

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

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Background: Increased levels of total serum IgE are a strong risk factor for the development of asthma. IgE is also involved in host defenses against parasites and fungi. Linkage of total serum IgE with markers located close to the 3 Mb cluster of cytokine genes in chromosome 5q31 has been reported. IL-4 or IL-13 are regarded as essential for IgE synthesis.

Objective: We tested whether polymorphisms in the IL-13 gene might explain the linkage between chromosome 5q31 and total serum IgE levels.

Methods: We used denaturing HPLC to detect polymorphisms in overlapping PCR fragments of the IL-13 gene including promoter and 3' untranslated regions. After sequencing was performed to identify the locations of the polymorphisms, PCR and primer-induced restriction site assays were used to genotype subjects in 3 unselected populations.

Results: We report here 7 polymorphisms (6 novel) in IL-13. Four of these polymorphisms are tightly linked to a variant in the terminal portion of the coding region of the gene that results in a predicted amino acid change in residue 130 (Arg130Gln). The Gln form is strongly associated ($P = .000002$) with increased serum IgE levels in 3 different populations comprising a total of 1399 children. Two additional polymorphisms in the promoter region of IL-13 are more loosely linked to Arg130Gln and are also less significantly associated with total serum IgE levels.

Conclusion: These data suggest that the Arg130Gln polymorphism in IL-13, or others in close linkage with it, is associated with the development of the elevated serum IgE phenotype. (J Allergy Clin Immunol 2000;105:506-13.)

Key words: IL-13, IgE, asthma, polymorphisms

Abbreviations used

DHPLC: Denaturing HPLC
IL-4R: IL-4 receptor
IL-13R: IL-13 receptor
MgCl₂: Magnesium chloride
ORF: Open reading frame
UTR: Untranslated region

Elevated total serum IgE levels have been found to be associated with asthma and atopy.¹ IgE is also involved in host defenses against parasites and fungi. Segregation analyses suggest that serum IgE levels are under genetic control and that basal IgE production is controlled by a major recessive or codominant gene.²⁻⁸ Genetic linkage has been reported between total IgE level and a region on chromosome 5q31-33 that encodes a cluster of cytokine genes.⁹⁻¹¹ This linkage was found to be more significant in a subset of nonatopic sibpairs, suggesting that the gene involved had a role in basal IgE regulation.⁹ Several ILs, including IL-3, IL-4, IL-5, IL-9, and IL-13, map to this area and have biologic functions that are involved in inflammation and IgE synthesis.

A polymorphism in the promoter region of IL-4 was found to be associated with a high IgE level and asthma,¹² but subsequent studies could not confirm this association in other populations.^{13,14} Significant association was found between total serum IgE and a marker in the IL-9 gene.¹⁵ However, a polymorphism that results in an amino acid substitution in IL-9 was not related to the IgE level or to other asthma-related phenotypes.¹⁶ Recently another candidate gene in the 5q region has been found to be associated with total serum IgE levels.¹⁷ A variation was identified in the 5' flanking region of the CD14 gene that was associated with circulating soluble CD14 levels and with total serum IgE.

Both IL-4 and IL-13 are T cell-secreted molecules known to participate in IgE synthesis in association with class switching to IgE by human B cells.¹⁸ They appear to share many (but not all) biologic activities. The relative roles of these 2 cytokines in IgE class switch have been difficult to elucidate in part because, in contrast to human B cells, mouse B cells do not express the IL-13

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receptor (IL-13R).¹⁸ Therefore, results using mouse models of IgE synthesis are not clearly analogous to the situation in humans. IL-13 is produced at high levels by human T_H2-like cells,¹⁹ but in contrast to IL-4, IL-13 is also produced both by T_H1-like and CD8⁺ cells.²⁰ Moreover, IL-13 is produced by naive CD45RA⁺ T cells that do not express IL-4,²¹ which suggests that IL-13 may play an important role in the initiation of IgE production.¹⁸ Studies also suggest that IL-13 may be a crucial determinant of bronchial hyperresponsiveness in animal models of allergic asthma, and this effect is independent of IL-4.^{22,23} Very recent studies with transgenic mice provide additional evidence for a role for one or both of these cytokines in regulation of IgE.²⁴ Mice that were transgenic for human IL-4 and IL-13 showed increased plasma IgE in response to antigen treatment.

We searched the IL-13 gene including 5' promoter and 3' untranslated region (UTR) for polymorphisms that could then be used to determine association with IgE regulation as well as other asthma-related traits. We report here 7 allelic variants, 6 of which have not been previously reported, and their correlation with serum IgE levels.

METHODS

Subjects

Three groups of children were included. The first group were participants in the Tucson Children's Respiratory Study, a large longitudinal study of asthma and allergies in an unselected population sample enrolled at birth.²⁵ The second group consisted of children who participated in a cross-sectional study of asthma in Munich, Germany,²⁶ whereas the third group of participants was from a similar study in Leipzig, in the former East Germany.²⁶ Both these German groups were unselected for asthma and allergies. All studies were approved by the local ethics committees and all parents of all subjects involved gave written informed consent for genetic studies.

Polymorphism detection in the IL-13 gene

Genomic DNA from 30 unselected subjects was screened for genetic variants with use of denaturing HPLC (DHPLC), a heteroduplex-based screening method. PCR products were generated with use of overlapping sets of primers designed to amplify all exons and introns of the IL-13 gene, including 1676 bp of promoter sequence. Table I lists the sequences of the primers for each PCR reaction and the specific magnesium chloride (MgCl₂) concentration and hybridization temperature. PCR reactions were carried out in a total volume of 25 μ L containing approximately 40 ng of genomic DNA, 10 mmol/L TRIS-hydrochloric acid (pH 8.3), 50 mmol/L potassium chloride, 200 μ mol/L of each deoxynucleotide triphosphate, 50 ng of each primer, and 1 unit of Taq DNA polymerase (Sigma Chemical, St Louis, Mo). Samples were denatured at 94°C for 2 minutes followed by 33 cycles of 94°C for 40 seconds, specific hybridization temperature for 40 seconds, and 72°C for 50 seconds and then a final extension for 10 minutes at 72°C. Fifteen microliters of each subject's PCR product was mixed with 15 μ L from one control subject. Products more than 500 bp were digested with a restriction enzyme to produce 2 fragments that could be analyzed simultaneously on the DHPLC column. The specific enzymes used are given in Table I.

DHPLC was carried out on the automatic WAVE DNA Fragment Analysis System (Transgenomic, Santa Clara, Calif) according to standard procedures.²⁷ PCR products were denatured for 3 minutes at 95°C, followed by a gradual reannealing from 95°C to 65°C over a period of 30 minutes. Samples were eluted with a linear acetonitrile

gradient of 2% per minute at a flow rate of 0.75 mL/min. To identify the location of the polymorphism, samples that produced a heteroduplex profile were sequenced in both directions with an ABI 373 automatic sequencer.

PCR Assays for genotyping

Subjects were genotyped for each polymorphism by PCR amplification of the region containing the polymorphism and selective restriction endonuclease digestion. The assay conditions for the Arg130Gln polymorphism are described in detail below and the modifications for the assays of the other polymorphisms are listed in Table II. A single PCR product (35 μ L) was generated, which included all three 3' UTR polymorphisms. Aliquots of this product were used for digestions at the site of each polymorphic nucleotide.

A 236-bp PCR fragment including the Arg130Gln polymorphism was generated with use of the primers 5'-CTTCCGTGAG-GACTGAATGAGACGTC-3' and 5'-GCAAATAATGAGCTTT-CGAAGTTTCAGTGGA-3'. The underlined bases were modified to create *Nla*IV restriction sites. PCR reactions were carried out in a total volume of 15 μ L containing approximately 24 ng of genomic DNA, 10 mmol/L TRIS-hydrochloric acid (pH 8.3), 50 mmol/L potassium chloride, 1.5 mmol/L MgCl₂, 200 μ mol/L of each deoxynucleotide triphosphate, 30 ng of each primer, and 0.6 units of Taq DNA polymerase (Sigma). Samples were denatured at 94°C for 2 minutes followed by 33 cycles of 94°C for 40 seconds, 55°C for 40 seconds, and 72°C for 50 seconds, and then a final extension for 10 minutes at 72°C. The PCR product was digested by addition of 0.25 units of *Nla*IV (New England BioLabs, Boston, Mass) and incubation at 37°C for 8 hours. *Nla*IV digests the PCR fragment 26 bp from the 5'-end, which serves as a control for assessing whether digestion was complete. It also digests 32 bp from the 3'-end of the fragment when the G nucleotide (Arg130) is present to produce a 178 bp fragment. The digested PCR products were electrophoresed on 3% agarose gels to separate the fragments.

Total serum IgE

In the Tucson sample total serum IgE levels were measured with the paper radioimmunosorbent test kit (Pharmacia Diagnostic, Piscataway, NJ). The assay threshold was set at 0.1 IU/mL, as described elsewhere.²⁸ In the German samples total serum IgE was measured with the Insulite system (DPC Biermann, Bad Nauheim, Germany) as described elsewhere.²⁶ Statistical analyses were performed with log-transformed values. Values are displayed as geometric mean and geometric 95% confidence intervals.

Allergy skin tests

In Tucson skin tests to 8 local aeroallergens (Bermuda grass, olive tree, mesquite tree, mulberry tree, careless weed, *Alternaria alternata*, cat dander, and *Dermatophagoides farinae*) were performed by the prick technique as described elsewhere.²⁹ In Munich the sensitivity to 6 local aeroallergens (*Dermatophagoides pteronyssinus*, *D farinae*, tree pollen, mixed grass pollen, *Alternaria tenuis*, and cat dander) was assessed with use of highly standardized extracts (ALK, Hørsholm, Denmark) and ALK lancets.²⁶ In Leipzig skin tests to 6 aeroallergens (*D pteronyssinus*, grass, birch and hazel pollen, cat and dog dander) were performed with a multitest device (Stallerkit, Stallergenes, Lyon, France) as described elsewhere.³⁰ Children were considered to be skin test positive if they showed at least one positive skin test result with diameter sums of more than 3 mm.

Statistical analysis

Standard ANOVA for quantitative traits was used to compare means. However, all available subjects were used to assess associations of the different polymorphism with IgE. To assess linkage dis-

TABLE I. Primers, PCR conditions, and restriction enzymes for DHPLC

| Primers | Location* | MgCl ₂ (mmol/L) | °C† | Restriction enzyme |
|------------------------------------|-----------|----------------------------|-----|--------------------|
| 5'-GGCAGGGCTTTTGGTGCCATG-3' | -1676 to | 1.5 | 60 | <i>Hae</i> II |
| 5'-GGCCATCCTTCTGGGAACTC-3' | -873 | | | |
| 5'-CCTGCAGAGACTGGTGAGCAAAGG-3' | -997 to | 1.5 | 60 | <i>Eae</i> I |
| 5'-GCAATGCTGCCCTGAGTTCCAG-3' | -316 | | | |
| 5'-CTAAGACTATCTGCTCAGCAC-3' | -443 to | 2.0 | 53 | |
| 5'-GGTGACGCGCTGACACCATGGTCTCTG-3' | -97 | | | |
| 5'-ATGCGACACTGGATTTTCCACAAAG-3' | -196 to | 1.5 | 42 | |
| 5'-CCTGACCTACAGGCATCTTAGGAC-3' | 241 | | | |
| 5'-GTGTCGGCTAGCCAGGGTCCTAGC-3' | 137 to | 1.5 | 60 | <i>Pst</i> I |
| 5'-CTGCTTTGAACAGCAGGCATAG-3' | 1031 | | | |
| 5'-CCTGACTATGGCAAGCCTTGCATG-3' | 941 to | 2.0 | 60 | <i>Hind</i> III |
| 5'-CCTCTACTGTACAGATGTGGAAATTGAG-3' | 1816 | | | |
| 5'-GGCTGAATATCCATGGTGTGTGTCC-3' | 1675 to | 1.5 | 60 | |
| 5'-GGCTGAGGTGCGCTAGGCTGAAGAC-3' | 2233 | | | |
| 5'-GGTAAAATTCCTAGCTTAGACCTC-3' | 2170 to | 1.5 | 48 | <i>Ssp</i> I |
| 5'-GCACCCCTACCTCGCTGTAATCC-3' | 3015 | | | |

*Sequence amplified by each pair of primers relative to first nucleotide of open reading frame (ORF).

†PCR hybridization temperature.

TABLE II. PCR primers, assay conditions, and restriction enzymes for genotyping

| Polymorphism | Primers (5', 3') | MgCl ₂ | °C* | Restriction enzyme units | Allele sizes | Fragment sizes |
|--------------|--|-------------------|-----|--------------------------|--------------|----------------|
| IL-13 | | | | | | |
| -1512 | 5'-CAACCGCCGCGCCAGCGCCTTCTC-3' | 1.5 | 54 | <i>Bst</i> UI (0.2) | A | 214 |
| | 5'-CCGCTACTTGGCCGTGTGACCGC-3' | | | | C | 192 |
| -1112 | 5'-GGAATCCAGCATGCCTTGTGAGG-3' | 1.5 | 54 | <i>Bst</i> UI (0.2) | C | 224 |
| | 5'-GTCGCCTTTTCTGCTCTTCCCGC-3' | | | | T | 247 |
| +1923 | 5'-GGCTGAATATCCATGGTGTGTGTCC-3' | 1.0 | 58 | <i>Bsa</i> AI (0.5) | C | 310, 249 |
| | 5'-GGCTGAGGTGCGCTAGGCTGAAGAC-3' | | | | T | 559 |
| +2044 | 5'-CTTCCGTGAGGACT GAATGAGACGGTC-3' | 1.5 | 55 | <i>Nla</i> IV (0.25) | G | 178 |
| | 5'-GCAAATAATGATGCTTTCGAAGTTTCAGTGGA-3' | | | | A | 210 |
| +2525 | 5'-GGACAGGGACCCACTTCACAC-3' | 2.0 | 44 | <i>Nhe</i> I (1) | G | 390 |
| | 5'-GCTAACATATTTAATATTTATGTAC-3' | | | | A | 253, 137 |
| +2580 | 5'-GGACAGGGACCCACTTCACAC-3' | 2.0 | 44 | <i>Ava</i> II (0.5) | C | 141, 192, 57 |
| | 5'-GCTAACATATTTAATATTTATGTAC-3' | | | | A | 141, 249 |
| +2749 | 5'-GGACAGGGACCCACTTCACAC-3' | 2.0 | 44 | <i>Bsr</i> GI (3) | C | 223, 167 |
| | 5'-GCTAACATATTTAATATTTATGTAC-3' | | | | T | 223, 142, 25 |
| IL-4 -589 | 5'-CACTAAACTTGGGAGAACATGGT-3' | 2.0 | 57 | <i>Ava</i> II (0.5) | C | 224 |
| | 5'-GAAGGGAGAGGCCACAGGGGT-3' | | | | T | 202 |
| IL-5 -746 | 5'-GCTCATGAACAGAATACGTA-3' | 1.5 | 52 | <i>Rsa</i> I (1) | T | 143 |
| | 5'-GAAGGTATTGGCTCATAGTAC-3' | | | | C | 124 |

*PCR hybridization temperature.

equilibrium, only subjects who were homozygous for the referenced polymorphism were used. This was justified by the fact that, when linkage disequilibrium between 2 markers is assessed, haplotypes can unambiguously be determined only for subjects who are homozygotes for 1 of the 2 markers. Because meiotic assortment occurs independently in both parents, the only potential source of bias for these calculations would be selective survival of individuals carrying certain haplotype combinations. This is unlikely to have occurred in our data. Levin's δ was used to assess the degree of linkage disequilibrium between the reference polymorphism and all other polymorphisms.³¹ This measure was chosen because it is more appropriate for case-control studies.

RESULTS

Identification of polymorphisms in the IL-13 gene

We used DNA samples from 30 unrelated volunteers to screen the IL-13 gene and its promoter region for polymorphisms with use of a DHPLC-based detection system. We found 7 polymorphisms, 6 novel and 1 recently reported by van der Pouw Kraan et al.³² These are identified according to their nucleotide number with respect to the first nucleotide of the ORF in Table III. The

TABLE III. Seven polymorphisms in IL-13 and its promoter region

| Polymorphism* | Location | Alleles (most common first) | Frequency of most common allele |
|----------------|-------------|-----------------------------|---------------------------------|
| –1512 (50012) | 5' Promoter | a/c | 0.78 |
| –1112 (49612) | 5' Promoter | c/t | 0.77 |
| +1923 (46578) | Intron 3 | c/t | † |
| +2044 (46457)‡ | Exon 4 | g/a | 0.77 |
| +2525 (45976) | 3' UTR | g/a | † |
| +2580 (45921) | 3' UTR | c/a | † |
| +2749 (45752) | 3' UTR | c/t | † |

*Nucleotide numbering begins at first nucleotide of IL-13 ORF. Nucleotide numbers in parentheses are from GenBank accession No. AC004039, which is reverse complement of transcribed sequence.

†These polymorphisms were in almost complete linkage disequilibrium with +2044 and were thus only genotyped in 60 children homozygous for +2044.

‡Arg130Gln.

polymorphism in nucleotide +2044 located in exon 4 leads to a predicted amino acid substitution of glutamine for arginine (Arg130Gln). Two polymorphisms were identified in the promoter region, one in the third intron, and three in the 3' UTR. The polymorphism identified as –1055 by van der Pouw Kraan et al³² corresponds to the –1112 polymorphism in the promoter region.

IL-13 genotypes of children in 3 populations

Next we genotyped 286 white children enrolled in a longitudinal study of asthma and allergies in Tucson, Ariz,²⁵ for polymorphisms –1512 and –1112, Arg130Gln, and 2 known variants in the promoter regions of IL-5³³ and IL-4,³⁴ which are located telomeric and centromeric to IL-13, respectively (Fig 1, *lower panel*). Allele frequencies for IL-4 –589 were not different from those reported in white populations by others.³⁴ Allele frequencies for 3 of the polymorphisms in IL-13 are presented in Table III. Because of their close proximity to Arg130Gln, only a group of 60 subjects who were homozygous for Arg130Gln polymorphisms were genotyped in the 3' UTR region and in intron 3. All 4 of these polymorphisms were in extremely tight linkage disequilibrium with Arg130Gln (Fig 1, *upper panel*). A significant degree of linkage disequilibrium ($\delta = 0.87$) was observed between IL-13 –1512 and IL-13 –1112, and both were in linkage disequilibrium with Arg130Gln (Fig 1, *upper panel*). Neither the polymorphism in the promoter region of IL-4 nor that in the promoter region of IL-5 was in linkage disequilibrium with any of the polymorphisms in the IL-13 gene.

Association of IgE with IL-13 genotypes

Next we assessed the relationship between total serum IgE (on a logarithmic scale) and IL-4 –589, IL-13 Arg130Gln, IL-13 –1112, and IL-13 –1512. This was done in the Tucson sample described above and also among 521 white children living in Munich, Germany, and 592 white children living in Leipzig, in the former East Germany. Genotype frequencies for these 3 polymorphisms were not significantly different in the German samples compared with the frequencies observed in the Tucson sample. Association (expressed as R^2) among each of these 4 polymorphisms and total serum IgE in skin test–positive and skin test–negative subjects for the

3 populations combined is shown in Fig 2. The strongest variance was observed for IL-13 Arg130Gln among skin test–negative subjects. Trends for an association were also observed among skin test positive–subjects, but these were only borderline significant for IL-4 –589 and for IL-13 Arg130 Gln.

Results for IL-13 Arg130Gln for each of the 3 populations separately (Table IV) and for skin test–positive and skin test–negative subjects (Table V) demonstrated very consistent results across populations. In all of them, the strongest effects were observed among skin test–negative subjects. A trend consistent with a codominant mode of inheritance was observed in all 3 samples, with heterozygotes having intermediate levels of IgE compared with both homozygote groups.

DISCUSSION

In this study we showed that a set of tightly linked polymorphisms in the IL-13 gene were associated with total serum IgE levels in 3 populations living in divergent social and geographic conditions. We found that all 7 polymorphisms that we detected in different regions of the IL-13 gene were in quite close linkage disequilibrium. This makes the identification of the polymorphism(s) or possible haplotypes responsible for the effect observed or possible haplotypic effects quite problematic. However, both for skin test–positive and skin test–negative subjects the strongest association was observed with IL-13 Arg130Gln.

The location of the Arg130Gln polymorphism within the D helix of IL-13, close to the C-terminal region of the molecule,³⁵ provides additional support for its role in determining the observed effects. Much like IL-4, the tertiary structure of IL-13 is characterized by a core of 4 antiparallel α -helices.³⁵ IL-13 interacts with its own IL-13 receptor complex, which consists of the 140-kd IL-4 receptor (IL-4R) α chain and an IL-13 binding protein that has been identified as IL-13R α 1.³⁶ Although the exact nature of the interaction between IL-13 and IL-13R has not been elucidated, insights come from knowledge of the interactions between IL-4 and the IL-4R. IL-4R consists of the same IL-4R α chain that is part of IL-13R and the common γ c chain, which is shared by the receptors for several ILs, including IL-2.³⁷ IL-4R is specific

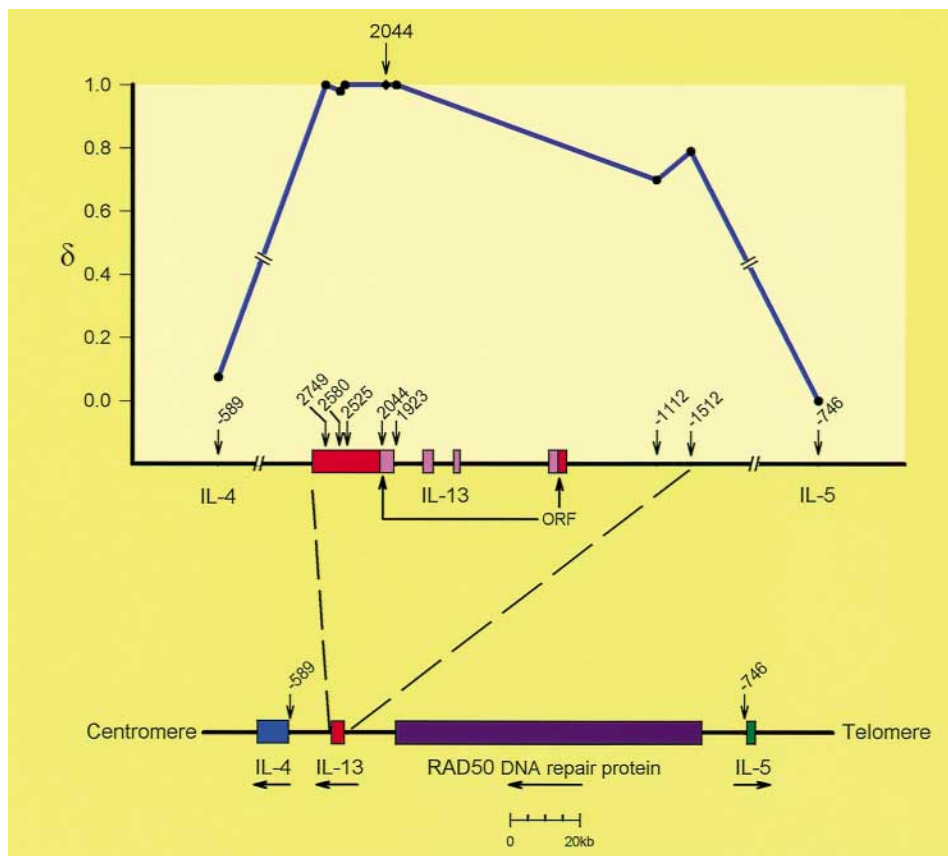


FIG 1. Chromosome location of IL-13 gene relative to 3 adjacent genes in chromosome 5. Lower panel, Arrows below each gene represent direction of transcription. Numbers above genes indicate location of relevant polymorphisms relative to first nucleotide of ORF of each gene. Upper panel, Linkage disequilibrium map of 7 novel polymorphisms in IL-13, 1 in IL-4, and 1 in IL-5 in Tucson population. The δ was calculated relative to Arg130Gln (2044) polymorphism. IL-13 exons are indicated by boxes and coding region by lighter pink.

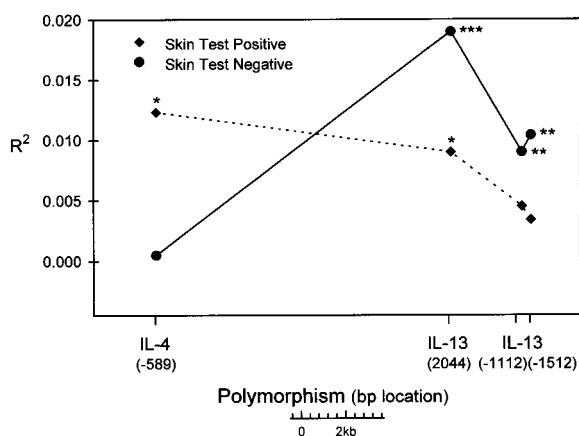


FIG 2. Association between polymorphisms in promoter region of IL-4 -589 and in coding region +2044 and promoter regions -1112 and -1512 of IL-13 and total serum IgE levels in skin test-positive and skin test-negative children in 3 populations (see Table V). R^2 is amount of variance in IgE explained by polymorphism. Asterisk, $P = .05$; two asterisks, $P \leq .005$; three asterisks, $P = .000015$.

for IL-4, but mAbs against the IL-4R α chain inhibit the biologic properties of both IL-4 and IL-13, indicating that IL-4R α is common to both receptors. The primary

binding determinants of IL-4 for IL-4R α are located in the A and C helices of IL-4,³⁸ whereas interaction between IL-4 and the γ c chain occurs in the D helix.³⁹ Some of the biologic functions of IL-4 are also mediated through the activation of IL-13R.⁴⁰ However, replacement of amino acid residue 124 in the D helix of IL-4 prevents activation of IL-13R both by IL-13 and by IL-4.⁴¹ These results suggest that the D helix of IL-4 may be responsible for its interaction with IL-13R α 1. It is thus possible that, by analogy, the D helix of IL-13 may also interact with IL-13R α 1 and that the Arg130Gln polymorphism may modify such interaction.

Although the location of IL-13 Arg130Gln is very suggestive of a potential biologic effect, it is important to stress that this polymorphism is in almost complete linkage disequilibrium with respect to 3 additional polymorphisms in the 3' UTR region of IL-13 and a fourth polymorphism in the third intron of IL-13. Although none of these 4 polymorphisms is located in consensus sequences that are known to regulate either gene splicing, gene expression, or IL-13 messenger RNA stability, we cannot exclude the possibility that at least some of the association observed may be the result of differences in biologic activities determined by one or more of these additional 4 polymorphisms. The 2 promoter polymorphisms may

TABLE IV. Geometric mean (95% confidence intervals) of serum IgE levels (International Units per milliliter) by genotype for Arg130Gln and by city of residence

| City | No. | ArgArg | ArgGln | GlnGln | R ² | Statistical significance |
|---------|------|------------------|-------------------|--------------------|----------------|--------------------------|
| Tucson | 286 | 43.3 (31.9-58.7) | 78.8 (56.4-110.1) | 173.0 (52.1-574.5) | 0.032 | <i>P</i> = .0023 |
| Leipzig | 592 | 47.6 (41.1-55.0) | 60.0 (49.6-72.5) | 80.2 (52.8-121.8) | 0.012 | <i>P</i> = .0081 |
| Munich | 521 | 45.8 (38.7-54.2) | 63.8 (50.8-80.0) | 81.8 (46.4-144.3) | 0.014 | <i>P</i> = .0069 |
| Total | 1399 | 46.0 (41.3-51.2) | 65.0 (56.8-74.3) | 89.4 (65.4-122.1) | 0.016 | <i>P</i> = .000002 |

TABLE V. Association of IL-13 Arg130Gln with total serum IgE by skin test reactivity to local aeroallergens

| | No. | Geometric mean serum IgE (IU/mL) | | | Statistical significance* |
|--------------------|-----|----------------------------------|--------|--------|---------------------------|
| | | ArgArg | ArgGln | GlnGln | |
| Tucson† | | | | | |
| Skin test–negative | 120 | 11.2 | 20.7 | 111.9 | <i>P</i> = .018 |
| Skin test–positive | 162 | 131.8 | 178.5 | 215.1 | <i>P</i> = .4 |
| Leipzig | | | | | |
| Skin test–negative | 430 | 37.9 | 46.8 | 68.4 | <i>P</i> = .019 |
| Skin test–positive | 107 | 102.8 | 125.5 | 140.4 | <i>P</i> = .37 |
| Munich | | | | | |
| Skin test–negative | 392 | 30.8 | 40.6 | 54.0 | <i>P</i> = .021 |
| Skin test–positive | 129 | 165.5 | 218.9 | 265.0 | <i>P</i> = .17 |

*For trend.

†Totals are less than those for the whole population because not all subjects had skin test results available.

also have a role in regulation of IgE, possibly modifying transcription control elements, but because of the strong linkage with the Arg130Gln variation it was impossible to find any additive effect resulting from haplotype (data not shown). Interestingly, the –1112 promoter polymorphism has been recently reported to be associated with increased risk of atopic asthma and with altered regulation of IL-13 production, but no association with IgE levels was found among asthmatic patients.³² Because these asthmatic subjects were atopic, they were less likely to demonstrate the association with total serum IgE (see below). No other IL-13 polymorphisms were either found or tested in this recent report,³² and it is thus not possible to know whether the reported association with atopic asthma is attributable to linkage disequilibrium between IL-13 –1112 and Arg130Gln. Clearly, additional functional studies are needed to elucidate the role of these different polymorphisms in IL-13 gene function or IL-13 receptor activation.

We found that the association described was stronger in all data sets among skin test–negative than among skin test–positive subjects. This is consistent with the original observations by Marsh et al² who reported that linkage of total serum IgE with markers located in close proximity to IL-13 was strongest among skin test–negative subjects in an Amish population. There are 2 possible explanations for this finding. Marsh et al² suggested that regulation of “basal” levels of IgE may be due to a different set of genes with respect to those that control specific IgE responses to local aeroallergens. It is also possible, however, that effects may be more easily detectable among skin test–negative subjects because their IgE levels may be less influenced by environmental factors. It is plausible to surmise that subjects who are skin test–positive to aeroal-

lergens that are quite abundant in the locale where they live may be prone to show changes in total serum IgE levels related to exposure to these aeroallergens. This could thus make total serum IgE more variable in these subjects and thus could add error to the determination. This explanation is supported by the fact that, although effects were less strong among skin test–positive subjects, the trend was the same as for the skin test–negative subjects.

Linkage studies first pointed to the cytokine-rich 5q31 region as a location for a gene involved in IgE regulation. We found no association with a previously reported polymorphism in the promoter region of the IL-4 gene (IL-4 –589) and total serum IgE levels. Association between IL-4 –589 and asthma-related phenotypes has been observed in some databases^{12,42} but not in others.^{13,14} A possible explanation for these discrepancies may be that IL-4 –589 is only a marker for the polymorphisms in IL-13 or another enhancer element. In this case, different degrees of linkage disequilibrium between the polymorphisms in IL-13 and IL-4 –589 may explain the different findings in these studies. It is also possible that IL-4 –589 may influence IL-4 transcription, but this influence may be affected by gene-gene interactions, which may be different in different populations. Further biologic studies with different combinations of the different genetic variants of IL-4 and IL-13 will be needed to elucidate this issue.

Recently, experiments in mice transgenic for human IL-4 and IL-13 confirm that an increased transcription of one or both of these genes can increase IgE levels.²⁴ The presence of several polymorphisms in the IL-13 gene and their association with total IgE, coupled with the lack of association with IL-4 variants, provide additional support for the role of IL-13 in IgE regulation. It is possible that variants in each of the genes that have been found to date

to affect IgE levels (CD14,¹⁷ IL-4R α ,⁴³⁻⁴⁵ Fc ϵ RI β ,⁴⁶ and IL-13) may make a small contribution to the overall level. The combination of these variants and those in other yet-to-be-described genes may contribute to the risk for asthma in the population.

Although functional studies are needed, our data strongly suggest that either the Arg130Gln variant or its associated polymorphisms play a role in regulation of total serum IgE levels in humans. These polymorphisms may also be important determinants of susceptibility to asthma and parasitic infections.

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