ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/12604106

Association Study of the Chromosomal Region Containing the FCER2 Gene Suggests It Has a Regulatory Role in Atopic Disorders

Article *in* American Journal of Respiratory and Critical Care Medicine · March 2000

DOI: 10.1164/ajrccm.161.3.9810056 · Source: PubMed

CITATIONS
55

READS
62

11 authors, including:

Conxi Lázaro
Catalan Institute of Oncology
450 PUBLICATIONS **13,532** CITATIONS

SEE PROFILE

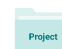
Paula Kauppi
Helsinki University Central Hospital
110 PUBLICATIONS **1,934** CITATIONS

SEE PROFILE

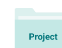
Josep M Antó
CREAL Center for Research in Environmental Epidemiology
582 PUBLICATIONS **38,902** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



TAPAS programme View project



NORdic Dataset for aSThmA Research (NORDSTAR) View project

All content following this page was uploaded by Rafael de Cid on 20 May 2014.

The user has requested enhancement of the downloaded file.

Association Study of the Chromosomal Region Containing the FCER2 Gene Suggests It Has a Regulatory Role in Atopic Disorders

TARJA LAITINEN, VESA OLLIKAINEN, CONXI LÁZARO, PAULA KAUPPI, RAFAEL de CID, JOSEP MARIA ANTÓ, XAVIER ESTIVILL, HEIKKI LOKKI, HEIKKI MANNILA, LAURI A. LAITINEN, and JUHA KERE

Department of Medical Genetics, Haartman Institute, and Department of Computer Science, University of Helsinki, Helsinki; Division of Pulmonary Medicine and Clinical Physiology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; Center for Medical and Molecular Genetics, Institut de Recerca Oncològica, Hospital Duran i Reynals, Barcelona; and Department of Epidemiology and Public Health, Institut Municipal d'Investigació Mèdica, Barcelona, Spain

On the basis of studies with animal models, the gene for the low-affinity receptor for immunoglobulin E (IgE) (FCER2, CD23) has been implicated as a candidate for IgE-mediated allergic diseases and bronchial hyperreactivity, or related traits. Given evidence for genetic complexity in atopic disorders, we sought to study two European subpopulations, Finnish and Catalonian. We studied three phenotypic markers: (*1*) total serum IgE level; (*2*) asthma; and (*3*) specific IgE level for a mixture of the most common aeroallergens in Finland. Altogether, eight polymorphic markers spanning a region of 10 cM around the FCER2 gene on chromosome 19p13 were analyzed in 124 families. The physical order of the markers and the location of the FCER2 gene were confirmed by using radiation hybrids. The allele and haplotype association study showed a suggestive haplotype association (signficance of $p \leq 0.03$ based on a permutation test) for a high serum IgE response. In a subset of chromosomes segregating with asthma in families with two or more affected members, a single haplotype was found to be highly enriched ($p = 8.3 \times 10^{-6}$). However, sequence polymorphisms, which would verify structural differences in the FCER2 gene, were not detected in the coding region of the receptor. Our results suggest that chromosome 19p13 might harbor a genetic determinant of IgE-related traits. Studies in other population samples are needed to verify this finding. Laitinen T, Ollikainen V, Lázaro C, Kauppi P, de Cid R, Antó JM, Estivill X, Lokki H, Mannila H, Laitinen LA, Kere J. Association study of the chromosomal region containing the FCER2 gene suggests it has a regulatory role in atopic disorders. AM J RESPIR CRIT CARE MED 2000;161:700–706.

The FCER2 gene encoding the low-affinity receptor for immunoglobulin E (IgE) (FceRII, CD23) has been mapped to chromosome 19p13.3 (1, 2). The human FCER2 gene spans approximately 13 kb and consists of 11 exons (3, 4). The receptor is a single-chain, 36-kD glycoprotein (321 amino acids) showing substantial homology with several lectins. As a result of alternative splicing of the first two exons, two proteins, with different amino termini in the cytoplasmic component, are translated (5). Their expression is regulated by two promoters that differ with respect to cell-type specificity and possibly in their response to extracellular stimuli.

On the basis of *in vitro* studies, CD23 has been proposed to have multiple functions, such as the regulation of IgE production and growth and differentiation of B cells, cellular adhesion, and antigen presentation (6, 8). Studies of mice deficient for CD23 indicate that the receptor may be involved in determining the basal level of IgE in serum and specific (T-cell–dependent) IgE responses (9–11). CD23 deficiency does not harm T- or B-cell development in mice (10). It is also apparent that CD23-deficient mice are able to develop specific and nonspecific IgE responses, bronchial eosinophilia, and bronchial hyperreactivity (12). In fact, $CD23^{-/-}$ mice develop a greater IgE response to ovalbumin when sensitized intraperitoneally or via the airways than do $CD23^{+/+}$ mice. This result indicates that CD23 may have a regulatory role in the negative feedback of T-cell–dependent, IgE-mediated immune responses.

(*Received in original form October 14, 1998 and in revised form June 29, 1999*)

Supported by grant BMH4-CT97-2486 from European Union BIOMED2 program (EU/BIOMED2), the Academy of Finland, Helsinki University Central Hospital Research Funds, the Association for the Pulmonary Disabled, the Finnish Lung and Health Association, the Sigrid Juselius Foundation, the Ida Montin Foundation, the Finnish Anti-Tuberculosis Association Foundation, the Servei Català de la Salut, grants FIS93/0393, FIS95/0050-01, and FIS98/0977 from the Fondo de Investigaciones Sanitarias, grants Comisión Interministral de Ciencia y Tecnologia (CICYT)/SAF96-1787-E and CICYT/IN95-0347 from the Ministerio de Educación y Ciencia by CEC/BIOMED2 (GENECT96-1364) and grants Comissió Interdepartamenteal de Recerca i Innovació Tecnològica (CIRIT)/GRQ93-9304, CIRIT/1995SGR00434 and CIRIT/1997SGR-00085 from the Generalitat de Catalunya.

Correspondence and requests for reprints should be addressed to Dr. Tarja Laitinen, Department of Medical Genetics, Haartman Institute, Box 21, Haartmaninkatu 3, 00014 University of Helsinki, Finland. E-mail: tarja.laitinen@helsinki.fi

Am J Respir Crit Care Med Vol 161. pp 700–706, 2000
Internet address: www.atsjournals.org

This is further supported by studies with IgE transgenic mice (13). In another study, high levels of serum IgE were accompanied by a high level of expression of membrane-bound CD23, whereas CD23 transgenic mice had a severely impaired capacity to produce IgE (14).

Given the evidence for the role of the FCER2 gene in upregulating IgE production, we decided to seek genetic evidence for a role of this gene in regulating serum IgE levels and in IgE-mediated atopic disorders in two isolated populations. Significant genetic heterogeneity is believed to exist in IgE regulation at the population level. However, this can be minimized when study subjects represent a population isolate. Populations that have expanded in isolation after a narrow genetic bottleneck are ideal for association studies that may well be more sensitive than linkage studies (15). We phenotyped individuals in 124 Finnish families for asthma, total serum IgE level as a marker of atopic susceptibility, and specific IgE level for eight aeroallergens as a potential marker of airway allergy. We genotyped these individuals for eight polymorphic markers on chromosome 19p13, and performed nonparametric linkage and association analyses by using two alternative models for haplotyping. In another restricted population, we studied patients coming from Catalonia, a region in the northeastern part of Spain. The Catalonian sample, consisting of patients and population controls (n = 382), was studied for allele associations. Our results suggest that FCER2 may be a genetic determinant of serum IgE levels.

METHODS

Physical Mapping

Published polymerase chain reaction (PCR) assays were used for markers D19S216, D19S567, D19S884 (Genethon), D19S534 (The Cooperative Human Linkage Center), D19S120, and D19S536 (Genome Data Base). A tetranucleotide (AAAT) repeat has been identified in the first intron of the FCER2 gene (4). Primers (5′-TGC TAA GAG CCC ATA ACC CA-3′ and 5′-GCA ACA GAG CGA GAC TCC AT-3′) were designed from the surrounding sequence to amplify this repeat. DNA samples from a radiation hybrid panel (GeneBridge 4; Research Genetics, Inc., Huntsville, AL) were amplified by PCR and the presence or absence of each gene or marker was scored. The Whitehead Institute network server (http://www.genome.wi.mit.edu) was used in the analysis of the hybrid mapping results. Physical distances were estimated through the conversion of 1 cR to 350 kb for the GeneBridge 4 panel (16).

Subjects and Families

Local radio and newspaper advertisements were used to recruit families with one or more asthmatic members in the eastern central part of Finland (Kainuu Province). The population history of this region and the clinical characteristics of the patients have been described previously in detail (17, 18). Blood samples for total and specific serum IgE measurements and DNA extraction were collected from members of nuclear families (proband, father, and mother; or proband, spouse, and at least one child; or proband, one parent, and at least one sibling) in order to establish haplotypes unambiguously. Additional family members (uncles, aunts, grandparents, further sibs, and cousins) were included when available.

Serum total IgE (kU/L) was determined with a solid-phase immunoassay (Diagnostics CAP fluorescence enzyme immunoassay [FEIA], Kabi Pharmacia, Uppsala, Sweden), and IgE-specific antibodies for the most common airway allergens in Finland (birch, mugwort, and timothy pollen; horse, cat, and dog dander; house dust mite, and *Cladosporium herbarum* mold) were quantitated with a Phadiatop CAP FEIA (Kabi Pharmacia). Although the serum total IgE level is known to slowly increase until young adulthood and gradually decrease during old age, it is very stable throughout adulthood (19). Study individuals represented adults (70% aged 35 to 65 yr) (17, 18). Therefore, age was not considered a confounding factor in the study.

The Catalonian patients were from a cohort of adult individuals who consulted Barcelona hospitals because of an acute asthma attack during a soybean asthma epidemic (20). DNA was obtained from 145 asthma patients. Control samples (n = 46) representing population-based controls were collected from anonymous blood donors from a hospital in the same region. Three microsatellite markers within the chromosome 19p region (D19S534, D19S922, and D19S884) were typed. The study was approved by the institutional committees on ethical practice of the participating Barcelona institutions, and all participants signed an informed consent agreement.

Genotyping

DNA was extracted from blood samples through a standard nonenzymatic method. PCR amplifications were done in 20 μl reaction volumes containing 50 ng of genomic DNA, 0.2 mM of each primer, 50 mM KCl, 1.5 M $MgCl_2$,10 mM Tris-HCl (pH 8.8 at 20° C), 0.2 mM of each deoxynucleotide triphosphate, and 0.3 U thermostable DNA polymerase (Dynazyme; Finnzymes, Espoo, Finland). The samples were denatured for 10 min at 93° C and subjected to 24 to 30 cycles each of 15 to 30 s at 93° C, 15 to 30 s at 55 to 60° C, and 15 to 30 s at 72° C, and were elongated for 8 min at 72° C. The samples were electrophoresed on denaturing 7 M urea/6% polyacrylamide gels, and the alleles were visualized by silver staining.

The following polymorphic microsatellites were typed: D19S216 (heterozygosity = 0.76), D19S567 (heterozygosity = 0.36), D19S884 (heterozygosity = 0.84), D19S534 (heterozygosity = 0.55), D19S922 (heterozygosity = 0.81), D19S120 (heterozygosity = 0.69), and D19S536 (heterozygosity = 0.68), and intronic polymorphisms within the FCER2 gene. For the FCER2 gene, three alleles were detected: 128 bp with a frequency of 0.26, 124 bp with a frequency of 0.72, and 120 bp with a frequency of 0.02. The observed heterozygosity was 0.368, and Mendelian segregation was confirmed. Typing results were read independently by two persons who were blind to the clinical data. In cases of ambiguity, the typing was repeated. Ninety-six percent of the alleles of the markers genotyped were called and verified to be constant with Mendelian segregation. In cases in which the phase of the alleles could not be determined, the allele was zeroed out from haplotype analysis.

Sequencing of the FCER2 Gene

The FCER2 gene was amplified by PCR in four selected individuals, using the following primer pairs: TTC CAA ATC AGC CAG AGC TGT G and CTG TGT GGT GTC CCA GTC TGG (exons 2, 3, and 4); TGC TTC TCC TGT GGC GTG AGG and AAT CTG CGT GGC TGT TTG CAG G (exons 3, 4, 5, and 6); GCG CAG AAA TCC CAG TGT GAG and ACT CAC CGC TGG ACA CCT GC (exons 5, 6, 7, and 8); and CGT CCG ACC CCT AGG CTT TG and GAA GGC AGG GGC CAT AGA GG (exons 9, 10, and 11). These amplicons covered all coding exons, and the unsequenced segments were in the middle of introns. Sequencing was performed with dye-terminator chemistry, using an ABI373A sequencer (PE Biosystems, Foster City, CA).

Statistical Analyses and Power Estimations

For linkage analysis, we studied the qualitative phenotypes of a high IgE level (> 100 kU/L), positive allergy screening results for aeroallergens, and asthma (17) by performing multipoint affected relative pair analysis with the computer package MAPMAKER/SIBS (Whitehead Institute, Cambridge, MA) (21), and nonparametric multipoint linkage analysis with the computer package GENEHUNTER (Whitehead Institute, Cambridge, MA) (22). We also studied IgE levels as a quantitative trait, using MAPMAKER/SIBS to perform the Haseman–Elston test.

For allele and haplotype association analysis, we used the chi-square test to compare the number of occurrences of each haplotype in phenotypic categories (high IgE versus low IgE, positive versus negative allergy screening results, and asthma versus no asthma). Only haplotypes with a frequency of $n \geqslant 5$ were considered. Since many tests were performed (every haplotype for every set of consecutive markers was examined), we used a permutation test (17) to estimate the significance of the overall observations. In the haplotype permutations, the observed haplotypes were used as fixed, whereas the affection status of

the chromosomes was randomized. The number of haplotypes in permutated data showing an association at the significance level of $p \leq 0.01$ was compared with the number of haplotypes in real data.

RESULTS

Physical Map

Since comprehensive maps were not available, we began by constructing a dense genetic and physical map of the 10-cM region of chromosome 19p, using the Genethon map (23) and the Genetic Location Database as starting points. To integrate the markers and the FCER2 gene into a single physically ordered map, we typed the entire set of markers in a radiation hybrid panel with an average resolution of 350 kb (Genebridge 4). An integrated physical map based on these results is shown in Figure 1.

Family Ascertaintment and Clinical Data

Families were collected from the Kainuu Province of Finland, and their origins were traced in order to avoid population stratification (17, 18). Families were selected through a proband (parent or child) with self-reported asthma. The entire data set (n = 124 families) included a total of 57 sibships and 35 first-degree and six second-degree cousin pairs, in most cases with complete sets of parents (488 subjects). There were two or more affected individuals in a family in 51 pedigrees for asthma, 44 pedigrees for a high IgE response, and 40 pedigrees for a positive allergy screening result. Each set of families was used in a corresponding linkage analysis. For association studies, the data set was extended with uniplex families (n = 73). These families consisted of an affected individual together with two relatives needed to determine the linkage phase: either an affected child with both parents or an affected parent with spouse and offspring.

Phenotyping of Subjects

Three traits clustering in asthmatic families were considered. Our diagnostic procedure for asthma and allergies has been described in detail (18). Both total and specific serum IgE values were determined from samples taken at the same time as samples for genetic studies, and were measured as a single laboratory batch. A specific IgE level of > 0.3 kU/L for a mixture of the eight aeroallergens was considered positive, and the total serum IgE level was considered high at > 100 kU/L.

Linkage Study

To determine the inheritance pattern of chromosome 19p13, we genotyped individuals in multiplex families for eight genetic markers in the 10-cM region spanning from D19S120 to D19S884. Multipoint linkage analysis was then performed by using the GENEHUNTER program (22). For the asthma phenotype, the nonparametric linkage (NPL) score was negative across the whole region, with an information content of 72 to 74%. For allergy screening and high IgE response, the NPL scores were positive but far below significance (NPL score < 1), with an information content of 72 to 74%. Serum IgE levels were also analyzed as a quantitative trait by using MAPMAKER/SIBS (21). No evidence for linkage was found (logarithm of the odds favoring genetic linkage [LOD] score < 0.1, t score < 1.5). We performed exclusion mapping for asthma phenotype (21). We were able to exclude at an LOD score of −2 the presence of a locus that would increase the sibling risk by threefold ($\lambda_s > 3$) compared with the risk in the population at large. A locus increasing the sibling risk by twofold ($\lambda_s > 2$) could still be excluded at an LOD score of −1. Loci of less than twofold effects could not be excluded.

Association Study

Our set of families, including mainly uniplex families, was primarily selected for a haplotype association study. If it is assumed that the study population contains only a limited number of disease-predisposing ancestral alleles, linkage disequilibrium mapping with a set of dense markers can be a more powerful tool than linkage for detecting a locus with low penetrance susceptibility. This assumption is valid when the study

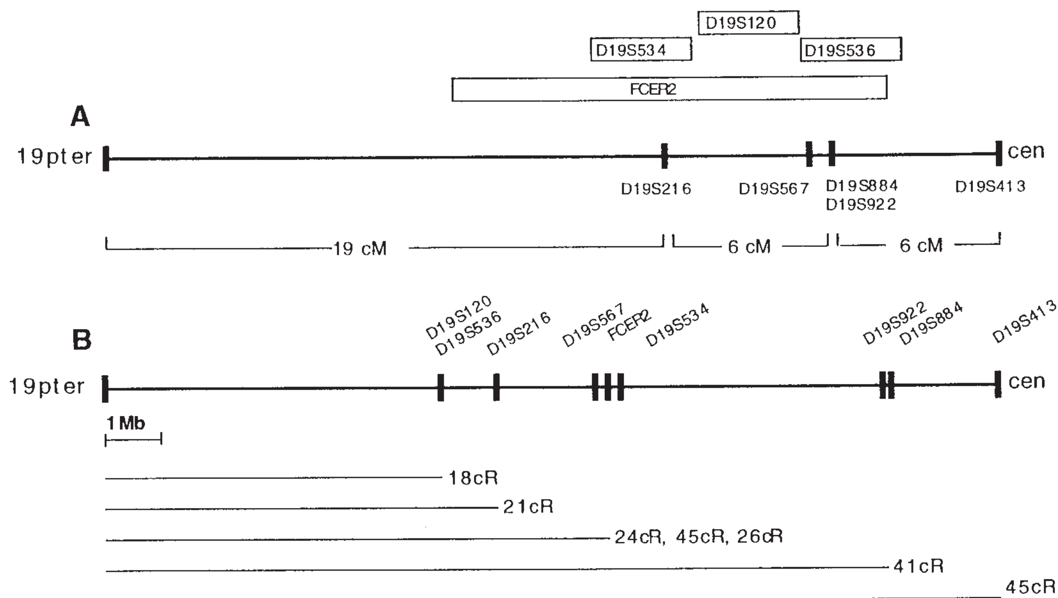


Figure 1. Refinement of the genomic map. (*A*) A previous genetic map (23) is shown on the line. The approximate locations of additional markers and the candidate gene are included in boxes above the line. (*B*) A physical map based on our radiation hybrid mapping shows the refined order of markers and genes. The estimated physical distances between eight markers are shown. The centromere is to the right.

families represent a relatively isolated and young population with a limited number of founders (24–26).

Haplotype analysis was based on all 124 families. In each family, the classification of chromosomes was done independently for all three traits investigated in the study. For the analysis, each chromosome that occurred in any affected family member was designated as trait-associated, and the remaining chromosomes (i.e., those occurring only in unaffected relatives) were designated as controls (Figure 2, Test 1). Each chromosome was counted only once per pedigree. The haplotype associations found at a significance level of $p \leqslant 0.01$ in chi-square tests for each trait are shown in Table 1. In the case of asthma, only one haplotype spanning the candidate gene was increased in affected chromosomes (D19S567*16–FCER2*2–D19S534*7–D19S922*8), with a 5-fold increased risk for asthma. For both a high serum IgE and positive allergy screening result, two different haplotype associations spanning the candidate gene were found. One of the haplotypes (D19S120*3–D19S536*4–D19S216*4–D19S567*16–FCER2*3–D19S534*7) was shared for the two traits. To correct for multiple testing errors, we used simulations to estimate the significance of a finding. We performed 10,000 permutations in which the haplotypes were fixed and the affection status of each chromosome was randomized while keeping the number of affected and control chromosomes fixed. In 1,163 such permutations for the asthma phenotype, the number of haplotypes that reached the significance level of $p \leqslant 0.01$ was higher in simulated than in actual data. Thus, based on the permutation test, it was unlikely that we had detected a true susceptibility haplotype for asthma ($p = 0.12$). The corresponding probability for detecting true susceptibility haplotypes for a high serum IgE was suggestive ($p = 0.038$), but for a positive allergy screening result was again not significant ($p = 0.19$). With this study design, our power to detect the presence of an ancestral chromosome with a frequency of 0.20 or 0.15 was high (94 to 96% and 84 to 89%, respectively), while that for detecting an ancestral chromosome with a frequency of 0.10 was moderate (54 to 65%), and that for detecting an ancestral chromosome with a frequency of 0.05 was low (< 19%). Rare susceptibility haplotypes (< 15%) could not be excluded.

If a putative predisposing gene acts in a dominant fashion, it is possible that the foregoing classification of haplotypes is too conservative. The pool of trait-associated chromosomes will become contaminated with as many as 50% of chromosomes that may not be associated with a predisposing allele. To test for this possibility, we considered those chromosomes that were present in at least two affected family members (i.e., segregated with the disease) (Figure 2, Test 2). The test also allowed for reduced penetrance in the parental generation. For example, if two affected sibs had the same chromosome but both parents were unaffected, the chromosome was still considered affected. The pool of control chromosomes remained unchanged. This test (Test 2) revealed a total of 49 affected chromosomes for the asthma phenotype. We investigated for whether any of the haplotypes found in Test 1 was enriched among these chromosomes, and found that the haplotype D19S567*16–FCER2*2–D19S534*7 was present in 12 of 49 (24.5%) of the affected chromosomes in Test 2, as compared with 18 of 316 (5.7%) of the affected chromosomes in Test 1 (chi-squared = 24, df 2, $p = 8.3 \times 10^{-6}$) and three of 244 (1.2%) of the control chromosomes (chi-squared = 50, df 2, $p = 1.6 \times 10^{-11}$). For high serum IgE, the haplotype D19-S216*4–D19S567*16–FCER2*3–D19S534*7 was slightly but not significantly enriched, to a frequency of six of 48 (12.5%) affected chromosomes compared with 24 of 255 (9.4%) ($p = 0.2$) affected chromosomes in Test 1 and 12 of 352 (3.4%) chromosomes among controls ($p = 0.004$). For allergy screening, none of the haplotypes was enriched.

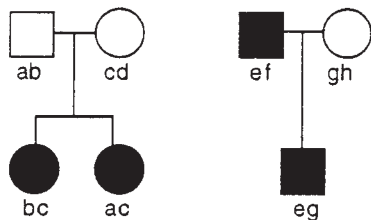


Figure 2. Haplotype designation in two example families. Test 1: a—affected, b—affected, c—affected, d—unaffected, e—affected, f—affected, g—affected, h—unaffected. Test 2: a—phenotype unknown, b—phenotype unknown, c—affected, d—unaffected, e—affected, f—phenotype unknown, g—phenotype unknown, h—unaffected.

Allele Associations for Asthma Phenotype among Finnish and Catalonian Asthma Patients

For the asthma phenotype, an allele association study was performed among the Catalonian asthma patients by using the polymorphic markers closest to the candidate gene (D19S534, D19S922, and D19S884). One allele (D19S922*8; allele size 240 bp) was associated with an increased risk of asthma in both populations: among the Finns the relative risk (RR) was 1.5 (70 of 285 asthma-associated chromosomes versus 38 of 226 control chromosomes, $p < 0.03$), and among the Catalonians the RR was 2.9 (37 of 290 asthma-associated chromosomes versus four of 92 control chromosomes, $p < 0.02$) in the chi-square test. The associations were not significant when the p values were corrected by the number of tests performed.

Sequencing of the FCER2 Gene

Since the first haplotype analysis showed suggestive significance for a high serum IgE level, and both haplotype analyses for asthma identified the same haplotype, we decided to sequence the entire coding region of the FCER2 gene. The gene was sequenced in four subjects having different combinations of three of the most strongly associated haplotypes (including a homozygote for the D19S567*16–FCER2*2–D19S534*7–D19-S922*8 haplotype and heterozygotes for the D19S567*16–FCER2*3–D19S534*7 and D19S216*10–D19S567*16–FCER2*3–D19S534*5 haplotypes) to search for a variant, and was also sequenced in one control to verify the published sequence. No sequence differences from the published sequence were found in affected or control chromosomes.

DISCUSSION

On the basis of findings in *in vitro* studies, and more recently in *in vivo* studies with CD23-deficient mice, it has been assumed that CD23 may have feedback-control for IgE-mediated responses (9–11). Therefore, the FCER2 gene has been implicated as a good candidate for producing the traits closely associated with IgE-mediated immune responses. Individuals with allergic disorders show increased expression of membrane-bound CD23 (27). They often also have increased levels of soluble CD23 in serum (28). Soluble CD23 may interfere with binding of IgE-containing immune complexes to membrane-bound CD23, thereby preventing an optimal feedback control loop in allergic individuals (7). Structural differences in genomic DNA in the gene or in the regulatory elements of the gene may, for example, increase the susceptibility to auto-

TABLE 1

COMPILATION OF HAPLOTYPES THAT SHOWED ASSOCIATION AT $p \leqslant 0.01$ WITH ASTHMA, HIGH SERUM IgE LEVEL, AND AIRWAY ALLERGY*

Haplotype Markers										No. of Haplotypes†	
D19S 120	D19S 536	D19S 216	D19S 567	FCE R2	D19S 534	D19S 922	D19S 884	Chi-square Value	Relative Risk Ratio	Asthma (*n = 316*)	Controls (*n = 244*)
2	2	7	16	3				10.3	0	0	8
							10	9.7	0.1	1	10
			16	3	7	5		9.4	0.3	5	16
				2	7	8		8.6	5	19	3
			16	2	7	8		7.8	4.7	18	3
2	2	7	16					7.6	0.2	2	10
						5		6.9	0.5	24	36
2	2	10						6.8	5.5	14	2
										No. of Haplotypes‡	
										High IgE Responder (*n = 255*)	Low IgE Responders (*n = 352*)
					7	6		11.2	3.3	21	9
		4	16	3	7			9.7	2.8	24	12
				2	7	6		9.0	4	14	5
	4	7	16	2	7			8.6	—	6	0
				3	9			8.4	0.1	1	15
3	4	4	16	3	7			8.1	5.2	11	3
				2	7	6	3	7.7	3.7	13	5
				3	7	4	4	7.3	—	5	0
			16	3	7	6		7.2	10.0	7	1
3	4	7	16	2	7			7.1	—	5	0
					9			6.7	0.2	3	18
										No. of Haplotypes§	
										Allergy Screening Positive (*n = 246*)	Allergy Screening Negative (*n = 370*)
						10	6	8.6	0.1	1	15
	4	10	16	3	5			7.8	10.3	7	1
3	4	10	16	3	5			7.6	10.3	7	1
		4	16	3	7			7.6	2.4	15	7
3	4	4	16	3	7			7.6	4.9	15	7
					7	6	3	7.0	3.2	10	3
3	4	4	16					6.9	3.2	20	12

* Results of permutation tests to assess significance are shown for each trait.

† In 1,163 of 10,000 permutation tests, the number of haplotype associations at a significance level of $p \leqslant 0.01$ was higher than in real data.

‡ In 383 of 10,000 permutation tests, the number of haplotype associations at a significance level of $p \leqslant 0.01$ was higher than in real data.

§ In 1,899 of 10,000 permutation tests, the number of haplotype associations at a significance level of $p \leqslant 0.01$ was higher than in real data.

proteolysis of membrane-bound CD23 into its soluble form, and may thus be involved in the development of allergic conditions. We genotyped a total of eight markers spanning a region of 10 cM flanking the FCER2 gene. We found no evidence of segregation in families with any of the phenotypes studied with either parametric or nonparametric linkage analysis. However, linkage as a method for identifying susceptibility loci in common multifactorial diseases such as asthma or atopy has not been very encouraging. Low penetrance of the susceptibility locus, phenocopies in the same pedigree, and genetic heterogeneity may dramatically decrease the power to detect linkage. Linkage programs, such as GENEHUNTER, which reconstructs multimarker haplotypes on the basis of maximum likelihood, are sensitive to a low genetic information content. In addition to a fairly moderate number of pedigrees in our data set for linkage analysis, genotype information was often missing for the older generation connecting younger, more distant affected family members. This reduced the power of the analysis, also for cases of possible true linkage.

For reasons mentioned earlier, families in our data set were primarily selected for a family-based haplotype association study. To test for actual significance of the haplotype associations, we performed permutation tests (17), because the right method for correcting for multiple testing was not obvious. Our results showed that a haplotype distribution as skewed as that observed for chromosomes present in individuals with high serum IgE level occurred only 383 times in 10,000 replicated simulations ($p = 0.038$). The result remained unchanged when the chi-square cutoff value used to select associated haplotypes was varied over a broad range.

To study further association between atopic disorders and FCER2, we extended our haplotype analysis. For a locus in-

herited in a recessive manner, our mode of haplotype analysis would be the most sensitive for identifying association. The mode of inheritance of the traits we studied is unknown, but dominant gene(s) with low penetrance, and genetic heterogeneity among families showing these traits, have been proposed. This mode of inheritance is supported by family studies showing similar parent–offspring and sibling risks in asthma and other atopic diseases (29–31). Moreover, multiplex families in our material were compatible with a dominant pattern of inheritance. Labeling all chromosomes that occur in affected individuals as "affected" might therefore dilute the pool of susceptibility-gene–carrying chromosomes. To avoid such dilution, only those chromosomes that were found in at least two affected individuals in the same pedigree were considered for possible enrichment of associated haplotypes. Indeed, this procedure led to the specific enrichment of an identical haplotype to that found to be associated with the asthma phenotype by our first test. The chi-square statistic gave highly significant p values for the association of this particular haplotype in enriched asthma-associated chromosomes as opposed to unenriched and control chromosomes. This further supports the biologic significance of our finding.

We also studied allele associations in a Catalonian population sample. Unfortunately, haplotypes could not be established in this data set. The corrected allele association tests remained nonsignificant for all loci across the chromosome 19p13 region. However, the same allele for the marker D19S922 (*8; 240 bp) showed an increased relative risk in both populations. The risk ratio was even higher in the Catalonian than in the Finnish sample (2.9 versus 1.5), reaching nominal significance without correction for multiple testing in both populations. The biologic significance of this observation remains unknown.

We conclude that our study provides suggestive evidence for an association between the FCER2 gene region in chromosome 19p13 and a high level of IgE-associated traits. Sequencing of the coding region of FCER2 in four patients selected on the basis of their haplotypes failed to reveal polymorphisms. This result, however, does not exclude FCER2 as a genetic determinant of IgE production. Intronic nucleotide polymorphisms, and especially those near exon–intron junctions, can modify gene transcription. It is also possible that some other, still functionally uncharacterized gene in the chromosome 19p13 region is responsible for the slight effect. Our data did not allow a more exhaustive study of the strength of the association between the FCER2 gene and a high level of IgE-associated traits, or of its cause. We propose that the chromosome 19p13 region be considered a candidate locus in other genetic studies of serum IgE regulation and asthma. Repeated studies in other population samples are warranted, especially because the relative importance of any locus may be stronger in other populations than in the Finnish and Catalonian subpopulations in our study. For these purposes, our study provides a set of highly polymorphic markers and an accurate physical map across the chromosome 19p13 region.

***Acknowledgment*:** The authors gratefully acknowledge Dr. Hannu Laitinen, Ms. Liisa Rajasalo, Ms. Päivikki Pajunen, and Ms. Sinikka Lindh for their help in organizing the sample collection drive. They thank Dr. Mark Daly for the initial analysis of the haplotypes; Dr. Ann-Christine Syvänen for her help in minisequencing; and Ms. Siv Knaappila and Ms. Merja Nissinen for their careful and skilled genotyping work.

References

1. Wendel-Hansen, V., M. Riviere, M. Uno, I. Jansson, J. Szpirer, M. Q. Islam, G. Levan, G. Klein, J. Yodoi, A. Rosen, and C. Szpirer. 1990. The gene encoding CD23 leukocyte antigen (FCER2) is located on human chromosome 19. *Somat. Cell Mol. Genet.* 16:283–286.
2. Trask, B., A. Fertitta, M. Christensen, J. Youngblom, A. Bergmann, A. Copeland, P. de Jong, H. Mohrenweiser, A. Olson, A. Carrano, and K. Tynan. 1993. Fluorescence in situ hybridization mapping of human chromosome 19: cytogenetic band location of 540 cosmids and 70 genes or DNA markers. *Genomics* 15:133–145.
3. Lüdin, C., H. Hofstetter, M. Sarfati, C. A. Levy, U. Suter, D. Alaimo, E. Kilchherr, H. Frost, and G. Delespesse. 1987. Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO J.* 6:109–114.
4. Suter, U., R. Bastos, and H. Hofstetter. 1987. Molecular structure of the gene and the 5′-flanking region of the human lymphocyte immunoglobulin E receptor. *Nucleic Acids Res.* 15:7295–7308.
5. Delespesse, G., M. Sarfati, and H. Hofstetter. 1989. Human IgE-binding factors. *Immunol. Today* 10:159–164.
6. Bonnefoy, J. Y., S. Lecoanet-Henchoz, J. P. Aubry, J. F. Gauchat, and P. Graber. 1995. CD23 and B-cell activation. *Cur. Opin. Immunol.* 7:355–359.
7. Lamers, M. C., and P. Yu. 1995. Regulation of IgE synthesis: lessons from the study of IgE transgenic and CD23-deficient mice. *Immunol. Rev.* 148:71–95.
8. Mudde, G. C., R. Bheekha, and C. A. Bruijnzeel-Koomen. 1995. Consequences of IgE/CD23-mediated antigen presentation in allergy. *Immunol. Today* 16:380–383.
9. Fujiwara, H., H. Kikutani, S. Suematsu, T. Naka, K. Yoshida, K. Yoshida, T. Tanaka, M. Suemura, N. Matsumoto, S. Kojima, T. Kishimoto, and N. Yoshida. 1994. The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 91:6835–6839.
10. Stief, A., G. Texido, G. Sansig, H. Eibel, G. Le Gros, and H. van der Putten. 1994. Mice deficient in CD23 reveal its modulatory role in IgE production but no role in T and B cell development. *J. Immunol.* 152: 3378–3390.
11. Yu, P., M. Kosco-Vilbois, M. Richards, G. Köhler, and M. C. Lamers. 1994. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* 369:753–756.
12. Haczku, A., K. Takeda, E. Hamelmann, A. Oshiba, J. Loader, A. Joetham, C. Irvin, H. Kikutani, and E. W. Gelfand. 1997. CD23 deficient mice develop allergic airway hyperresponsivness allowing sensitization with ovalalbumin. *Am. J. Respir. Crit. Care Med.* 56:1945–1955.
13. Adamczewski, M., G. Köhler, and M. C. Lamers. 1991. Expression and biological effects of high levels of serum IgE in epsilon heavy chain transgenic mice. *Eur. J. Immunol.* 21:617–626.
14. Texido, G., H. Eibel, G. Le Gros, and H. van der Putten. 1994. Transgene CD23 expression on lymphoid cells modulates IgE and IgG1 responses. *J. Immunol.* 153:3028–3042.
15. Liu, L., C. Forsell, L. Lilius, K. Axelman, E. H. Corder, and L. Lannfelt. 1996. Allelic association but only weak evidence for linkage to the apolipoprotein E locus in late-onset Swedish Alzheimer families. *Am. J. Med. Genet.* 67:306–311.
16. Hudson, T. J., L. D. Stein, S. S. Gerety, J. Ma, A. B. Castle, J. Silva, D. K. Slonim, R. Baptista, L. Kruglyak, S-H. Xu, X. Hu, A. M. E. Colbert, C. Rosenberg, M. P. Reeve-Daly, S. Rozen, L. Hui, X. Wu, C. Vestergaard, K. M. Wilson, J. S. Bae, S. Maitra, S. Ganiatsas, C. A. Evans, M. M. DeAngelis, K. A. Ingalls, R. W. Nahf, L. T. Horton, Jr., M. O. Anderson, A. J. Collymore, W. Ye, V. Kouyoumjian, I. S. Zemsteva, J. Tam, R. Devine, D. F. Courtney, M. T. Renaud, H. Nguyen, T. J. O'Connor, C. Fizames, S. Faure, G. Gyapay, C. Dib, J. Morissette, J. B. Orlin, B. W. Birren, N. Goodman, J. Weissenbach, T. L. Hawkins, S. Foote, D. C. Page, and E. S. Lander. 1995. An STS-based map of the human genome. *Science* 270:1945–1954.
17. Laitinen, T., P. Kauppi, J. Ingatius, T. Ruotsalainen, M. J. Daly, H. Kääriäinen, L. Kruglyak, E. S. Lander, A. de la Chapelle, L. A. Laitinen, and J. Kere. 1997. Genetic control of serum IgE levels and asthma: linkage and linkage disequilibrium studies in an isolated population. *Hum. Mol. Genet.* 6:2069–2076.
18. Kauppi, P., L. A. Laitinen, H. Laitinen, J. Kere, and T. Laitinen. 1998. Verification of self-reported asthma and allergy in subjects and in their family members volunteering for gene mapping studies. *Respir. Med.* 92:1281–1288.
19. Barbee, R. A., M. Halonen, W. Kaltenborn, M. Lebowitz, and B. Burrows. 1987. A longitudinal study of serum IgE in a community cohort: correlations with age, sex, smoking, and atopic status. *J. Allergy Clin. Immunol.* 79:919–927.
20. Soriano, J. B., G. Ercilla, J. Sunyer, F. X. Real, C. Lásaro, M. J. Rodrigo, X. Estivill, J. Roca, R. Rodríguez-Roisín, F. Morell, and J. M. Antó. 1997. HLA Class II Genes in Soybean Epidemic Asthma Patients. *Am. J. Respir. Crit. Care Med.* 156:1394–1398.

21. Kruglyak, L., and E. S. Lander. 1995. Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am. J. Hum. Genet.* 57:439–454.
22. Kruglyak, L., M. J. Daly, M. P. Reeve-Daly, and E. S. Lander. 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am. J. Hum. Genet.* 58:1347–1363.
23. Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Hazan, E. Seboun, M. Lathrop, G. Gyapay, J. Morisette, and J. Weissenbach. 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–154.
24. de la Chapelle, A. 1993. Disease gene mapping in isolated human populations: the example of Finland. *J. Med. Genet.* 30:857–865.
25. Hästbacka, J., A. de la Chapelle, M. M. Mahtani, G. Glines, M. P. Reeve-Daly, M. Daly, B. A. Hamilton, B. Trivedi, A. Weaver, A. Coloma, M. Lovett, A. Buckler, I. Kaitila, and E. S. Lander. 1994. The diastrophic dysplasia gene encodes a novel sulfate transporter, positional cloning by fine-structure linkage disequilibrium mapping. *Cell* 78:1073–1087.
26. Höglund, P., P. Sistonen, R. Norio, C. Holmberg, A. Dimgerg, K.-H. Gustavson, A. de la Chapelle, and J. Kere. 1995. Fine mapping of the congenital chloride diarrhea gene by linkage disequilibrium. *Am. J. Hum. Genet.* 57:95–102.
27. Melewicz, F. M., R. S. Zeiger, M. H. Mellon, R. D. O'Conner, and H. L. Spielberg. 1981. Increased peripheral blood monocytes with Fc receptors for IgE in patients with severe allergic disorders. *J. Immunol.* 126: 1592–1595.
28. Boccafogli, A., L. Vicentini, D. Lambertini, and R. Scolozzi. 1997. Soluble CD23 is increased in allergy. *Allergy* 52:357–358.
29. Jenkins, M. A., J. L. Hopper, and G. G. Giles. 1997. Regressive logistic modeling of familial aggregation of asthma in 7,394 population-based nuclear families. *Genet. Epidemiol.* 14:317–332.
30. Laitinen, T., M. Räsänen, J. Kaprio, M. Koskenvuo, and L. A. Laitinen. 1998. Importance of genetic factors in adolescent asthma—a population based twin-family study. *Am. J. Respir. Crit. Care Med.* 157:1073–1078.
31. Räsänen, M., T. Laitinen, J. Kaprio, M. Koskenvuo, and L. A. Laitinen. 1998. Hay fever—a nationwide study of adolescent twins and their parents. *Allergy* 53:885–890.

View publication stats