

High serum IgE concentrations: Association with HLA-DR and markers on chromosome 5q31 and chromosome 11q13

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Background: Linkage studies mapped a locus regulating total serum IgE concentrations in a noncognate fashion to chromosome 5q31 and a locus for atopy to chromosome 11q13. In contrast, antigen-driven IgE production seems to be largely controlled by major histocompatibility complex class II genes.

Objective: We therefore analyzed the association between the phenotype of high IgE serum levels and six microsatellite markers on chromosomes 5q31 and 11q13, as well as HLA-DRB1, in a random sample of the adult East German population.

Methods: One hundred twenty-nine persons identified as "cases" (serum IgE level >200 kU/L) and 266 control subjects (serum IgE level ≤200 kU/L) were genotyped for five 5q31 microsatellites (D5S436, D5S393, D5S210, IL-4, and IL-9) and an 11q13 microsatellite (FCERIB). Cases and controls were also typed for HLA-DRB1. Allele frequencies were compared between cases and controls by means of a two-sided Fisher's exact test.

Results: None of the markers was significantly associated although a weak association to the markers within the IL-9 gene and the FCERIB gene and to the HLA-DRB1*01 allele was found when specific IgE-positive cases were compared with negative controls.

Conclusions: The weak associations observed after stratification for specific IgE might point to a contribution of genes in these regions to the development of allergy. (*J Allergy Clin Immunol* 1997;99:828-36.)

Key words: Association, IgE, allergy, atopy, interleukins, HLA-DR, simple repetitive sequence, microsatellites, genetic marker

In human beings, a high serum IgE level is considered a potent predictor for the development of asthma.^{1,2} In this respect, the combination of a susceptibility to asthma and allergy with high serum IgE concentrations,

Abbreviations used

6-FAM:	6-carboxyfluorescein
BHR:	Bronchial hyperresponsiveness
DNA:	Deoxyribonucleic acid
HEX:	6-carboxy 2',4',7',4,7-hexachlorofluorescein
PCR:	Polymerase chain reaction
ROX:	6-carboxyrhodamine
SD:	Standard deviation
TAMRA:	6-carboxy-tetramethylrhodamine

positive skin-prick test results, or specific IgE (by RAST) to common allergens is usually referred to as atopy. From twin studies it has been inferred that the total serum IgE concentration is largely determined by genetic factors.³ Though most of the family studies done favor the possibility of a major gene controlling the serum IgE level, the mode of inheritance is still a matter of controversy.⁴⁻⁸ Nevertheless, other determinants such as sex, age, race, exposure to environmental allergens such as tobacco smoke, and occupational exposure also appear to influence the total serum IgE concentration.⁹⁻¹¹ Additionally, total IgE serum concentrations can dramatically increase, for example, after helminthic parasite infestation and in advanced stages of acquired immunodeficiency syndrome.^{12,13}

The production of IgE is tightly regulated. Whereas the synthesis of antigen-specific IgE antibodies is strongly determined by major histocompatibility complex class II molecules (HLA-DR and DQ),^{14,15} the regulation of the overall serum IgE level may involve cells expressing the high-affinity IgE receptor (FcεRI), including basophils and mast cells, as well as T helper lymphocytes.¹⁶⁻¹⁹ The latter play a crucial role in the immune response, determining its outcome by differentiating into type 1 or 2 phenotypes (T_{H1} and T_{H2}). The secretion of IL-4 and IL-13 by T_{H2} cells induces Ig heavy chain class switching to the ε isotype in the final steps of B cell activation,²⁰⁻²³ a process that is enhanced by IL-5.²⁴ In addition, these cytokines have a positive feedback on the development of T helper cells toward

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the T_H2 phenotype. In this context, it is noteworthy that the stimulation of Fc ϵ RI, expressed on mast cells, triggers the release of cytokines such as IL-4 that promote the commitment of T cells to the T_H2 lineage and thus the production of IgE by B cells. In addition, it is also conceivable that basophils directly induce the production of IgE by B cells in a noncognate fashion by secreting IL-4 and interacting with CD40 via their CD40 ligand.¹⁶ This process might be triggered by the antigen cross-linking of specific IgE molecules bound to Fc ϵ RI at the cell surface of basophils.

Given this regulatory network, IL-4 may be considered a major candidate for IgE responsiveness and atopy. It maps, like other candidate genes for atopic diseases including IL-5 and IL-13, within the "IL-4 cytokine-gene cluster" on chromosome 5q31. Indeed, linkage of an IgE regulatory locus to this region was demonstrated by two groups in independent samples with a maximum lod score (logarithm of the likelihood ratio for linkage) of 3.56 ($\theta = 0.09$) for the marker D5S436.^{25, 26} In addition, an association of a functional promoter polymorphism of the IL-4 gene with elevated serum IgE levels has been reported.²⁷ Other groups have demonstrated linkage of a gene predisposing to atopy with markers on chromosome 11q13,²⁸⁻³¹ although four other studies excluded linkage of this region to atopy.³²⁻³⁵ Recently, for two variants of the β subunit of the high-affinity IgE receptor (Fc ϵ RI β), which maps to chromosome 11q13, association with atopy has been reported.³⁶⁻³⁸ In addition, other studies have implicated HLA-D on 6q21,^{15, 39, 40} the T cell antigen receptor A/D locus on chromosome 14q11.2,⁴¹ and yet to be defined loci on chromosome 12q,⁴² 7, and 16⁴³ in the regulation of total serum IgE concentration.

In an attempt to identify and narrow down the genomic region potentially harboring a major gene causing the high IgE serum level atopy phenotype, we performed an association study with use of microsatellites located in the IL-4 cytokine-gene cluster on chromosome 5q31 and within the gene encoding Fc ϵ RI β (FCERIB). We also analyzed a possible association of HLA-DR with high IgE serum concentrations.

METHODS

Recruitment of probands

As part of the European Community Respiratory Health Survey in the period 1990 to 1992, 4990 subjects 19 to 65 years old were drawn at random from the offices of population census in Erfurt, an industrial and commercial city located in a shallow basin in the southwestern region of what was formerly East Germany with a total population of about 210,000. Full details of this study have been published elsewhere.⁴⁴ In brief, this cross-sectional study used a two-stage approach. In stage I, the screening questionnaire (standardized within the European Community Respiratory Health Survey) was mailed to the selected probands. Of the stage I responders, a random sample (40.9%) was invited by letter or home visit for stage II tests. Results reported here are all from stage II, which comprised a detailed questionnaire, spirometric measurements, methacholine or bronchodilator inhalation tests, skin prick testing, and

determination of total and specific IgE. By this protocol, 1150 probands were recruited. Of these probands, all persons ($n = 129$) with a total serum IgE level >200 kU/L ("cases") and a random sample approximately twice this size ($n = 266$; "controls") of the remaining 1021 probands were included in the current study. The study protocol was approved by the local ethics committee, and written consent was obtained from all participants.

The questionnaire with 71 items was developed from preexisting questionnaires. Questions that related to respiratory symptoms and medical history were taken from the International Union Against Tuberculosis and Lung Disease questionnaire^{45, 46} and those related to smoking were taken from the American Thoracic Society questionnaire.⁴⁷

Sera and blood samples treated with ethylene diaminetetraacetic acid were taken between September 1991 and April 1992 and stored at -80°C until analyzed in February 1995. Total and specific serum IgE against *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, cat, timothy grass, and birch were measured centrally by Pharmacia (Uppsala, Sweden) with use of the Pharmacia CAP system.

Isolation of deoxyribonucleic acid

Blood samples were rapidly thawed and 500 μl was removed. From this aliquot, deoxyribonucleic acid (DNA) was immediately extracted by the QIAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation.

Microsatellite typing

The nomenclature of the dinucleotide repeat loci investigated and of the primers used for their amplification, as well as the sequences of the latter, were obtained from the Online Mendelian Inheritance in Man database (<http://www3.ncbi.nlm.nih.gov/omim/>) except those of IL-4²⁵ and FCERIB.⁴⁸ The microsatellites were either anonymous markers (D5S436, D5S393, and D5S210) or were located within genes (IL-4, IL-9, and FCERIB). The primers were synthesized; labeled with one of the following fluorophores as indicated: 6-carboxyfluorescein (6-FAM), 6-carboxy 2',4',7',4,7-hexachlorofluorescein (HEX), or 6-carboxy-tetramethylrhodamine (TAMRA); and purified by reversed-phase high performance liquid chromatography at Applied Biosystems (Weiterstadt, Germany). The following dinucleotide repeat loci and polymerase chain reaction (PCR) primers were used: locus D5S436 was amplified by the primers AFM263wg5m: 5'-GTCTCCACCCACAGCAGG-3' labeled with 6-FAM and AFM263wg5a: 5'-TATGTGCCAGGCAT-TACGC T-3'; locus D5S393 was amplified by the primers AFM042xd12m: 5'-CATTCCTCATTCCCTCATTCC-3' labeled with 6-FAM and AFM042xd12a: 5'-TTCTACCTGACCTTTCCTCT-3'; locus D5S210 was amplified by the primers Mfd122CA: 5'-ATGCAGAATCTACAAGGACC-3' labeled with 6-FAM and Mfd122GT: 5'-CTTTAATCATCCTTTAA-CAGC-3'; locus IL-4 (located in the second intron of the IL-4 gene) was amplified by the primers IL-4-R1A: 5'-TGCACCTGGGCAACAGTTTA-3' labeled with HEX and IL-4-R1B: 5'-GTTGGATGGACT TGGAGATT-3'; locus IL-9 (located in the fourth intron of the IL-9 gene) was amplified by the primers IL-9-RP1A: 5'-AGGCTTTCTCTAATGCAGAG-3' labeled with HEX and IL-9-RP1B: 5'-GGTGGTTGACCTCAAAT-TGG-3'; locus FCERIB (located in the fifth intron of the FCERIB gene) was amplified by the primers FCERIB-A: 5'-ATCTACTGCAAGTGACGATC-3' labeled with TAMRA and FCERIB-B: 5'-CATCTCCCTACCATCTGACC-3'.

Three separate multiplex PCRs were done with the marker

combinations: D5S436 and D5S393, D5S210 and IL-4, and IL-9 and FCERIB. A reaction volume of 15 μ L, containing 40 ng of genomic DNA, 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 8.3, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxyribonucleoside triphosphate, 5 pmol of each primer, and 0.6 U Taq polymerase (Perkin Elmer, Weiterstadt, Germany), was amplified in a GeneAmp PCR System 9600 thermocycler (Perkin Elmer) with the following conditions: 5 minutes at 94°C; followed by 10 cycles of 30 seconds at 94°C, 30 seconds at 57°C, and 45 seconds at 72°C; followed by 20 cycles of 30 seconds at 89°C, 30 seconds at 57°C, and 45 seconds at 72°C; and finally 10 minutes at 72°C. For each DNA sample, the three reactions were pooled and stored at 4°C until analysis. Markers that failed to give an amplification product for a DNA sample in the multiplex PCR were reamplified individually by the same protocol. In this case, the amplicate of the marker was quantitated by electrophoresis in a 2.5% agarose gel stained with ethidium bromide before analysis on a polyacrylamide gel. If no marker could be amplified from a DNA sample, the DNA was extracted by phenol/chloroform and ethanol precipitated before reanalysis according to the stated protocol.

Two microliters of formamide, 0.5 μ L 0.5% (wt/vol) blue dextran, and 0.5 μ L of the 6-carboxyrhodamine (ROX) labeled size standard Genescan-350 (Applied Biosystems) were added to 3 μ L of pooled PCR reactions and heated to 95°C for 5 minutes before loading onto a polyacrylamide gel.

Electrophoresis was done with 6% acrylamide/8.3 mol/L urea on 12 cm Well-to-Read gels (24 wells), run for 4 to 5 hours at a limiting power of 23 W, using a 373A Stretch automated DNA analysis system (Applied Biosystems) set to filter wheel A. With this filter set 6-FAM-labeled loci appear in blue, those labeled with HEX in green, and those labeled with TAMRA in yellow, whereas the ROX-labeled marker is given in red. The label together with the expected allele size range for every marker as obtained from the Online Mendelian Inheritance in Man database allows the unambiguous identification of the alleles belonging to each locus.

Analysis was done with GENESCAN 672 software (version 1.2.2-1) (Applied Biosystems) as described in the manufacturer's manual. Specific procedures done were as follows: (1) gel lane tracking was checked manually; (2) local Southern method sizing options were used to calculate peak sizes (to 1 one-hundredth of a base); (3) size standard peaks were user defined; (4) sizing of the ROX-labeled size standard peaks was checked manually for all lanes; and (5) when necessary, individual lanes were used to define the size standard peaks and reanalyzed.

The Genotyper software was used as described (Genotyper manual, version 1.1, Applied Biosystems) for the semiautomatic "allele calling." Specific procedures done were as follows: (1) for each locus the range of expected alleles was defined as category; (2) peaks were labeled with peak size (to 1 one-hundredth of the base); (3) the alleles were determined with use of the filter labels option and default parameters; (4) the allele assignment was checked manually for ambiguities and artifacts, which were removed; and (5) the data were tabulated and exported to the EXCEL program (version 4.0).

After a "global binning" for each locus, the allele sizes were converted to discrete alleles on the basis of an iteration of 2 bp.

DRB1 typing

PCR amplification of the DRB1 gene and subsequent typing by hybridization with the use of sequence specific oligonucleotides was done as described elsewhere.⁴⁹

Statistical analysis

Each allele was examined separately for association in a 2×2 contingency table by case/control status in a two-sided Fisher's exact test. In a second step, the study population was stratified according to the presence or absence of at least one positive RAST result for specific IgE. Differences between allele frequencies in these subgroups were assessed by the same statistical methods. The data were reanalyzed after adjustment for the covariates gender and smoking habit by adding the sample mean of $\log[\text{total IgE}]$ to the residuals of the multiple regression. Values of p were Bonferroni corrected for each marker by multiplication by the number of alleles. A p value < 0.05 was regarded as significant.

Linkage disequilibria for the markers on chromosome 5q31 were calculated as described.⁵⁰

The statistical analyses were done with the SAS 6.07 system.

RESULTS

Assignment and description of cases and controls

The mean age of the 1150 participants was 41.6 ± 12.5 (standard deviation [SD]) years, with a range from 19 to 65 years. In this population-based sample, the distribution of the $\log[\text{total IgE}]$ showed an approximately normal distribution, characteristic of a quantitative trait with a mean of 3.65 and an SD of 1.42 (Fig. 1) and with men having a higher mean $\log[\text{total IgE}]$ (3.89 ± 1.45) than women (3.40 ± 1.36 , $p = 0.0001$). In linkage studies, atopy is frequently defined as a qualitative trait by means of a threshold for the total IgE serum concentration of 100 to 400 kU/L, despite its normal distribution within a population.^{36, 48, 51} To set a cut-off beyond the influences of gender, age, and smoking habits on serum IgE levels in our population, we considered persons with total serum IgE levels > 200 kU/L ($\log[\text{total IgE}] = 5.3$, 88th percentile) as "cases." This reflects a phenotype definition closely related to its clinical relevance. By this definition, 129 cases (64.3% male) could be extracted from the whole population sample. A control group comprising 266 persons (52.6% male) was randomly selected from the remaining persons in the available population sample (Table 1). The mean age of the persons in the group of cases (40.9 ± 11.5 [SD] years) was not significantly higher than that of the control subjects (39.8 ± 12.1 years). The cases had a mean $\log[\text{total IgE}]$ of 6.13 ± 0.63 (SD) and the respective value for the controls was 3.32 ± 1.19 (SD). Specific IgE for *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, cat, timothy grass, or birch was detectable in 75.2% (of which 66% were male) of the cases as compared with 26.7% (of which 46.5% were male) of the controls. Of the 24.8% of cases without a single positive RAST result, 40.5% were male, compared with 54.9% male subjects within 73.3% of RAST-negative controls. Of the cases, 5.4% had a history of asthma and 23.3% a history of rhinitis, compared with 0.4% and 9.8%, respectively, in the control group. This reflects the fact that there were significantly more atopic subjects in the case group. Finally, 49.6% (71.9% male)

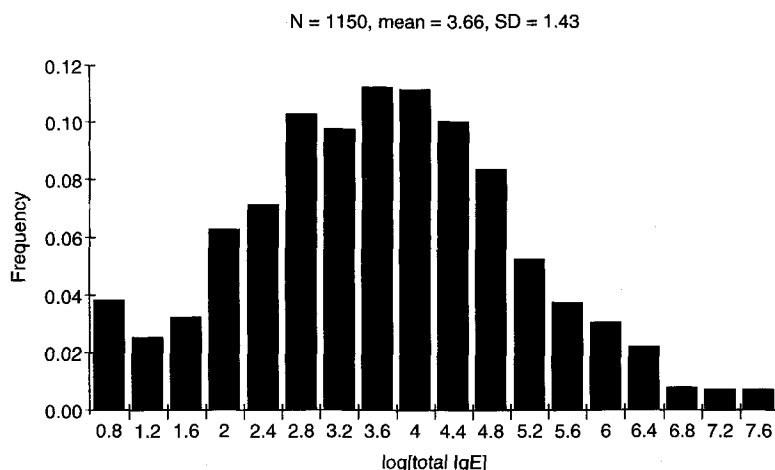


FIG. 1. Distribution of log[total IgE] in the entire population cross-sectional sample recruited by European Community Respiratory Health Survey in area of Erfurt, Germany, shown as histogram. For each class, the midpoint is given. Cut-off log[total IgE] for assignment to either case or control group was 5.3.

of the cases were active smokers compared with 35.3% (54.3% male) of the controls.

Microsatellites

The 395 subjects were genotyped for the chromosome 5q31 microsatellite markers: IL-4, IL-9, D5S393, D5S210, and D5S436 (Fig. 2), as well as for a microsatellite within the gene FCER1B on chromosome 11q13. The allele frequencies of the microsatellites investigated are given in Table II. Because none of the markers showed a significant depression of the observed heterozygosity compared with the calculated heterozygosity, we ruled out the possibility of null alleles for the loci investigated in the study population. This interpretation is further supported by similar heterozygosity values reported for these markers by other investigators. Therefore PCR amplifications resulting in no detectable product for a locus were considered as technical failures rather than null alleles and were not included in the calculations.

Three lines of evidence support the assumption that the mistyping rate was very low. First, the tolerance of the allele typing of DNA from families revealed a tolerance lower than ± 1 bp for each of the markers investigated. Second, approximately 50% of the subjects were typed twice with identical results. Finally, marker allele frequencies for FCER1B were similar to those of a similarly sized panel of 366 chromosomes,⁵² although a direct comparison was not possible because different methods were used in each case. The size of microsatellites determined by radioactive techniques used by Daniels and Shirakawa⁵² might differ from those obtained by us with the Genotyper software but the bimodal distribution of the FCER1B marker allele frequencies reported by Daniels and Shirakawa⁵² was similar to our observation. Therefore the alleles A6 (116 bp), A4 (120 bp), and A3 (122 bp) as designated by Daniels and Shirakawa⁵² probably correspond to alleles

5 (120 bp), 7 (124 bp), and 8 (126 bp), respectively, in our study.

On comparison of cases and controls, four uncorrected *p* values (IL-4 allele 3, D5S210 allele 2, and D5S436 alleles 7 and 14) were <0.05 but exceeded the significance level after correction (Table II). Moreover, none of these exhibited considerable overrepresentation in the case group, which would be expected if a nearby major gene controlling IgE production was present. Also, comparison of the genotype frequencies of the six loci resulted in no significant difference between cases and controls (data not shown), nor did the comparison of the phenotype frequencies (data not shown).

In a limited British study a Leu181 variant of FcεRIβ was shown to be associated with atopy, whereas in a larger Australian population sample a Leu181/183 variant of the receptor was found to be associated with atopy.^{36,37} In our attempt to confirm these results we were unable to detect any subject carrying the atopy-related Leu181/183 or Leu181 mutation of the FcεRIβ chain, although the former was expected to be present in the population at a level of approximately 4.5%, as has been reported for a population sample from Busselton, western Australia.³⁷ This failure to reproduce the association between the Leu181/183 variant of the FcεRIβ chain and atopy was also described by another group.⁵³

Linkage disequilibrium of the microsatellite loci on chromosome 5q31 could not be observed.

DRB1

The results of the DRB1 typing are given in Table III. There was an overrepresentation of the DRB1*01 allele in the case group, giving an uncorrected *p* value of 0.043, which exceeded the significance level on correction. When cases and controls were stratified according to the presence (RAST positive) or absence (RAST negative) of specific IgE, the comparison of RAST-positive cases with RAST-negative controls by Fisher's exact test re-

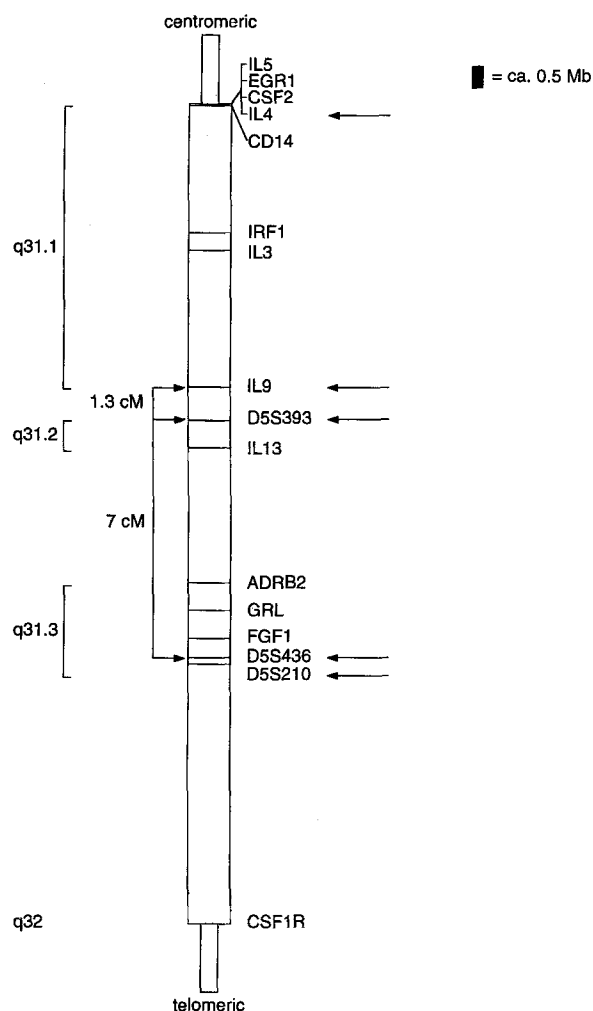


FIG. 2. Combined physical and genetic map of the human chromosome 5q31-q32 region (summary map obtained from the Genetic Location Database [LDB₁] http://cedar.genetics.soton.ac.uk/public_html/). In addition to IL genes indicated, the following genes also map to this region: early growth factor response-1 (*EGR1*); granulocyte-macrophage colony-stimulating factor-2 (*CSF2*); cluster of differentiation antigen 14 (*CD14*); interferon regulatory factor-1 (encoding transactivator of IFN- α and IFN- β and other IFN-inducible genes) (*IRF1*); β_2 -adrenergic receptor (*ADRB2*); glucocorticoid receptor (*GRL*); fibroblast growth factor-1 (acidic) (*FGF1*); and colony stimulating factor-1 receptor (*CSF1R*). Mapping of genes and markers to chromosomal bands is given on left. Markers used in this study are indicated by arrows.

sulted in a reduction of the uncorrected p value to 0.015 for DRB1*01, which did not remain significant after correction. In contrast, this difference was not observed when RAST-negative cases were compared with RAST-negative controls (data not shown). When phenotype frequencies were analyzed, the overrepresentation of the DRB1*01 allele in the case group became only nominally significant ($p = 0.021$, not significant on correction) when RAST-positive cases were compared with RAST-negative controls (cases: 31.3%; controls: 18.2%).

TABLE I. Description of cases and controls

	Cases	Controls
N	129	266
Mean age $\pm$ SD	40.9 $\pm$ 11.5	39.8 $\pm$ 12.1
Female/male (%)	35.7/64.3	47.4/52.6
Active smokers (%)	49.6	35.3
Female/male (%)	28.1/71.9	45.7/54.3
Mean log[total IgE] $\pm$ SD	6.13 $\pm$ 0.63	3.32 $\pm$ 1.19
RAST positive (%)*	75.2	26.7
Female/male (%)	34/66	53.5/46.5
RAST negative (%)†	24.8	73.3
Female/male (%)	40.6/59.4	45.1/54.9

*Subjects with at least one positive RAST result for *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, cat, grass, or birch.

†Subjects with no positive RAST results for *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, cat, grass, or birch.

DISCUSSION

From a cross-sectional study we extracted all persons with a total serum IgE concentration >200 kU/L (88th percentile) and from the remaining subjects we selected a random control group. All subjects were genotyped for the chromosome 5q31 microsatellites IL-4, IL-9, D5S210, D5S393, and D5S436, as well as for a microsatellite in the FCER1B gene and HLA-DRB1.

None of the six markers investigated was significantly associated with the trait of elevated total serum IgE concentration. Because recombination frequencies vary at different points throughout the genome, this finding does not exclude the possibility that genes involved in the regulation of serum IgE levels are located close to the markers investigated. The testing of numerous markers decreased the likelihood that a candidate gene for elevated serum IgE levels was present within the regions investigated. This holds particularly true for the narrow spacing of markers used for the chromosome 5q31 region.

Several nongenetic determinants have been shown to influence the serum IgE level.¹⁰ In contrast to age, for which cases and controls did not differ significantly, other factors that increase serum IgE levels such as male sex and smoking were represented to a higher degree in the case group (Table I). To rule out a loss of association caused by confounding effects of gender and smoking habits, we reanalyzed the data after adjustment for these covariates. There was no substantial alteration to the previous results of Fisher's exact test after this procedure (data not shown), except that the overrepresentation of DRB1*01 did not remain significant. Therefore genetic factors are likely to account for the majority of this difference.

A limitation of population-based association studies using microsatellites might be the relatively high mutation rate of microsatellites (ca. 0.001⁵⁴), which diminishes the strength of an association, although certain microsatellite loci have been reported to be evolutionarily very stable.⁵⁵ However, linkage disequilibrium de-

TABLE II. Comparison of microsatellite allele frequencies between cases and controls

Locus and allele size (bp)	Allele designation	Frequency in percentage (N)*		p Value†
		Cases	Controls	
IL-4				
152	1	3.7 (9)	6.7 (32)	0.015
154	2	76.6 (187)	71.7 (344)	
156	3	2.0 (5)	6.3 (30)	
158	4	1.2 (3)	0.4 (2)	
164	5	0.4 (1)	0.2 (1)	
168	6	0.8 (2)	0.6 (3)	
170	7	0.4 (1)	0.6 (3)	
172	8	3.7 (9)	3.5 (17)	
174	9	2.4 (6)	2.9 (14)	
176	10	2.4 (6)	1.5 (7)	
178	11	4.1 (10)	4.6 (22)	
180	12	2.0 (5)	0.8 (4)	
182	13	0.0 (0)	0.2 (1)	
Overall		100.0 (244)	100.0 (480)	
IL-9				
103	1	12.2 (27)	9.6 (50)	0.021
105	2	7.9 (20)	5.2 (27)	
107	3	0.8 (2)	1.3 (7)	
109	4	0.0 (0)	0.2 (1)	
115	5	0.0 (0)	0.2 (1)	
117	6	0.4 (1)	1.3 (7)	
119	7	1.2 (3)	1.0 (5)	
121	8	5.5 (14)	5.0 (26)	
123	9	12.2 (31)	11.9 (62)	
125	10	22.0 (56)	26.4 (138)	
127	11	23.6 (60)	22.4 (117)	
129	12	10.6 (27)	12.5 (65)	
131	13	2.8 (7)	2.1 (11)	
133	14	0.8 (2)	1.0 (5)	
Overall		100.0 (254)	100.0 (522)	
D5S393				
156	1	0.0 (0)	0.4 (2)	0.021
162	2	1.3 (3)	1.3 (6)	
164	3	12.9 (31)	12.6 (60)	
166	4	27.5 (66)	26.8 (128)	
168	5	9.6 (23)	10.0 (48)	
170	6	4.2 (10)	4.6 (22)	
172	7	11.7 (28)	9.2 (44)	
174	8	22.9 (55)	22.6 (108)	
176	9	5.4 (13)	5.6 (27)	
178	10	2.9 (7)	3.6 (17)	
180	11	0.4 (1)	1.7 (8)	
182	12	1.3 (2)	1.3 (6)	
184	13	0.4 (1)	0.4 (2)	
Overall		100.0 (240)	100.0 (478)	
D5S210				
208	1	2.0 (5)	1.8 (8)	0.021
212	2	0.8 (2)	4.0 (18)	
214	3	19.4 (48)	22.9 (104)	
216	4	3.6 (9)	3.3 (15)	
218	5	19.0 (47)	17.2 (78)	
220	6	29.8 (74)	27.8 (126)	
222	7	1.6 (4)	2.4 (11)	
224	8	10.5 (26)	9.9 (45)	
226	9	1.6 (4)	1.8 (8)	
228	10	7.7 (19)	6.2 (28)	
230	11	4.0 (10)	2.9 (13)	
Overall		100.0 (248)	100.0 (454)	

TABLE II. Cont'd

Locus and allele size (bp)	Allele designation	Frequency in percentage (N)*		p Value†
		Cases	Controls	
D5S436				
232	1	0.0 (0)	0.2 (1)	0.045
234	2	1.3 (3)	1.2 (5)	
236	3	1.3 (3)	4.0 (17)	
248	4	24.2 (58)	24.1 (103)	
240	5	5.4 (13)	5.1 (22)	
242	6	5.8 (14)	5.1 (22)	
244	7	8.3 (20)	13.8 (59)	
246	8	13.3 (32)	14.7 (63)	
248	9	15.0 (36)	15.4 (66)	
250	10	9.2 (22)	6.8 (29)	
252	11	8.3 (20)	6.1 (26)	
254	12	5.0 (12)	2.6 (11)	
256	13	1.3 (3)	0.9 (4)	
258	14	1.7 (4)	0.0 (0)	
Overall		100.0 (240)	100.0 (428)	
FCER1B				
110	1	0.4 (1)	0.2 (1)	0.033
114	2	0.0 (0)	0.2 (1)	
116	3	0.8 (2)	1.0 (5)	
118	4	0.8 (2)	2.0 (10)	
120	5	37.1 (89)	37.0 (187)	
122	6	2.1 (5)	3.6 (18)	
124	7	39.2 (94)	35.0 (177)	
126	8	16.7 (40)	17.6 (89)	
128	9	0.0 (0)	0.6 (3)	
130	10	0.8 (2)	0.2 (2)	
132	11	0.4 (1)	0.6 (3)	
134	12	1.7 (4)	2.2 (11)	
Overall		100.0 (240)	100.0 (506)	

*The frequencies are given on the basis of the number of chromosomes for which an amplificate could be obtained. Therefore *N* for cases and controls varies among the different markers.

†Uncorrected *p* value of the two-sided Fisher's exact test. Only *p* values less than the significance level of 0.05 are shown.

terminated by computer simulation will not be significantly affected up to a mutation rate of 0.001 per generation for a genetic distance between marker and locus of less than 1 cM (I. Tomlinson, personal communication). Because the genetic distances between the 5q31 markers investigated all exceed 1 cM the observed lack of linkage disequilibria for these markers is not surprising, assuming an average recombination rate.

Linkage of markers on chromosome 5q31 to total serum IgE concentrations have been demonstrated in two independent studies by sib-pair analysis.^{25, 26} Both studies mapped a major IgE control locus to the same overall chromosomal region, although in the study of Marsh et al.²⁵ evidence for linkage peaked at the IL-4 microsatellite whereas in the study of Meyers et al.²⁶ linkage was strongest to the D5S436 marker. This difference may reflect the different ascertainment strategies used in the two studies and the different genetic backgrounds of the probands. Moreover, the involvement of at least one other locus regulating total serum

TABLE III. Comparison of DRB1 allele frequencies between cases and controls

Allele	Frequency in percentage (N)		<i>p</i> Value*
	Cases	Controls	
DRB1*01	16.5 (42)	11.0 (56)	0.043
DRB1*02	16.9 (43)	15.5 (79)	
DRB1*03	9.1 (23)	7.6 (39)	
DRB1*04	12.6 (32)	16.3 (83)	
DRB1*06	2.4 (6)	4.1 (21)	
DRB1*07	11.0 (28)	12.0 (61)	
DRB1*08	3.9 (10)	3.3 (17)	
DRB1*09	1.6 (4)	1.4 (7)	
DRB1*10	0.8 (2)	1.0 (5)	
DRB1*11	14.2 (36)	14.7 (75)	
DRB1*12	2.4 (6)	2.4 (12)	
DRB1*13	5.1 (13)	8.4 (43)	
DRB1*14	3.5 (9)	2.4 (12)	
Overall	100.0 (254)	100.0 (510)	

*Uncorrected *p* value of the two-sided Fisher's exact test. Only *p* values less than the significance level of 0.05 are shown.

IgE levels and unlinked to chromosome 5q31 has been proposed.⁵⁶ This might explain why in a genome-wide screen to map traits related to asthma chromosome 5q31 was neither linked to an IgE regulatory locus nor to atopy.⁴³ Thus there may be a considerable degree of genetic heterogeneity or polygenic inheritance in the genetics of IgE serum levels, as suggested by the conflicting results obtained in family studies, which would preclude association with any of the markers investigated (reviews in Meyers et al.⁸ and Lawrence et al.⁵⁷). That the studies of Marsh et al.²⁵ and Meyers et al.²⁶ map an IgE control locus to the same overall region may be because of the clustering of several cytokine genes involved in immunoglobulin heavy chain switching to the ϵ isotype. The possible importance of this genomic region in allergic diseases was further supported by the mapping of a single dominant locus controlling T_H1/T_H2 differentiation and thus IgE production to a syntenic region in the mouse.⁵⁸

The study of Meyers et al.²⁶ ascertained families through a proband with symptomatic asthma. Therefore an interference of the IgE phenotype with asthma cannot be excluded. Because the case group in our study contained almost no subjects with asthma (only 7 subjects or 5.4% reported symptomatic asthma), it is possible that we investigated a different phenotype. Therefore genes responsible for the combination of high serum IgE concentrations and asthma might have been absent from the sample. On the other hand, a further restriction of the phenotype by excluding the subjects with symptomatic asthma did not reveal an association between high IgE levels and any of the markers investigated (data not shown), which thus rules out a loss of association caused by the heterogeneity introduced to the case group by the presence of persons with asthma.

In the study of Marsh et al.,²⁵ when sib pairs with

specific IgE were excluded from the calculation, the evidence for linkage of IL-4 and IRF1 with log[total IgE] was further enhanced. When specific IgE-positive subjects were excluded from our computations, none of the alleles was significantly overrepresented in the case group. Only for the IL-4 marker did allele 12 show a nominally significant overrepresentation in the case group (uncorrected *p* = 0.046), and this lost significance on correction.

Linkage of atopy to chromosome 11q13 has been shown by some studies, but has not been confirmed by other groups.^{28, 29, 32-35, 48, 59, 60} We were unable to detect an association between high IgE levels and a chromosome 11q13 marker present in the gene FCERIB, which has been shown to be linked to severe atopy³¹ and to an IgE regulatory locus.⁴³ To correspond to the definition of severe atopy of Shirakawa,³¹ we compared cases with specific IgE to controls negative for specific IgE (data not shown). In this comparison, four markers showed nominally significant uncorrected *p* values for at least one allele (IL-4 alleles 1 [*p* = 0.009] and 3 [*p* = 0.024]; IL-9 allele 2 [*p* = 0.036]; D5S436 alleles 3 [*p* = 0.044], 7 [*p* = 0.044], and 14 [*p* = 0.035]; and FCERIB allele 7 [*p* = 0.043]), but only the alleles of FCERIB and IL-9 showed a considerable excess in the case group, which might point to a relevance of these genes in the development of a more severe form of atopy.

In previous studies, the genetic linkage of atopy to three chromosome 11q13 markers (D11S97, cCI11-319ca, and the FCERIB marker used in this study), located within an 8 cM interval, was found exclusively for maternally derived alleles.^{48, 61} Because the ascertainment of subjects for this study did not allow us to consider this epigenetic phenomenon, an association with the chromosome 11q13 marker might have remained undetectable.

In addition, a considerable overlap between the subsets of atopy and bronchial hyperresponsiveness (BHR), especially in the asthmatic population, may confound genetic analysis. A recent study by Postma et al.,⁶² which used the same set of families used in the study of Meyers et al.,²⁶ suggested that the linkage to D5S436 may partly depend on the underlying proportion with BHR, though they provided statistical evidence for coinheritance of BHR with a major gene for atopy. In addition, the linkage of the FCERIB marker to atopy has recently been ascribed to coincident BHR.⁶³ Such a bias tends to increase when probands are recruited from clinics, whereas in a population-based approach such as we used the overlap with BHR might be considerably lower.

Finally, we observed an excess of DRB1*01 in the cases, though this was not significant after correction for multiple testing (Table III). This excess became even more pronounced when RAST-positive cases were compared with RAST-negative controls (*p* = 0.015) and was absent when RAST-negative cases were compared with RAST-negative controls (data not shown). Although these results need to be confirmed with a larger inde-

pendent panel, they might point to an important role of DRB1*01 in determining the level of antigen-driven IgE production.

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