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Association of corticotropin releasing hormone receptor 2 (CRHR2) genetic variants with acute bronchodilator response in asthma

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Abstract

Objective—Corticotropin - releasing hormone receptor 2 (CRHR2) participates in smooth muscle relaxation response and may influence acute airway bronchodilator response to short – acting β_2 agonist treatment of asthma. We aim to assess associations between genetic variants of *CRHR2* and acute bronchodilator response in asthma.

Methods—We investigated 28 single nucleotide polymorphisms in *CRHR2* for associations with acute bronchodilator response to albuterol in 607 Caucasian asthmatic subjects recruited as part of the Childhood Asthma Management Program (CAMP). Replication was conducted in two Caucasian adult asthma cohorts – a cohort of 427 subjects enrolled in a completed clinical trial conducted by Sepracor Inc. (MA, USA) and a cohort of 152 subjects enrolled in the Clinical Trial of Low-Dose Theopylline and Montelukast (LODO) conducted by the American Lung Association Asthma Clinical Research Centers.

Results—Five variants were significantly associated with acute bronchodilator response in at least one cohort (p-value ≤ 0.05). Variant rs7793837 was associated in CAMP and LODO (p-value = 0.05 and 0.03, respectively) and haplotype blocks residing at the 5' end of *CRHR2* were associated with response in all three cohorts.

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Conclusion—We report for the first time, at the gene level, replicated associations between CRHR2 and acute bronchodilator response. While no single variant was significantly associated in all three cohorts, the findings that variants at the 5' end of *CRHR2* are associated in each of three cohorts strongly suggest that the causative variants reside in this region and its genetic effect, although present, is likely to be weak.

Keywords

Asthma; genetics; corticotrophin releasing hormone receptor 2; CRHR2; bronchodilator response; polymorphism; β_2 adrenergic receptor agonist

INTRODUCTION

β_2 – adrenergic receptor (β_2 AR) agonists are the most widely used class of medication in the treatment of asthma worldwide [1], [2]. Variability of the acute bronchodilator response in asthmatic individuals exists [3]. Findings from genetic studies of candidate genes (e.g. beta-2 adrenergic receptor (*ADRB2)* [4], [5], [6], [7], [8], [9], [10] and adenylate cyclase 9 (*ADCY9)* [11]) and bronchodilator response have provided evidence of a genetic component in inter-individual variability.

In this study, we hypothesize that genetic variants of *CRHR2* are associated with acute airway bronchodilator response upon β_2 AR agonists administration in asthmatic individuals. The logic in looking at the CRHR2 gene as an indicator of bronchodilator response to beta agonists comes from 2 lines of evidence: (1) Similar smooth muscle relaxation mechanism between the beta-2 agonist and CRHR2 pathways. Agonists produce bronchodilation in asthmatics by binding to and directly simulating the β_2 AR on airway smooth muscle cells. The interaction of agonist and receptor leads to multiple downstream signals, one of which is the activation of adenylyl cyclase via stimulatory G proteins, which in turn increases cyclic adenosine monophosphate (cAMP) production and activates protein kinase A (PKA). PKA phosphorylates several target proteins, decreases intracellular calcium cations, and leads to smooth muscle relaxation [2]. Similar to β_2 AR, CRHR2 belongs to the family of G protein coupled – receptors (GPCRs). CRHR2 and its homologue, corticotropin releasing hormone receptor 1 (CRHR1), signal mainly through G proteins to increase adenylyl cyclase activity and cAMP levels. In mice, CRHR1 and CRHR2 have been found to be expressed in airway epithelium and smooth muscle relaxation response was induced by urocrotin (UCN) III – CRHR2 mediated cAMP elevation [12]. In rats, UCN mRNA was expressed in lung tissues in non-allergically sensitized animals and was expressed more pronouncedly in their sensitized counterparts [13]. By interacting with its ligands (UCN-I, II, III and CRH), CRHR2 may initiate various downstream signaling pathways (e.g. cAMP/PKA and MLC_{20} phosphorylation) to elicit contractile/relaxation responses in airway smooth muscles. (2) Interactions between beta-2 agonist and corticosteroid pathways. In vitro study has shown that β2AR mRNA level is elevated in the presence glucocorticoid [11]. CRHR2 signaling pathways regulate the hypothalamic-pituitary-adrenocortical axis [14]. Animal models have suggested an important role of the corticotropin-releasing hormone (CRH) system in airways [12], [13]. Since CRHR2 modulates the release of corticosterone [14], [15], asthmatic individuals with different genetic variants of CRHR2 may have different endogenous levels of corticosterone, leading to different degrees of interaction with beta-2 agonist in the airways.

The *CRHR2* gene is located on genomic region 7p21-p15 [16] and spans roughly 50 thousand base-pairs (Kbp) [17]. The gene consists of 12 exons; alternate splicing of exon 1 gives rise to 3 isoforms (CRHR2α, β and γ)[18]. Animal and human expression studies suggested that CRHR2α and CRHR2β are expressed in both the brain and the periphery such

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as smooth muscle and CRHR2γ predominantly expressed in the brain (reviewed in [19]). Our aim is to identify genetic variants of *CRHR2* contributing to variability in acute bronchodilator response in asthmatic individuals.

METHODS

Primary study population

The study population consisted of asthma patients enrolled in the Childhood Asthma Management Program (CAMP), a multicenter, randomized, double-blinded clinical trial testing the safety and efficacy of inhaled steroid versus non-steroid anti-inflammatory agent versus placebo over a mean of 4.3 years ([20], [21]). Briefly, children ages 5 to 12 years who suffered from mild to moderate asthma were recruited based on criteria including (1) asthma symptoms and/or (2) medication use for ≥ 6 months in the previous year and (3) at least a 20% reduction in FEV_1 after the administration of methacholine at a concentration less than or equal to 12.5 mg/ml ($PC_{20} \leq 12.5$mg/ml). Pulmonary function including bronchodilator responses and airway responsiveness was measured before randomization. For this study, acute bronchodilator response at baseline (i.e. before randomization) was the phenotype of interest, measured as the percent change in FEV_1 after the administration of 2 puffs of albuterol, compared with FEV_1 measured immediately prior to albuterol administration. Subjects were asked to cease all bronchodilator medications for at least 12 hours before the measurement of responsiveness. Data on acute bronchodilator response prior to randomization to different treatment arms from 607 Caucasian subjects were analyzed for genetic association. Approval by the Institutional Review Board was obtained and informed consents from all subjects were received.

Replication study populations

The first replication cohort consisted of asthma patients enrolled in an adult study of β_2 – agonist conducted by Sepracor Inc (Marlborough, MA), hereafter named Sepracor. A total of 427 subjects, who were over the age of 18, Caucasians, and had the full range of asthma severity were assessed for associations [22], including spirometry and bronchodilator responsiveness. The second replication cohort consisted of individuals enrolled in a completed trial conducted by the American Lung Association Asthma Clinical Research Centers (ALA-ACRC) to evaluate the effectiveness of low dose Theophylline as add-on treatment in asthma (the LODO trial), as described previously [23]. Briefly, the LODO trial was a multi-center, randomized, double-masked and placebo-controlled trial. Inclusion criteria include 15 years of age of older, a history of physician-diagnosed asthma, daily asthma medications use for 1 year or more, an FEV_1 of 50% or more of predicted value, and poor asthma control as defined by a score of 1.5 of greater on the Asthma Control Questionnaire [24], [25]. Exclusion criteria include use of oral corticosteroid, leukotriene antagonists, or theophylline within 4 weeks before enrollment, and current or former smokers with 20 packyears or more smoking history. For the purpose of replication, data from 152 Caucasian subjects were analyzed for associations. Similar to the dosage used to measure acute bronchodilator response in CAMP, acute bronchodilator response at baseline prior to randomization were obtained by measuring the change in FEV_1 after administration of 2 inhalations (90μg) of albuterol by metered dose inhaler. Approval by the Institutional Review Board was obtained and informed consents from all subjects in the two replication cohorts were received.

SNPs genotyping and statistical analyses

We investigated 39kb of genomic DNA harboring *CRHR2*, spanning from chromosome 7 position 30697689 to 30658511 on build 36.1 genome assembly released by the National Center for Biotechnology Information. A total of 28 SNPs were selected from public

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databases, prior to the availability of the HapMap database, for investigation in CAMP in order to cover the genomic region of *CRHR2* based on physical location and compatibility with genotyping methods. Subsequent investigation of the HapMap Release 22 data showed that there are 7 SNPs which tag (tagging SNPs) the region with $r^2 \geq 0.8$ in the CEU population, and these tagging SNPs were in the original 28 SNPs panel. SNP genotyping was performed using standard protocol for the iplex assay on Sequenom MassARRAY MALDI-TOF mass spectrometer (26) (Sequenom, CA, USA) and TaqMan assays (27) (Applied Biosystems, CA, USA).

Statistical analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC). Multivariate associations between individual SNP and acute bronchodilator response in the presence of potential confounders (height, age, gender, and baseline FEV_1 pre-bronchodilator administration) were tested using general linear model. Genotypes were coded for association testing under an additive model. SNP characteristics (e.g. allele frequency, Hardy – Weinberg equilibrium) for each SNP were assessed using the software Haploview [28].

Haplotype analyses were conducted using the software *haplo.stats* [29]. To narrow the genomic region (e.g. lengths of haplotype blocks) within the 32kb region that was associated with the phenotype, the sliding window option was used to test for associations between haplotypes of length 2 to 10 SNPs and acute bronchodilator response, adjusted for potential confounders. All p-values were obtained from simulation with default values of 1000 replicates. Simulated p-value is the number of times the simulated score statistic, calculated from a permutated-reordering of the trait and covariates and the original ordering of the genotyping matrix, exceeds the observed, divided by the total number of simulations.

RESULTS

Populations Baseline characteristics

A total of 607 Caucasian CAMP subjects with the mean age of 8.86 years (standard deviation (sd) = 2.13 years) were analyzed in this study (Table 1). The mean age of onset was 3.06 years (sd = 2.44 years). The male to female ratio was 1.5:1.0. The mean bronchodilator response was 10.75% (sd = 10.13%). In Sepracor, a total of 427 Caucasian asthmatic subjects with the mean age of 32.55 years (sd = 13.72 years) were analyzed. The male to female ratio was 1:1 and the mean bronchodilator response was 40.36% (sd = 20.98%). In LODO, a total of 152 Caucasian asthmatic subjects with the mean age of 42.84 years (sd = 14.77 years) were analyzed. The male to female ratio was 0.33:1 and the mean bronchodilator response was 9.68% (sd = 11.13%). We have confined our analyses to Caucasians to avoid potential confounding due to population substructures. In addition, power to detect associations in other racial groups is low due to small sample sizes.

SNP Characteristics

A total of 28 SNPs were investigated in CAMP (Figure 1, Table 2). Minor allele frequencies range from 1.9% to 39.0%. The average frequency of missing genotypes for the 28 SNPs was 4.0% (ranges from 1.15 - 11.37% (rs733453)). In Sepracor, 26 of the 28 SNPs tested in CAMP were analyzed. Minor allele frequencies range from 1.9% to 40.4% (Table 2). The average missing genotype rate was 3.1% (0 – 11.2% (rs2014663)). In LODO, 23 of the 28 SNPs were analyzed. Minor allele frequencies range from 0.70% to 48.4% (Table 2). The average missing genotype rate was 6.1% (0 - 13.1% (rs2284217)). Across all 3 cohorts, only 4 variants (rs733453, rs917195, rs929377 and rs1076291) were marginally out of Hardy-Weinberg equilibrium ($0.03 \leq$ p-value ≤ 0.05), as expected by chance given the number of SNPs tested.

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Associations between SNPs and acute bronchodilator response

In CAMP, variants rs255100, rs7793837 and rs2267715 were statistically associated with acute bronchodilator response under an additive model (p-value ≤ 0.05) (Table 3). Minor alleles rs255100A, rs7793837T and rs2267715G were associated with reduced bronchodilator response. Haplotypes of length ranging from 2 to 10 SNPs were analyzed for associations and a total of 2 haplotype blocks of lengths 2 and 4 SNPs demonstrated significant associations under an additive model (simulated global p-value < 0.05) (Figure 2A). Of the 3 associated SNPs, rs255100 and rs2267715 are in strong linkage disequilibrium (r^2 = 0.88), rs255100 and rs7793837, and rs7793837 and rs2267715 are in moderate linkage disequilibrium (r^2 = 0.48 and 0.40, respectively) (data not shown). Haplotypes of each associated blocks were further assessed for associations and a common haplotype (rs7793837A-rs4723002A-rs1003929C-rs2284220A (frequency = 0.60)) demonstrated the most significant association (simulated p-value = 0.026) (Figure 2B).

In Sepracor, none of the associated variants in CAMP was associated with acute bronchodilator response. A different variant, rs2284220, was associated with the phenotype (p-value = 0.03) (Figure 3A). The minor allele (rs2284220G) was associated with reduced bronchodilator response (Table 3). A total of 3 haplotype blocks of length 2, 7 and 8 SNPs demonstrated significant associations (simulated global p-value < 0.05) (Figure 3A). Further assessment for associations within each block showed that haplotype rs4723002A-rs1003929T (frequency = 0.13) was associated with the phenotype with the most statistical significance (simulated p-value = 0.003), followed by haplotype rs255102A-rs255100A-rs917195C-rs7793837A-rs4723002A-rs1003929T-rs2284220G (frequency = 0.10, simulated p-value = 0.008) (Figure 3B). In LODO, variants rs7793837 and rs2267716 were associated with acute bronchodilator response (p-value ≤ 0.05) (Figure 4A). The minor alleles (rs7793837T and rs2267716C) were associated with reduced bronchodilator response (Table 3). The two SNPs are in linkage disequilibrium (r^2 = 0.87). A 2-SNP haplotype block was associated with the phenotype (simulated global p-value = 0.05) and further assessment of individual haplotypes within this block showed that haplotype rs4723002A-rs7793837A (frequency = 0.75) was the most significantly associated (simulated p-value = 0.02, respectively) with acute bronchodilator response (Figure 4B).

Discussions

This is the first study to report gene-based replicated associations between the *CRHR2* gene and acute bronchodilator response upon short-acting β2 agonist administration in asthmatic subjects. In our primary and two replication cohorts, different SNPs of *CRHR2* were found to be associated with acute bronchodilator response to albuterol, however, the physical proximity of these SNPs with each other (between exon β1a and exon 3) suggests that the causative variant(s) may reside towards the 5' end of the gene. By comparing the haplotypes showing the most significant associations in each cohort, the associated region spans approximately 8.7kb between introns β1b and 2 (Table 4). The haplotype analyses detected the common haplotypes (frequency between 60% - 78%) to be associated with acute bronchodilator response in the three cohorts. The haplotypes are of different length, yet in CAMP and LODO, allele rs7793837A resides in almost all the associated common haplotypes. In Sepracor, the associated haplotype with a frequency of 78% consisted of 2 neighboring SNPs (rs4723002 and rs1003929) 1 and 7 kb downstream of variant rs7793837. We speculated that variant rs7793837 was associated in Sepracor because (1) it is not the causative variant but in LD with the causative variant, and (2) Sepracor is phenotypically different from CAMP and LODO. This cohort has a higher mean bronchodilator response due to the recruitment requirement of subjects with >15% bronchodilator response (Sepracor: -40.98% (standard deviation of 20.98), CAMP:10.75 (sd 10.13) and LODO: 9.67 (sd 11.07)). A different causative variant, also residing at 5' end of CRHR2 may be

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responsible for the association observed in Sepracor. This 5' region encodes the N-terminal extracellular domain of CRHR2, where interactions with ligands occur (reviewed in [19]). In addition to encoding the N-terminal, this region houses multiple exons 1 (β1b, γ1 and α1), where various isoforms of CRHR2 are translated depending on the splicing transcripts being translated [19]. Additional sequencing of the 5' end of *CRHR2* was carried out to identify the causative variant(s). Approximately 32 kb of genomic regions (exons β1a, β1b, γ1, α1, 2 and flanking intronic regions) were sequenced in 48 CAMP subjects, revealing 3 novel intronic variants located 199bp upstream and 26 and 74bp downstream of exon β1a, respectively. Two of the 3 novel variants were chosen for their potential functions and were genotyped in a subset of CAMP subjects, but no association was observed with acute bronchodilator response (data not shown). Although the original 28 SNPs panel did not cover exon β1a, subsequent sequencing and genotyping efforts ensured exon β1a and surrounding intronic regions were investigated.

The rationale for testing for associations in both pediatric and childhood asthma cohorts is to establish generalization of the associations between *CRHR2* genetic variants and acute bronchodilator response across different age groups; to determine whether the association was a developmental, an aging, or both a developmental and aging phenomenon in the lungs. Our findings that different SNPs were associated with acute bronchodilator response in the three cohorts and that these SNPs reside at the 5' end of *CRHR2* suggest that the associations observed are likely due to linkage disequilibrium between associated SNPs and the true phenotype causing variant(s), in both childhood and adult asthma.

The 5' end of *CRHR2* is of great biological interest. The CRHR2 protein has 3 isoforms (CRHR2α, CRHR2β, and CRHR2γ) which differ at the N termini, and are encoded by the 5' end exons of *CRHR2*. Translations of exon β1a and exon β1b encode the N terminus of the CRHR2β isoform, and exon 1α and 1γ encode the N termini of CRHR2α and CRHR2γ, respectively [18]. *In vitro* study has demonstrated that the three isoforms have different downstream signaling capacities [18]. We speculate that the causative variant(s) may influence acute bronchodilator response through regulating translations of *CRHR2* into various isoforms.

How genetic variations in *CRHR2* function contribute to differential responses to β2-agonist remains to be investigated. Literature has presented complex interactions between β2 AR and other signaling pathways, based on which we propose three molecular mechanisms that could potentially explain the association of *CRHR2* variants with bronchodilator response. First, CRHR2 signaling may desensitize β2 AR function in asthmatics. β2 AR signaling can be desensitized not only by its own activation but also by signaling through other G protein-coupled receptors. This cross-talk, which is referred to as heterologous desensitization (versus homologous desensitization by its own signaling), has been observed between β2 AR and PGE2 receptors [30]. This mechanism predicts that individuals with higher CRHR2 signaling capacity may have reduced bronchodilator response due to stronger cross-desensitization. A second mechanism takes into consideration the documented anti-inflammatory role of CRHR2 [12]. In this case, reduced CRHR2 function may lead to increased production of pro-inflammatory cytokines, many of which will down-regulate the signaling through β2 ARs [31]. The last mechanism concerns the possible synergistic interaction between CRHR2 and β2 AR since both of them relax smooth muscles by stimulating cAMP production. If synergistic contribution from CRHR2 is pivotal for β2 agonists to achieve effective bronchoprotection in asthmatics, patients carrying CRHR2 alleles that have lower biological activities will have dampened therapeutic response to β2 agonists. Synergy between G-protein coupled receptors (GPCRs) has been demonstrated in many biological systems where activation of one GPCR can amplify the signaling events in a parallel but separate pathway [32], [33]. To determine which one of these three proposed

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paradigms is valid, we need to perform physiological studies on mice that were engineered to be CRHR2-deficient as well as biochemical analysis of signaling interaction between CRHR2 and β2 AR in primary airway smooth muscle cells.

This study has limitations. First, we did not detect a single SNP that was associated across the 3 cohorts. Second, the significant associations detected would not reach significance level when corrected for multiple testing. However, in spite of these limitations, we felt that by detecting associations with SNPs covering a region within the gene across 3 populations, the findings that CRHR2, as a gene, is associated with acute bronchodilator response is likely to be valid. The lack of any single SNP being associated in all three cohorts suggests that the genetic effect of CRHR2, although present, is likely to be weak. Being a complex trait, the number of genetic variants explaining inter-individual variations in bronchodilator response is likely to be high, with each variant exerts low to moderate effect. Our findings suggests that genetic variants of *CRHR2*, in addition to the widely studied *β2AR* and many yet to be characterized genetic variants, influence acute bronchodilator response to short acting β2A. Despite our subsequent sequencing and genotyping effort of exons at the 5' end of *CRHR2*, we did not detect any causative variants, hence, the causative variants are likely to be in untranslated regions. Additional exploration of the region will be needed to identify the causative variants affecting acute bronchodilator response so that subsequent screening tests can be developed to predict in advance the efficacy of administering β_2 agonists as an asthma treatment in asthmatic individuals.

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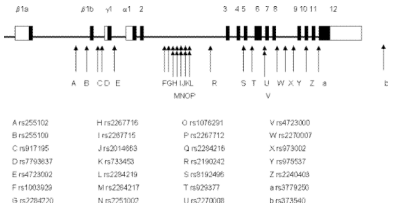

Figure 1. Genomic organization of *CRHR2*

Exons are represented by black boxes connected by straight line representing introns. White boxes representing untranslated regions of exons. Location of exons are described elsewhere [17]. Positions and names of the 28 SNPs analyzed are represented by arrows and letters below the gene structure.

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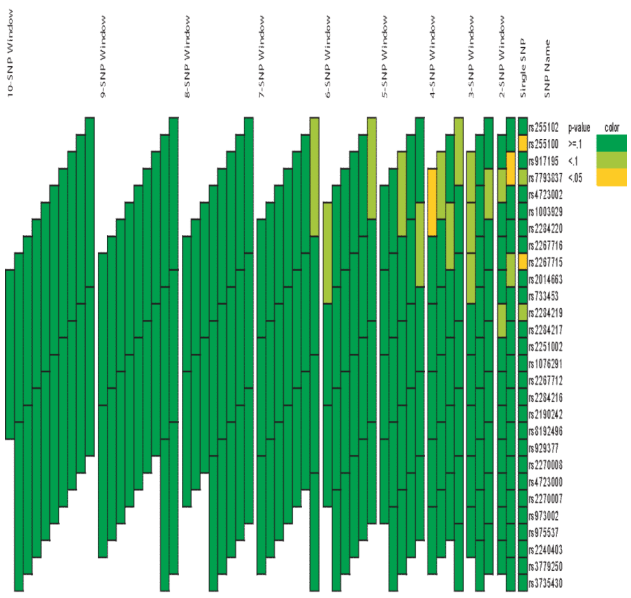
A



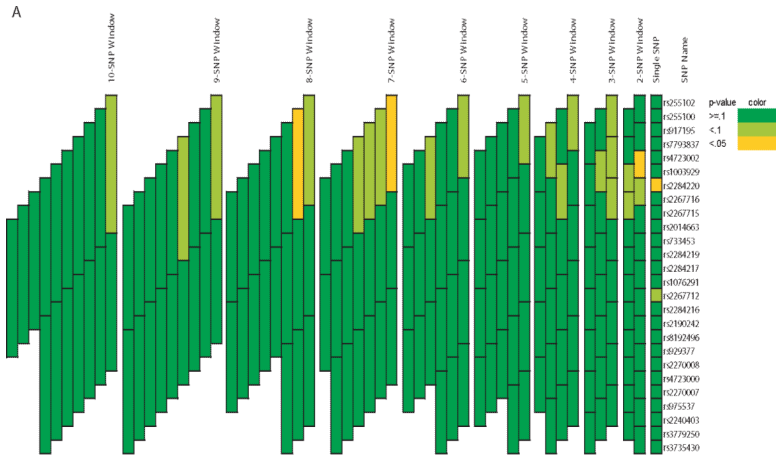
B

5′end

rs917195	rs7793837	rs4723002	rs1003929	rs2284220	frequency	hap-score	p-value
C	A				0.76	2.08	0.040
C	T				0.02	-2.09	0.034
	A	A	C	A	0.60	2.29	0.026
	T	A	C	A	0.15	-2.15	0.033

Figure 2. Association analyses of *CRHR2* variants and acute bronchodilator response in CAMP

(A) Significance of association, as indicated by simulated global p-values, between genetic variants of *CRHR2* (individual SNPs and haplotypes) and phenotype is represented by color and constructed using the graphical tool GRASP[33]. The SNPs making up each haplotype block can be found by matching the upper and lower boundaries of each block to the SNP name on the ‘SNP Name’ column. (B) Haplotypes within the associated blocks were further assessed for association. Hap-scores are the score statistics calculated from *Haplo.stats*. Only haplotypes with significant (simulated) p-values were listed.



B

5′end

255102*	255100	917195	7793837	4723002	1003929	2284220	2267716	2267715	freq.**	hap_score	p-value#
				A	C				0.78	2.37	0.019
				A	T				0.13	-2.70	0.003
A	A	C	A	A	T	G			0.10	-2.75	0.008
A	A	T	T	A	C	A			0.05	2.44	0.015
	A	C	A	A	T	G	T	G	0.13	-1.99	0.046

Figure 3. Association analyses of *CRHR2* variants and acute bronchodilator response in SEPRACOR

See legend of figure 2. *SNP rs number **Frequency

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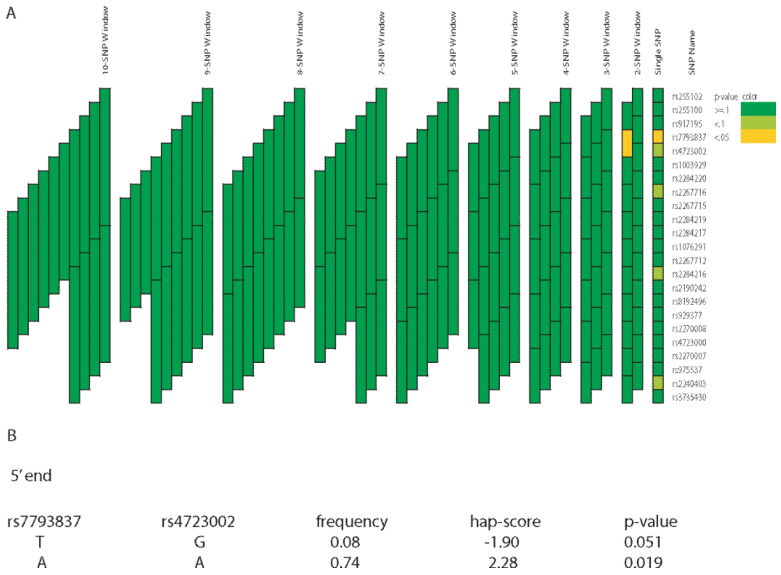

Figure 4. Association analyses of *CRHR2* variants and acute bronchodilator response in LODO
See legend of figure 2.

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Table 1

Population characteristics

	CAMP	SEPRACOR	LODO
Number of subjects	607	427	152
Age (yrs), mean (sd)	8.86 (2.13)	32.55 (13.72)	42.88 (14.74)
Age (yrs), range	5.17-13.24	12-80	15-76
Age at first asthma symptoms (yrs), mean (sd)	3.06 (2.43)	not available	not available
Male gender count (frequency)	364 (0.60)	214 (0.50)	38 (0.25)
Female gender count (frequency)	243 (0.40)	213 (0.50)	114 (0.75)
Baseline pre - bronchodilator FEV1 percent predicted (sd)	94.52 (14.06)	54.95 (7.76)	68.07 (14.31)
Baseline post - bronchodilator FEV1 percent predicted (sd)	103.55 (12.51)	76.58 (12.65)	73.91 (13.78)
Baseline brochodilator response % (sd)	10.75 (10.13)	40.36 (20.98)	9.67 (11.07)

CAMP – Childhood Asthma Management Program, LODO – Low Dose Theophylline and Montelukast trial, FEV_1 – forced expiratory volume in 1 second, sd – standard deviation

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Table 2

SNP characteristics

SNP rs#	chromosome position	MA	MAF^{CAMP}	$MAF^{SEPRACOR}$	MAF^{LODO}	genomic location, downstream of exon
rs255102	30697689	T	0.33	0.30	0.38	intronic, exon β1a
rs255100	30695433	A	0.39	0.40	0.39	intronic, exon β1a
rs917195	30694977	T	0.22	0.24	0.23	intronic, exon β1b
rs7793837	30693302	T	0.24	0.26	0.26	intronic, exon β1b
rs4723002	30692226	G	0.09	0.10	0.08	intronic, exon γ1
rs1003929	30685574	T	0.16	0.13	0.15	intronic, exon 2
rs2284220	30684628	G	0.15	0.14	0.13	intronic, exon 2
rs2267716	30683168	C	0.23	0.24	0.23	intronic, exon 2
rs2267715	30682612	G	0.37	0.37	0.36	intronic, exon 2
rs2014663	30682098	C	0.15	0.12	NT	intronic, exon 2
rs733453	30681298	G	0.36	0.38	NT	intronic, exon 2
rs2284219	30680961	A	0.37	0.38	0.37	intronic, exon 2
rs2284217	30680133	A	0.22	0.21	0.19	intronic, exon 2
rs2251002	30679369	G	0.35	NT	NT	intronic, exon 2
rs1076291	30679120	A	0.35	0.35	0.30	intronic, exon 2
rs2267712	30678759	A	0.15	0.14	0.13	intronic, exon 2
rs2284216	30678486	T	0.09	0.10	0.09	intronic, exon 2
rs2190242	30676000	C	0.23	0.20	0.20	intronic, exon 2
rs8192496	30671630	G	0.29	0.29	0.27	intronic, exon 4
rs929377	30670684	A	0.31	0.31	0.29	intronic, exon 5
rs2270008	30668769	T	0.12	0.13	0.14	intronic, exon 6
rs4723000	30668439	A	0.12	0.12	0.13	intronic, exon 6
rs2270007	30666497	G	0.17	0.16	0.15	intronic, exon 8
rs973002	30665429	G	0.17	NT	NT	intronic, exon 8
rs975537	30663882	T	0.22	0.22	0.19	intronic, exon 8
rs2240403	30661727	T	0.09	0.09	0.09	exonic, exon 10
rs3779250	30660785	C	0.35	0.35	NT	intronic, exon 11
rs3735430	30658511	T	0.02	0.02	0.01	intronic, exon 12

rs – refSNP number, MA – minor allele, MAF – minor allele frequency, CAMP – Childhood Asthma Management Program, LODO – Low Dose Theophylline and Montelukast trial, NT – not tested

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Table 3

Significant associations of *CRHR2* genotypes and acute bronchodilator response in CAMP and replication cohorts

	CAMP			SEPRACOR			LODO		
Variants	**Count (freq)**	**mean (se)**	**p-value**	**Count (freq)**	**mean (se)**	**p-value**	**Count (freq)**	**mean (se)**	**p-value**
rs255100									
TT	221 (0.36)	11.70 (0.54)	0.02	151 (0.35)	40.49 (2.33)	0.78	61 (0.40)	11.23 (1.35)	0.38
TA	287 (0.47)	10.82 (0.48)		209 (0.49)	39.91 (1.32)		71 (0.47)	8.43 (1.15)	
AA	88 (0.15)	9.22 (0.87)		67 (0.16)	40.93 (1.55)		20 (0.13)	10.09 (2.13)	
missing	11 (0.02)			0 (0.00)			0 (0.00)		
rs7793837									
AA	349 (0.57)	11.33 (0.44)	0.05	235 (0.55)	39.04 (1.23)	0.24	75 (0.49)	11.46 (1.08)	0.03
AT	207 (0.34)	10.07 (0.57)		155 (0.36)	41.98 (1.52)		55 (0.36)	7.34 (1.26)	
TT	40 (0.07)	9.62 (1.29)		31 (0.07)	40.41 (3.40)		8 (0.05)	8.34 (3.35)	
missing	13 (0.02)			6 (0.01)			*14 (0.09)		
rs2284220									
AA	402 (0.66)	10.82 (0.40)	0.9	311 (0.73)	41.08 (1.06)	0.03	111 (0.73)	9.34 (0.92)	0.52
AG	141 (0.23)	11.57 (0.68)		103 (0.24)	37.97 (1.84)		27 (0.18)	11.88 (1.91)	
GG	10 (0.02)	6.67 (2.69)		5 (0.01)	20.86 (8.40)		5 (0.03)	8.07 (4.37)	
missing	54 (0.09)			8 (0.02)			9 (0.06)		
rs2267716									
TT	361 (0.60)	11.23 (0.43)	0.1	246 (0.58)	40.04 (1.22)	0.84	82 (0.54)	11.34 (1.11)	0.05
TC	203 (0.33)	10.09 (0.57)		151 (0.35)	41.25 (1.56)		53 (0.35)	7.11 (1.38)	
CC	33 (0.05)	9.91 (1.41)		26 (0.06)	38.83 (3.78)		5 (0.03)	10.43 (4.71)	
missing	10 (0.02)			4 (0.01)			*12 (0.08)		

rs # - refSNP number, count – genotype count, freq – frequency, means (se) –means bronchodilator response estimate obtained from linear regression (standard error), p – p-value obtained from linear regression under an additive model, CAMP – Childhood Asthma Management Program, LODO – Low Dose of Theophylline and Montelukast trial. Only variants with significant association (p-value ≤ 0.05) in at least 1 cohort are shown.

*The mean bronchodilator response of subjects with missing genotypes is similar to the mean of those with genotypes (10.10% for rs7793837 and 9.46% for rs2267716 versus 9.68%); hence data can be considered missing at random.

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Table 4

Haplotypes demonstrating the most significant association with acute bronchodilator response in CAMP, SEPRACOR and LODO

cohort	rs7793837	rs4723002	rs1003929	rs2284220	frequency	hap-score	simulated p-value
CAMP	A	A	C	A	0.6	2.29	0.026
SEPRACOR		A	T		0.13	-2.7	0.003
LODO	A	A			0.74	2.28	0.019

CAMP – Childhood Asthma Management Program, LODO – Low Dose of Theophylline and montelukast trial, Hap-score – the score statistics calculated from Haplo.stats.