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Variation in the Interleukin 4–Receptor α Gene Confers Susceptibility to Asthma and Atopy in Ethnically Diverse Populations

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Summary

After a genomewide screen in the Hutterites was completed, the *IL4RA* gene was examined as the 16p-linked susceptibility locus for asthma and atopy. Seven known variants and one novel variant, representing all nonsynonymous substitutions in the mature protein, were examined in the Hutterites; on the basis of studies in the Hutterites, outbred white, black, and Hispanic families were genotyped for selected markers. All population samples showed evidence of association to atopy or to asthma (*P* values .039–.0044 for atopy and .029–.0000061 for asthma), but the alleles or haplotypes showing the strongest evidence differed between the groups. Overall, these data suggest that the *IL4RA* gene is an atopy- and asthma-susceptibility locus but that variation outside the coding region of the gene influences susceptibility.

Introduction

Genomewide screens for loci conferring susceptibility to asthma and atopy have recently been completed in the Hutterites, a founder population of European origins (Ober et al. 1998, 1999). Evidence for linkage between D16S401 and an atopic phenotype—that is, positive skin-prick test (+SPT) to at least one allergen—was reported (Ober et al. 1999; $P = .00027$) by use of an association-based test, the transmission/disequilibrium test (TDT) of Spielman et al. (1993). A +SPT is an indication of previous exposure and of an IgE response to the specific allergen. D16S401 was also linked to specific IgE measured in serum in German families (Deichmann et al. 1998). D16S401 is located on 16p12, ~5 cM from the gene encoding the α-chain of the interleukin (IL)–4 receptor, which also serves as the α-chain of the IL-13 receptor (Mak and Simard 1998). IL-4 and IL-13 are pleiotropic T helper cell type 2 cytokines, whose functions overlap and include mediating isotype switching to IgE.

Thirteen single-nucleotide polymorphisms (SNPs) were previously identified in the coding region of the *IL4RA* gene, including seven SNPs that result in amino acid substitutions (Deichmann et al. 1997; Hershey et al. 1997; Kruse et al. 1999; fig. 1). Associations between atopic phenotypes and *IL4RA* SNPs were reported in two case-control studies: Mitsuyasu et al. (1998) reported an association between the ile50 allele and atopic asthma, and Hershey et al. (1997) reported an association between the arg551 allele and hyper-IgE syndrome and atopic dermatitis. (Hershey et al. referred to "arg551" as "arg576" in their study; two systems have been used for numbering the amino acids and nucleotides in this gene [for examples, see Deichmann et al. 1997; Hershey et al. 1997]. In the present study, we followed the recommendations of Shirakawa et al. [in press] and numbered the amino acids beginning with the mature protein.) Both polymorphisms were associated with functional alterations in cellular assays (Hershey et al. 1997; Mitsuyasu et al. 1998, 1999). After a linkage study (Deichmann et al. 1998), Kruse et al. (1999) reported significant associations between the pro478 and arg551 alleles (referred to as "pro503" and "arg576" in their study) and low IgE levels and further demonstrated that these two variants act synergistically to influence signal-transduction pathways through the *IL4RA* gene. Thus, at least three of the seven known amino acid polymorphisms in this gene are associated with functional alterations of the receptor. However, be-

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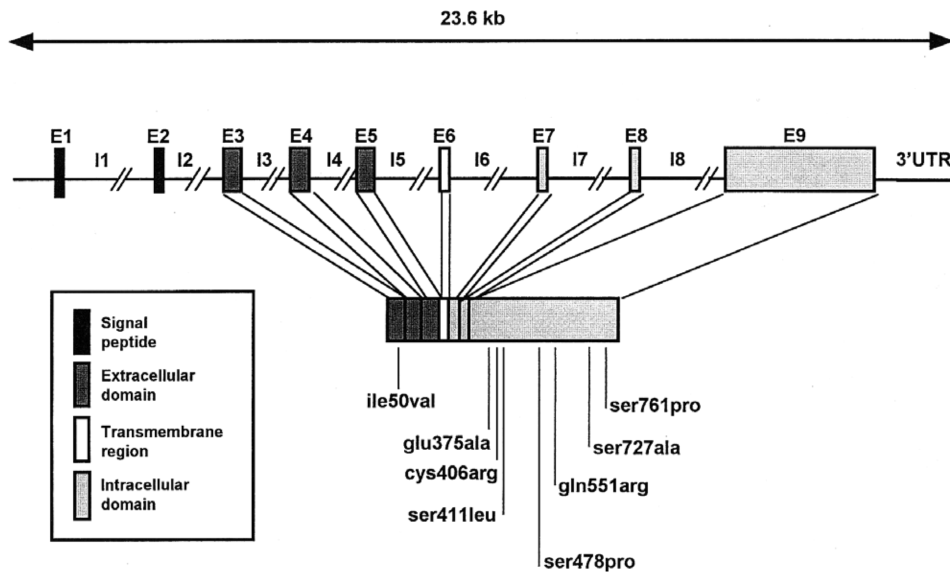

Figure 1 Genomic organization of the *IL4RA* gene on chromosome 16p12 (not drawn to scale). The genomic organization of the gene was determined from the genomic (GenBank accession number AC004525) and cDNA sequences (Idzerda et al. 1990). Exons (E1–E9) are shown as rectangles, and introns (I1–I8) are shown as broken horizontal lines. The sizes of the exons and introns are as follows: E1, 70 bp; E2, 39 bp; E3, 152 bp; E4, 152 bp; E5, 157 bp; E6, 100 bp; E7, 79 bp; E8, 50 bp; E9, 1,579 bp; I1, 1,847 bp; I2, 2,609 bp; I3, 1,446 bp; I4, 5,920 bp; I5, 3,111 bp; I6, 3,007 bp; I7, 1,768 bp; and I8, 1,436 bp. Amino acid polymorphisms in the mature protein are shown. The polymorphism at amino acid 727 was reported in this study; others are from Deichmann and colleagues (Kruse et al. 1990; Deichmann et al. 1997) and Hershey et al. (1997).

cause it is both likely that there is considerable linkage disequilibrium among these polymorphic sites within each population and possible that additional variation exists within this gene, studying only one or two *IL4RA* polymorphisms will not provide definitive information regarding the amino acid (or combination of amino acids) that accounts for the associated functional changes and resulting atopic phenotypes.

We considered the *IL4RA* to be an excellent positional candidate for the D16S401-linked atopy-susceptibility allele in the Hutterites. To evaluate the role of variants in this gene in conferring susceptibility to atopic phenotypes in the Hutterites, we genotyped this sample for the seven SNPs that are associated with amino acid substitutions (i.e., nonsynonymous substitutions) and sequenced all the exons encoding the translated portion of the protein in Hutterites with different *IL4RA* haplotypes. On the basis of results of studies in the Hutterites, we genotyped selected *IL4RA* polymorphisms in ethnically diverse outbred populations that were collected as part of the Collaborative Study on the Genetics of Asthma (CSGA; CSGA 1997). We report here (1) significant associations between variation in the *IL4RA* gene and atopic phenotypes in Hutterites, outbred whites, blacks, and Hispanics and (2) the identification of additional variation in the *IL4RA* gene.

Material and Methods

Sample Composition and Clinical Evaluation

Subjects in all study samples were evaluated by use of the CSGA protocol, described elsewhere (CSGA 1997). In brief, the diagnoses of asthma were based on the following criteria: (1) at least two of three symptoms—cough, wheeze, and/or dyspnea; (2) either (*a*) bronchial hyperresponsiveness, characterized by a fall in baseline forced expiratory volume in 1 s (FEV_1) by ≥20% at ≤25 mg/methacholine ml or (*b*) bronchodilator reversibility, defined as a ≥15% increase in baseline FEV_1 after bronchodilator use; and (3) <3 pack years of cigarette exposure. Skin-prick testing was performed for 14 standardized allergens, including house dust mites (*Der pteronyssinus* and *D. farinae*), cockroaches (*Blattella germanica* and *Periplaneta americana*), molds (*Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus*), pollens (*Lolium perenne*, *Ambrosia artemisifolia*, *Artemisia vulgaris*, *Quercus alba*, and *Betula verrucosa*), and animal dander (*Felis domesticus* and *Canis familiaris*), and to both a negative control (saline) and a positive control (histamine). Purified allergen extracts were provided to all participating CSGA centers by ALK A/S. A skin-prick–test result was considered

positive if the largest diameter of the wheal size was ⩾3 mm larger than that of the negative control (saline).

The Hutterite sample was studied during field trips to colonies in South Dakota. All members of nine colonies who were age ⩾6 years and were home at the time of our visit were included in our study ($n = 694$). This sample included 315 individuals with atopy (i.e., at least one +SPT) and 77 individuals with asthma. Thirty-seven individuals had both asthma and atopy. In the Hutterite sample, there were a maximum of 282 trios (affected child + parents) available for the TDT tests for atopy, and 77 trios were available for the TDT tests for asthma. The CSGA families were ascertained through two affected sibs; families were extended, with no skipping through more than one unaffected (nonasthmatic) relative (Blumenthal et al. 1995). This sample included 73 white families and 56 black families ascertained in Chicago and Baltimore and 30 Hispanic families ascertained in New Mexico. For the purpose of analysis, one affected child with asthma and one affected child with at least one +SPT were randomly selected from each family. The affected child and his or her parents (trios) were included in the TDT. In 72 of the 159 trios, the same child (with both asthma and atopy) was selected as the proband in both analyses.

Genotyping

In the Hutterite and Chicago CSGA samples, genotypes for SNPs in the *IL4RA* gene were determined in dot-blotted DNA hybridized to allele-specific–oligonucleotide probes. Eight SNPs were examined in the Hutterites: nt 148A/G (ile50val), nt 1124A/G (glu375ala), nt 1216T/C (cys406arg), nt 1232C/T (ser411leu), 2281T/C (ser761pro [Deichmann et al. 1997]), 1432T/C (ser478pro), nt 1652A/G (gln551arg [Hershey et al. 1997]), and 2179T/G (ser727ala [identified in this study]). DNA was amplified by use of primers for the extracellular domain from Mitsuyasu et al. (1998) and for the intracellular domain from Hershey et al. (1997) Allele-specific probes were as follows: ile50 5′-ACACGTGTATCCCTGAGA-3′; val50, 5′-ACACGTGTGTCCCTGAGA-3′; glu375, 5′-GGAAGGGAGGGCATTGTG-3′; ala375, 5′-GGAAGGGCGGGCATTGTG-3′; cys406, 5′-CGGRAGAAGRCATGACTCCC-3′ (where R = A and G); arg406, 5′-GGGAGTCACGYCTTCTYCCG-3′ (where Y = C and T); ser411, 5′-CCACCTTCGGGAAGTAC-3′; leu411, 5′-GTACTTCCCAAAGGTGG-3′; gln551, 5′-GTGGCTATCAGGAGTTTG-3′; ser478, 5′-GCTCAGGGAGTTGCTGAA-3′; pro478, 5′-TTCAGCAACCCCCTGAGC-3′; arg551, 5′-CAAACTCCCGATAGCCAC-3′; ser 727, 5′-GGGCGAGGACCTGTCTCC-3′; ala 727, 5′-GGGCGAGGCCCTGTCTCC-3′; ser761, 5′-CTTCTCTGAGATGCCCGA-3′; pro761 5′-TCGGGCATCCCAGAGAAG-3′.

In the Maryland and New Mexico samples, SNPs at glu375Ala and cys406Arg were genotyped by RFLP analysis (with *Cac*8I and *Tsp*45I, respectively), and SNPs at ser478pro and gln551arg were genotyped by allele-specific PCR (FAS-PCR), by use of fluorescent dyes and automated sequencer technology, as described elsewhere (Howard et al. 1999). The ser478-specific primer was (TET) 5′-TGCTTACCGCAGCTTCAGCAACT-3′, and the pro478-specific primer was (FAM) 5′-CTTACCGCAGCTTCAGCAACC-3′. The common reverse primer was 5′-TTTCTGGCTCAGGTTGGGGC-3′. The gln551 allele was (TET) 5′-GGCCCCCACCAGTGGCTATCA-3′, and the primer specific for the arg551 allele was (FAM) 5′-CCCCCACCAGTGGCTATCG-3′. The same reverse primer, 5′-CCAGTCCAAAGGTGAACAAGGGG-3′, was used to detect each of the allele-specific products.

Sequencing Studies and Denaturing High-Performance Liquid Chromatography (DHPLC) Analyses

Exons 3–9 of the *IL4RA* gene were sequenced in nine Hutterites who were homozygous for the four most common haplotypes ($n = 8$) or heterozygous for the 2111111/2221221 haplotypes ($n = 1$). PCR reactions for direct sequencing of genomic DNA were conducted by use of the following primer pairs: exon 3 (364 bp), forward 5′-AAGTCTGATGCGGTTCCTG-3′ and reverse 5′-GCGGCTTCCTCCTGCTGTTG-3′; exon 4 (813 bp), forward 5′-CATACACTTTGCCTTCACTG-3′ and reverse 5′-AGCGGCATGCAGAGATAACT-3′; exon 5 (375 bp), forward 5′-CAGTTACAGAGGTGGCAAGC-3′ and reverse 5′-ACAGGGGAAGAATGGAGAGT-3′; exon 6 (514 bp), forward 5′-GATGAGGTGGAGGGGTGGTC-3′ and reverse 5′-AGGCGGAGGTTACTGTTAGG-3′; exon 7 (419 bp), forward 5′-GGCCTCACTCTCCTCATCTG-3′ and reverse 5′-TCTCCCCTGCAACCCTCCTG-3′; exon 8 (343 bp), forward 5′-CTGCTCTTTCATTGGCTGTC-3′ and reverse 5′-CACGGCTGCTCTGGTTGTTA-3′; exon 9a (1,076 bp), forward 5′-GCCCACACTGGAAGAATTGTCTTAC-3′ and reverse 5′-TTTCTGGCTCAGGTTGGGGC-3′; exon 9b (766 bp), forward 5′-CCGAAATGTCCTCCAGCATG-3′ and reverse 5′-TTTTGGGGGTCTGGCTTGAG-3′. Thermal cycling conditions were 94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C (exons 5 and 6) or 59°C (exons 3, 4, 7, and 8) for 30 s, and 72°C for 30 s; then the final cycle extension, at 72°C for 10 s. Exons 9a and 9b were amplified as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 15 s, 60°C for 1 min 20 s, and 72°C for 15 s. The last PCR cycle was followed by extension for 10 min at 72°C. PCR products were purified by use of QAIquick PCR Purification Kit (Qiagen). Purified product was sequenced by use of ABI Prism BigDye Terminator Cycle Sequencing Ready Re-

action Kit according the manufacturer's directions (PE Applied Biosystems), except that the 50°C cycle was extended to 10 s. All exons were sequenced in both the 5′ and 3′ directions. The 5′ and 3′ PCR primers were used as sequencing primers in addition to the following primers: exon 4, forward 5′-GATACGGGTTGCTCCAG-GTC-3′ and reverse 5′-GGCCGCTTCTCCCGCAG-TGA-3′; exon 9, forward 5′-AAGTCCTCCTGCCA-GCCCGA-3′ and reverse 5′-CCAGTCCAAAGGTGA-ACAAGGGG-3′ and reverse 5′-AAAAGCCCCCAT-TCTCCTCT-3′. Sequences were analyzed by use of SEQUENCER 3.1 software (Gene Codes).

DHPLC (Oefner and Underhill 1995) was used to confirm the lack of variation in all amplicons that were homozygous by sequencing. For these experiments, DNA from all possible pairs of the nine individuals used in the sequencing studies were mixed 1:1 and were analyzed by DHPLC. DNA was amplified by use of the primer pairs described earlier. Amplified DNA was denatured at 95°C and was reannealed to allow heteroduplex formation. Five microliters of each sample were injected into a Dynamax high-performance liquid chromatograph (Varian Chromatography Systems) and were run through an acetylnitrile gradient.

Statistical Analysis

The evidence for linkage and association between each *IL4RA* variant or haplotype with asthma or atopy was evaluated by use of the TDT (Spielman et al. 1993; Spielman and Ewens 1996). Because the Hutterite sample is part of a single large family, the TDT was used as a test of linkage and not association in the Hutterites, as was the case in our previous studies (Ober et al. 1998; 1999). In the CSGA samples, one affected child was randomly selected from each family for inclusion in the TDT.

Allele-specific TDTs were performed by use of alleles at each biallelic polymorphic site. Global TDTs were calculated at each locus for each multiallelic haplotype. In these analyses, transmissions for haplotypes with <10 transmission events were pooled into a single category. The χ^2 values were summed over all haplotype categories at each locus, and the degrees of freedom equaled the number of haplotype categories minus 1. *P* values were not adjusted for multiple comparisons for the following reasons: first, prior evidence from linkage studies in the Hutterites and candidate gene studies in other samples suggested that the *IL4RA* gene was an atopy-susceptibility gene in the Hutterites; second, the individual *IL4RA* alleles and haplotypes are not independent of each other; and third, the outbred samples were used to replicate our findings in the Hutterites and to better define the location of the susceptibility allele(s).

In the Hutterites, haplotypes composed of alleles at the seven sites were constructed by direct observation of the variants segregating in families. In the CSGA families, haplotypes composed of two or more loci were constructed by use of the program HAPLO version 1.00 (Sobel et al. 1996). Linkage disequilibrium between SNP loci was measured by *D'* by use of the program LINKD (Kidd et al. 1998). *D'* was chosen as a measure of linkage disequilibrium because it is relatively insensitive to allele frequencies (Devlin and Risch 1995). Because the Hutterites are all related to each other, it was not possible to determine the number of independent chromosomes in this sample, and, therefore, the significance of *D'* could not be assessed in the Hutterites.

Results

IL4RA *Alleles, Haplotype Frequencies, and Linkage Disequilibrium*

The frequencies of alleles at each of the seven published SNPs in the study samples are available at our Web site (Complex Trait Mapping in the Hutterites; fig. 1). The pro761 allele was rare (frequency .009) in the Hutterites and therefore was neither genotyped in any of the CSGA families nor included in subsequent analyses. In addition, on the basis of results of the TDT analyses (discussed later), the CSGA families from the University of Maryland, which included a Hispanic sample from New Mexico, were not genotyped for SNPs at amino acids 50 and 411. All loci were in Hardy-Weinberg equilibrium in unrelated subjects from the three outbred samples ($P > .10$ in all samples at all loci). Because the Hutterites are all related to each other and it is not possible to determine the number of independent chromosomes in the Hutterite sample, Hardy-Weinberg estimations were not performed in the Hutterites.

Six-locus haplotypes in the Hutterites and in the black and white CSGA families from Chicago are shown in table 1. Seventeen different haplotypes were present in the sample, but only eight were common to all three groups. All haplotypes observed in the Hutterites were present in at least one of the other samples, whereas the blacks had one and the whites had two private haplotypes. In the Hutterite and outbred whites, the most common haplotype (table 1) was the haplotype carrying the allele in the reference sequence (this allele, 111111, was referred to as the "wild-type allele") at each locus, and the second most common haplotype was 211111, which differs from 111111 by having a valine instead of an isoleucine at amino acid 50. Haplotype 111111 was predominant in the Hutterites (haplotype 111111, frequency .466; haplotype 211111, frequency .292), whereas the two haplotypes were at nearly equal frequencies in the outbred whites (haplotype 111111, frequency .382; haplotype 211111, frequency .376). In the blacks, 211111 was the most common (frequency .194) and 111111 the second most common (frequency .129) haplotype . The leu411 allele occurred at low frequencies

Table 1

Frequencies of Six-Locus Haplotypes in the Hutterites and Chicago CSGA Families

Haplotype	Frequency of Six-Locus Haplotypes in[a]		
		Chicago CSGA Families	
	Hutterites (*n* = 1,044)	Blacks (*n* = 108)	Whites (*n* = 170)
111111	.466	.129	.382
111112	.023	.111	.029
111121	.003	.019	.029
111222	.002	.028	.006
121112	.002	.056	.0
121122	.002	.037	.006
122122	.035	.074	.041
211111	.292	.194	.376
211112	.023	.019	.012
211121	.0	.018	.029
211122	.0	.064	.0
211222	.051	.0	.053
221112	.003	.102	.0
221121	.0	.0	.006
221122	.015	.148	.0
222121	.0	.0	.006
222122	.084	.0	.024

[a] Frequencies in the Hutterites are based on the entire sample; frequencies in the CSGA families are based on unrelated individuals. Allele "1" corresponds to the allele in the reference sequence (ile50, glu375, cys406, ser411, ser478, and gln551), and "2" corresponds to the alternative allele (val50, ala375, arg406, leu411, pro478, and arg551). *n* = no. of chromosomes in each sample.

in all samples and was present on only two haplotypes in the Hutterites (haplotype 111222, frequency .002; haplotype 211222, frequency .051). Therefore, this polymorphism was not included in further analyses.

Linkage disequilibrium between alleles at the SNP loci in the outbred samples are shown in table 2. There was little evidence for linkage disequilibrium between alleles at the extracellular polymorphism (amino acid 50) and those at the remaining polymorphisms in the intracellular domain in blacks and whites, with the exception of alleles at amino acids 50 and 406 in blacks ($P = .005$). A potential explanation for this exception is the fact that the arg406 allele is present on only a single haplotype (122122) in the black sample but is present on three haplotypes in whites. In contrast, combinations of alleles at all pairs of intracellular SNPs in the CSGA samples and at all pairs of SNPs except amino acids 406 and 551 in blacks were in significant linkage disequilibrium.

Associations with IL4RA *SNPs and Haplotypes in Hutterites*

Evidence for linkage to atopy and asthma in the Hutterites was examined by use of the TDT (table 3). Alleles at amino acids 50, 375, and 406 were nonrandomly transmitted to subjects with atopy ($P = .025$, .021, and .0051, respectively), and alleles at amino acids 375 and 406 were nonrandomly transmitted to subjects with asthma ($P = .0060$ and .011, respectively). The ile50 allele was undertransmitted to affected individuals, whereas, at all other amino acids, the wild-type allele was overtransmitted to affected individuals.

To determine whether amino acids at more than one position interact to confer susceptibility, we examined associations with pairwise combinations of loci. The *P* values are presented in table 4; the number of transmissions and nontransmissions for each pair of SNPs is available at our Web site (Complex Trait Mapping in the Hutterites). In the Hutterites, nearly all pairwise combinations of alleles, other than those including alleles at gln551arg, showed modest overtransmission to individuals with atopy ($P < .05$; table 4, upper half). None of these combinations of alleles were more significantly associated with atopy than was the cys406 allele by itself. For example, the transmitted alleles:nontransmitted alleles (TR:NT) ratio for the cys406 allele was 60:33 (1.8:1), whereas the TR:NT ratios for the most associated two-locus haplotypes were 120:91 (1.3:1) for the val50–glu375 haplotype and 67:46 (1.5:1) for the glu375–cys406 haplotype. Furthermore, no other combinations of haplotypes composed of alleles at three to five adjacent SNPs were more significantly associated with atopy than was the cys406 allele in the Hutterites (data not shown).

Pairwise combinations of alleles at all loci were highly significantly associated with asthma in the Hutterites, with global *P* values as small as 6.1×10^{-6} for the glu375-ser478 haplotype, and were much more significantly associated with asthma than were any of the in-

Table 2

Linkage Disequilibrium between All Pairs of Loci

Ethnic Group and Amino Acid	Linkage Disequilibrium[a] in Amino Acid (D')				
	50	375	406	478	551
Blacks:					
50		.120	1.0[*]	.107	.092
375			1.0[*]	.314[**]	.598[*]
406				1.0[*]	.376
478					.521[*]
Whites:					
50		.144	.166	.169	.028
375			1.0[*]	1.0[*]	.943[*]
406				1.0[*]	1.0[*]
478					.753[*]
Hispanics:					
375			1.0[*]	.843[*]	.903[*]
406				1.0[*]	1.0[*]
478					.918[*]

[a] Calculated for 92 unrelated blacks, 177 unrelated whites, and 56 unrelated Hispanics.

[*] $P = .001$.

[**] $P = .005$.

Table 3

TDT Results with Alleles at Polymorphic Sites in the Hutterites

IL4RA Allele	TR:NT	*P*
Atopy:		
val50	125:92	.025
glu375	52:31	.021
cys406	60:33	.0051
ser478	38:35	>.05
gln551	75:66	>.05
Asthma:		
val50	34:30	>.05
glu375	20:6	.0060
cys406	22:8	.011
ser478	14:7	>.05
gln551	23:12	>.05

dividual alleles (tables 3 and 4). The significant associations between nearly all pairs of loci in Hutterites are likely caused by the extensive linkage disequilibrium that is present in this founder population. However, no other combinations of haplotypes composed of alleles at three to six adjacent SNPs were more significantly associated with asthma in the Hutterites than were the two-locus haplotypes shown in table 4 (e.g., see table 5; data on other haplotypes not shown).

The results of the TDT for the six-locus haplotypes with asthma in the Hutterites are shown in table 5. The haplotype with a valine at amino acid 50 and with wild-type alleles at all other sites (211111) was significantly overtransmitted to affected individuals ($P = .0095$). All other haplotypes with a valine (or "2" allele) at amino acid 50 were significantly undertransmitted to affected individuals ($P < .05$). This would suggest that asthma susceptibility is associated with one or more wild-type alleles at the intracellular polymorphic sites. However, random transmission of haplotype 111111 to affected individuals (32 transmissions, 32 nontransmissions) suggests that the "1" alleles at the known intracellular sites are not asthma-susceptibility allele(s) per se but must be in linkage disequilibrium with the true susceptibility allele. Furthermore, these studies indicate that the asthma-susceptibility variant likely resides on haplotype 211111 in the Hutterites.

Sequencing Studies in the IL4RA *Gene*

To identify additional variation in the *IL4RA* gene, DNA from distantly related Hutterites who were homozygous for haplotypes 111111 ($n = 2$), 211111 ($n = 2$), 222122 ($n = 2$), or 211222 ($n = 2$) or who were heterozygous for haplotypes 211111/222122 ($n = 1$) were selected for sequencing studies. Exons 3–9 of the *IL4RA* gene, corresponding to the mature protein, were sequenced in these samples (fig. 1). One additional polymorphic site that resulted in an amino acid substitution (ser727ala) was identified in exon 9, at nt 2179 (T/G). No additional novel variation in the other exons was identified by sequencing or by DHPLC (Oefner and Underhill 1995). The frequency of the ala727 allele was .053 in the Hutterite sample, and this allele was present on the following three haplotypes in table 1: 111111 (frequency .001), 111222 (frequency .002), and 221122 (frequency .050). The low frequency of this allele in the Hutterite sample excluded it as an asthma-susceptibility allele in this population.

Studies in Outbred Samples

None of the alleles at the five SNPs were significantly overtransmitted to atopic offspring in any of the individual or pooled outbred samples (table 6), although alleles at the intracellular loci showed a modest degree of overtransmission in the white and Hispanic samples. In the black families, there is nearly equal transmission of alleles at each locus. In the pooled sample, there was overtransmission of the glu375 allele, but this did not reach statistical significance ($P = .063$). Among the individuals with asthma, there was significant overtransmission of the gln551 allele in the small Hispanic sample ($P = .034$) and in the pooled sample ($P = .039$). The ser478 allele was modestly overtransmitted in all samples but was not statistically significant in any of the individual or pooled samples.

The two-locus haplotypes provided stronger evidence of association to atopy and asthma in the CSGA samples (table 7). The results for the individual ethnic groups are available at our Web site (Complex Trait Mapping in the Hutterites). In the pooled outbred sample, all combinations of intracellular SNPs showed significant evidence of overtransmission to individuals with atopy, and all combinations except glu375-cys406 showed evidence of overtransmission to asthma. On the basis of both the *P* values and the actual transmission ratios, the two-locus haplotypes were nearly equally associated with atopy and asthma in the outbred families, but the association with atopy was stronger in the outbred families than in the Hutterites, whereas the associations with asthma were stronger in the Hutterites than in the outbred families.

Discussion

Elucidating the genetic basis of common complex diseases, such as asthma and atopy, is currently one of the major challenges in human genetics. Unlike single-gene, Mendelian traits, in which highly penetrant, rare mutations with large phenotypic effects are causally related to disease, susceptibility to complex diseases is caused by the small individual effects of many low-penetrant,

Table 4

***P* Values Corresponding to Global TDT Analysis of Two-Locus *IL4RA* Haplotypes (over All Haplotype Transmissions), for Each Pair of Loci in the Hutterites**

	P (No. of Transmission Events) for				
Amino Acid	50	375	406	478	551
50		.039 (596)	.023 (604)	.022 (596)	NS
375	.0018 (158)		.028 (226)	.025 (290)	NS
406	.01 (160)	.000084 (52)		NS	NS
478	.00013 (156)	.0000061 (68)	.00002 (74)		NS
551	.0028 (170)	.00044 (80)	.00031 (80)	.000012 (78)	

Note.—Transmission ratios for all haplotypes are available on our Web site (Complex Trait Mapping in the Hutterites). NS denotes $P > .05$.

common alleles. Extensive variation in human genes that are associated with common diseases has been reported elsewhere (Deichmann et al. 1997; Nickerson et al. 1998; Rieder et al. 1999), and it is likely that these levels of variation will be characteristic of many loci in the human genome that are associated with disease. As indicated in the present study and by Clark et al. (1998), variants within single genes are also likely to be in linkage disequilibrium in human populations, making it particularly difficult to sort out the effects that individual variants have on disease susceptibility. Furthermore, disease-associated variants may be on different haplotype backgrounds in different populations because of human population history and structure.

Variants in the *IL4RA* gene had previously been reported to be associated with atopic phenotypes in three case-control studies (Hershey et al. 1997; Mitsuyasu et al. 1998; Kruse et al. 1999). In these studies, only one (Hershey et al. 1997; Mitsuyasu et al. 1998) or two (Kruse et al. 1999) *IL4RA* variants were examined. The present study examined all known nonsynonymous substitutions in the exons encoding the mature protein and has provided further insights into the genetic basis of atopy and asthma. We found significant associations between *IL4RA* alleles or haplotypes and asthma and atopy in Hutterites and in outbred samples, indicating that variation in this gene influences these phenotypes. The fact that linkage disequilibrium did not extend beyond the intracellular domain in the outbred families indicates that the association is likely with variation in the *IL4RA* gene per se and not with variation in a nearby gene. However, the results of this study further suggest that variation outside the coding region of the *IL4RA* gene is likely contributing to disease susceptibility. This is supported by two observations. First, no individual SNP or combination of SNPs is primarily associated with asthma or atopy in all samples studied. Second, examination of the transmission of the six-locus haplotypes in the Hutterites suggests that the asthma-susceptibility variant(s) is on haplotype 211111 but is not any of the SNPs comprised by this haplotype (table 5). Furthermore, no additional variation was detected, in exons 3–9 of the gene, that could account for the observed associations in the Hutterites, indicating that disease risk is associated with variation in other parts of this gene, such as the 5′-promoter region, the 3′UTR, or the introns. Studies are under way to assess variation in these regions. Nonetheless, these findings illustrate the necessity of examining all variation within candidate genes in multiple population samples, to obtain the complete spectrum of variation and disease susceptibility.

Table 5

Results of TDT with Six-Locus Haplotypes and Asthma in the Hutterites

Haplotype[a]	TR:NT	χ^2	*P*
111111	32:32	.0	1.0
211111	30:12	6.72	.0095
222122	4:13	4.76	.029
Other	8:16	4.00	.046
Global		15.48[b]	.0014

[a] For an explanation of the haplotype designations, see legend of table 3. Alleles at intracellular SNPs are underlined. All haplotypes with <10 TR + NT events ($n = 6$) are included in "other."

[b] 3 df.

Not unexpectedly, there was little linkage disequilibrium between the extracellular SNP at amino acid 50 and SNPs in the intracellular region in outbred samples (table 2), regions that are separated by ≈24 kb. However, it is noteworthy that associations with asthma or atopy were not detected with most SNPs in the intracellular domain (exon 9) in these samples, despite the fact that significant linkage disequilibrium exists between these SNPs (table 2). Thus, an SNP map containing only 100,000 markers (Collins et al. 1997), which would be spaced, on average, every 33,000 bp, could have missed identification of associations with variation in the *IL4RA* gene in the outbred samples. For example, the gln551arg locus, which had been associated with atopic phenotypes in previous studies (Hershey et al. 1997; Kruse et al. 1999), was not by itself associated with

Table 6

TDT Results with *IL4RA* Alleles at Polymorphic Sites in the Outbred Samples

	Blacks		Whites		Hispanics		Pooled Samples	
IL4RA Allele	TR:NT	*P*	TR:NT	*P*	TR:NT[a]	*P*[b]	TR:NT	*P*
Atopy:								
ile50[c]	9:11	>.05	18:20	>.05	NG	…	27:31	>.05
glu375	18:15	>.05	12:6	>.05	10:4	>.05	40:25	>.05
cys406	5:6	>.05	12:6	>.05	8:4	>.05	25:16	>.05
ser478	16:16	>.05	21:11	>.05	9:5	>.05	46:32	>.05
gln551	14:12	>.05	15:12	>.05	8:1	NI	37:25	>.05
Asthma:								
ile50[c]	11:10	>.05	21:18	>.05	NG	…	32:28	>.05
glu375	17:18	>.05	11:8	>.05	10:3	.052	38:29	>.05
cys406	4:9	>.05	11:8	>.05	8:2	.057	23:19	>.05
ser478	22:17	>.05	20:15	>.05	8:4	>.05	50:36	>.05
gln551	17:14	>.05	21:13	>.05	9:2	.034	47:29	.039

[a] NG = not genotyped.
[b] NI = not informative (<10 transmission events).
[c] Polymorphisms at AA50 were genotyped in Chicago families only.

either atopy or asthma in the Hutterites or outbred whites or blacks (tables 3 and 6). If we had focused only on this variant in a single sample, we could have incorrectly excluded this gene as an asthma- or atopy-susceptibility locus. Even in the Hutterites, a founder population with few founders and recent origins (Ober et al. 1998), associations could have been missed by use of 100,000 SNPs spaced evenly across the genome. In contrast, two-locus SNP haplotypes were more informative for association studies and provided much better evidence for linkage to allergic and asthmatic phenotypes in the Hutterites and in the outbred samples than did the individual SNPs. Thus, identification of multiple SNPs per gene, for association-based studies, may indeed be a reasonable strategy for identification of common variants that have small effects on disease risk (Risch and Merikangas 1996), as has been suggested by computer simulation studies (Kruglyak 1999).

Last, our studies in the Hutterites provide some clues about the relationship between the related phenotypes of +SPT and asthma. These two phenotypes are highly associated in individuals and in families (Burrows et al. 1989; Platts-Mills et al. 1991). However, it is not clear whether they are variable expressions of the same primary defect or whether they share common pathways but have distinct primary molecular etiologies. Our data in the Hutterites suggest the latter. The Hutterite sample is particularly useful for sorting out these differences, because only ~50% of Hutterites with asthma are atopic (Ober et al. 1999), whereas 75%–85% of the CSGA subjects with asthma are atopic (CSGA 1997). This likely explains why the same two-locus haplotypes show similar evidence of association with both atopy and asthma in the CSGA families (table 7). In the Hutterites, however, different patterns of variation are seen with each phenotype. For example, there were relatively equal and modest nonrandom transmissions, to atopic individuals, of haplotypes at all pairs of loci, except those including the gln551arg alleles, with *P* values of .02–.04 (table 4). The cys406 allele by itself showed the most evidence for overtransmission to atopic individuals ($P = .0051$). In contrast, the most significant nonrandom transmissions to asthmatic individuals were with haplotypes composed of alleles at the glu375ala or the ser478pro loci and other loci, including the gln551arg locus, with *P* values $<1 \times 10^{-4}$. The most significant result was for the two-locus haplotype composed of alleles at these two loci ($P = 6.1 \times 10^{-7}$; table 4). Furthermore, the same pattern of overtransmission is present in both the atopic and nonatopic asthmatics (data not shown), indicating that the variants conferring risk for asthma are not atopy susceptibility alleles per se. We predict, on the basis of these results, that the underlying molecular lesions in the *IL4RA* gene that are associated

Table 7

***P* Values Corresponding to Global TDT Analysis of Two-Locus Haplotypes (Overall Haplotype Transmissions) for Each Pair of Loci in Outbred Families**

Amino Acid	*P* (No. of Transmission Events) for			
	375[a]	406	478	551
375		.0050 (114)	.0091 (158)	.038 (130)
406	NS		.038 (138)	.018 (124)
478	.029 (160)	.0063 (136)		.0074 (120)
551	.041 (134)	.020 (134)	.028 (138)	

Note.—The transmission ratios for all haplotypes are available at our Web site (Complex Trait Mapping in the Hutterites).
[a] NS denotes $P > .05$.

with atopy are not identical to those associated with asthma. This hypothesis is plausible, given the highly pleiotropic nature of the IL-4 and IL-13 cytokines, both of whose receptors use a common α-chain encoded by the *IL4RA* gene. IL-4 and IL-13 play a major role in the regulation of IgE, a marker of atopic disease, and polymorphisms in the *IL4* gene on chromosome 5q have been associated with atopic phenotypes (Marsh et al. 1994; Rosenwasser et al. 1995). However, recent studies in mouse models have suggested that the expression of IL13 in the lung may be involved specifically in the pathogenesis of asthma, through an IL-4–independent pathway (Wills-Karp et al. 1998; Zhu et al. 1999). Thus, it is possible that variation in the *IL4RA* gene influences atopy through an IL-4–mediated pathway and influences asthma through an IL-13–mediated pathway. Variants influencing both phenotypes may be present—more often than would be expected by chance—on the same *IL4RA* haplotypes because of linkage disequilibrium, thus contributing to the clinical associations between atopic phenotypes and asthma.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Complex Trait Mapping in the Hutterites, http://www.genes.uchicago.edu/hutterite

GenBank, http://www.ncbi.nlm.nih.gov/Genbank (accession number AC004525)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for IL4RA-associated atopy and asthma [MIM 147781])

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