among the remaining children, unexposed status was defined as having a nonsmoking mother for at least 4 of 6 time points during the longitudinal follow-up period. Ambiguous exposure situations were coded as missing.

Indoor allergen exposure. House dust samples were obtained within a 1-month interval at the ages of 6, 18, 36, and 60 months, as described previously.^{5,6} Only the sample collected at the age of 5 years included the measurement of house dust concentrations in both mattress and carpet samples. Carpets were present in all homes in this study.

These dust samples were analyzed quantitatively for Der p 1, Der f 1, and Fel d 1 by using a sandwich ELISA (ALK, Copenhagen, Denmark). The detection limit was 2 ng/g dust.

The Der p 1 and Fel d 1 concentrations from carpet dust samples for the first 2 measurements (6 months, 18 months, or both) were used to represent the allergen exposure levels for each child during early life. We assigned the lowest limit of detectability to a household with undetectable allergen exposures. If both 6- and 18-month values were available, the arithmetic mean of these 2 exposure levels was used. Demarcation between low and significant exposure levels for Der p 1 and Fel d 1 were based on the cutoff points of 2000 ng/g dust and 8000 ng/g dust, respectively.⁵²

Genotype analysis

DNA samples were genotyped by using melting curve analysis, as described previously.⁵¹ Sequences of PCR primers and fluorescence resonance energy transfer probes, as well as PCR conditions, are available on request (renate.nickel@charite.de). Fluorescence resonance energy transfer probes were designed by Olfert Landt (Tib Molbiol, Berlin, Germany).

Statistical analysis

Univariate associations between genotype and case-control status were estimated by using simple logistic regression models. The most commonly used genetic models from previous studies were implemented. When variants C-1055T and Arg130Gln in the *IL13* gene showed substantial effects on the risk of sensitization to specific allergens, the total serum IgE levels at age 1 year were adjusted to test whether these variants caused the sensitization through mechanisms other than increased total serum IgE levels. Both of these variants have been associated with high total serum IgE levels,⁵¹ which might be a potential intermediate variable between gene expression and specific IgE response. Multiple logistic regression models were used to test for gene-gene and gene-environment interaction by adding an interaction term between indicator variables for the genotypes or observed environmental factors. LRTs and Wald tests were used to examine the significance of such multiplicative interaction effects. Odds ratios (ORs) and 95% CIs are reported for all analyses. No corrections for multiple comparisons were made in this study. Case-control analyses were performed with STATA (Version 7; Stata Statistical Software, College Station, Tex). Family-based association studies were performed by using a log-linear model.⁵³

The program SNPDM⁵⁴ was used for haplotype analyses. This software uses an E-M algorithm to obtain maximum likelihood estimates for haplotype frequencies among patients and control subjects and then performs a test of heterogeneity between patient and control subject frequency profiles through an LRT. Empiric *P* values were based on 1000 random permutations.

RESULTS

Baseline characteristics, including sex, study center, parent education level, number of siblings, birth month, and duration of breast-feeding, were not significantly associated with both disease status and each of the tested SNPs (data not shown), and therefore these variables were not considered in the following 4 case-control studies.

Case-control study for sensitization to food allergens

A total of 176 cases were sensitized to food allergens, and 306 controls had no detectable specific IgE antibodies. When compared with genotypes CC and CT, genotype TT at position -1055 in the *IL13* gene showed a statistically significant association with the risk of food sensitization (OR, 3.49; 95% CI, 1.52-8.02; Table I). This association decreased slightly after adjustment for total serum IgE levels (OR, 2.81; 95% CI, 1.06-7.48). Although marginally significant interactions between variants C-1055T in the *IL13* gene and the 2 variants (Ser478Pro and Gln551Arg) in the *IL4RA* gene were observed (Table II), small cell sizes for the presence of alleles Pro478 and Arg551 made the estimation of ORs imprecise. No significant genotypic effects on the risk of food sensitization for the other 5 SNPs in this case-control study were found (Table I), and no statistically significant evidence for interaction effects between maternal smoking and these 6 SNPs were seen (data not shown).

Case-control study for sensitization to mite allergen

A total of 108 children sensitized to mite allergen were identified as cases, and 432 children nonsensitized to mite allergen served as controls. We found no significant association between mite sensitization and any of these 6 genetic variants (Table I). No statistically significant evidence for interaction between these SNPs and exposure to maternal smoking or gene-gene interactions were observed (data not shown).

For children who carried the T-590 allele, the ORs of mite sensitization were different among those exposed to high (OR, 3.76; 95% CI, 1.42-9.77) and low (OR, 0.74; 95% CI, 0.40-1.34) concentrations of Der p 1 allergen. The OR of the interaction is 8.2 ($3.76/[0.74 \times 0.62]$), which is statistically significant on the basis of both the Wald test and the LRT (Table III). Der p 1 exposure levels significantly varied among study centers ($P < .0001$), but the interaction estimate did not change after adjustment for this variable (see note in Table III). The interaction between variant C-590T and Der p 1 still existed but with less significance levels when a cutoff point of the 75th percentile (375 ng/g) of the distribution of allergen exposure ($P = .01$) or a categorical variable reflecting quartiles ($P = .05$) was used.

Case-control study for sensitization to cat allergens

There were 64 cases and 472 controls who met our criteria for cat sensitization. Univariate analyses showed

that none of the 6 SNPs were associated with sensitization to cat (Table I). There was a decreased risk of cat sensitization among individuals carrying the *IL4RA* Arg551 allele in the absence of prenatal exposure to maternal smoking, with an OR of 0.72 (95% CI, 0.32-1.51). However, children who were exposed to prenatal maternal smoking and carried the Arg551 allele showed an increased risk for cat sensitization, with an OR of 2.64 (95% CI, 1.03-6.29; Table IV). Both the LRTs and Wald tests indicated statistically significant interaction between Arg551 carriers and prenatal exposure to maternal smoking ($P = .044$ and $P = .045$, respectively). Postnatal exposure to maternal smoking also showed a substantial modifier effect on the apparent effects of variant Gln551Arg, but this was not statistically significant. Children exposed to prenatal maternal smoking were more likely to be exposed to postnatal maternal smoking, and therefore it is impossible to distinguish between the effects of prenatal and postnatal exposure. We did not observe any significant interaction effects between any variants and cat allergen exposure levels or any gene-gene interactions (data not shown).

Case-control study for sensitization to outdoor allergens

A total of 196 cases with outdoor allergen sensitization and 344 nonsensitized controls were identified. Univariate analyses of the 6 SNPs showed that only variant C-1055T of the *IL13* gene was substantially associated with risk of sensitization to outdoor allergens, with an OR of 2.27 (95% CI, 1.04-4.94; Table I). This variant might be associated with sensitization to outdoor allergens either through its apparent increase in total serum IgE levels or through its effect on food sensitization because the IgE-adjusted OR decreased to 1.42 (95% CI, 0.56-3.6; Table I) and the OR among children without food sensitization became 1.33 (95% CI, 0.15-11.7). There was no significant evidence for interaction between genotypes and maternal smoking or gene-gene interactions on the risk of outdoor allergen sensitization (data not shown).

Additional tests of association

Both 2-locus and 3-locus haplotype analyses were performed for sensitization to food, mite, cat, or outdoor allergens. Omnibus test statistics were not statistically significant for any of these phenotypes (data not shown). Additionally, for those patients with parental DNA available, family-based association tests for variant C-1055T of the *IL13* gene were conducted. No evidence of linkage and disequilibrium for this variant by using patients sensitized to food (93 case-parent trios) or outdoor allergens (108 case-parent trios) was found (data not shown).

DISCUSSION

It is evident that allergic sensitization to specific antigens is a complex trait controlled by both genetic and environmental factors. Each of the sensitization pheno-

TABLE I. ORs and 95% CIs for 6 variants of the genes *IL4*, *IL13*, and *IL4RA* in 4 case-control studies

SNPs	Genotype	Food sensitization			Mite sensitization		
		Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
<i>IL4</i> (C-590T)	CC	123	211	Reference	73	294	Reference
	CT, TT	48	89	0.93 (0.61~1.40)	31	129	0.97 (0.61~1.55)
<i>IL13</i> (C-1055T)	CC, CT	159	294	Reference	102	407	Reference
	TT	17	9	3.49 (1.52~8.02)*	5	21	0.95 (0.35~2.58)
<i>IL13</i> (Arg130Gln)	Arg/Arg	99	197	Reference	65	267	Reference
	Arg/Gln	64	97	1.31 (0.88~1.95)‡	40	135	1.22 (0.78~1.90)
	Gln/Gln	12	10	2.39 (0.99~5.72)‡	2	25	0.33 (0.08~1.42)
<i>IL4RA</i> (Ile50Val)	Val/Val, Val/Ile	127	207	Reference	73	294	Reference
	Ile/Ile	47	93	0.82 (0.54~1.25)	34	130	1.05 (0.67~1.66)
<i>IL4RA</i> (Ser478Pro)	Ser/Ser	128	219	Reference	77	311	Reference
	Pro/Pro, Ser/Pro	46	85	0.93 (0.61~1.41)	30	116	1.04 (0.65~1.68)
<i>IL4RA</i> (Gln551Arg)	Gln/Gln	121	201	Reference	75	283	Reference
	Arg/Arg, Gln/Arg	54	102	0.88 (0.59~1.31)	32	144	0.84 (0.53~1.33)

*After the adjustment of total IgE levels at year 1: OR, 2.81; 95% CI, 1.06-7.48.

†After the adjustment of total IgE levels at year 1: OR, 1.42; 95% CI, 0.56-3.6.

‡P = .09 for LRT.

TABLE II. Case-control study of sensitization to food allergens: Interaction between variant C-1055T in the *IL13* gene and variants Ser478Pro and Gln551Arg in the *IL4RA* gene

	TT	Cases	Controls	OR*	95% CI
Pro478					
+	+	3	5	1.13	0.26-4.80
+	–	43	79	1.02	0.66-1.58
–	+	14	4	6.57	2.11-20.42
–	–	114	214	1.00	Reference
Arg551					
+	+	3	5	1.10	0.26-4.69
+	–	51	96	0.97	0.64-1.47
–	+	14	4	6.41	2.06-19.96
–	–	107	196	1.00	Reference

*Pro478 Wald test and LRT for interaction term: P = .06 and P = .05, respectively. Arg551 Wald test and LRT for interaction term: P = .07 and P = .06, respectively.

types considered here is likely caused by different but overlapping sets of genes and environmental factors. Therefore in this study we tested the relationship between 6 potentially functional variants in the genes *IL4*, *IL13*, and *IL4RA* and sensitization to food, mite, cat, and outdoor allergens separately by using a case-control design. Interaction effects between variants and exposure to maternal smoking (prenatal and postnatal) were evaluated for each sensitization outcome. Also, interaction effects between variants and indoor allergen exposure levels were examined for outcomes of mite and cat sensitization. Several findings should be noted. First, the variant C-1055T in the *IL13* gene significantly increased the risk of sensitization to both food and outdoor allergens. Second, the effects of the TT genotype on the risk of food sensitization might be modified by 2 variants (Ser478Pro and Gln551Arg) in the *IL4RA* gene. Third,

the effects of the promoter variant in the *IL4* gene on the risk of mite sensitization might depend on levels of Der p 1 allergen obtained from carpet dust samples. Fourth, there was significant evidence of interaction between prenatal exposure to maternal smoking and the variant Gln551Arg in the *IL4RA* gene on the risk of cat sensitization. There was no significant evidence of individual or interactive effects for the other 2 variants (Arg130Gln in *IL13* and Ile50Val in *IL4RA*) for risk of any sensitization. Finally, haplotype analyses by using these SNPs did not provide extra evidence for association among these 3 candidate genes and the sensitization phenotypes used here. The lack of evidence for global differences in haplotype frequencies between groups might indicate that the specific SNP C-1055T is directly involved in risk, and therefore inclusion of surrounding haplotype information does not add to the association results.

Two variants in the *IL13* gene were associated with increased total serum IgE levels in our previous study,⁵¹ but in this study only the promoter polymorphism increased the risk of sensitization to food and outdoor allergens. This suggests that total serum IgE levels and each sensitization are controlled by different genetic and environmental factors, although there might be some overlap. The observed substantial effect of the homozygous genotype for allele T-1055 in the *IL13* gene on the risk of food sensitization both before and after adjustment for total serum IgE levels at the age of 1 year suggests that the genetic effect of this variant on food sensitization might be independent of increased total serum IgE levels. However, the observed association between the TT genotype and sensitization to outdoor allergens might be through its effect on either increased total serum IgE levels or sensitization to food allergens, which are earlier signs of sensitization to inhalant allergens.⁵⁵

Previous studies have found a few positive effects for these 6 tested SNPs on specific IgE response or atopy.^{32,38,41,43,45} Because only one SNP was generally

Cat sensitization			Outdoor allergen sensitization		
Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
45	323	Reference	133	245	Reference
16	138	0.83 (0.45~1.52)	60	88	1.26 (0.85~1.86)
58	448	Reference	181	328	Reference
6	20	2.32 (0.90~6.01)	15	12	2.27 (1.04~4.94)†
40	292	Reference	111	220	Reference
19	153	0.91 (0.50~1.62)	69	104	1.31 (0.90~1.92)
4	22	1.32 (0.44~4.05)	15	15	1.98 (0.94~4.20)
43	322	Reference	140	229	Reference
20	143	1.05 (0.60~1.84)	54	109	0.81 (0.55~1.19)
46	336	Reference	135	246	Reference
18	131	1.00 (0.56~1.79)	61	93	1.20 (0.81~1.76)
42	309	Reference	125	227	Reference
22	158	1.02 (0.59~1.78)	71	112	1.15 (0.80~1.66)

considered at a time, the true genetic effect might be masked by other genes or by environmental factors in some studies. The present study illustrates this limitation. In our study we observed no marginal effects of Arg551 (*IL4RA*) carriers on the risk of sensitization to cat allergen. On the contrary, this variant might decrease the risk among children not exposed to prenatal maternal smoking but appeared to increase the risk of cat sensitization among smoke-exposed children. This apparent gene-environment interaction was statistically significant in our data. Similar phenomena were seen in tests for interaction between C-590T in *IL4*, with high concentrations of Der p 1 exposure levels on risk of sensitization to mite. However, we did not observe any such interaction effects between Fel d 1 allergen exposure level and any SNP on the risk of cat sensitization. Health selection bias and unavoidable cat exposure in the community⁵⁶ might make indoor carpet Fel d 1 exposure levels unrepresentative of true exposure to these inhaled allergens, biasing our test for interaction. Another reason could simply be small sample sizes because only 64 patients were available for these interaction tests.

Small sample size also limits the precision of tests for interaction between variant C-1055T and 2 variants in the *IL4RA* gene on sensitization to food allergen, although these marginal gene-gene interaction effects were quite consistent with the recent report in Dutch asthmatic patients.⁵⁷ Another limitation of our study is multiple comparisons. Tests for relationships between 4 different phenotypes and 6 different SNPs, as well as their interaction effects, were performed without correction for multiple tests, and therefore the positive results seen here might be spurious. Confounding because of population stratification could also account for some positive results from case-control comparisons presented here. The case-parent trio analysis would not be subject to this confounding; however, our limited number of trios make these tests less powerful.

TABLE III. Case-control study of sensitization to mite: Interaction between high concentration of Der p 1 exposure and variant C-590T in the *IL4* gene

T-590	Der p 1	Cases	Controls	OR*	95% CI
+	+	11	12	3.76	1.58-8.93
+	–	19	105	0.74	0.42-1.30
–	+	5	33	0.62	0.23-1.66
–	–	60	246	1.00	Reference

*Wald test and LRT for interaction term: $P = .003$ and $P = .0018$, respectively. After adjustment for study center, the OR for interaction is 8.56 ($P = .002$).

TABLE IV. Case-control study of sensitization to cat: Interaction between prenatal exposure to environmental tobacco smoke and variant Gln551Arg of the *IL4RA* gene

Arg551	Prenatal		Cases	Controls	OR	95% CI
	ETS					
+	+	10	27	2.64*	1.17-5.98	
+	—	12	119	0.72	0.36-1.45	
—	+	10	67	1.06	0.50-2.28	
—	—	31	221	1.00	Reference	

ETS, Environmental tobacco smoke.

*Wald test and LRT for interaction term: $P = .045$ and $P = .044$, respectively.

There are 4 major advantages to the present study. First, we used a more restricted case and control definition than used previously. To avoid possible ambiguous phenotypes caused by single measurement variation, CAP class II (0.7 kU/L) was chosen as the cutpoint for definition of sensitization, control subjects were those who had at least 4 measurements of specific IgE, and none of the available values showed detectable IgE anti-

bodies (0.35 kU/L). This strict definition of control subjects minimizes misclassification bias. Second, recall bias for environmental factors seemed unlikely in the present study because of prospective collection of maternal smoking exposure data and dust allergen exposure levels. Third, haplotype analyses with omnibus LRTs should have more power than single-locus analyses to detect association, especially when allelic heterogeneity is quite possible for the 2 candidate loci in this study, although no significant results were obtained. Finally, this study not only tested the individual effects of each SNP but also evaluated gene-environment interaction on each specific serum IgE response. By using these methods, a variant with a weak effect is less likely to be missed than when an individual gene is considered.

In summary, we found variants in the genes *IL4*, *IL13*, and *IL4RA* that individually or interactively play an important role in controlling specific IgE response to certain allergens in German children. Interactions identified in this study might improve efforts to prevent allergic disease by pinpointing risk factors that could be avoided among genetically susceptible persons. Additional studies with larger sample sizes will be needed to confirm the interaction effects presented here.

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