Chapter 7 | Fine-mapping of an increased Total IgE susceptibility gene on chromosome 2q: Analysis of CTLA-4 and CD28

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Abstract

Asthma and allergy are complex diseases caused by an interaction between host susceptibility factors and environmental exposures. Genetic studies have identified several chromosomal regions that may contain genes that contribute to asthma, atopy, or both. Evidence for linkage of high total serum IgE levels and other allergy-associated phenotypes has been observed to a region on chromosome 2q in multiple populations. Two candidate genes in this region are CTLA-4 and CD28, which function together to regulate a key control point for IgE synthesis and regulation. T-cells recognize antigen-presenting cells by the antigen bound to MHC class II molecules, but this binding alone is insufficient to activate T-cells. Co-stimulation by other receptor-ligand complexes facilitates efficient and appropriate activation of T-cells. Two of the main co-stimulation complexes are the B7-1 (CD80) and B7-2 (CD86) ligands and CD28 and CTLA-4 receptors. We have sequenced the coding region of both of these genes and identified two novel SNPs in CTLA-4 and three in CD28. These polymorphisms, in addition to the two existing SNPs in CTLA-4, were analyzed in a well-characterized population of Dutch families ascertained through a proband with asthma that was initially studied 25 to 35 years ago. Using the probands and their spouses from this population in a case-control study design, we observed significant associations of atopy and asthma related phenotypes with two of the CTLA-4 polymorphisms. Significant evidence for an association was observed with the –1147 C/T SNP to asthma and BHR ($p = 0.005 – 0.007$), but not to the allergy-related phenotypes. The Thr17Ala SNP (+49 A/G) in exon 1 was significantly associated with all four phenotypes examined in this population, driven by individuals that were homozygous for the Thr (A) allele. These data suggest that the co-stimulation pathway, and specifically the role of CTLA-4, is important in the development of atopy and asthma related phenotypes.

Introduction

Asthma and allergy are complex diseases caused by an interaction between host susceptibility factors and environmental exposures. Bronchial hyperresponsiveness and elevated total serum IgE levels are phenotypes that predispose individuals to the development of allergy and asthma.[1-4] Genetic studies have identified several chromosomal regions that may contain genes that contribute to asthma, atopy, or both. Evidence for linkage of high total serum IgE levels and other allergy associated phenotypes have been observed in different populations: chromosomes 2q [5-8], 5q [9-11], 11q [11-13] and 12q.[14,15] Each of these chromosomal regions contains several important candidate genes that are biologically relevant to IgE regulation.

A key control point for IgE synthesis and regulation is the necessity for co-stimulation and activation of T-cells. T-cells recognize antigen-presenting cells by the antigen bound to MHC class II molecules, but this binding alone is insufficient to activate T-cells. Co-stimulation by other receptor-ligand complexes facilitates efficient and appropriate activation of T-cells. Two of the main co-stimulation complexes are the B7-1 (CD80) and B7-2 (CD86) ligands with CD28 and cytotoxic T-lymphocyte associated gene 4 (CTLA-4) receptors. CD28 is constitutively expressed on T-cells, and acts as a positive co-stimulator of T-cell activation. CTLA-4 is only expressed on activated T-cells, and acts as a negative feedback regulator of T-cell activation. For instance, CTLA-4 deficient mice have elevated immunoglobulin levels.[16] Therefore, these two receptors maintain a homeostasis in T-cell activation

Because of its role in activation and the downstream effects of this process, alteration of the co-stimulatory pathway could result in susceptibility to immunologic diseases. A polymorphism in the promoter[17]or the first exon (referred to as the +49 A/G or T17A) have been shown to be associated with type 1 diabetes[18-21], autoimmune thyroid disease[18], celiac disease[22], Grave's disease[23], and multiple sclerosis.[24;25] These studies suggest a major involvement of CTLA-4 in the pathogenesis of different immunologic diseases. In addition, CTLA-4 maps within the candidate region on chromosome 2q33 that we have reported with total serum IgE levels in a Dutch asthma cohort.[5] Association studies with a number of immunologic diseases suggest that CTLA-4 may be a susceptibility gene in the development of T-cell inflammatory responses in allergic asthma.

In a cohort of 200 Dutch families that was originally ascertained for asthma, we have performed fine-mapping on chromosome 2q with microsatellite genetic markers and analyzed CTLA-4 and CD28 as candidate genes for asthma and allergy phenotypes. In addition to previously described polymorphisms, we have identified two novel SNPs in the 5' putative promoter region of CTLA4 and three novel SNPs in the 5' region (promoter and exon 1) of CD28. We have determined and evaluated the contribution of CTLA-4 and CD28 on asthma and allergic phenotypes in this population.

Material and Methods

Population

This population has been described in detail previously.[5,9,26,27] Families were ascertained through a proband with clinical asthma initially characterized between 1962-1975. Between 1990 and 1998, 200 probands with asthma (together with their spouses, children and available grandchildren) were restu died. Briefly, all individuals underwent spirometry, bronchodilator reversibility to 800 mg albuterol, and bronchial responsiveness testing to histamine using a 30 second inhalation protocol.[27,28] For atopy, adult subjects had intracutaneous skin testing with 16 common aeroallergens and total serum IgE levels were measured. In the first 92 families, total serum IgE levels were measured by solid-phase immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). Duplicate measurements were made and the mean for each subject was used. If the duplicate samples differed by more than five percent the test was repeated. In the second set of 108 families, total IgE levels were measured by enzyme linked fluorescence assay (Mini Vidas, Biomerieux, Inc.). Although entire families were ascertained for linkage studies, the probands and spouses represent an appropriate cohort for this case-control association study. Probands and spouses are similar in age, removing the confounding effect of age on changes in BHR, IgE and atopy. This study was approved by the Medical Ethics Committee at the University of Groningen and the Institutional Review Board at Wake Forest University School of Me dicine. All subjects provided written informed consent.

Molecular Methods

CTLA-4 was sequenced in 32 unrelated African-American individuals with mild and severe asthma. All four exons and 1200 bp of the 5′ putative promoter region were sequenced, using primers (Table 1) designed from the available genomic sequence (GenBank accession number AF225900). Sequencing was performed using BigDye terminator chemistry (ABI) and an ABI 3700 DNA Analyzer (ABI, Inc., Foster City, CA). Potential 5′ promoter binding sites were determined using MatInspector from Genomatix (genomatix.gsf.de/cgi-bin/matinspector/matinspector.pl) with both of the –994 and –1147 alleles. CD28 was sequenced using similar techniques and three novel SNPs were detected. Since all three of these SNPs were in linkage disequilibrium in the individuals that were sequenced, only one of these (-824 A/G) was genotyped in the entire proband-spouse population.

Microsatellite genetic markers were genotyped using fluorescently labeled oligonucleotide primers. PCR products were pooled, run on a 3700 DNA Analyzer (ABI, Inc.), and scored using Genotyper software (ABI, Inc.). A modified version of Linkage Designer (dnalab www.uia.ac.be/dnalab /ld.html) was used to bin alleles and check for inheritance inconsistencies. The output from Linkage Designer was then analyzed further for any inconsistencies by running the LINKAGE software without disease information. The final check that was performed on the data was to run CRIMAP[29] to de-

termine the order and length of the chromosomal map and to detect double recombinants. SNPs were genotyped using PCR and RFLP analysis. The primers and annealing temperatures for each specific SNP are reported in Table 1.

Table 1. Primers for sequencing and genotyping CTLA4

Sequencing primers

Name	Sequence	Location
CTLA4PR1F	5'-GCTGAGGTGTGGACAATGG-3'	5'
CTLA4PR1R	5'-TCAGGTGTTCTTAAAAGCCTTAAC-3'	
CTLA4PR2F	5'-CTTGAATCATTTGGGTTGGC-3'	5'
CTLA4PR2R	5'-AAGTGGAGACTTGGAGAATTTCC-3'	
CTLA4PR3F	5'-TGGTTAAGGATGCCCAGAAG-3'	5'/exon 1
CTLA4PR3R	5'-AGGTAGGAGAAACACCTCCTCC-3'	
CTLA4E2AF	5'-AAGCTAGAAGGCAGAAGGGC-3'	exon 2
CTLA4E2AR	5'-CACCCACAATAAGCAAGGCT-3'	
CTLA4E3AF	5'-ATGTTGGGGACTAGAGCCCT-3'	exon 3
CTLA4E3AR	5'-TCCTTCCTCTTCATTTATTGCC-3'	
CTLA4E4AF	5'-ATTTTTAACCAGCTAGGGACCC-3'	exon 4
CTLA4E4AR	5'-CATTTCGGCTATAAACGTCTCA-3'	

Genotyping Primers

SNP	Primer Sequences	Annealing Temp.	Restriction Enzyme	Reference
-1147 C/T	5'-GCTGAGGTGTGGACAATGG-3' 5'-GTTAAGGCTTTTAAGAACACCTGA-3'	60°C	*Fok* I	This report
-994 A/G	(same as -1147 C/T)	60°C	*Hsp* 92II	This report
-318 C/T	5'-AAATGAATTGGACTGGATGGT-3' 5'-TTACGAGAAAGGAAGCCGTG-3'	62°C	*Mse* I	Deichmann et al. 1996
+49 A/G (A17T)	5'-AAGGCTCAGCTGAACCTGGT-3' 5'-CTGCTGAAACAAATGAAACCC-3'	65°C	*BstE* II	Marron et al. 1997
3' UTR TA rpt	5'-fam-GCCAGTGATGCTAAAGGTTG-3' 5'-AACATACGTGGCTCTATGCA-3'			

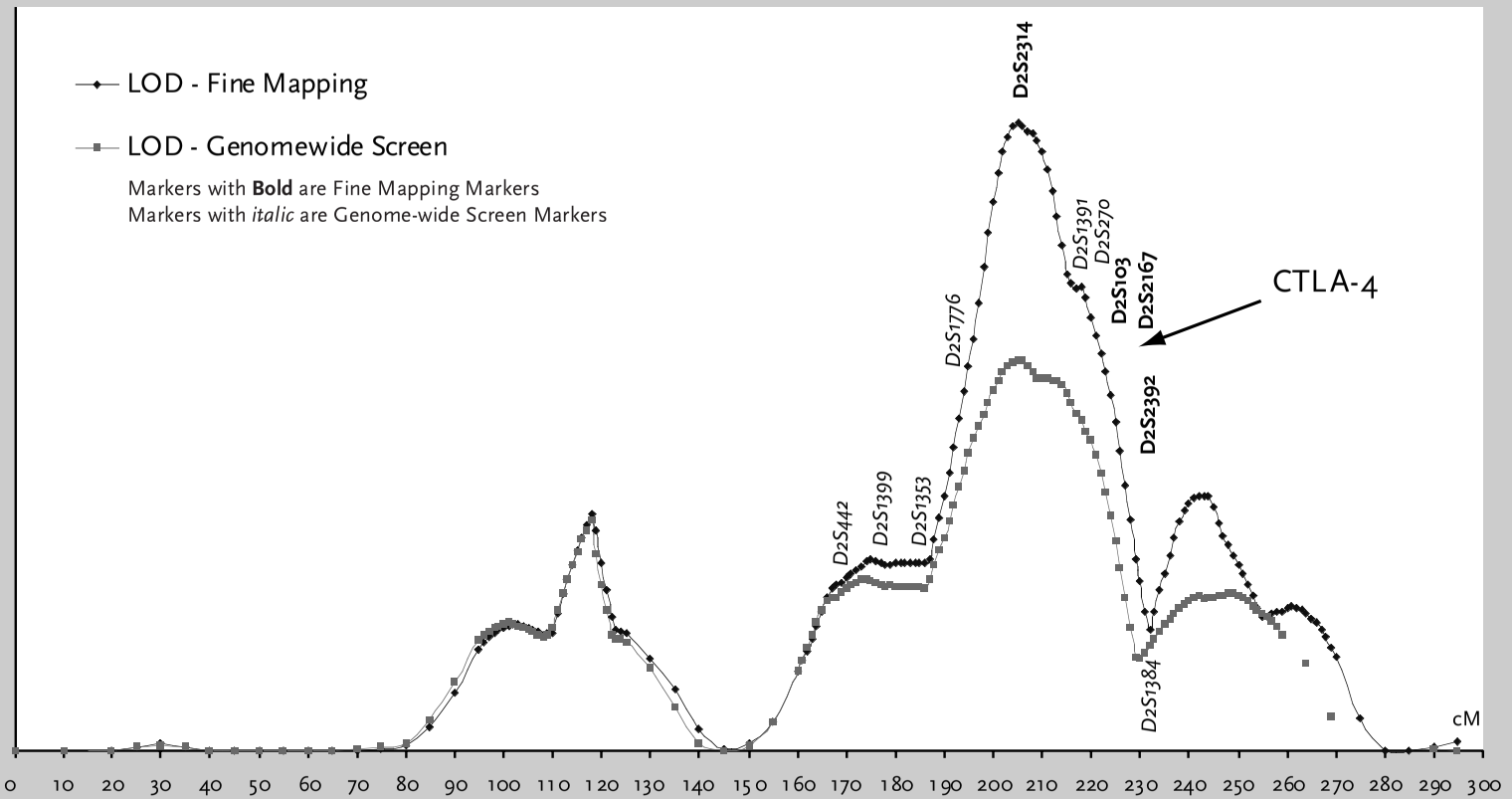
Genetic Analysis

Fine mapping on chromosome 2 was performed using an additional 9 markers and the SNPs genotyped in both CTLA-4 and CD28. Variance component linkage analysis was performed using the computer program package Sequential and Oligogenic Linkage Analysis Routines (SOLAR). The same analysis was performed for the genome wide screen for total serum IgE levels and is described in more detail in Xu et al, 2000.

Because of the previous associations of CTLA-4 with immunologic diseases, its role in co-stimulation of T-cell activation, and our linkage data suggesting an allergy susceptibility gene on chromosome 2q33 in this same population, our primary hypothesis was that polymorphisms in this gene would alter allergy phenotypes. We therefore investigated association with total serum IgE levels and skin test responsiveness to common allergens, but also examined the closely related phenotypes asthma and BHR. As described previously, all of the probands met published criteria for a diagnosis of asthma.[27] Total serum IgE was logarithm-transformed to approximate a normal distribution and analyzed as a quantitative trait. Differences between groups were tested with ANOVA, t-test, and multiple regression. Individuals were considered responsive to an allergen skin test if one or more test showed a mean wheal diameter of ≥ 5mm. For the asthma phenotype, only probands were included in the case group, whereas for BHR, cases were defined as original probands and spouses with a $PC_{20} \leq 32$ mg/ml histamine. The control group for both BHR-positive and asthma cases consisted of BHR-negative spouses ($PC_{20} > 32$ mg/ml). Each of the biallelic polymorphisms was analyzed by comparing differences in genotype frequencies between cases and controls. Chi-square tests assuming a dominant model were performed, due to the small number of homozygotes for the rare allele. No corrections were made for multiple comparisons for two reasons. First, the phenotypes tested (asthma, BHR, total serum IgE levels, and skin test response) are strongly associated with each other in this population and, therefore, the statistical analyses do not represent independent tests. Second, we performed tests for association with phenotypes that have been observed by other investigators, both to confirm previous results and to better characterize susceptibility to asthma and atopic phenotypes in our population.

Linkage disequilibrium testing between SNPs was performed using an exact test assuming multi-nominal probability of the multi-locus genotype, conditional on the single-locus genotype.[30] A Monte Carlo simulation was used to assess significance, by permuting the single-locus genotypes among individuals in the sample to simulate the null distribution. The empirical p-va lues of the LD for each pair of SNPs was based on 10,000 replicate samples.

Figure 1 Major Gene regulating age and sex adjusted Log(IgE) Levels in 200 Dutch Asthma Families.



Results

Our previous genome screen in this population revealed evidence for linkage of total serum IgE levels to several chromosomal regions, including 2q31-q33.[5] Nine additional genetic markers were added to this region and reanalyzed. The evidence for linkage on chromosome 2q33 to total serum IgE levels increased from 1.96 with the genome screen data to 3.16 with the addition of the new markers (Figure 1). With this new genetic data, the chromosome 2 linkage accounts for 36% of the total variance in total serum IgE levels in these Dutch families.

Table 2. Clinical characteristics of dutch proband/spouse population*

	Probands	Spouses
Sex, M:F	124:76	76:125
Age, mean ± SD	52.1 ± 8.4	51.0 ± 9.2
IgE		
Total IgE, IU (geometric mean)	93.0	26.2
% ≥ 100 IU/ml	72.5	15.4
Skintests		
% with ≥ 1 positive skintest	81.9	31.0
% positive with specific IgE, house dust mite	75.7	30.0
FEV_1		
% Predicted Pre-Medication (mean)	69.6	98.4
% Predicted Post-Medication (mean)	82.4	103.9
Reversibility		
%, ≥ 15% (baseline)	59.4	6.5
%, ≥ 9% (predicted)	62.9	18.9
Airway Obstruction		
% FEV_1 /VC ≤ 70% and FEV_1 ≤ 75%	51.0	3.0
BHR †		
PC_{20} ≤ 32 mg/ml, %	88.2	25.6

* Total sample population consisted of 200 probands and 201 spouses. Different numbers for the SNPs in the following tables are due to missing genotype data.
†Thirty probands were not retested due to an FEV_1 that was too low to be tested safely (FEV_1 ≤ 40% predicted).

CTLA-4 is located between the genetic markers D2S103 and D2S2392 based on data from The SNP Consortium (snp.cshl.org), placing it on the distal shoulder of the chromosome 2 linkage peak (Figure 1). Due to the functional and positional information available for CTLA-4, we evaluated it as a candidate gene for regulating total serum IgE levels. Sequencing of 1200 bp of the putative 5′ promoter region and all four exons revealed two novel single nucleotide polymorphisms, both located in the 5′ region of CTLA-4. These SNPs are identified as –994 A/G and –1147 C/T, relative to the translation start site (+1) (Figure 2). The –994 A/G substitution polymorphism was detected in the African-American asthma population (used for sequencing only), but was not detected in the Dutch probands and spouses presented in this study. The substitution of C for T at –1147 creates consensus binding sites for serum response factor (SRF) and the human CCAAT displacement protein (CDP) (Figure 3).

Figure 2 Structure of CTLA-4 gene

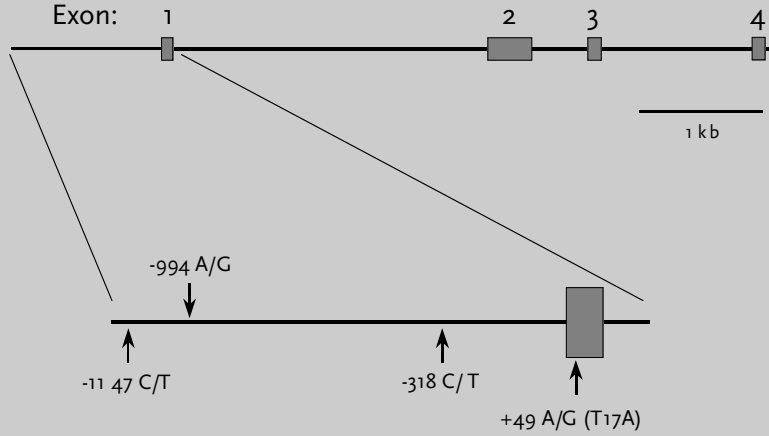


Figure 3 Substitution of -1147 A/T creating consensus binding sites

CTGAGGTGTG GACAATGGGA AACCATGGAT GGACTGGAGT AGGCAAATGT CATATTCCCT

Ik1 /2 SRF

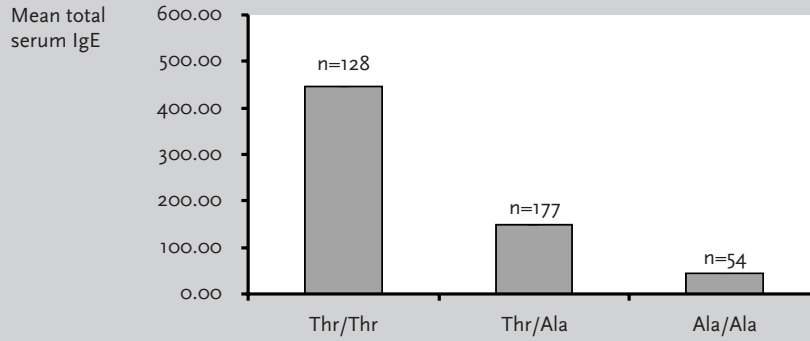
NFAT Cut

CTGAGGTGTG GACAATGGGA AACCATGGAC GGACTGGAGT AGGCAAATGT CATATTCCCT

Ik1 /2

NFAT

Figure 4 Association of the Thr17Ala snp with mean total serum IgE



The probands and spouses from the 200 Dutch families were analyzed for the three SNPs in CTLA-4. As a quantitative trait, total serum IgE levels (logarithm transformed) were significantly associated with the Thr17Ala (+49 A/G) polymorphism (p=0.0006), with Thr/Thr homozygotes having the highest total serum (log) IgE (1.84 ±0.77), Ala/Ala homozygotes the lowest (1.46 ±0.66), and Thr/Ala individuals intermediate between the two (1.65 ±0.68) (Figure 4). The overall results for the closely associated traits examined (asthma, BHR, skin test response, and total serum IgE levels) are reported in Table 3 and Figure 4. Significant evidence for an association was observed with the –1147 C/T SNP to asthma and BHR (p = 0.005 – 0.007), but not to the allergy-related phenotypes. The Thr17Ala SNP (+49 A/G) in exon 1 was significantly associated with all four phenotypes examined in this population, driven by individuals that were homozygous for the Thr (A) allele (recessive effect). Forty-six percent of individuals with total serum IgE levels of at least 100 IU/ml were Thr/Thr homozygotes, compared with 31% of individuals with total serum IgE levels less than 100 IU/ml (p=0.007). In addition, 41% of individuals with at least one positive skin test response, compared to 30% of those with none were AA homozygotes (p=0.031). A similar association was observed with asthma and BHR, where 43% of the original probands for the study were AA, compared to only 29% of their unaffected spouses (p=0.012). Similar analyses were performed for the SNP genotyped in CD28. No evidence for a significant association with allergy or asthmatic phenotypes were observed (Table 3).

Table 3. Frequency of CTLA-4 and CD28 genotypes within asthma and allergy phenotypes

	Asthma		BHR		Skin Test	
SNPs	Probands	Unaff. Spouses	$PC_{20} \leq 32$mg/ml	$PC_{20} > 32$mg/ml	≥1	0
CTLA-4						
-1147 C/T	*n=156*	*n=117*	*n=201*	*n=117*	*n=175*	*n=143*
CC	0.69	0.83	0.69	0.83	0.72	0.76
CT	0.30	0.15	0.30	0.15	0.27	0.22
TT	0.01	0.02	0.01	0.02	0.01	0.02
	p= 0.007		**p= 0.005**		p=ns	
-318 C/T	*n=176*	*n=131*	*n=176*	*n=148*	*n=195*	*n=158*
CC	0.82	0.88	0.85	0.86	0.85	0.85
CT	0.17	0.11	0.14	0.12	0.15	0.13
TT	0.01	0.02	0.01	0.01	0.00	0.02
	p=ns		p=ns		p=ns	
+49 A/G (T17A)	*n=177*	*n=134*	*n=179*	*n=151*	*n=197*	*n=162*
AA	0.43	0.29	0.41	0.30	0.41	0.30
AG	0.46	0.54	0.44	0.55	0.47	0.51
GG	0.11	0.17	0.15	0.15	0.12	0.19
	p= 0.012		**p= 0.038**		**p= 0.031**	
CD28						
-824 A/G	*n=173*	*n=129*	*n=218*	*n=129*	*n=191*	*n=156*
11	0.15	0.16	0.15	0.16	0.17	0.14
12	0.43	0.41	0.42	0.41	0.43	0.39
22	0.42	0.43	0.43	0.43	0.40	0.47
	p=ns		p=ns		p=ns	

Discussion

We have refined a region on chromosome 2q33 with evidence for linkage to total serum IgE levels. Two candidate genes for IgE regulation, CTLA-4 and CD28, are key homeostatic regulators of T-cell activation and map to this region. Two novel SNPs were identified in the 5′ putative promoter region of CTLA-4 and three novel SNPs were identified in the 5′ region of CD28. Association studies with these and two previously reported polymorphisms (-318 C/T and +49 A/G) suggest that the –1147 C/T and +49 A/G (Thr17Ala) variation in CTLA-4 are involved in the development of allergy and asthma related phenotypes in the Dutch asthma families evaluated.

Co-stimulation of T-cell activation is necessary for the downstream response of the immune response to antigen. A defective or improperly regulated co-stimulatory pathway may contribute to an allergy phenotype. Since allergy and asthma are closely related conditions, we hypothesized that the co-stimulation pathway, and specifically CTLA-4 and CD28, may play a role in the clinical characteristics of individuals in a Dutch family study that were ascertained based on a diagnosis of asthma.
Association and linkage-based studies with CTLA-4 polymorphisms have previously been performed in a variety of immunologic diseases, including asthma and atopy. In two studies, no association to either asthma or atopic asthma phenotypes was observed with polymorphisms in either CTLA-4 or CD28 in Japanese[31] or German [32]populations. In one of these studies, however, individuals were ascertained independent of their atopic phenotype[32], resulting in only 55 asthma patients compared to 205 controls. Therefore, this group may not be appropriate for evaluation of candidate genes for asthma.

In studies of other immune-related phenotypes, an association with the +49 A/G (Thr17Ala) variation has been observed. In a multi-ethnic collection of insulin-dependent diabetes mellitus (IDDM) families, the increased transmission of the G (Ala) allele to diabetic children was ethnicity dependent[20] This unequal transmission was mostly observed in Mediterranean-European (Italy, Spain and France) ($p= 10^{-5}$) and Mexican-American (p=0.002) populations. The most significant results were observed when these two groups were combined ($p=10^{-7}$).

Association of CTLA-4 with IDDM is particularly interesting because of the differences between IDDM and atopic diseases. IDDM is caused by a Th1-mediated immune response, whereas atopic diseases are mediated by a Th2 response. In addition, the majority of the reports with the +49 A/G SNP in CTLA-4 involve over-transmission or association of the G (Ala) allele with the allergy phenotype. In this study, we also observed an association with an allergy related phenotype, but with the A (Thr) allele. Association with the A (Thr) allele has also been observed in Celiac disease[33], but not with any other immune diseases. It is possible that the +49 A/G polymorphism is not the causative variant of the reported associations in these studies, but the observations are due to a nearby SNP in lin kage disequilibrium. Another possibility is that Celiac disease and elevated total serum IgE levels have a similar molecular etiology, which differs from that of autoimmune diseases. The total serum IgE levels and Celiac disease are both triggered by an environmental antigen, whereas autoimmune diseases are caused by intrinsic, organ-specific autoantibodies. Further investigation into this possibility is warranted.

Family-based linkage studies have identified the CTLA-4/CD28 region on 2q33 as containing an IgE-related gene. It is interesting to note that while allergy and asthma-related phenotypes (including total serum IgE levels) have been analyzed in numerous populations, evidence for linkage at 2q33 occurs consistently in studies utilizing European and Hispanic populations. We have recently reported evidence for linkage of total serum IgE to 2q33 in our Dutch population[5], and this region was observed with the "asthma" phenotype in our Hispanic population of the Collaborative Study on the Genetics of Asthma.[34,35] In the Hutterites, a founder population in the United States that originated in the Tyrolean Alps in the 1500s, evidence for linkage to 2q (peak marker D2S2944) was observed with positive skin test response to cockroach and house dust mite.[7] An (AT) repeat polymorphism in the 3' UTR of CTLA-4 was evaluated as a candidate gene for this region, but no association was observed to any phenotype. Analysis of the +49 A/G SNP in this population may further identify the potential role of CTLA-4. Evidence for linkage was also observed to this region of chromosome 2q with total serum IgE levels (p=0.0016) in a population of mostly German families (83 German, 5 Swedish, and 9 other nationality families).[6] A study from the French Epidemiological Study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA) also reported evidence for linkage of total serum IgE levels to 2q33.[8] Using a two-stage analysis with two sets of families, this region was significant in the first set of 46 families but was not replicated in the second set of 61.

The –1147 substitution of T for C creates a consensus binding site for the serum response factor (SRF) and the CCAAT displacement protein/Cut-like protein (CDP) (Figure 3). SRF is one component of a complex that leads to transactivation of the promoter[36]; an additional protein in this complex is one or more Ets family member. Interestingly, two genes recently described for asthma susceptibility in a Tristan da Cunha and Toronto cohort of asthma families were also Ets-related family members, referred to as ASTH1I and ASTH1J (US patent # 6,087,485). These data suggest that the SRF complex may be involved in the regulation of asthma or allergy related genes. A second potential binding site that is created by the C to T substitution is for CDP/cut, the human homologue of Drosophila cut[37], that has recently been shown to be a repressor of specific MHC class I genes.[38] If the potential CDP/cut binding site in the CTLA-4 5' region is an active site, then binding of CDP/cut may decrease transcription of CTLA-4. Down-regulation of CTLA-4 would lead to decreased repression of T-cell activation, since CTLA-4 is a negative regulator of this process. Therefore, T-cells in stimulated individuals would remain in the activated state for longer periods of time, potentially leading to allergy or asthma phenotypic expression. This is consistent with our data, where the T allele (which creates the CDP/cut site) is more common in individuals with BHR (Table 3). It is interesting to note that the CDP/cut gene is located on chromosome 7q22, the region with the highest lod score for total serum IgE levels.[5]It is possi-

ble that polymorphisms in this gene may also contribute to allergy or asthma phenotypes, either independently or by interacting with variations in other genes, such as CTLA-4.

In summary, we have identified a region on chromosome 2q33 that contains one or more susceptibility genes for asthma. Fine-mapping with microsatellite genetic markers in a Dutch population ascertained by a proband with asthma has localized this region to an interval containing multiple candidate genes, including CTLA-4 and CD28. By sequencing these two genes we have identified five new SNPs (two in CTLA-4 and three in CD28) and evaluated a subset of these SNPs for association with asthma and allergic phenotypes. Significant association was observed to polymorphisms in CTLA-4 that may regulate the expression levels, the function of the CTLA-4 protein, or both. Further analysis of this gene is necessary to determine its role in the susceptibility to asthma in this and other populations.

References

1 Burrows B, Martinez FD, Halonen M, Barbee R, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989; 320(5):271-277.

2 Burrows B, Sears MR, Flannery EM, Herbison GP, Holdaway MD. Relations of bronchial responsiveness to allergy skin test reactivity, lung function, respiratory symptoms, and diagnoses in thirteen-year-old New Zealand children. J Allergy Clin Immunol 1995; 95(2):548-556.

3 Halonen M, Stern D, Taussig LM, Wright A, Ray CG, Martinez FD. The predictive relationship between serum IgE levels at birth and subsequent incidences of lower respiratory illnesses and eczema in infants. Am Rev Respir Dis 1992; 146(4):866-870.

4 Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. N Engl J Med 1991; 325(15):1067-1071.

5 Xu J, Postma DS, Howard TD, Koppelman GH, Zheng SL, Stine OC et al. Major genes regulating total serum immunoglobulin E levels in families with asthma. Am J Hum Genet 2000; 67(5):1163-1173.

6 Wjst M, Fischer G, Immervoll T, Jung M, Saar K, Rueschendorf F et al. A genome-wide search for linkage to asthma. Genomics 1999; 58:1-8.

7 Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. Am J Hum Genet 2000; 67(5):1154-1162.

8 Dizier MH, Besse-Schmittler C, Guilloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J et al. Genome screen for asthma and related phenotypes in the french EGEA study. Am J Respir Crit Care Med 2000; 162(5):1812-1818.

9 Meyers DA, Postma DS, Panhuysen CI, Xu J, Amelung PJ, Levitt RC et al. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. Genomics 1994; 23(2):464-470.

10 Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 1994; 264(5162):1152-1156.

11 Doull IJ, Lawrence S, Watson M, Begishvili T, Beasley RW, Lampe F et al. Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial hyperresponsiveness. Am J Respir Crit Care Med 1996; 153(4 Pt 1):1280-1284.

12 Folster Holst R, Moises HW, Yang L, Fritsch W, Weissenbach J, Christophers E. Linkage between atopy and the IgE high-affinity receptor gene at 11q13 in atopic dermatitis families. Hum Genet 1998; 102(2):236-239.

13 Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet 1989; 1(8650):1292-1295.

14 Nickel R, Wahn U, Hizawa N, Maestri N, Duffy DL, Barnes KC et al. Evidence for linkage of chromosome 12q15-q24.1 markers to high total serum IgE concentrations in children of the German Multicenter Allergy Study. Genomics 1997; 46(1):159-162.
15 Barnes KC, Neely JD, Duffy DL, Freidhoff LR, Breazeale DR, Schou C et al. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. Genomics 1996; 37(1):41-50.
16 Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 1995; 270(5238):985-988.
17 Deichmann K, Heinzmann A, Bruggenolte E, Forster J, Kuehr J. An Mse I RFLP in the human CTLA4 promotor. Biochem Biophys Res Commun 1996; 225(3):817-818.
18 Abe T, Takino H, Yamasaki H, Ozaki M, Sera Y, Kondo H et al. CTLA4 gene polymorphism correlates with the mode of onset and presence of ICA512 Ab in Japanese type 1 diabetes. Diabetes Res Clin Pract 1999; 46(2):169-175.
19 Lee YJ, Huang FY, Lo FS, Wang WC, Hsu CH, Kao HA et al. Association of CTLA4 gene A-G polymorphism with type 1 diabetes in Chinese children. Clin Endocrinol (Oxf) 2000; 52(2):153-157.
20 Marron MP, Raffel LJ, Garchon HJ, Jacob CO, Serrano-Rios M, Martinez Larrad MT et al. Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. Hum Mol Genet 1997; 6(8):1275-1282.
21 Badenhoop K. CTLA4 variants in type 1 diabetes: some stirrups serve better backing endocrine autoimmunity. Clin Endocrinol (Oxf) 2000; 52(2):139-140.
22 Naluai AT, Nilsson S, Samuelsson L, Gudjonsdottir AH, Ascher H, Ek J et al. The CTLA4/CD28 gene region on chromosome 2q33 confers susceptibility to celiac disease in a way possibly distinct from that of type 1 diabetes and other chronic inflammatory disorders. Tissue Antigens 2000; 56(4):350-355.
23 Donner H, Rau H, Walfish PG, Braun J, Siegmund T, Finke R et al. CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. J Clin Endocrinol Metab 1997; 82(1):143-146.
24 Ligers A, Teleshova N, Masterman T, Huang WX, Hillert J. CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. Genes Immun 2001; 2(3):145-152.
25 Harbo HF, Celius EG, Vartdal F, Spurkland A. CTLA4 promoter and exon 1 dimorphisms in multiple sclerosis. Tissue Antigens 1999; 53(1):106-110.
26 Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI et al. Genetic susceptibility to asthma-bronchial hyperresponsiveness coinherited with a major gene for atopy. N Engl J Med 1995; 333(14):894-900.
27 Panhuysen CIM, Bleecker ER, Koëter GH, Meyers DA, Postma DS. Characterization of obstructive airways disease in family members of probands with asthma; an algorithm for the diagnosis of asthma. Am J Respir Crit Care Med 1998; 157:1734-1742.

28 de Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch All 1962; 20:93-101.
29 Lander ES, Green P. Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci U S A 1987; 84(8):2363-2367.
30 Zaykin D, Zhivotovsky L, Weir BS. Exact tests for association between alleles at arbitrary numbers of loci. Genetica 1995; 96(1-2):169-178.
31 Nakao F, Ihara K, Ahmed S, Sasaki Y, Kusuhara K, Takabayashi A et al. Lack of association between CD28/CTLA-4 gene polymorphisms and atopic asthma in the Japanese population. Exp Clin Immunogenet 2000; 17(4):179-184.
32 Heinzmann A, Plesnar C, Kuehr J, Forster J, Deichmann KA. Common polymorphisms in the CTLA-4 and CD28 genes at 2q33 are not associated with asthma or atopy. Eur J Immunogenet 2000; 27(2):57-61.
33 Djilali-Saiah I, Schmitz J, Harfouch-Hammoud E, Mougenot JF, Bach JF, Caillat-Zucman S. CTLA-4 gene polymorphism is associated with predisposition to coeliac disease. Gut 1998; 43(2):187-189.
34 CSGA. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). Nat Genet 1997; 15(4):389-392.
35 Xu J, Meyers DA, Ober C, Blumenthal MN, Mellen B, Barnes KC et al. Genomewide screen and identification of gene-gene interactions for asthma-susceptibility loci in three U.S. populations: Collaborative Study on the Genetics of Asthma. Am J Hum Genet 2001; 68(6):1437-1446.
36 Cahill MA, Janknecht R, Nordheim A. Signalling pathways: jack of all cascades. Curr Biol 1996; 6(1):16-19.
37 Blochlinger K, Bodmer R, Jack J, Jan LY, Jan YN. Primary structure and expression of a product from cut, a locus involved in specifying sensory organ identity in Drosophila. Nature 1988; 333(6174):629-635.
38 Snyder SR, Wang J, Waring JF, Ginder GD. Identification of CCAAT displacement protein (CDP/cut) as a locus- specific repressor of major histocompatibility complex gene expression in human tumor cells. J Biol Chem 2001; 276(7):5323-5330.