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IL10 Gene Polymorphisms Are Associated With Asthma Phenotypes in Children

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Abstract

IL10 is an anti-inflammatory cytokine that has been found to have lower production in macrophages and mononuclear cells from asthmatics. Since reduced IL10 levels may influence the severity of asthma phenotypes, we examined IL10 single-nucleotide polymorphisms (SNPs) for association with asthma severity and allergy phenotypes as quantitative traits. Utilizing DNA samples from 518 Caucasian asthmatic children from the Childhood Asthma Management Program (CAMP) and their parents, we genotyped six IL10 SNPs: 3 in the promoter, 2 in introns, and one in the 3' UTR. Using family-based association tests, each SNP was tested for association with asthma and allergy phenotypes individually. Population-based association analysis was performed with each SNP locus, the promoter haplotypes and the 6-loci haplotypes. The 3' UTR SNP was significantly associated with FEV₁ as a percent of predicted (FEV₁PP) ($P=0.0002$) in both the family and population analyses. The promoter haplotype GCC was positively associated with IgE levels and FEV₁PP ($P=0.007$ and 0.012 , respectively). The promoter haplotype ATA was negatively associated with lnPC₂₀ and FEV₁PP ($P=0.008$ and 0.043 , respectively). Polymorphisms in IL10 are associated with asthma phenotypes in this cohort. Further studies of variation in the IL10 gene may help elucidate the mechanism of asthma development in children.

Keywords

interleukin 10 (IL10); single nucleotide polymorphism (SNP); genetic association; family-based association test (FBAT); haplotype; promoter; 3'; untranslated region (3'UTR)

Introduction

Interleukin-10 (IL10) is an anti-inflammatory cytokine that has been found to have lower in vitro production from macrophages and mono-nuclear cells from asthmatics [John et al., 1998; Lim et al., 1998]. This variability in production of IL10 has been demonstrated to have a genetic basis by twin concordance studies and an estimated heritability of 74% [Westendorp et al., 1997]. The IL10 gene is located on chromosome 1q31-q32 and spans

about 5.2 kb of genomic DNA containing 5 exons, a region not found to be linked to asthma or pulmonary function in published linkage analysis. [Palmer et al. 2003]. The protein product is a 36-kDa homodimeric cytokine that was originally given the name “cytokine synthesis inhibitory factor” due to its ability to inhibit the secretion of other cytokines [Moore et al., 2001].

IL10 is a candidate gene in the pathophysiologic mechanism of auto-immune/inflammatory disease since it has been shown to regulate both cellular and humoral immunity. It down-regulates macrophage proinflammatory cytokines IL1, IL6, IL8, granulocyte-macrophage-colony-stimulating-factor, and tumor-necrosis-factor- α , and, consequently, has an immunosuppressive effect on T cells, monocytes, and macrophages. While it suppresses the Th-1 response, it also promotes B cell activation, regulates immunoglobulin class switching, and inhibits B cell apoptosis [Moore et al., 2001]. In the presence of IL4 in vitro, IL10 has been demonstrated to regulate IgE production, and reduce IgE switching [Jeannin et al., 1998] and while not involved in switching directly, serves as a control point for modulation of the effects of inflammatory cytokines [Moore et al., 2001].

In the lung, mononuclear cells are the primary source of IL10 protein, and expression studies done in bronchial alveolar lavage cell pellets showed lower levels of mRNA and decreased production of IL10 in asthmatics compared to normal subjects [Rosenwasser and Borish, 1997]. In healthy airways, IL10 is constitutively expressed, contributing to the decreased expression of B7 molecules on alveolar macrophages, thereby suppressing their antigen presenting capability and consequently inflammation [Rosenwasser and Borish, 1997]. Murine studies show constitutive IL10 transcription and post-transcriptional regulation, allowing for very rapid control of this cytokine [Moore et al., 2001]. IL10 release has been shown to be proportional to mRNA levels, further suggesting post-transcriptional regulation [Huizinga et al., 2000; Powell et al., 2000].

To explore the contribution of sequence variation in the IL10 gene to asthma intermediate phenotypes, we genotyped six IL10 SNPs in a large outbred population of children with mild to moderate asthma to look for association with several intermediate quantitative phenotypes of asthma, including severity, bronchodilator responsiveness, and atopy. Association was measured in family trios by the Family Based Association Test (FBAT) [Horvath et al., 2001]. Allelic and haplotypic associations were also tested using population-based methods.

Subjects And Methods

Our work focuses on six of the SNPs in the IL10 gene previously described by Lazarus et al. [2002]. The SNP locations are shown in Table I. Three promoter SNPs –1117A/G, –854C/T, and –627C/A (numbered from the ATG start) were genotyped along with two intronic and one 3' UTR SNP.

Population

Study participants were a subset of the Childhood Asthma Management Program (CAMP) study population. CAMP is a multi-center, randomized, double-masked clinical trial designed to determine the long-term effects of anti-inflammatory medications in children with mild to moderate asthma [CAMP Group, 1999]. The results of the original clinical trial have been previously reported [CAMP Group, 2000]. Baseline phenotypic data were collected on 1,041 participants and DNA samples were obtained from 968 of those participants and 1,518 of their parents in the CAMP Genetics Ancillary Study. Each center was granted approval by their Institutional Review Board. Informed consent was obtained from the parents and assent was obtained from each child. Complete family trios were

available for 704 probands in 646 families, of which 478 were identified as Caucasian American, 66 as African American, 48 as Hispanic American, and 54 as other races. Analysis was limited to the Caucasian families: 441 trio families, 34 families with two children with asthma, and 3 families with three children with asthma, for a total of 518 trios. The 518 individual CAMP children constituted our population-based cohort. The mean age of the affected children was 8.07 (± 2.09) years. Mendelian inconsistencies were detected with the Ped-check program and the genotype data for all loci of the 12 families were excluded on this basis [O'Connell and Weeks, 1998].

Phenotype Data

Phenotypic data on each subject were obtained during the screening period of the study. Screening was done with four visits and included a 28-day period when the children were treated with beta-agonist therapy only for asthma symptoms. Total IgE was measured by radio immunoabsorbant testing from blood samples collected during the screening period of the CAMP study. Eosinophil counts were performed on blood samples collected during the screening period of the CAMP study. All IgE values and eosinophil counts were analyzed as $\log_{10}$ scale.

Skin prick tests were performed according to the CAMP protocol during the screening period [CAMP Group, 1999]. All participants were tested with a core group of ten antigens that included *Dermatophagoides pteronyssinus*, *Dermatophagoides farinai*, cat and dog dander, American cockroach, German cockroach, *Penicillium*, *Aspergillus*, Timothy grass, and short ragweed. A test was considered positive if the prick site developed a wheal with a mean diameter ≥ 3 mm and/or a flare with a mean diameter ≥ 10 mm. The total number of positive core skin tests was considered in this analysis.

Pulmonary function tests were performed by CAMP-trained technicians using a volume displacement spirometer in accordance with American Thoracic Society Criteria [CAMP Group, 1994; ATS, 1995]. Pre- and post-bronchodilator measurements were performed and adjusted for age, height, gender, and race [Wang et al., 1993; CAMP Group, 1999]. The bronchodilator albuterol was administered by metered dose inhaler with a spacer and at least 15 min elapsed before post-bronchodilator measurements were obtained. The FEV₁ measured after bronchodilator administration as a percent of predicted FEV₁ (FEV₁PP) was the phenotype used to assess lung function [Knudson et al., 1983; Coultas et al., 1988; Wang et al., 1993; Weiss et al., 2000]. The bronchodilator responsiveness phenotypes analyzed were absolute change in FEV₁ before and after bronchodilator in mLs (ABS_{BD}), the absolute change as a percent of predicted FEV₁ (Pred_{BD}), and the absolute change as a percent of baseline FEV₁ (FEV_{BD}). To assess asthma severity, a methacholine challenge was administered using increasing concentrations of methacholine and the Wright nebulizer-tidal breathing technique [Weiss et al., 2000]. Testing was performed at least 4 hours after use of a short-acting and 24 hours after use of a long-acting bronchodilator [CAMP Group, 1999]. The concentration of methacholine that causes a 20% decline in FEV₁ (in mg/mL) was measured for each subject and was natural log transformed for analysis (lnPC₂₀) and used as a measure of asthma severity.

Dna Extraction And Genotyping

Blood samples were collected as part of the CAMP protocol at each local center. DNA was extracted with Puregene kits (Gentra Systems). SNP genotyping for the six SNPs in the IL10 gene was done using the length-polymorphism single base extension (LP-SBE) technique [Lindblad-Toh et al., 2000]. Primer specifications and reaction conditions are available at the IIPGA website (www.innateimmunity.net). Multiplex PCR was performed on MJ Research DNA Engine tetrads. Five microliters of PCR product were purified using 2 ml of

EXOSAP-IT (USB Corp.) The LP-SBE reactions were done in multiplex fashion using the SnaPshot Multiplex Kit (Applied Biosystems) according to the manufacturer's protocol except for dilution of the SnaPshot mix with HalfBD BigDye sequencing dilution buffer (Genpak) in a 1:1 ratio. Ten microliters of the reaction product were then treated with 2 ml shrimp alkaline phosphatase and incubated at 37°C for 90 min. The reaction product was separated on an ABI 3100 capillary electrophoresis system and allele determination was performed using ABI Prism Genotyper software. The genotype tracings were checked for accuracy manually. Linkage disequilibrium patterns were established using a likelihood-ratio test whose empirical distribution was obtained by a permutation procedure as implemented in the Arlequin software package [Slatkin and Excoffier, 1996; Schneider et al., 2000].

Statistical Analysis

Transformation Of Phenotypes

Since most of the eight phenotype distributions were skewed, all phenotypes were transformed and their corresponding normal scores used in the analysis.

Family-based analysis. Single-locus association analysis—Family-based association tests (FBATs) were used to test each SNP for association with the 8 selected phenotypes (see Table II) [Laird et al., 2000]. Testing 6 loci for association with 8 phenotypes implies that 48 univariate FBATs have to be computed. Since adjusting for multiple testing with 48 tests leads to extremely conservative corrections of the P values, we utilized conditional power calculations for FBATs to reduce the number of tests [Lange and Laird, 2002a,b]. The data in the non-informative families were used to regress the phenotypes on the genotype information of the offspring, obtaining an estimate of the effect sizes for each phenotype. Since only non-informative families were used in this regression and FBATs are based on informative families, the subsequent FBAT results do not have to be adjusted for this procedure. The conditional power for each phenotype was estimated using PBAT (see Electronic-Database Information) [Lange et al., 2002]. For each locus, effect size estimates were used to compute the conditional power for all 8 univariate FBATs and the 3 most powerful phenotypes for each marker were selected. These phenotypes were then tested by univariate FBATs at an overall significance level of 1%, i.e., an adjusted significance level of $1/(3*6)\% = 0.056\%$ or $P < 0.0006$.

Population-based analysis. Single-locus association analysis—We also applied the population-based approach for multivariate association mapping proposed by Lange and Laird [2002a,b] using the genotype data from the asthmatic probands. This approach is based on the generalized estimating equation (GEE) approach by Liang and Zeger [1986] and allows one to test a set of phenotypes for association with a SNP locus. The phenotypes of the study participants were modeled as a function of the locus score, i.e., $Y = \mu + a * x$, where Y is the phenotype and x is the locus score. The phenotypic variance matrix is assumed to be unstructured. The null-hypothesis of no association between the locus and the phenotype is equivalent to the null-hypothesis of the mean parameter, “a” being equal to zero. We estimated all mean and variance parameters by the hybrid GEE-approach [Lange and Laird, 2002a,b]. The mean parameters were then tested for association by Wald tests using the robust estimates for the standard errors provided by GEE. No a priori alpha level was set.

Haplotype association analysis: Associations between the IL10 haplotypes and the intermediate phenotypes were tested using a modification of the population-based method proposed by Schaid et al. [2002]. In this method, score tests derived from generalized linear models are used for global tests of association, as well as haplotype-specific tests. Linkage

phase ambiguity (inherent in methods that infer haplotypes from unphased marker data) is addressed by computing the conditional distribution of haplotypes given the observed marker data for all individuals in the study. These conditional distributions serve as weights in the construction of the score tests. We modified the method to include data from individuals with partially missing marker information.

Results

The location of the IL10 SNPs we genotyped and their frequency in the study population is shown in Table I and shown graphically in Figure 1. The linkage disequilibrium patterns are shown graphically in Figure 2. SNP pairs –1117A/G with 4299T/C ($R^2=0.96$) and –627C/A with –854C/T ($R^2=0.94$) are essentially in complete linkage disequilibrium. The phenotype values for the probands are summarized in Table II. The genotypes of the probands at each of the six loci were in Hardy-Weinberg equilibrium, but for the parents SNPs –1117A/G and 4299T/C were not in Hardy-Weinberg equilibrium (P values of 0.004 and 0.003, respectively).

Single Locus Analysis

Family-based association tests were performed for each phenotype and SNP. The only phenotype-SNP comparison that reaches significance after adjustment for conditional power (defined as $P < 0.0006$) is FEV₁PP with SNP 4299T/C ($P=0.0002$). Population-based analysis of the proband cohort was performed and detected the same association between SNP 4299T/C and FEV₁PP ($P=0.0002$), that was robust even with a conservative Bonferroni correction of the significance level, i.e., $1/48\%=0.0002$. In the population-based analysis, there were other less significant associations for three loci with FEV₁PP including SNPs –1117A/G, –627C/A, 1668T/A, and 2866T/C with P values of 0.001, 0.038, 0.01, and 0.038, respectively. The population-based analysis also detected association between IgE level and the three promoter SNPs (0.001, 0.019, and 0.001, respectively) as well as for SNP 4299T/C ($P=0.005$). No associations were detected with the three bronchodilator responsiveness phenotypes.

The effect of the 4299T/C genotype on FEV₁PP is displayed graphically for each genotype in Figure 3. There appears to be an additive effect in that the minor allele C is associated with a higher FEV₁PP. The mean FEV₁PP for participants with the TT genotype is 101.5% ($\pm 13\%$), for the TC genotype is 103.4% ($\pm 11\%$), and for the CC genotype is 106.0% ($\pm 13\%$) showing a 2-3% increase with the addition of each C allele.

Haplotype Analysis

Haplotype association tests were used to attempt to replicate the previously reported associations between promoter haplotypes and asthma severity and to further explore the association between SNP 4299T/C and FEV₁PP. We tested all intermediate phenotypes with the promoter haplotypes ATA, ACC, and GCC and the six loci haplotypes using the Schaid method [Schaid et al., 2002]. Haplotypes with estimated frequencies below 0.05 were not included in either analysis. Table III displays the inferred promoter haplotypes, their estimated frequencies, and the haplotype association test P values. The global test of haplotype association between FEV₁PP and the promoter haplotypes shows a score test statistic of 7.99 and a P value of 0.046. The GCC haplotype is positively associated with FEV₁PP (score test statistic 2.52, P value 0.012), while the ATA haplotype is negatively associated with FEV₁PP (score test statistic –2.02, P value 0.043). For each child, the promoter haplotypes were reconstructed by selecting the promoter haplotype that had the highest conditional probability given the estimated haplotype frequency. Using the

reconstructed haplotypes, the effect of the GCC and ATA haplotypes on FEV₁PP is illustrated graphically (Fig. 4).

Another asthma severity phenotype, the LnPC20 (natural log of the concentration of methacholine that causes a 20% decline in FEV₁ in mg/mL) showed similar findings with a global score test statistic of 7.84 and a *P* value of 0.049 as the GCC haplotype was positively associated (score test statistic 2.32, *P* value 0.020) and the ATA haplotype was negatively associated (score test statistic -2.64, *P* value 0.008) for this phenotype. The GCC haplotype was also associated with IgE levels with a score statistic of -2.68 and *P* value of 0.007. The global score test of the promoter haplotypes with the IgE phenotype was 8.54 with a *P* value of 0.036.

Tests of association using the 6-loci haplotypes also provided evidence of an association between FEV₁PP and the haplotypes including ATA and GCC with score test statistics of 2.24 and -2.00, respectively (*P* values of 0.025 and 0.045). The *P* values for the haplotype-specific tests were not significant after correction for multiple comparisons, but the directions of the association match the directions observed in the promoter haplotypes.

Discussion

We tested for associations between intermediate phenotypes of asthma and six IL10 SNPs using a family-based analysis of parent proband trios and a population-based analysis of the asthmatic probands in those families. We found a significant association between post bronchodilator FEV₁ as a percent of predicted (FEV₁PP) and SNP 4299T/C in the family-based and the population-based analyses. The population-based analysis also suggested associations between several SNPs and the atopy phenotype, IgE level. We tested the haplotypes defined by these loci and the previously described promoter haplotypes for association with the asthma phenotypes. The haplotype population-based tests showed an association of two promoter haplotypes with the FEV₁PP phenotype, as well as an association with two of the 6-loci haplotypes. The C allele at SNP 4299T/C in the 3' UTR was associated with a significant increase in FEV₁PP, and this allele is in linkage disequilibrium with the GCC promoter haplotype that was also associated with an increase in FEV₁PP. In both instances, the values of FEV₁PP were > 100%. This is to be expected in children with mild to moderate asthma who show a range of FEV₁PP up to 120%. FEV₁PP was used as a quantization of lung function, rather than a measure of the severity of asthma symptoms at the time of the measurement, and therefore, reflects a gradation in lung function for children with the noted genotypes and haplotypes. Since lung function is a complex trait, it is unlikely that a single gene would determine enough variation to cause a clinically significant change in FEV₁PP. We established a statistically significant difference in FEV₁PP, implicating IL10 gene variation in the development of lung function in asthmatic children. The subjects chosen in the CAMP study were classified as having mild to moderate asthma. Thus, it is possible that our results do not apply to children with more severe disease.

Much of the interest in IL10 as a candidate gene in disease processes has focused on the promoter region as a way of explaining the variation in IL10 levels by alterations in transcription. Eleven SNPs [Turner et al., 1997; Lazarus et al., 2002] and two microsatellite (AC)ⁿ repeats have been reported in the promoter [Eskdale and Gallagher, 1995; Kube et al., 1995; Eskdale et al., 1996, 1997]. Three promoter SNPs at -1,082, -819, and -592 (corresponding to SNP numbers -1117A/G, -854C/T, and -627C/A in our analysis) have been shown by several groups to form three common haplotypes in Caucasian populations: GCC, ACC, and ATA [Turner et al., 1997; Eskdale et al., 1999; Asadullah et al., 2001; Helminen et al., 2001]. Promoter association studies with these three haplotypes have shown

significant association in inflammatory diseases such as lupus erythematosus, psoriasis, and multiple sclerosis [Lazarus et al., 1997; Asadullah et al., 2001; Myhr et al., 2002]. Variation at the -592 site has also been associated with increased risk of HIV-1 infection and disease progression [Shin et al., 2000].

The GCC haplotype has been found to be associated with high IL10 production while the ATA haplotype was associated with low IL10 production in stimulated lymphocytes [Turner et al., 1997; Hajeer et al., 1998]. It has been suggested that these polymorphisms could interfere with transcription factor binding. The -1082G/A SNP occurs within a putative ETS-like transcription factor binding site [Kube et al., 1995]. The -819 SNP may affect an estrogen receptor element and the -592 SNP has been demonstrated to be in a region of negative regulatory function [Kube et al., 1995; Lazarus et al., 1997]. However, overall variation in secretion of IL10 could not solely be attributed to variation in the promoter haplotype, and additional factors were thought to be necessary [Eskdale et al. 1998].

Our promoter haplotype analysis (Table III) shows that the ATA haplotype was associated with a lower FEV₁PP and lower lnPC₂₀ while the GCC haplotype was associated with a higher FEV₁PP and higher lnPC₂₀. These phenotypes represent lung function and asthma severity, respectively. More severe asthmatics have a lower FEV₁PP and lnPC₂₀ [Weiss et al., 2000] and previous studies have demonstrated a higher frequency of the ATA haplotype and lower IL10 levels in severe asthmatics compared to controls [Lim et al., 1998; Tomita et al., 2002]. This is also consistent with in vitro data in stimulated lymphocytes where the GCC haplotype has been found to be associated with high IL10 production and the ATA haplotype with low IL10 production [Turner et al., 1997; Hajeer et al., 1998]. A recent study using IL10 deficient mice found that IL10 is involved in the development of hyperresponsiveness, but not inflammation [Makela et al., 2003]. Pulmonary hyperresponsiveness in our study was represented by lnPC₂₀, a phenotype that was found to be associated with the promoter haplotypes, but not the individual SNPs.

Our analysis suggested a negative association between the GCC haplotype and one of the atopy phenotypes, log IgE. We also observed an association of the promoter SNPs and the 4299T/C SNP individually in our population-based analysis.

One of these SNP associations has been previously reported by Hobbs et al. [1998], who found higher IgE levels associated with the -571C/A (-627 C/A in our report) variant in 97 wild type (C/C) 34 heterozygotes (C/A) and 13 homozygotes (A/A) with asthma.

A recent study by Karjalainen et al. [2003] reported an association of the ATA haplotype with eosinophil counts in asthmatics and IgE level in male asthmatics. While this study had 245 adult asthmatics and 405 controls, there were only 9 asthmatic and 14 control subjects with ATA/ATA haplotypes. They did not find an inverse association with the GCC haplotype in asthmatic individuals, yet found that the GCC haplotype to be associated with lower IgE levels in male controls. Our study did not parallel these results, and in fact demonstrated an inverse relationship between the GCC genotype and IgE in asthmatic individuals. While our analysis did not stratify by gender, our study population was larger, and focused on a homogeneous group of children (Caucasian patients with mild-moderate asthma). Karjalainen et al. [2003] did not find any association of IL10 variation with lung function in asthmatics. It is possible that the pathologic mechanism resulting in asthma from IL10 variation effects lung function more strongly in the young subjects and inflammatory markers more strongly in older patients.

With the availability of DNA samples from CAMP participants and their parents, we chose a family-based study design by using FBATs, which are robust and resistant to population stratification. However, they are less powerful than population-based tests, and in our

analysis FBATs detected association with asthma phenotypes at fewer loci. Our results in the population-based tests for each locus showed suggestion of association for other phenotypes including log IgE at four loci (−1117A/G, −854C/T, −627C/A, and 4299T/C) and FEV₁PP with four loci (−1117A/G, −627C/A, 1668T/A, and 2866T/C) in addition to 4299T/C. In this analysis, the locus score is the predictor variable and an environmental correlation matrix is applied to account for correlation of the phenotypes in order to gain power. The stronger associations seen in the population-based study may be due in part to this adjustment and the sensitivity of the family-based method to missing data. An a priori level of significance was not defined for the population-based tests, yet after adjusting the *P* values of the population-based analysis with Bonferroni corrections, the association between the SNP 4299T/C and FEV₁PP still reached overall significance.

The genotype frequencies of the six IL10 loci were in Hardy-Weinberg equilibrium (HWE) for the probands, but SNPs −1117A/G and 4299T/C were not in HWE for the parents. Test statistics that incorporate deviations from HWE as evidence of association have been proposed for application in the family-based design [Whittemore and Tu, 2000] and the population-based design [Hoh et al. 2001]. These methods are restricted to binary outcomes and, therefore, not applicable to our study. FBAT methods are known to be robust to deviation from HWE [Lazzeroni and Lange, 1998; Rabinowitz and Laird, 2000].

Since SNPs −1117A/G and 4299T/C were in complete linkage disequilibrium, we expected to see an association for both or neither of these loci. In the family-based association, testing with FEV₁PP, SNP −1117A/G showed a non-significant *P* value of 0.026. The association for this SNP was not as strong as for SNP 4299T/C (*P*=0.0002) due to a smaller number of informative families and a higher percentage of missing genotypes. The decreased number of alleles yielded less power to detect the associations by transmission disequilibrium testing, but in general the family-based and population-based analyses demonstrated the same trends.

We have shown that both the promoter and the six-loci haplotypes are associated with pulmonary function and possibly atopy in asthmatic children, but the functional variant cannot be discerned with these methods. It is possible that the alteration in IL10 response is due to synergy between the promoter and the 3'UTR, and clarification of the molecular mechanism will require further studies. This is the first reported association of the 3'UTR SNP with an asthma phenotype, and combined with the evidence of mRNA half-life determining IL10 level, could support a post-transcriptional alteration of function with this SNP [Huizinga et al., 2000]. Functional studies of the promoter SNPs and the 3'UTR SNP may elucidate the modulation of the inflammatory response by IL10 and demonstrate an important control point for potential therapeutic intervention in asthma.

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Fig. 1.
Position of SNPs within the IL10 gene. The location of the SNPs genotyped in this analysis is shown with numbering according to Table I. Minor allele frequency is shown in parentheses.

Fig. 2.
Pair-wise linkage disequilibrium plot for IL10 SNPs of Caucasian CAMP proband's parents.
The intensity of shading denotes the R^2 value.

Fig. 3.

Box plot of post-bronchodilator FEV₁ as a percent of predicted for the CAMP children by genotype, with means below the plot. The C allele is the minor allele. There is a 4.5% overall increase in FEV₁PP in an additive genetic model, comparing the CC homozygotes (n=124) to the TT homozygotes (n=130) Heterozygotes TC (n=263).

Fig. 4. Box plot of post-bronchodilator FEV₁ as a percent of predicted for the CAMP children by imputed promoter haplotype. Homozygotes with the GCC promoter haplotype (n=117) had an overall increase in FEV₁PP of 4.5% compared to homozygotes for the ATA haplotype (n=34). Mean post-bronchodilator FEV₁ for GCC haplotyped participants was 105.8% and for ATA haplotyped participants was 101.3%.

TABLE I
IL10 SNPs genotyped in 518 Caucasian CAMP study participants

Position ^a	Location	Frequency of minor allele	
		Parents	Probands
-1117A/G	Promoter	0.49	0.50
-854C/T	Promoter	0.24	0.25
-627C/A	Promoter	0.24	0.25
1668T/A	Intron 3	0.25	0.26
2866T/C	Intron 3	0.07	0.06
4299T/C	3'UTR	0.49	0.49

^aPosition calculated based upon genomic sequence numbering in relation to the A of the ATG start codon.

TABLE II
Phenotypic characteristics of the 518 Caucasian CAMP study participants

Phenotype	Mean (SD)	Mean in boys (N=319)	Mean in girls (N=199)
FEV₁PP (%)	103.5 (12.8)	102.2 (12.9)	105.5 (12.3)
ABS_{BD} (mL)	0.160 (0.13)	0.161 (0.14)	0.158 (0.13)
Pred_{BD} (%)	9.05 (7.23)	9.06 (7.36)	9.04 (7.03)
FEV_{BD} (%)	10.38 (0.53)	10.65 (10.15)	9.93 (8.45)
Log Eosinophils	2.57 (0.38)	2.57 (0.36)	2.57 (0.41)
Log IgE (IU)	2.58 (0.70)	2.58 (0.68)	2.56 (0.73)
Positive skin tests	3.38 (2.66)	3.54 (2.70)	3.13 (2.57)
LnPC20	0.04 (0.51)	0.06 (0.51)	0.02 (0.53)

TABLE III

Population-based promoter haplotype association analyses^a

Haplotype (frequency)	Eosinophils	IgE	Positive skin tests	FEV _{BD} [*]	ABS _{BD} [*]	P _{predBD} [*]	LogPC ₂₀	FEV ₁ PP
GCC (0.49)	0.073	0.007 (↓)	0.149	—	—	—	0.020 (↑)	0.012 (↑)
ACC (0.26)	0.062	0.157	0.572	—	—	—	0.974	0.299
ATA (0.24)	0.755	0.066	0.158	—	—	—	0.008 (↓)	0.043 (↓)
global	0.212	0.036	0.094	—	—	—	0.049	0.046

^aThe haplotypes formed by the loci -1117A/G, -854C/T, and -627C/A and the observed frequencies in >5% of Caucasian CAMP participants. P-values for the tests of each haplotype with each intermediate phenotype are given. Arrows indicate the direction of the effect of the haplotype on the phenotypes.

^{*} Indicates the three bronchodilator response phenotypes showed $P > 0.1$; data not shown.