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Association of Vascular Endothelial Growth Factor polymorphisms with childhood asthma, lung function, and airways responsiveness

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Abstract

Vascular endothelial growth factor (VEGF) is an angiogenic factor implicated in asthma severity. The objective of this study was to determine whether VEGF single nucleotide polymorphisms (SNPs) are associated with asthma, lung function, and airways responsiveness.

We analyzed 10 SNPs in 458 white families in the Childhood Asthma Management Program (CAMP). Tests of association with asthma, lung function, and airways responsiveness were performed using PBAT. Family and population-based repeated measures analysis of airflow obstruction were conducted. Replication studies were performed in 412 asthmatic children and their parents from Costa Rica.

Associations with asthma, lung function, and airways responsiveness were observed in both cohorts. SNP rs833058 was associated with asthma in both cohorts (CAMP p=0.004, Costa Rica p=0.01). This SNP was also associated with increased airways responsiveness in both populations (CAMP p=0.01, Costa Rica p=0.03). An association of rs4711750 and its haplotype with FEV¬11/FVC ratio in both cohorts (CAMP p=0.01, Costa Rica p=0.01) was observed. Longitudinal analysis in CAMP confirmed an association of rs4711750 with FEV1/FVC decline over approximately 4.5 years of observation (p=0.03).

VEGF polymorphisms are associated with childhood asthma, lung function, and airways responsiveness in two populations, suggesting that VEGF polymorphisms influences asthma susceptibility, airflow obstruction, and airways responsiveness.

Key Words: Airflow obstruction, Asthma, Single Nucleotide Polymorphisms, Vascular endothelial growth factor

Abbreviations:

Childhood Asthma Management Program (CAMP)

Family-based association test (FBAT)

Forced Expiratory Volume in One Second (FEV_1)

Forced Vital Capacity (FVC)

Minor Allele Frequency (MAF)

Ratio of Forced Expiratory Volume in One Second/Forced Vital Capacity (FEV_1/FVC)

Single Nucleotide Polymorphisms (SNP)

Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor receptor (VEGFR)

Introduction

Exaggerated T-helper type 2 cell (T_H2)- mediated inflammation, producing airflow obstruction, is one of the pathologic cornerstones of asthma. Although this airflow obstruction is typically reversible with bronchodilator use, progressive, irreversible airflow obstruction can develop in some patients with persistent asthma, resulting in long-term disability[1]. This progressive obstruction is often associated with the characteristic histopathologic changes of airway remodeling, which include subepithelial fibrosis, neovascularization, and increased smooth muscle deposition, all leading to a decrease in the caliber of the small airways[2]. Angiogenesis has been found to be an important histologic feature of the airway wall in asthma. Bronchial biopsies from asthmatic patients demonstrate increased vascularity, with notable increases in both the subepithelial vascular surface area and overall vessel size[2].

Vascular endothelial growth factor (VEGF) is the critical angiogenic factor implicated in neovascularization in response to tissue injury and repair. Several lines of evidence suggest that VEGF contributes to the development of asthma, airways responsiveness, and airway remodeling. Animal models have demonstrated the importance of VEGF in antigen-induced T_H2- mediated airway inflammation in asthma[3]. Transgenic mouse models have also demonstrated that over expression of VEGF leads to increased vascularity in the airway epithelium, airway inflammation, and airway hyperresponsiveness, all of which are largely attenuated by VEGF antagonism[4]. Pathologic findings are similar in human subjects with asthma. Simcock et al. recently showed that levels of proangiogenic factors including VEGF are higher in the bronchoalveolar lavage fluid (BAL) of patients with mild asthma than in non-atopic

healthy controls[5]. In humans, VEGF expression is significantly higher in the BAL of asthmatic subjects than in that of healthy controls[6]. Among asthmatics, VEGF expression in BAL is inversely correlated with lung function[2]. Another recent study in asthmatic human subjects confirms that increased VEGF and VEGF receptor (VEGFR) expression within airway epithelial cells was correlated with airway remodeling in histologic samples, airflow obstruction on spirometry, and increased airways responsiveness to methacholine[7]. In this study, treatment with budesonide/fomoterol for six months decreased VEGF and VEGFR expression and decreased airway remodeling noted on histologic specimens[7]. Together, these data implicate VEGF as a plausible molecular determinant of asthma susceptibility, airway remodeling, airways responsiveness, and progressive lung function decline.

Given these observations, we hypothesized that VEGF gene sequence variation influences asthma susceptibility, progressive airflow obstruction, and airways responsiveness in children with asthma. The human VEGF gene (located on chromosome 6p21) harbors at least 140 known single nucleotide polymorphisms (SNPs), several of which have been associated with clinical phenotypes[8-10]. However, no studies of VEGF associations with asthma, lung function, or airways responsiveness have been reported to date. To determine whether VEGF variants contribute to asthma susceptibility, airflow obstruction, and airways responsiveness, we performed family-based genetic association studies in two childhood asthma cohorts.

Methods:

Study Population

The Childhood Asthma Management Program (CAMP) was a multicenter, randomized, double-blind, placebo-controlled trial to investigate the long-term effects of inhaled corticosteroids and inhaled nedocromil. Of the 1,041 children randomized in the clinical trial, 968 children and 1,518 of their parents contributed DNA samples as part of the genetic ancillary study of CAMP. DNA was sufficient for all family members for 470 nuclear families of self-reported non-Hispanic white ancestry studied previously[11]. Thirty-two of these families had more than one asthmatic offspring, resulting in a total of 503 asthmatic children available for analysis.

Children enrolled in CAMP had mild to moderate persistent asthma based on demonstration of airway hyperresponsiveness to methacholine with a PC_{20} (provocative concentration causing a 20% fall in FEV_1) less than or equal to 12.5mg/ml, and at least two of the following: asthma symptoms at least two times per week, the use of inhaled bronchodilator at least twice weekly, or the use of daily asthma medication for at least six months in the year prior to screening[12]. Follow-up clinic visits with spirometry occurred at two and four months and every four months thereafter. Spirometry performance was required to meet American Thoracic Society (ATS) criteria for acceptability and reproducibility. At least three spirometric maneuvers were performed, with at least two reproducible maneuvers required for each test. Post-bronchodilator spirometric values were obtained at least fifteen minutes after the administration of 2 puffs of albuterol (90mcg/puff). Complete trial design, methodology, and primary outcomes analysis of the CAMP study have been previously published[13].

Replication Population

Replication studies were performed in 412 parent-child trios recruited as part of the Genetic Epidemiology of Asthma in Costa Rica cohort between February of 2001 and March of 2005. Details on subject recruitment and study protocols have been published elsewhere[14]. In brief, children ages 6 to 14 years were included in the study if they had asthma (a physician's diagnosis of asthma and ≥ 2 respiratory symptoms or asthma attacks in the previous year) and a high probability of having ≥ 6 great-grandparents born in the Central Valley of Costa Rica (as determined by our study genealogist on the basis of the paternal and maternal last names of each of the child's parents). This requirement increased the likelihood that children would be descendants of the founder population of the Central Valley[15]. All children completed a questionnaire, pulmonary function testing (meeting ATS criteria for acceptability and reproducibility), methacholine challenge testing, and measurements of serum total IgE and peripheral blood eosinophils.

Approval was obtained from the Institutional Review Boards of Brigham and Women's Hospital (Boston, MA), the Hospital Nacional de Niños (San José, Costa Rica), and each of the CAMP participating institutions. Informed consent was obtained from parents of participating children, and the child's assent was obtained prior to study enrollment.

Genotyping

SNPs were selected from the HapMap (http://www. www.hapmap.org) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP) databases to tag all common VEGF haplotype blocks and to achieve a physical density of ~5kb/SNP. SNP genotyping was performed using the Illumina Golden Gate platform (Illumina Inc, San Diego, CA).

Duplicate genotyping was performed on approximately 5% of the sample to assess the quality of genotyping. The pedigree data were assessed for evidence of parent-offspring genotype incompatibility using PedCheck[16]. Hardy-Weinberg Equilibrium was tested in parents at each locus using an exact method[17]. Genotype data quality was assessed by genotype completion rates, discordance in the duplicate genotyping, and evidence of Mendelian inconsistencies in the data.

Statistical Analysis

Family-based tests of association (FBAT) were conducted using the PBAT software version 5.3, as implemented in the GoldenHelix package (http://www.goldenhelix.com). The asthma association analyses were performed without covariate adjustment[18]. The analyses of lung function phenotypes (post bronchodilator forced expiratory volume in one second or FEV_1 and the ratio of FEV_1/ forced vital capacity (FVC) were conducted with covariate (age, gender, and height) adjustment. The analysis of airways responsiveness was adjusted for age, gender, height, and use of inhaled steroids. Standard phenotypic residuals were obtained by regressing the phenotype on non-genetic covariates that are thought to be strong predictors of the phenotype. The goal is to remove the variability in the phenotype that is due to non-genetic factors (listed above for each phenotype tested). Standard phenotypic residuals were then used as the outcome for the quantitative trait analysis. Additive and dominant genetic models were considered. To account for multiple comparisons, results were deemed statistically significant (study-wide alpha < 0.05) when similar associations (i.e. same allele, same phenotype, same direction of genetic effect given the same genetic model) were observed in both the CAMP and Costa Rica populations with a Fisher's

combined p-value < 0.0008. This threshold accounts for testing of 8 effectively independent markers (determined using the Nyholt SNPSpD method - see on-line supplement S1[19,20], four non-independent phenotypes, under two genetic models).

Haplotype block structure was defined by the Gabriel method[21] using Haploview(http://www.broad.mit.edu/mpg/haploview/), and haplotype-based tests of association were performed using PBAT. Global tests of haplotype association were performed using the multiallelic haplotype test with a cut-off frequency of 0.01. Individual haplotype block associations were then assessed using haplotype-specific tests of association.

Given the availability of longitudinal data on the children in CAMP, we were able to assess the effect of SNP rs4711750 on progressive airflow obstruction. A longitudinal analysis was performed in CAMP for FEV_1/FVC using mixed models implemented in the Proc MIXED procedure in SAS (version 9.1; SAS Institute, Cary, NC). The longitudinal analysis was adjusted for age, gender, treatment group, and height. Corroborative family-based tests of association with repeated spirometric measures used a principal component family-based associations test (FBAT-PC)[22].

The CAMP study design, as a randomized, placebo controlled clinical trial, enabled testing whether treatment with inhaled corticosteroids modifies the effect of VEGF polymorphisms on longitudinal lung function. We thus performed gene-by-treatment group tests for interaction using the family-based association tests of interaction (FBAT-I) implemented in PBAT[23]. The Costa Rican population was not studied in the context of randomized treatment assignments, precluding similar analysis in this cohort.

Results

Phenotypic and genetic comparability of the CAMP and Costa Rican cohorts

The baseline characteristics of the index cases in both the CAMP and Costa Rican trios genotyped for this study are presented in Table 1. Despite the differences in geographic and ancestral origin and methods of sample ascertainment, the baseline characteristics of the CAMP and Costa Rican probands were very similar. There were more boys in both cohorts, in keeping with the known increased prevalence of childhood asthma among boys.

Of the 470 white families in the CAMP study, 13 were removed from this analysis because of Mendelian inconsistencies, and 10 probands had inadequate genotyping. Of the 426 trios participating in the Genetic Epidemiology of Asthma in Costa Rica study, 14 were excluded because of Mendelian inconsistencies (n=9) or inadequate genotypic data for VEGF (n=5). Seventeen SNPs in VEGF were thus genotyped in 458 non-Hispanic white families (493 probands) in CAMP and 412 Costa Rican trios. The quality of the genotypic data was high for both study populations included in the analysis, with an average completion rate of 99% and no discrepancies between the initial genotyping and the 5% of samples that underwent repeat genotyping. Parental genotypes in both populations were in Hardy-Weinberg equilibrium at all loci.

Figure 1A presents the patterns of linkage disequilibrium (LD) and haplotype block structure in both asthma cohorts. Despite differing ancestral histories, regional LD surrounding the VEGF gene was similar, with the 10 SNP used in this analysis organizing into four discrete haplotype blocks in both populations. Block structure was nearly identical between cohorts, in that block boundaries and the haplotypes within each block were similar. However, notable differences in the distribution of haplotype

frequencies were found (Figure 1B). For example, block 4 was defined by three adjacent SNPs in both cohorts and harbors the same four common haplotypes, yet the most common haplotype in CAMP (TCG, frequency 0.517) was much less common in Costa Rica (0.122). Similar but less extreme differences in haplotype distributions were noted in other blocks, particularly block 2. Thus, while LD and haplotype structure are virtually identical between these cohorts, haplotype frequencies vary somewhat.

Association of VEGF with Asthma and Lung Function Phenotypes in CAMP and Costa Rica

We next tested VEGF genetic variation for association with asthma, airflow obstruction, and airways responsiveness. To reduce the number of statistical comparisons, we limited association testing to 10 of the 17 SNPs genotyped by excluding 7 SNPs with MAF < 10% (due to limited statistical power).

The results of family-based association testing using dominant genetic models between the 10 VEGF polymorphisms and asthma affection status in both populations are shown in Table 2. There was evidence of association of rs833058 with asthma in CAMP (p=0.004). Notably, this association was replicated in the Costa Rican trios (p=0.01). In both populations the T allele was overtransmitted to individuals with asthma (Fisher's combined p=0.0004), which was statistically significant after correction for multiple comparisons[24]. Another VEGF variant demonstrated suggestive evidence of association with asthma in CAMP (rs7748962, p=0.02), but this association did not replicate in the Costa Rican cohort.

Given the role of VEGF in mouse models of airway remodeling, we next examined whether VEGF polymorphisms affect FEV_1 in childhood asthma. Suggestive associations in CAMP included one SNP with FEV_1 (rs4711750, p=0.01), but this association did not replicate in the Costa Rican trios. In the Costa Rican trios, we also found suggestive evidence of association of SNP rs2146323 with post-bronchodilator FEV_1 (p=0.02).

Since airway remodeling has been associated with progressive airflow obstruction, we next sought to determine whether VEGF variation influenced airflow obstruction as measured by FEV_1/ FVC at the time of randomization in the clinical trial. Of note, SNP rs4711750, located in the 3' untranslated region of VEGF, was associated with FEV_1/FVC (p=0.01) in CAMP, with the major A allele being overtransmitted to individuals with higher FEV_1/ FVC ratios. The association of SNP rs4711750 with FEV_1/ FVC in CAMP was also found in the Costa Rican cohort (p=0.02), albeit with opposite direction (the minor A allele was undertransmitted to individuals with higher FEV_1/ FVC).

VEGF expression has been associated with airways responsiveness in the general population[25]. Therefore, we examined whether VEGF polymorphisms affect airways responsiveness in childhood asthma. The asthma-associated SNP rs8833058 was also associated with increased airways responsiveness in both populations, with carriers of the T allele demonstrating increased airways responsiveness in both CAMP (p=0.01) and Costa Rica (p=0.03, Fisher's combined p=0.003, Table 2). No additional SNP associations with airways responsiveness were observed.

Haplotype block associations with asthma and lung function phenotypes in CAMP and Costa Rica

Based on the replicated findings of the single SNP analysis demonstrating an association with asthma, FEV_1/ FVC, and airways responsiveness in both populations, the haplotype blocks containing these SNPs were subsequently tested for association with these phenotypes. Using the block structure established in Haploview, block 1 was tested for association with asthma affection status and airways responsiveness in both populations. The association with asthma was confirmed, with the AT haplotype being overtransmitted in both CAMP and Costa Rica (p=0.02 and p=0.005 respectively, Fisher's combined p=0.001). The AT haplotype in block 1 was also associated with increased airways responsiveness albeit with weaker affect (CAMP p=0.03, and Costa Rica (p=0.03, Fisher's combined p= 0.007). Furthermore, block 4, which is the terminal 3-SNP haplotype block containing rs4711750, was tested for association with FEV_1/ FVC. The ACA haplotype was associated with FEV_1/ FVC in both populations. The ACA haplotype was overtransmitted to individuals with higher FEV_1/ FVC values in CAMP and undertransmitted in the Costa Rican cohort (p=0.01 and 0.003, respectively).

Longitudinal Analysis of Airflow Obstruction in CAMP

To assess the effect of rs4711750 on airflow obstruction over time, we performed a population-based longitudinal analysis of post-bronchodilator FEV_1/FVC over ~4.5 years of observation during the CAMP clinical trial. In order to exclude the potential effects of population stratification, confirmatory family-based association testing using repeated measures of FEV_1/FVC was performed using FBAT-PC. A significant

relationship between rs4711750 genotype and FEV_1/FVC was noted (p=0.03 with both analyses). As illustrated in Figure 2, TT homozygotes developed more severe airflow obstruction over time as compared to AT or AA subjects. Of interest, inhaled corticosteroids did not alter the development of progressive airflow obstruction over the time course of the clinical trial (p=0.11). Similar longitudinal data are not available in the Costa Rica cohort, precluding assessment of replication of this finding.

Modification of the Effect of VEGF SNPs on Longitudinal Lung Function by Treatment with Inhaled Corticosteroids in CAMP

Given previous evidence for the modification of the effect of VEGF on airway remodeling by treatment with inhaled corticosteroids for six months[7], we tested for SNP-by-treatment group assignment interactions, using FBAT-I[23]. These analyses revealed evidence for interaction between SNP rs2146323 and treatment group on post-bronchodilator FEV_1/FVC (p for interaction = 0.02): individuals on inhaled corticosteroids carrying at least on copy of the A allele had higher mean FEV_1/FVC after four years of treatment with inhaled corticosteroids compared to subjects not treated with inhaled corticosteroids (85.1 ± 5.6 vs. 82.5 ±7.3). In contrast, subjects who did not carry the A allele demonstrated similar FEV_1/FVC ratios regardless of treatment assignment (83.3 ±7.5 vs. 84.2 ± 6.7). There was no additional evidence for SNP-treatment interactions for the other VEGF SNPs.

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Discussion

A substantial proportion of children with persistent asthma (including those with mild disease) have reduced lung function as compared to non-asthmatic, healthy age-matched controls and fail to attain their maximal predicted lung growth[26]. Although the pathologic changes characteristic of airway remodeling are often attributed to persistent allergic inflammation, sustained treatment of childhood asthma with inhaled corticosteroids at conventional doses does not appear to influence long- term lung function[12]. Thus, identifying the molecular determinants underlying the development of asthma and airway remodeling is of great importance, since understanding novel pathways that contribute to airways dysfunction may ultimately have profound therapeutic implications.

In this family-based association study, we found that a VEGF polymorphism (rs833058) was associated with asthma in two independently ascertained and ethnically distinct populations. This replicated association is significant after study-wide correction for multiple comparisons (see Supplement S1 for details). Of note, this SNP was also associated with increased airways responsiveness in both childhood asthma populations. We demonstrated that VEGF variants also affect airflow obstruction: rs4711750 was associated with FEV_1/ FVC in both populations and was also associated with progressive airflow obstruction over more than four years of follow-up in the CAMP study, suggesting that this variant (or others in linkage disequilibrium with it) may contribute to airway remodeling. Furthermore, we demonstrated that four years of treatment with inhaled corticosteroids in the CAMP clinical trial modified the effect of SNP rs2146323 on FEV_1/ FVC after 4 years of observation.

Substantial evidence exists demonstrating a role for VEGF in the development of airways responsiveness. Recently, Wang and colleagues demonstrated the correlation of airways responsiveness (as measured by methacholine response) with bronchial epithelial cell VEGF and VEGFR mRNA expression in asthmatics.[7]. Genetic association of VEGFR polymorphisms with airways responsiveness in the general population have been reported[25]. To our knowledge, the results presented here are the first to suggest association of a VEGF polymorphism with airways responsiveness in asthmatics.

Animal models of lung development have demonstrated the importance of the VEGF pathway in lung morphogenesis, with specific contribution to airway development *in utero*[30]. Substantial evidence from murine models of development suggests that epithelial expression of VEGF during early lung development regulates epithelial branching morphogenesis. We demonstrate associations of VEGF polymorphisms with lung function and airways responsiveness in two asthmatic populations, which may be related to the implications of VEGF in the context of airway development.

Like other intermediate asthma phenotypes, airway remodeling is likely to be a complex process, resulting from the interplay of genetic and environmental factors. An important environmental factor is cigarette smoke exposure (both active and passive), but longitudinal studies of lung function in adult asthmatics have shown that the accelerated decline often observed in asthmatics cannot be explained by smoking alone[31]. The evidence supporting a genetic contribution to this process is considerable. In addition to numerous studies demonstrating heritability of lung function in otherwise healthy populations, heritability estimates of lung function in asthmatic populations also support an important genetic contribution. For example, the heritability of post-bronchodilator

FEV_1/ FVC was estimated as 15.4% (SD 6.6%; p=0.001) in Costa Ricans, and subsequent genome-wide linkage analysis of pulmonary function in extended pedigrees from this population identified modest linkage of post-bronchodilator FEV_1/FVC on chromosome 6p in non-smokers[32]. Linkage to this region, which includes the VEGF locus, has also been detected in a second asthma population[33]. It is conceivable that genetic variation at the VEGF locus contributes to these linkage results.

Bronchial biopsy specimens in patients with asthma demonstrate increased basement membrane thickening and increased vascularity when compared to healthy controls[34]. Several studies suggest that these pathologic changes diminish after treatment with inhaled corticosteroids[7, 34]. Based on these observations, we tested for interaction of inhaled corticosteroids on lung function after four years of treatment with inhaled corticosteroids. We have shown that treatment with inhaled corticosteroids results in higher lung function at four years in patients with at least one copy of the A allele for SNP rs2146323, which is supported by the previous research. We caution that this pharmacogenetic analysis was performed post-hoc and that the results were not amenable to replication in the Costa Rica cohort. Therefore, it is unclear whether these latter findings can be generalized to other populations.

Unlike the association of rs833058 with asthma, for which similar genetic effects were noted in both cohorts, the associations of rs4711750 with FEV_1/ FVC are in opposing directions in the CAMP and Costa Rica cohorts. There have been several other 'flip-flop' associations documented in the literature[35-38]. Though possibly representing two false-positive associations, such flip-flops are occasionally noted when there are subtle differences in genetic architecture, suggesting that differences in LD between the

typed marker and with non-genotyped functional SNP may explain the conflicting associations[39]. Other possible explanations for this 'flip-flop' association include differences in environmental exposures, epistatic interactions, or gene-by environment interactions (not modeled in this analysis) between the two cohorts.

In summary, to our knowledge this is the first time that genetic variation in the VEGF gene has been associated with asthma and airways responsiveness in two cohorts of children with mild-moderate persistent asthma, suggesting a direct role of VEGF in the development of asthma. We also demonstrate preliminary associations of VEGF variation with lung function in both the CAMP and Costa Rica cohorts, though additional fine mapping of this region, along with additional replication studies in other populations, will be needed to resolve the directionality issues noted. If the observed effects of VEGF genetic variation on lung function are corroborated in future studies, the functional markers may serve as potential biomarkers for the identification of susceptible individuals who could benefit from novel therapies that target VEGF pathways.

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Legends for Figures

Figure 1A: Linkage disequilibrium structure of VEGF in CAMP and Costa Rica

Linkage Disequilibrium (LD) structure based on D' in the CAMP (left) and Costa Rica (right). The 17 SNPs genotyped are represented from 5' (top) to 3' (bottom). Haplotype blocks defined using the method outlined by Gabriel, et al[4]. **Bold** indicates SNP tested for association in CAMP and Costa Rica. The red blocks indicated SNPs with high LD, while regions of low LD are shown in light blue and white.

Figure 1B: Comparison of haplotype block structure across the VEGF locus in CAMP and Costa Rica

A comparison of the haplotype block structure, defined by the Gabriel method, of the CAMP and Costa Rican populations. SNP organize into four discrete haplotype blocks with similar boundaries in both populations. Haplotypes are similar in the two populations within each block, but the differences in haplotype frequencies between the two populations are shown here. Theta represents the recombination frequency between adjacent haplotype blocks.

Figure 2: Longitudinal FEV_1/FVC ratio by genotype for SNP rs4711750

Effect of VEGF SNP rs4711750 on rate of lung function decline in CAMP cohort over four years of observation as measured by post-bronchodilator FEV_1/FVC. Solid line = CAMP subjects that are homozygous or heterozygous for major allele. Dashed line = minor allele homozygotes.

Figure 1A: Linkage disequilibrium structure of VEGF in CAMP and Costa Rica

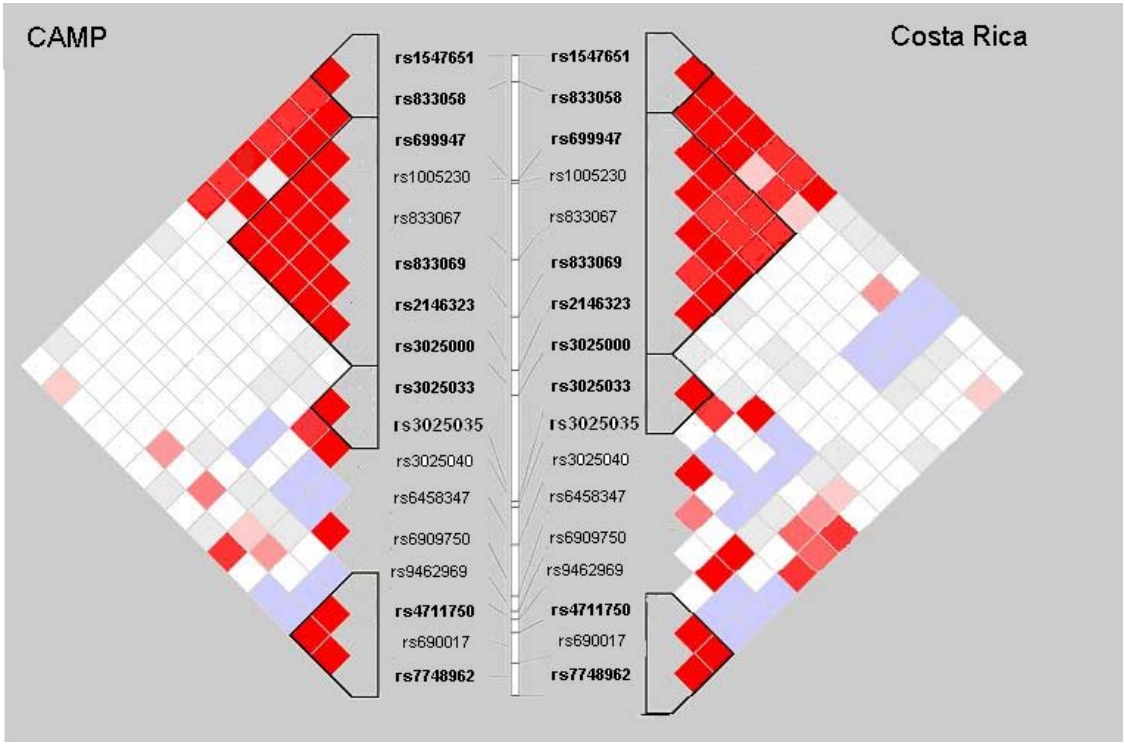

Figure 1B. Comparison of haplotype block structure in CAMP and Costa Rica.

							Haplotype Frequencies:	CAMP	Costa Rica
Block 1:									
	rs1547651	*rs833058*							
Hap 1	A	C						0.452	0.315
Hap 2	A	T						0.389	0.536
Hap 3	T	C						0.157	0.146
Theta								0.64	0.79
Block 2:									
	rs699947	*rs1005230*	*rs833067*	*rs833069*	*rs2146323*	*rs3025000*			
Hap 1	A	T	C	T	A	C		0.327	0.279
Hap 2	C	C	T	C	C	T		0.318	0.279
Hap 3	C	C	T	T	C	C		0.185	0.246
Hap 4	A	T	C	T	C	C		0.15	0.074
Hap 5	C	C	T	C	C	C		0.013	0.62
Theta								0.24	0.24
Block 3:									
	rs3025033	*rs3025035*							
Hap 1	G	C						0.147	0.206
Hap 2	A	C						0.79	0.695
Hap 3	A	T						0.063	0.099
Theta								0.38	0.34
Block 4:									
	rs4711750	*rs6900017*	*rs7748962*						
Hap 1	T	C	G					0.517	0.122
Hap 2	A	C	A					0.226	0.488
Hap 3	T	C	A					0.185	0.323
Hap 4	T	T	A					0.069	0.068

Figure 2: Longitudinal FEV_1/FVC ratio by genotype for SNP rs4711750

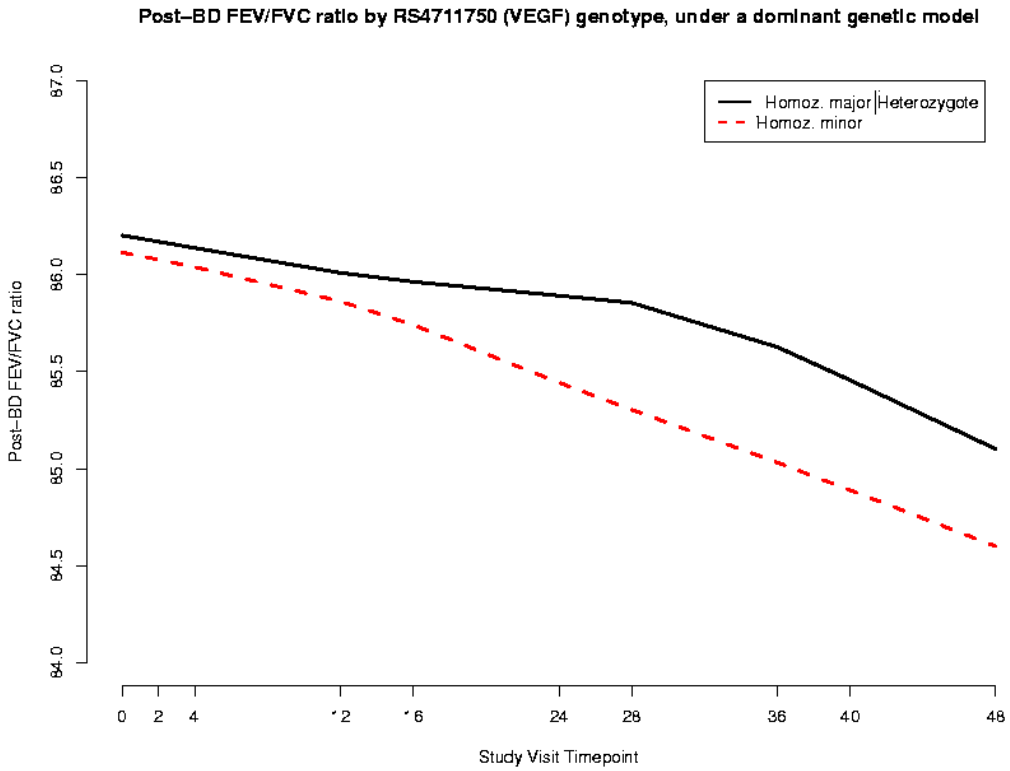

Table 1: Baseline phenotypic characteristics of index cases in CAMP and Costa Rica

Variable	CAMP Non-Hispanic white n=493	Costa Rica n=412
Mean age (years)*	8.7 (7.0-10.5)	9.2(7.8-10.4)
Sex, female	187(38%)	152 (37%)
Mean Baseline Post-Bronchodilator FEV_1 (L)	1.81 (0.5)	1.84 (0.5)
Mean Baseline Post-Bronchodilator FVC (L)	2.13 (0.6)	2.15 (0.6)
Mean Baseline Post-Bronchodilator FEV_1/ FVC	85.62 (6.3)	85.63(6.4)
Median IgE Level IU/L †	405.0 (171.0-1061.0)	422.0 (118.0-975.0)
Median PC_{20} (mg/ml)	1.1 (0.5-2.8)	1.2 (0.7-1.4)
Median Eosinophil Count (cells/mm^3)	411.0 (229.0-700.0)	530.0 (290.0-805.0)

Definitions of abbreviations: CAMP= Childhood Asthma Management Program; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1
* Median (interquartile range)
† Mean (standard deviation)
** Lung function phenotypes presented in the table are unadjusted values.

Table 2: Suggestive and Replicated Associations in CAMP and Costa Rica Under Dominant Genetic Models*

Phenotype	dbSNP rs#	CAMP					Costa Rica				
		Allele	Allele Frequency	# Informative Families	Z Score	p value	Allele	Allele Frequency	# Informative Families	Z Score	p value
Asthma	rs833058	T	0.40	178	2.72	0.004	T	0.53	183	2.36	0.01**
	rs7748962	G	0.23	254	-2.36	0.01	G	0.12	149		p>0.05
FEV_1	rs4711750	A	0.52	227	2.36	0.01	A	0.49	247		p>0.05
	rs2146323	A	0.33	228		p>0.05	A	0.32	233	2.36	0.01
FEV_1/FVC	rs4711750	A	0.53	225	2.36	0.01	A	0.49	247	-2.36	0.01
Airways Responsiveness	rs833058	T	0.40	241	-2.32	0.01	T	0.53	180	-2.12	0.03

* Lung Function phenotypes are post-bronchodilator values

**Combined p value=0.001, which is significant after study-wide correction for multiple comparisons