PRKCA: A Positional Candidate Gene for Body Mass Index and Asthma

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Asthma incidence and prevalence are higher in obese individuals. A potential mechanistic basis for this relationship is pleiotropy. We hypothesized that significant linkage and candidate-gene association would be found for body mass index (BMI) in a population ascertained on asthma affection status. Linkage analysis for BMI was performed on 657 subjects in eight Costa Rican families enrolled in a study of asthma. Family-based association studies were conducted for BMI with SNPs within a positional candidate gene, *PRKCA*. SNPs within *PRKCA* were also tested for association with asthma. Association studies were conducted in 415 Costa Rican parent-child trios and 493 trios participating in the Childhood Asthma Management Program (CAMP). Although only modest evidence of linkage for BMI was obtained for the whole cohort, significant linkage was noted for BMI in females on chromosome 17q (peak LOD = 3.39). Four SNPs in a candidate gene in this region (*PRKCA*) had unadjusted association p values < 0.05 for BMI in both cohorts, with the joint p value for two SNPs remaining significant after adjustment for multiple comparisons (rs228883 and rs1005651, joint p values $= 9.5 \times 10^{-5}$ and 5.6×10^{-5}). Similarly, eight SNPs had unadjusted association p values < 0.05 for asthma in both populations, with one SNP remaining significant after adjustment for multiple comparisons (rs11079657, joint p value $= 2.6 \times 10^{-5}$). *PRKCA* is a pleiotropic locus that is associated with both BMI and asthma and that has been identified via linkage analysis of BMI in a population ascertained on asthma.

Introduction

Asthma affects an estimated 300 million individuals worldwide.[1] A number of large cross-sectional[2–6] and prospective[7–10] epidemiologic studies have independently noted an elevated risk of asthma in association with increasing body mass index (BMI). This association has often been reported only[8,9] or more strongly in females.[2,4,7] For instance, the population attributable risk of asthma resulting from obesity has been estimated to be as high as 28% in postpubertal females[11] but to be not significant in postpubertal males.

A variety of mechanisms have been proposed to link obesity with asthma. We have previously postulated that common genetic pathways may contribute, given that both obesity and asthma are complex traits with significant heritability and previous studies have shown linkage to both obesity and asthma for the same genomic regions.[12] Although twin studies have verified that a significant amount of the covariation between obesity and asthma is caused by shared genetic risk factors for both conditions,[13,14] to our knowledge, no gene has been associated with both obesity and asthma.

In this study, we report the results of a genome-wide linkage analysis of BMI in eight extended families of Costa Rican children with asthma. Because of known modification of the effect of BMI on asthma by sex and the potential for sex-specific genetic effects on both asthma and obesity,[15–18] we report both overall and sex-stratified linkage results. We show significant linkage to BMI on chromosome 17q24 in females. Although multiple interesting candidate genes are located within this linkage peak, several of these, including somatostatin receptor 2 (*SSTR2*, [MIM 182452]), galanin receptor 2 (*GALR2*,[MIM 603691]), and growth factor receptor-bound protein 2 (*GRB2*, [MIM 108355]) have been previously tested for association with obesity phenotypes, with negative results.[19] In addition, a knockout mouse model of the regulatory II beta isoform subunit in protein kinase C α (*PRKCA* [MIM 176960]), results in almost no white adipose tissue.[20] We therefore chose to conduct an association study for *PRKCA*, a candidate gene located within the linkage region of interest. We first demonstrate an association between single-nucleotide polymorphisms (SNPs) in *PRKCA* and BMI in two independent family-based cohorts of children with asthma. Given the relationship between asthma and obesity, we also present evidence for association between *PRKCA* SNPs and asthma affection status in these populations.

Subjects and Methods

Study Populations

Costa Rica

Eight families of Costa Rican children ascertained on asthma affection status were recruited as previously described.[21] In brief, in addition to asthma affection status, inclusion criteria for the eight probands included age $\geq$ 6 years but $\leq$ 14 years, $\geq$ 1 sibling with physician-diagnosed asthma, increased airway responsiveness (a dose of methacholine causing a 20% reduction in FEV_1 [PD_{20}] $\leq$ 8.58 μmol of methacholine) and $\geq$ 6 great-grandparents born in the Central Valley of Costa Rica.

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DOI 10.1016/j.ajhg.2009.06.011.

The family-based association study of *PRKCA* and BMI was conducted in an independent set of 415 Costa Rican children with asthma and their parents (parent-child trios). Index children in the trios were recruited on the basis of age (6 to 14 years), asthma (defined as physician-diagnosed asthma and either at least two 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.

All study participants (with the exception of parents in trios, who only provided a blood sample) completed a protocol that included a questionnaire and collection of blood samples for DNA extraction and measurements of height and weight. Height was measured with a stadiometer to the nearest half inch. Weight was measured with a professional scale (Tanita Corp., Arlington Heights, IL).

Written consent was obtained from adults. Written parental consent was obtained for children, for whom written assent was also obtained. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, MA).

The Childhood Asthma Management Program

The Childhood Asthma Management Program (CAMP) was a clinical trial evaluating the effects of anti-inflammatory medications in children with mild to moderate asthma over a period of 4-6 years.[22,23] Inclusion criteria included doctor's diagnosis of asthma in conjunction with presence of asthma symptoms and/or low peak flow readings during a run-in when taking albuterol, as well as evidence of increased airway responsiveness ($PC_{20} \leq$ 12.5 mg/mL of methacholine). This analysis was restricted to 493 non-Hispanic white participants and their parents because of the small sample size of other ethnic groups. CAMP was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the other CAMP study centers.

Phenotypes

Primary Outcome—BMI

For all subjects, BMI was calculated as kg/m^2.

Secondary Outcome—Asthma

To enhance comparability of the asthma phenotype among Costa Rican children in trios with that used in CAMP, we defined asthma as (1) physician-diagnosed asthma and either two respiratory symptoms or asthma attacks in the prior year and (2) increased airway responsiveness ($PD_{20} \leq 16.81$ μmol of methacholine) or (for children unable to undergo methacholine challenge testing) increased bronchodilator response (improvement in baseline FEV_1 of 12% or greater [at least 200 ml] after albuterol administration).[24] In CAMP, all probands were classified as having asthma.

Genotyping

The Genome Quebec Innovation Centre performed genome-wide microsatellite genotyping in 671 individuals from the eight extended pedigrees in CR (Costa Rica) by using Applied Biosystems (AB) 3700 and 3730 analyzers with DNA that was extracted from blood samples with Puregene Kits (Gentra Systems). A total of 380 autosomal short-tandem repeat (STR) markers with an average spacing of 8.2 cM were genotyped.

RELPAIR was used for determining pedigree relationships on the basis of the genome scan marker data.[25,26] Mendelian inconsistencies at individual markers were resolved with PEDCHECK.[27] Pedigree genotype inconsistencies were observed on average <0.5% per STR.

Additional SNP genotyping was performed in the 415 Costa Rican trios with Illumina Golden gate technology. We initially selected 39 SNPs from the *PRKCA* gene by using the following criteria: reported minor allele frequency (MAF) ≥ 10%, validated in Golden Gate, and location within the gene every ~10 kb. After the initial analysis using these 39 SNPs, we extended coverage of *PRKCA* by using data from European Americans (CEU) in the International HapMap project[25] and applying a linkage disequilibrium (LD)-tagging algorithm[26] (MAF ≥ 10% and $r^2 > 0.8$) to capture common variation in *PRKCA* and its 50 kb flanks. We used the "force include" option to include the 39 previously genotyped SNPs, when possible. This process led to selection of an additional 137 SNPs in *PRKCA*, which were successfully genotyped in Costa Rican trios with the SEQUENOM iPLEX platform[27] (Sequenom, San Diego, CA). Replication genotyping was performed on the INITIAL 39 SNPs in 493 CAMP families with the Illumina Golden-Gate genotyping assay (Illumina, San Diego, CA);[28] the remaining LD-tag replication SNPs were genotyped in 400 CAMP families as part of a larger experiment with the Illumina Infinium HumanHap550 genotyping chip (Illumina, San Diego, CA).[29] In both Costa Rica and CAMP, duplicate genotyping was performed on ~5% of the samples. Genotype quality control was assessed by <1% discordances, <5 Mendelian inconsistencies, and genotype completion rates of >98.5% for all loci.

Statistical Analyses

Linkage Analysis

Multipoint linkage analysis was conducted with the variance components approach implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program (version 2.1.5).[30] SOLAR uses identity-by-descent sharing to segment the observed phenotypic variance into genetic and nongenetic components. The multipoint identity-by-descent matrices were estimated with the Loki program.[31]

Because of potential sexual dimorphism in BMI, a sex-stratified analysis was conducted in addition to an analysis of the overall sample. The variance components models in SOLAR were adjusted for age, age squared, and sex (in the overall cohort only). Covariates with a p value < 0.05 were retained in the final model. Because of the nonnormality of the BMI distribution (resulting in residual kurtosis estimates ranging from 1.0–2.3), robust LOD scores were calculated.[32] We obtained the robust LOD scores by applying an adjustment factor to the observed LOD scores. In brief, to calculate the adjustment factor, we generated an empirical distribution (based on 10,000 simulations) of LOD scores under the null hypothesis, by using simulated, fully informative markers that are unlinked to the trait. The adjustment factor is the coefficient obtained from a regression of the simulation-based LOD scores on the LOD scores that would be expected under a normally distributed trait. The LOD adjustment factors were 0.99 in the overall group, 0.59 in males, and 1.18 in females. Robust LOD scores ≥ 3.3 were considered significant.[33]

Association Analysis

Family-based association tests (FBATs) of BMI and asthma were conducted in the Costa Rican and CAMP families with PBAT[34] (version 3.5), in both the overall set of families and sex-stratified groups, under additive, dominant, and recessive genetic models. The PBAT approach provides a general framework that can accommodate family-based analyses with missing parental genotypes, additional siblings, quantitative phenotypes, extended pedigrees, and multivariate traits.[34–38] The quantitative BMI analysis was adjusted for age, age-squared, and sex. For quantitative traits,

Table 1. Baseline Characteristics of Members of Extended Pedigrees of Costa Rican Children with Asthma

Family	N	Gender, Female (%)	Mean BMI (SD)	Mean Age (SD)	Asthma (%)
1 (5001)	34	16 (47.1)	23.4 (4.5)	34.9 (19.3)	12 (35.3)
2 (5002)	20	11 (55.0)	24.9 (4.7)	33.7 (19.3)	8 (40.0)
3 (5003)	233	135 (57.9)	23.8 (5.6)	27.9 (17.1)	59 (25.3)
4 (5004)	102	61 (59.8)	24.8 (5.7)	32.0 (17.9)	17 (16.7)
5 (5005)	8	7 (87.5)	27.8 (5.6)	36.0 (20.5)	2 (25.0)
6 (5006)	107	60 (56.1)	23.2 (5.4)	29.1 (17.1)	8 (7.5)
7 (5007)	23	10 (43.5)	24.8 (4.9)	32.2 (19.0)	5 (21.7)
8 (5008)	130	56 (43.1)	22.9 (5.3)	28.1 (18.9)	24 (18.5)
Total	657	301 (45.8)	23.8 (5.5)	29.6 (17.9)	135 (20.6)

evidence for association is suggested when the transmission of an allele is strongly correlated with phenotypic values. The analysis of asthma did not adjust for any covariates; previous studies have shown that the inclusion of covariates in a family-based analysis of a binary trait may reduce power.[39] In an analysis of trios in which all offspring are affected (i.e., asthma), without adjustment for covariates, under an additive model, the family-based association test is equivalent to the transmission disequilibrium test (TDT).[40] Evidence of association is suggested when an allele is systematically over- or undertransmitted more than would be expected (conditioning on parental mating types and assuming Mendelian transmission) by chance alone.

Although longitudinal data are available for CAMP subjects (with a maximum of 14 measurements over a 4 year time period), BMI was measured at only one time point in the Costa Rican families. Thus, the randomization visit (RZ) was selected as the time point for the primary replication analysis in CAMP. This visit was selected so that the number of missing subjects and the impact of any asthma treatment intervention on BMI could be minimized. A secondary, multivariate, longitudinal analysis of BMI was also conducted in the CAMP subjects, with a population-based approach. We conducted the population-based analysis in SAS (version 9.1) by using mixed models, with fixed effects for the SNP, subject age at baseline, age at baseline squared, gender, and time under study. Random intercepts and slopes were modeled for each subject.

In assessing joint evidence for association, p values were combined across study populations with Fisher's combined probability method.[41] In combining the p values, all hypothesis tests in the replication population had one-sided alternatives (based on the direction of the association in the testing population), so that combined test statistics from association tests in opposite directions would not produce inappropriately small p values. For comparison, p values reflecting two-sided alternatives in both populations were also calculated. After calculating joint p values, the results were compared against an overall significance threshold that was Bonferroni-corrected for the overall number of comparisons conducted per phenotype (528 comparisons: 176 SNP × 3 genetic models). For both the BMI and asthma analyses, a SNP with a Bonferroni adjusted p value $< 9.5 \times 10^{-5}$ (0.05/528) was considered significant.

Results

Characteristics of Extended Families Contributing to Linkage Analysis

Of the 667 members of the extended pedigrees of Costa Rican children with asthma, 657 subjects had a measured BMI and were thus included in the multipoint linkage analysis.[30] Table 1 shows the characteristics of the members included in the linkage analysis. There was variability among participating families with regard to number of members, sex, BMI, and percentage of subjects with asthma.

Genome-wide Linkage Analysis of BMI

Figure 1 displays the main results of the genome-wide linkage analysis of BMI in extended Costa Rican families. There was only modest evidence of linkage for BMI for the entire cohort (peak robust LOD = 1.67) and little

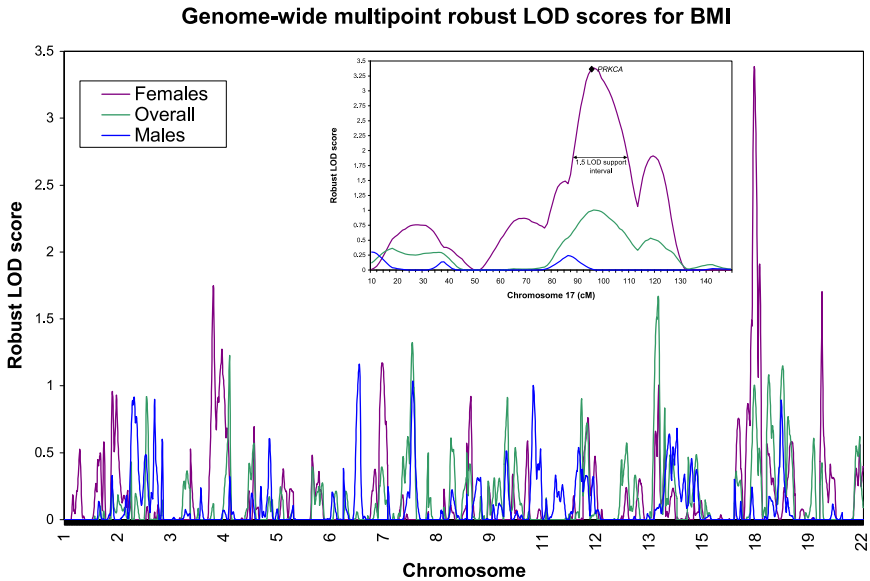

Figure 1. Genome-wide Linkage of Body Mass Index in 657 Costa Rican Subjects from Eight Families

The families were ascertained via an asthmatic proband. Significant linkage is noted for BMI in females on chromosome 17q, with a peak LOD score of 3.39. The inset demonstrates the approximate location of *PRKCA* within the linkage peak.

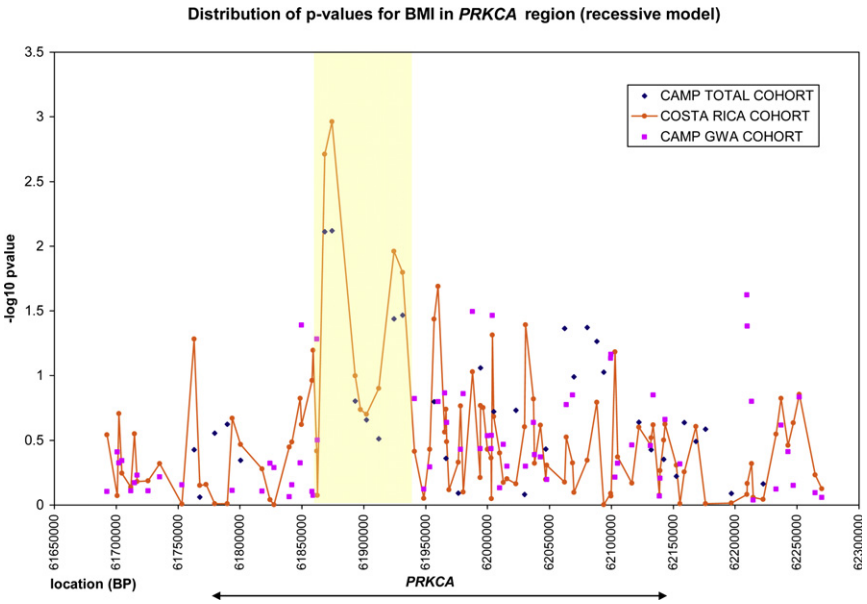

Figure 2. Distribution of p Values from Family-Based Association Tests of Body Mass Index in the Costa Rican and CAMP Cohorts
The highlighted region contains four SNPs significantly associated in both cohorts.

evidence for linkage to BMI in males in the sex-stratified analysis (peak robust LOD = 1.16). However, there was significant linkage to BMI in females on chromosome 17, with a peak robust LOD score of 3.39, as well as modest evidence for linkage to BMI in two other regions [chromosome 3 (1.48); chromosome 17 (1.44)]. The 1.5 LOD unit support interval for the linkage peak on chromosome 17 fully encompasses chromosome17q23.2-q25.1, the location of the *PRKCA* gene (Figure 2). Thus, we moved forward with association testing in the *PRKCA* region.

Characteristics of the Populations Contributing to Association Analyses

Table 2 shows the characteristics of the probands in the populations that were analyzed for association between SNPs in *PRKCA* and BMI. After excluding subjects missing BMI data, the primary test population for the BMI analysis included 415 parent-child trios participating in a study of the genetics of asthma in Costa Rica. Of the 415 Costa Rican probands, 311 (~75%) had asthma with either increased airway responsiveness or bronchodilator responsiveness (31 had missing data and 73 did not meet criteria for stringent asthma) and were thus included in the analysis of asthma. After removing subjects missing BMI measurements, the replication population comprised 457 white families with 493 offspring participating in the CAMP clinical trial. Overall, the groups were relatively balanced with respect to gender and BMI, but there were significant differences in the mean age (9.1 versus 8.8 years, p = 0.01) and percent predicted pre-bronchodilator FEV1 (99.8 versus 94.0, $p = 1.5 \times 10^{-8}$) of the participants in CAMP and Costa Rica.

Table 2. Baseline Characteristics of Children in Nuclear Families in Costa Rica and CAMP

Characteristic	Costa Rica	CAMP	p Value[a]
n	415	493	NA
Age in years, mean (SD)	9.1 (1.8)	8.8 (2.1)	0.01
Gender, female (%)	158 (38.1)	191 (38.7)	0.89
BMI, mean (SD)	17.8 (3.4)	17.8 (3.1)	0.79
Prebronchodilator FEV1% predicted, mean (SD)	99.8 (15.9)	94.0 (13.8)	1.5×10^{-8}

[a] This column reflects the p value from either a chi-square test of independence for binary traits or student's t test for continuous traits.

Association Studies of *PRKCA* and BMI

Family-Based Analysis in Costa Rica and CAMP

In the analysis of the Costa Rica population, we initially tested 39 SNPs in *PRKCA* for association with BMI in the Costa Rica cohort by using FBATs. Of these, four SNPs (rs228883, rs1005651, rs228875, and rs2244497) were significantly associated with BMI under a recessive genetic model in the overall group, with (unadjusted) p values ranging from 0.016 to 0.001. For all four SNPs, transmission of two copies of the minor allele was associated with increased BMI. On the basis of the strong evidence of association, these four SNPs were tested in the CAMP cohort. All four SNPs replicated in CAMP under a recessive genetic model and demonstrated association in the same direction, with p values of similar magnitude (one sided p values ranged from 0.018 to 0.004). The results from both the Costa Rica and CAMP populations are presented in Table 3. In this table, genetic effect sizes are also presented. Across all four SNPs, the observed increase in BMI ranges from 1.21 to 2.45 across the two populations.

The joint p value (from Fisher's combined probability method[41]) was compared against a significance threshold (9.5×10^{-5}) that was Bonferroni corrected for the overall number of comparisons (528) conducted in the primary analysis of the BMI phenotype. Although this association with BMI was observed among the 39 SNPs initially genotyped, we adjusted for the final number of comparisons conducted for this phenotype, thus including the additional 137 LD-tagging SNPs. Of the four SNPs, two (rs228883 and rs1005651) were significantly associated with BMI after correction for multiple comparisons, with joint p values of 5.6×10^{-5} and 9.5×10^{-5}, respectively. However, given the consistency in association and

Table 3. Evidence for Association of *PRKCA* with BMI in Costa Rica and CAMP

Marker	Location (BP)[a]	Minor Allele	Allele Frequency		Number of Informative Families[b] (number of offspring with 0/1 recoded genotype)		Effect Size[c]		CR p Value[d,e]	CAMP Replication p Value[d,e] (two-sided)	Joint p Value[f] (CR, CAMP two-sided)
			CR	CAMP	CR	CAMP	CR	CAMP			
rs228883	61874457	T	0.27	0.33	91 (67/24)	110 (80/39)	2.45	1.60	+0.0011	+0.0038 (+0.0076)	5.6×10^{-5}** (1.0×10^{-4})
rs1005651	61868473	C	0.26	0.33	83 (60/23)	113 (83/39)	2.27	1.60	+0.0019	+0.0039 (+0.0077)	9.5×10^{-5}** (1.8×10^{-4})
rs228875	61924337	A	0.29	0.35	101 (70/31)	129 (92/46)	1.71	1.22	+0.0109	+0.0182 (+0.0364)	0.0019 (0.0035)
rs2244497	61931405	C	0.31	0.36	120 (86/34)	136 (98/47)	1.69	1.21	+0.0160	+0.0171 (+0.0341)	0.0025 (0.0046)

[a] SNP positions from dbSNP build 129. *PRKCA* is located on chr17:61,729,388-62,237,324, with the ATG start site located at position 61,729,432,(hg18).
[b] Number of informative families for the FBAT statistic under a recessive model (i.e., both parents had at least one copy of the minor allele and at least one parent was heterozygous). The number of offspring in CAMP families may be greater than the number of informative families, given that some families had more than one offspring.
[c] The genetic effect size is obtained from the following model: BMI~ $(x - Ex) + age + age^2 + sex$, where x reflects the coding of the minor allele of the SNP under the recessive model (i.e., 0 for 0/1 copies, 1 for 2 copies), and Ex is the expected transmission under the null hypothesis of no linkage/association.
[d] From the FBAT statistic, adjusting for age, age^2, and sex. In Costa Rica, p values reflect a two-sided alternative hypothesis, whereas the replication p values in CAMP are one sided. The p value in parentheses under the CAMP replication reflects a two-sided alternative hypothesis. CAMP and CR p values are unadjusted for multiple comparisons.
[e] A plus sign (+) indicates that transmission of two copies of the minor allele (in comparison to zero or one copy) is associated with an increase in BMI.
[f] The joint p value reflects the combination of a two-sided alternative hypothesis in CR and a one-sided alternative hypothesis in CAMP. The p values in parentheses are the combination of a two-sided alternative hypothesis in both CR and CAMP. Double asterisks (**) indicate that the joint p value was significant after Bonferroni correction for the number of comparisons conducted (528, 176 SNP × 3 genetic models) in the Costa Rican cohort. The threshold for significance is 9.5×10^{-5} (0.05/528).3

significant (unadjusted) p values in each cohort, we considered SNPs rs228875 and rs224497 to be suggestive of association and included them as markers of interest in the longitudinal analysis in CAMP, our replication population. It also should be noted that if p values from two-sided alternative hypotheses were used for both Costa Rica and CAMP (instead of two-sided in Costa Rica and one-sided in CAMP) when calculating the joint test statistic p values (given in parentheses in Table 3), the joint p values (1.0×10^{-4} and 1.8×10^{-4} for rs228875 and rs224497, respectively) would not have met the significance criteria after our conservative adjustment for multiple comparisons, which ignored linkage disequilibrium in this region and incorporated adjustment for testing under multiple genetic models in both the initial and replication population.

As noted above, after observing the initial results for these four SNPs, the additional 137 LD-tag SNPs in Costa Rica and the CAMP families were tested for association. None of the additional tests demonstrated convincing evidence of association (Figure 2). The highlighted region contains the four SNPs that were significant in both the Costa Rica and CAMP populations. Additionally, in the gender-stratified analysis, females did not demonstrate stronger evidence of association than males (data not shown).

Longitudinal Analysis of BMI in CAMP

To extract the most information from the CAMP study, we also conducted a population-based longitudinal analysis of BMI for the four SNPs of interest. Each marker was analyzed separately. All four SNPs were significant predictors (with unadjusted p values between 0.0048 and 0.0007) of BMI in the mixed linear models and demonstrated a consistent increase in mean BMI over time in probands with two copies of the minor allele. The coefficients for the markers ranged from 1.29 (rs1005651) to 0.99 (rs2244497), reflecting an overall mean BMI increase of 0.99 to 1.29 in subjects with two copies of the minor allele. An exemplar profile plot (rs228883) is shown in Figure 3. At each follow-up visit, the mean BMI of probands with two copies of the minor allele is approximately 1.2–1.8 units higher than probands with one or two copies of the wild-type allele.

Association of *PRKCA* SNPs and Asthma

Family-Based Association Analysis in Costa Rica and CAMP

Given that both our primary test and replication populations were ascertained on asthma, we tested for association between the genotyped *PRKCA* SNPs and asthma affection status in both the CAMP and Costa populations. Two SNPs, rs732191 and rs9895580, demonstrated association at $p < 0.05$ in both populations under a recessive genetic model (Table 4). These SNPs are within 10 Kb of each other with an $r^2 = 0.99$.

Additional testing of the 137 LD-tag SNPs in the Costa Rica and CAMP cohorts revealed that the asthma-associated region extended far beyond the two originally identified SNPs. Within a 170 KB region surrounding the two initially

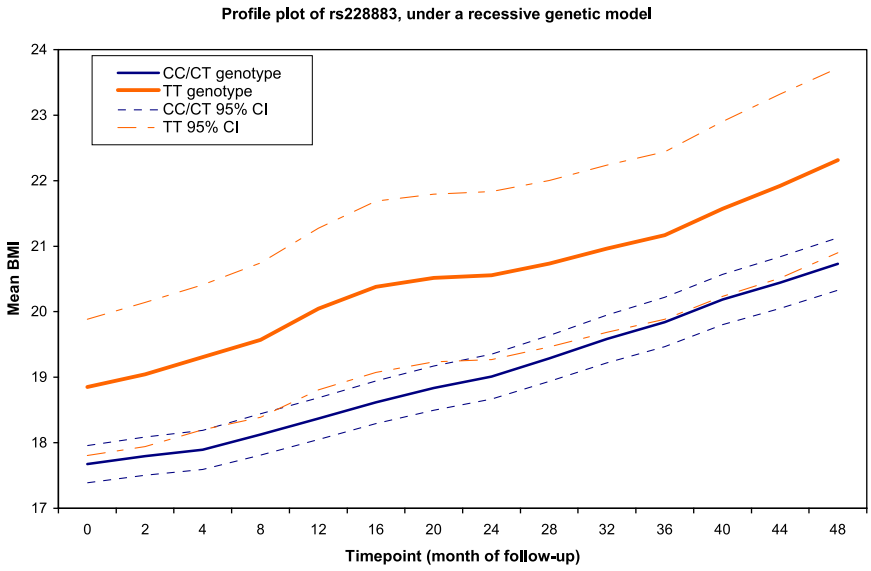

Figure 3. *PRKCA* SNP rs22883 Influences BMI Both at Baseline and longitudinally in the CAMP population
At each time point, subjects homozygous for the mutant allele have at least a one unit increase in their BMI. The dashed lines reflect point-wise 95% confidence intervals.

identified SNPs, there were an additional 13 SNPs with unadjusted p values < 0.05 in Costa Rica; eight of these SNPs also had (unadjusted) one-sided p values < 0.025 in the CAMP population (Table 4). These results included the two markers initially identified, rs732191 and rs9895580. As done for the BMI analysis, the p values displayed for CAMP are one-sided p values. The direction of the association was identical across CAMP and Costa Rica (shown in front of p values in Table 4 as a plus sign for overtransmission of the minor allele and as a negative sign for undertransmission of the minor allele). For the CAMP population, association p values reflecting both one-sided and two-sided (in parentheses) alternative hypotheses are given. The transmitted-to-untransmitted ratios of the minor allele, presented in Table S1 available online, also demonstrate the consistency of the direction of the association across the two populations.

Although exploration of the association between BMI and SNPs in *PRKCA* was considered our primary analysis, we also considered multiple comparisons adjustment when calculating the joint p value (Table 4) for asthma. There were 176 SNP in total analyzed for asthma (39 + 137), tested under three genetic models; thus we considered any joint p value $< 9.5 \times 10^{-5}$ (0.05/(176 × 3)) significant at the alpha = 0.05 level. One SNP, rs11079657,

Table 4. Evidence for Association of *PRKCA* with Asthma in Costa Rica and CAMP

			Allele Frequency		Number of Informative Families[b] (number of offspring with 0/1 recoded genotype)				
Marker	Location (BP)[a]	Minor Allele	CR	CAMP	CR	CAMP	Costa Rica p Value[c,d]	CAMP Replication p Value[c,d] (two-sided)	Joint p Value[e] (CR, CAMP two-sided)
rs732191	61779673	G	0.46	0.35	168 (117/51)	141 113/43	−0.0194	−0.0214 (−0.0428)	0.0036 (0.0067)
rs9895580	61789701	C	0.47	0.35	168 (117/51)	141 114/43	−0.0171	−0.0160 (−0.0320)	0.0025 (0.0047)
rs4411531	61793662	A	0.29	0.12	88 (70/18)	25 (24/1)	−0.0058	−0.0058 (−0.0117)	0.0004 (0.0007)
rs8080771	61824330	G	0.46	0.35	164 (116/48)	108 (90/29)	−0.0161	−0.0070 (−0.0140)	0.0011 (0.0021)
rs11652956	61839798	G	0.29	0.12	83 (65/18)	23 (22/1)	−0.0101	−0.0111 (−0.0222)	0.0011 (0.0021)
rs7221968	61848731	C	0.27	0.11	79 (63/16)	18 (17/1)	−0.0122	−0.0216 (−0.0432)	0.0024 (0.0045)
rs7405806	61862056	A	0.49	0.31	164 (109/55)	90 (77/20)	−0.0309	−0.0009 (−0.0018)	0.0003 (0.0006)
rs11079657	61862528	A	0.38	0.23	129 (94/35)	60 (56/8)	−0.0092	−0.0002 (−0.0004)	2.6×10^{-5}** (5.0×10^{-5}**)

[a] SNP positions from dbSNP build 129. *PRKCA* is located on chr17:61,729,388-62,237,324, with the ATG start site located at position 61,729,432,(hg18).
[b] Number of informative families for the FBAT statistic under a recessive model (i.e., both parents had at least one copy of the minor allele and at least one parent was heterozygous). The number of offspring in CAMP families may be greater than the number of informative families, given that some families had more than 1 offspring.
[c] From the FBAT statistic. The Costa Rica p values reflect a two-sided alternative hypothesis, whereas the replication p values in CAMP are one sided. The p value in parentheses under the CAMP replication reflects a two-sided alternative hypothesis. CR and CAMP p values are unadjusted for multiple comparisons.
[d] A minus sign (−) indicates that the minor allele is undertransmitted to subjects with asthma.
[e] The joint p value reflects the combination of a two-sided alternative hypothesis in CR and a one-sided alternative hypothesis in CAMP. The p values in parentheses are the combination of a two-sided alternative hypothesis in both CR and CAMP. Double asterisks (**) indicate that the joint p value was significant after Bonferroni correction for the number of comparisons conducted (528, 176 SNP × 3 genetic models) in the Costa Rican cohort. The threshold for significance is 9.5×10^{-5} (0.05/528).

demonstrated significant association after this correction, with a joint p value of 2.6×10^{-5}. This SNP was also significant (5.0×10^{-5}) when applying a two-sided alternative hypothesis (and p value) to the results in the CAMP population. None of the other eight SNPs met the criteria for adjusted significance, although the consistency and strength of association in each population is suggestive of an association.

Discussion

In the genetic evaluation of BMI in families ascertained via asthma, we have sequentially identified a region on chromosome 17q that was significantly linked to BMI, investigated, and replicated the association of the positional candidate gene *PRKCA* with BMI, and confirmed the pleiotropic nature of *PRKCA* via its association with asthma in two populations. The description of significant linkage and replicated associations with a positional candidate is noteworthy.

Among female members of large Costa Rican families, there was significant evidence of linkage to BMI on chromosome 17q22-q24, with a peak LOD score of 3.39. This genomic region has been previously linked to obesity-related phenotypes in several other studies,[19,42–44] including a replicated linkage scan in individuals of Hispanic (predominantly Mexican) descent.[19,42] Although the other scans in this region were not sex stratified, the finding of a linkage primarily within the female members of our pedigrees may be related to the ascertainment scheme, given that the genetic liability to obesity in asthma may be significantly greater in females.[13] Our linkage results for BMI are the first reported in families of asthmatics and provide increased confidence that a gene or genes in the 17q22-q24 region truly influences BMI. This region is adjacent to a previously identified area of possible linkage to asthma in the same Costa Rican pedigrees.[21]

We report an association of SNPs within the protein kinase-Cα gene (*PRKCA*) with BMI in two populations. As longitudinal profiling demonstrates, the effects of this gene on BMI do not appear confined to early childhood (Figure 3). *PRKCA* was investigated because of its location centrally within our linkage peak for BMI on chromosome 17 (*PRKCA* maps to 17q22-q23.2), its biologic plausibility as an obesity gene, and our concurrent interest in the gene in asthma pathogenesis and drug treatment response. Protein kinase-C represents a family of closely related serine and threonine protein kinases that regulate a wide variety of biological events within the cell.[45] Protein kinase-C α is felt to be the most ubiquitously expressed protein kinase C and has been widely investigated for its roles in cellular proliferation and differentiation; cell cycle regulation; cell adhesion, survival, and apoptosis; and cellular transformation.[46] Although *PRKCA* has not previously been associated with BMI or obesity in humans, Zucker obese (fa/fa) rats have decreased expression of protein kinase-Cα compared to Zucker lean control (fa/−) animals.[46] Moreover, protein kinase-C α is a negative feedback inhibitor of adipocyte differentiation and insulin signaling,[47–53] both of which are crucial determinants of human obesity. Therefore, it is entirely plausible that *PRKCA* genetic variation, such as that observed in our cohorts, can lead to decreased protein kinase-Cα expression, thereby leading to increased insulin-related fat deposition, adipocyte differentiation, and subsequent increases in body mass.

Twin studies have estimated that ~8% of the genetic component of obesity is shared with asthma.[13,14] However, to our knowledge, no gene has previously been shown to be associated with both obesity and asthma within the same study populations. In addition to its association with BMI, we also report the pleiotropic association of *PRKCA* with asthma in two populations. As noted above, 17q has been previously linked to asthma and asthma-related phenotypes.[21,54–58] Given the ubiquitous expression and diverse cellular roles of protein kinase-Cα, it is not surprising that expression of this gene has also been associated with smooth muscle proliferation.[59,60] Moreover, increased protein kinase-Cα has also been demonstrated to sensitize smooth muscle to contraction,[61–64] leading to a postulated therapeutic effect related to *PRKCA* inhibition in asthma.[61] Additional evidence that *PRKCA* may contribute to asthma pathogenesis has been reported: increases in protein kinase-C α may induce nitric oxide induced airway inflammation and mucous production,[65] regulate matrix metalloproteinase-9 associated with airway remodeling,[66] and help mediate leukotriene D4 signaling.[66]

Although we report significant linkage to BMI in females only, our association analyses did not reveal results specific to females. The most likely explanation for this disparity is that the association populations consisted of children, whereas the linkage pedigrees consisted of mostly adults. In cohort studies, the association between asthma and obesity has been consistently reported as stronger in adult females;[2,4,7,11] the gender-specific relationship is less convincing in children.[5,6] Therefore, our genetic analyses of BMI in populations ascertained on asthma affection status may also be influenced in a sex-specific manner according to age.

The issue of multiple testing must also be addressed when attempting to identify associations within linked regions.[67] For both BMI and asthma, our findings were considered statistically significant if they were replicated at the SNP level (for the same phenotype and in the same direction) after Bonferroni correction for the number of comparisons for each phenotype.

We note that *PRKCA* as a positional candidate gene is associated with two distinct, but partially correlated, phenotypes and therefore ascribe pleiotropy at the level of the gene. However, we acknowledge that the SNPs associated with BMI differ from those associated with asthma. We further acknowledge that, because the asthma associations within the gene occur with SNPs that lie just 5′ of the SNPs associated with BMI, it is not possible to

distinguish a truly pleiotropic locus from that of two very closely linked loci, given the currently available data. Additionally, although we have completely covered the *PRKCA* gene for known common variants via LD tagging, each of our significant associations was with an intronic SNP. Further work, including deep resequencing to identify both common and rare variants that may help to explain the linkage disequilibrium patterns and functional basis for these associations, is clearly needed. Because protein kinase C is well known to demonstrate cell-specific differences in expression,[68,69] additional functional work could be directed at determining whether the SNPs associated with BMI and those associated with asthma could be in linkage disequilibrium with a variant or variants that alter *PRKCA* expression, each in a tissue-specific manner.

In conclusion, by exploring the genetic influences on BMI through cohorts ascertained on asthma affection status, we have confirmed a locus on 17q influencing BMI and identified the positional candidate gene, *PRKCA*, as a gene associated with BMI in two populations. Moreover, we have also noted that *PRKCA* is a pleiotropic locus associated with asthma susceptibility in addition to BMI. The search for genetic factors influencing a given trait in populations ascertained by way of a different but biologically related phenotypic characteristic may yield new insights into identifying both positional and pleiotropic loci.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.ajhg.org/.

Acknowledgments

We thank all subjects for their ongoing participation in this study. We acknowledge the CAMP investigators and research team, supported by NHLBI, for collection of CAMP Genetic Ancillary Study data. All work on data collected from the Genetics of Asthma in Costa Rica and the CAMP Genetic Ancillary Study was conducted at the Channing Laboratory of Brigham and Women's Hospital under appropriate CAMP policies and human subject's protections. The CAMP Genetics Ancillary Study is supported by grants U01 HL075419, U01 HL65899, P01 HL083069, R01 HL 086601, and T32 HL07427 from the NHLBI of the National Institutes of Health (NIH). The Genetics of Asthma in Costa Rica study is supported by grants K01 HL04370 and R37 HL66289 from the NIH. K.G.T. is supported by K23 HG3983 from the NHGRI of the NIH. S.W. is a consultant for Genentech, Inc. and the Chair of the Genentech TENOR advisory board.

Received: March 9, 2009
Revised: May 5, 2009
Accepted: June 16, 2009
Published online: July 2, 2009

Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov.ezp-prod1.hul.harvard.edu/sites/Omim
PBAT software, http://www.biostat.harvard.edu/~clange/default.htm
PEDCHECK software, http://watson.hgen.pitt.edu/register/docs/pedcheck.html
RELPAIR software, http://csg.sph.umich.edu/boehnke/relpair.php
SOLAR software, http://solar.sfbrgenetics.org/download.html

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