

An *IL13* coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German Multicenter Atopy Study (MAS-90)

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Background: Allergic diseases are one of the major causes of morbidity in the developed countries today, and the prevalence of these diseases is increasing steadily. Study of total serum IgE level is important in understanding the genetics of allergic diseases because IgE levels are considered to be a crucial pathogenic component. IL-13 plays an important role in the induction of IgE synthesis and in the pathogenesis of allergic diseases.

Objective: We sought to examine potential variation at the *IL13* gene and estimate its effect on elevated IgE level and atopic dermatitis (AD).

Methods: We conducted mutational analyses of the *IL13* gene by using single-stranded conformation polymorphism and DNA sequencing. Case control studies for high-IgE phenotype and AD were performed by using subjects from the German MAS-90 cohort.

Results: A novel *IL13* coding region variant at 4257 bp (G to A, fourth exon) was identified. Case control studies of a German sample from the MAS-90 cohort showed significant associations between the presence of the A allele and two atopic phenotypes: high IgE (odds ratio, 2.38; 95% confidence interval, 1.35-4.21; $P = .0026$) and AD (odds ratio, 1.77; 95% confidence interval, 1.06-2.96; $P = .03$).

Conclusion: This *IL13* coding region variant may be involved in the pathogenesis of AD and high total serum IgE level in a study population of white subjects. (*J Allergy Clin Immunol* 2000;106:167-70.)

Key words: IL-13, polymorphism, IgE level, atopic dermatitis

Abbreviations used

95% CI: 95% Confidence interval
AD: Atopic dermatitis
OR: Odds ratio
SSCP: Single-stranded conformation polymorphism

Allergic disease is one of the major causes of morbidity in the developed countries, and its prevalence is increasing steadily.¹⁻⁴ High total serum IgE level is a characteristic seen in a number of disorders related to atopy, such as asthma and atopic dermatitis (AD). Study of total serum IgE level, a well-characterized quantitative trait, is thus important in understanding the molecular genetic basis of these allergic diseases. Total serum IgE level is thought to be regulated by a complex array of genes and environmental factors that most likely interact with one another. In the search for the genetic basis of IgE levels and allergic diseases, association studies have been used to test polymorphic candidate genes for significant relationship with disease. Association analysis in case control studies can be a more powerful tool than linkage analysis in families when searching for genes with weak and moderate effects underlying complex phenotypes.^{5,6} To date, associations between total serum IgE concentration and polymorphism in several candidate genes have been reported. Those genetic variants with positive results include the genes encoding IL-4 (C-590T),⁷ IL-10 (C-571A),⁸ transforming growth factor β (C-509T),^{8,9} CD14 (C-159T),¹⁰ β_2 -adrenergic receptor (Arg16Gly and Glu27Gln),¹¹ FC ϵ R1 β (RsaI_{ex7} polymorphism^{12,13} and CA repeat,¹² RsaI_{in2}^{13,14}), HTM4 (TaqI),¹⁴ IFN- γ receptor 1 and 2,¹⁵ IL-4R α chain (S503P and Q576R,¹⁶ Ile50Val^{17,18}), TNF (LT α NcoI),¹⁹ and IL-9 (allele 118).²⁰

The *IL13* gene is located in the chromosome 5q31-q33 region, where both linkage and association studies have previously provided evidence for susceptibility genes for bronchial hyperreactivity, asthma, and total serum IgE levels.^{10,21-36} The region in 5q31-q33 is rich in candidate genes, particularly within a 1-mb region of 5q31, which includes clustered cytokine genes, such as *IL4* and *IL13*, that are known to be important in class switching to IgE

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and in the differentiation of naive T cells into T_{H2}/T_{H0} cells.³⁷ Recently, both animal models and clinical studies have indicated a critical role for *IL13* in the pathogenesis of allergic disease.³⁸⁻⁴³ Thus it will be informative to determine whether variant alleles at this gene influence the regulation of IgE responsiveness. Very recently, an *IL13* promoter polymorphism (C to T) at the position -1055 (relative to the transcription start site) was identified and was associated with allergic asthma.⁴⁴ However, two reports showed the absence of sequence variants in the *IL13* proximal promoter region.⁴⁵ In this communication we report a novel sequence variant in the *IL13* coding region and demonstrate a significant association of this allele with both high total serum IgE level and AD in a white study population.

METHODS

Study population

The German Multicenter Atopy Study (MAS-90) initially included 1314 unrelated healthy newborns from 5 German cities. The cohort has been described in detail previously.⁴⁶ Total serum IgE levels were determined at ages 1, 2, 3, 5, 6, and 7 years. High- and low-IgE phenotypes were defined as individuals with the sex-adjusted serum log (IgE) level above the 85th percentile (1.62, 2.04, 2.20, 2.34, 2.40, and 2.47 log kU/L at age 1, 2, 3, 5, 6, and 7 years, respectively) and below the 15th percentile (0.34, 0.62, 0.77, 1.00, 1.09, and 1.16 log kU/L for age 1, 2, 3, 5, 6, and 7 years, respectively) for at least two of 6 different time points. We conducted a modified case control study by selecting these two extreme phenotypes in the study population. One hundred patients with high IgE levels and 111 control subjects with low IgE levels were selected.

We also conducted a population-based association study for AD with 187 patients with AD and 98 control subjects without AD selected from the MAS-90 study population. The diagnosis of AD was generated from 3 sources (examining doctor, family physician, and computer algorithm from relevant symptoms) and defined as a combination of dry skin and at least 3 of 9 typical signs (eg, itching, erythema, and red papules) at 3 or more of 26 typical sites.⁴⁷ Children were examined once a year (every birthday $\pm$ 4 weeks), and those who were diagnosed with AD at two or more time points were selected for the present study. All children with AD were first diagnosed for AD before age 4 years, most of them before the age of 3 years.

Single-stranded conformation polymorphism and DNA sequencing

We conducted mutational analyses of the *IL13* gene by using single-stranded conformation polymorphism (SSCP) and DNA sequencing. For SSCP, 30 ng of genomic DNA from each study subject was used in a 5- μ L PCR. PCR primers were designed on the basis of published genomic DNA sequence (GenBank accession No. U31120) and synthesized (Genosys, The Woodlands, Tex). For amplification of exon 4 sequences, the primer sequences are as follows: 5' primer (position 4127-4146), 5'-TTCTACTCACGTGCTGACCT-3'; and 3' primer (position 4280-4299), 5'-CTGTCTCTGCAAATAATGAT-3'. The length of the amplified product is 173 bp. PCR was performed for 37 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 45 seconds at 72°C. The PCR products were denatured by heating to allow strand separation in denaturing buffer and fractionated on polyacrylamide gels. Two different conditions (6% acrylamide with 10% glycerol and 4.5% acrylamide with 5% glycerol plus 0.5 $\times$ Tris-borate-EDTA buffer) were used to ensure the detection of possible mutations. Gels were run at room temperature at low

power (6 W) for 16 hours, dried, and autoradiographed. DNA sequencing was done at the Genetics Resources CORE Facility (Johns Hopkins University, Baltimore, Md).

Statistical analysis

The Pearson χ^2 and Fisher exact tests were used to compare genotype frequencies. Under the assumption that the observed genotype is the cause of the high-IgE phenotype, ratios of penetrance to one another can describe a tentative mode of inheritance for the study marker while providing evidence for allelic or genotypic association.⁴⁸ Analyses were done by using Stata 6 software.⁴⁹ The Fisher exact test is preferred for comparing differences in genotypic distribution between high- and low-IgE phenotypes caused by the small expected value for genotype AA (less than 5).

RESULTS

Using a combination of SSCP detection and sequencing approach, we identified a novel *IL13* coding region polymorphism at 4257 bp (G to A; Fig 1). This point mutation results in a change of the amino acid residue 130 from arginine to glutamine. Genotyping of 604 German children shows a frequency (A allele) of 0.21, and this polymorphism is under the Hardy-Weinberg equilibrium with a *P* value of .8. The penetrance ratio suggests a dominant effect of this *IL13* variant in our German sample for both high-IgE phenotype and AD. Statistically significant differences in genotypic distribution were found between high- and low-IgE phenotypes (Pearson's $\chi^2_{(2)} = 10.97$, *P* = .004; Fisher's exact *P* = .003). Compared with the low-IgE children, the odds ratio (OR) of high-IgE children with respect to the presence or absence of this *IL13* variant was 2.38 (*P* = .0026).

The genotypic distribution for patients with AD and control subjects without AD shows only marginal difference (*P* = .06; Table I), but a significant association between the presence of the A allele and AD with an OR of 1.77 (95% confidence interval, 1.06-2.96; *P* = .03) was indicated, assuming a dominant mode of inheritance.

DISCUSSION

We found a novel variant in the coding region for the *IL13* gene at 4257 bp. This polymorphism is significantly associated with high IgE levels and AD in both genotypic and allelic association studies in a German study population. Evidence for association with high IgE levels was also confirmed by an additional 51 Swedish subjects selected from the International Study of Asthma and Allergies in Childhood cohort (OR, 4.00; 95% confidence interval, 1.18-13.42).

A modified case control study was used for the IgE association study because the two extreme phenotypes (ie, high-IgE patients and low-IgE control subjects) were highly selected, providing more power than a simple *t* test on the mean of a quantitative trait in genotypic groups. Also, this extreme sampling minimizes the misclassification bias. In the MAS-90 cohort the prevalence of asthma and allergic rhinitis has been increasing at each time point, and thus selection of an unaffected control group at any one visit has the risk of misclassification.

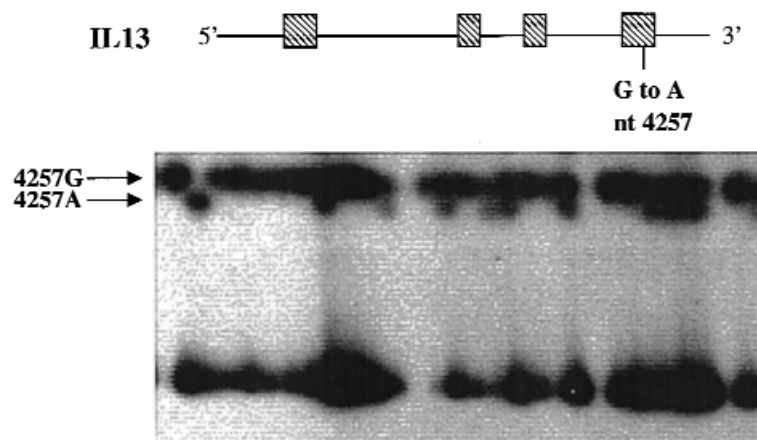


FIG 1. The point mutation (G to A) was located at 4257 bp. The *slashed boxes* indicate the codon of the *IL13* gene and the SSCP of the PCR-amplified 173-bp fragment of the *IL13* coding region (bp -4127 to -4299). Two different bands labeled by 4257G and 4257A refer to the wild-type and mutant alleles, respectively, in both the MAS-90 and International Study of Asthma and Allergies in Childhood cohorts.

TABLE I. Genotypic distribution of the *IL13* 4257G/A polymorphism

Phenotype	AA*	AG*	GG*	Pearson χ^2 test (P value)	Fisher exact test P value	OR (95% CI) [†]
High IgE	6	42	52	10.97 (.004)	.003	2.38 (1.35-4.21)
Low IgE	1	30	80			
AD	10	72	105	5.67 (.059)	.057	1.77 (1.06-2.96)
Non-AD	6	24	68			

CI, Confidence interval.

*AA, AG, and GG represent the 4257A/4257A, 4257A/4257G, and 4257G/4257G polymorphisms, respectively.

[†]Exposure refers to the presence or absence of the *IL13* 4257A allele.

This statistical association between the polymorphism at the *IL13* gene could reflect a direct effect of the marker allele on IgE phenotype and possibly AD, or alternatively, the *IL13* variant may be in tight linkage disequilibrium with a putative susceptibility gene in the 5q31 region. It is interesting that no significant association was found for a coding region polymorphism in the *IL9* gene in this same study population (data not shown), which was recently shown to be involved in IgE regulation⁵⁰ and in allergic inflammation in a mouse model for asthma.⁵¹ Extended population- and family-based studies, combined with the assessment of environmental risk factors, such as increased exposure to house dust mite allergens, should provide more information for future studies. Additional comparisons of wild-type and mutant alleles in vitro and in vivo with regard to binding to its ligand are needed.

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