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Corticosteroid pharmacogenetics: association of sequence variants in *CRHR1* with improved lung function in asthmatics treated with inhaled corticosteroids

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Corticosteroids mediate a variety of immunological actions and are commonly utilized in the treatment of a wide range of diseases. Unfortunately, therapy with this class of medications is associated with a large proportion of non-responders and significant side effects. Inhaled corticosteroids are the most commonly used asthma controller therapy. However, asthmatic response to corticosteroids also varies widely between individuals. We investigated the genetic contribution to the variation in response to inhaled corticosteroid therapy in asthma. The association of longitudinal change in lung function and single nucleotide polymorphisms from candidate genes crucial to the biologic actions of corticosteroids were evaluated in three independent asthmatic clinical trial populations utilizing inhaled corticosteroids as the primary therapy in at least one treatment arm. Variation in one gene, corticotropin-releasing hormone receptor 1 (*CRHR1*) was consistently associated with enhanced response to therapy in each of our three populations. Individuals homozygous for the variants of interest manifested a doubling to quadrupling of the lung function response to corticosteroids compared with lack of the variants (*P*-values ranging from 0.006 to 0.025 for our three asthmatic populations). As the primary receptor mediating the release of adrenocorticotropic hormone, which regulates endogenous cortisol levels, *CRHR1* plays a pivotal, pleiotropic role in steroid biology. These data indicate that genetic variants in *CRHR1* have pharmacogenetic effects influencing asthmatic response to corticosteroids, provide a rationale for predicting therapeutic response in asthma and other corticosteroid-treated diseases, and suggests this gene pathway as a potential novel therapeutic target.

INTRODUCTION

Corticosteroids mediate a variety of immunological actions and are commonly utilized in the treatment of a diverse number of diseases. However, focused evaluation of the literature surrounding therapy with corticosteroids demonstrates a variable response, with a substantial number of individuals that fail to respond to this class of medication. For example,

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Table 1. Population characteristics[a]

	Adult Study (primary)	CAMP (replicate)	ACRN (second replicate)
N	470	311	336
Inhaled corticosteroid used	Flunisolide	Budesonide	Triamcinolone
Age	39.4 ± 13.4	9.0 ± 2.1	33.2 ± 11.6
Sex, *n* (%)			
Male	195 (41.5)	181 (58.2)	139 (41.4)
Female	275 (58.5)	130 (41.8)	197 (58.6)
Race, *n* (%)			
Caucasian[b]	415 (88.5)	201 (64.6)	224 (66.7)
African American	34 (7.0)	44 (14.1)	63 (18.8)
Hispanic	12 (2.6)	32 (10.3)	25 (7.4)
Other	9 (1.9)	34 (10.9)	24 (7.1)
Mean baseline FEV_1[c] (%)	72.2 ± 16.2	93.6 ± 14.4	77.8 ± 15.9
Mean change in FEV_1[d] (%)	7.0 ± 19.3	8.3 ± 14.1	6.7 ± 19.7

[a]Plus–minus values are means ± standard deviations.
[b]Owing to concerns over possible population stratification and small numbers of subjects in other racial groups, only genotypic information from Caucasians were analyzed.
[c]As a percent of predicted.
[d]Change in FEV_1 while on inhaled corticosteriods evaluated at 8 weeks in the Adult Study and CAMP and 6 weeks in ACRN.

approximately one-third of patients in recent studies of Crohn's disease (1) and nephrotic syndrome (2) failed to respond to initial therapy with corticosteroids. Moreover, corticosteroid treatment in these studies was associated with a significant incidence of adverse side effects (1,2). In asthma, corticosteroids taken by the inhalational route are the most effective and commonly used drugs for the treatment of asthma but may also be associated with serious adverse effects (3–5). Large inter-individual variation, including a significant number of non-responders, exists in the treatment response to each of the major classes of asthma medications (6,7), including corticosteroids. In one study of asthmatics, 22% of individuals taking inhaled beclomethasone had decrements in their forced expiratory volume at 1 s (FEV_1) (6) after 12 weeks of therapy, while in a second study 38% of patients randomized to either budesonide or fluticasone demonstrated FEV_1 improvements of $<5\%$ over the course of 24 weeks (7).

Given the significant numbers of individuals that fail to respond to therapy with corticosteroids, as well as the potential morbidity attributable to this class of medications, the identification of those individuals most likely to demonstrate a significant response to corticosteroids would be invaluable. Since the individual response to inhaled corticosteroid treatment in patients with asthma is highly repeatable (8), it is reasonable to postulate a genetic basis for this heterogeneity in therapeutic response. Therefore, we hypothesized that sequence variants in the genes controlling the pharmacokinetics (uptake, synthesis or degradation) or pharmacodynamics (site of action) of corticosteroids would be associated with the therapeutic response to this class of asthmatic drugs. Using three independent clinical therapeutic trials involving asthmatics on inhaled corticosteroids, we tested this hypothesis using a pathway candidate gene association approach. We analyzed the association between single nucleotide polymorphisms (SNPs) in the genes and the longitudinal response to inhaled corticosteroid treatment, measured as the change in FEV_1. The FEV_1 is a standardized and widely accepted measure of lung function; increased FEV_1 indicates improved lung function.

Here we show that variation in one gene, corticotropin-releasing hormone receptor 1 (*CRHR1*) was associated consistently with enhanced response to therapy in each of our three populations, as manifested by a doubling to quadrupling of the longitudinal FEV_1 response to corticosteroids, compared with lack of the variation. These findings are consistent with the known physiologic role of *CRHR1* in that variations of this gene would be expected to alter basal levels of endogenous corticosteroid secretion providing for the opportunity for an enhanced response to exogenous corticosteroid administration.

RESULTS

Populations

We studied three different clinical trial populations: 470 adult asthmatics (termed Adult Study), 311 childhood asthmatics (termed CAMP for Childhood Asthma Management Program) and 336 adult asthmatics (termed ACRN for Asthma Clinical Research Network). Clinical characteristics of the three populations are shown in Table 1. Our analyses were confined to Caucasians, owing to concerns about possible population stratification and the small numbers of subjects in other racial groups. In addition to age, gender distribution and type of inhaled corticosteroid used, the baseline severity of the populations (as denoted by mean FEV_1 at enrollment) differed, with the two adult populations composed of moderate to severe asthmatics and the pediatric population, of mild to moderate asthmatics.

The primary outcome measure of the association analyses was percent change in FEV_1 over time in response to inhaled corticosteroids, defined as the FEV_1 difference from baseline to 8 weeks for the Adult Study and CAMP, and to 6 weeks in ACRN, divided by the baseline value. The mean FEV_1 percent change was 7.0 ± 19.3% in the Adult Study, 6.8 ± 13.8% in CAMP and 6.7 ± 19.7% in ACRN. Although each of these changes represented significant improvements in lung function from baseline ($P < 0.05$), there was wide inter-individual variability in these responses (Fig. 1).

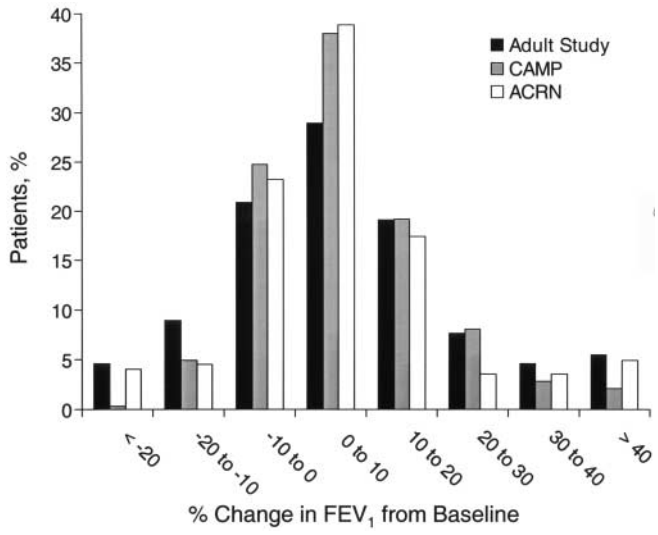

Figure 1. Heterogeneity of response to inhaled corticosteroids at 8 weeks (Adult Study and CAMP) and 6 weeks (ACRN). The distribution of responses within each population is approximately normal and suggests that other factors, including genetic, may be contributing to the therapeutic response.

Screening of first population and initial replication

In the Adult Study, we screened 131 SNPs in 14 genes (Supplementary Material). Utilizing a cutoff value of $P < 0.05$, we identified four SNPs (rs242941, rs1990975, rs889182 and rs6191) from three genes, *CRHR1* (NM_004382), *FCER2* (NM_002002) and *NR3C1* (NM_000176), associated with the 8 week response to inhaled corticosteroids. We recognized that false positive results could occur in these analyses because the significance threshold was not corrected for multiple comparisons, but viewed these screening results as providing an initial list of candidates for further replication testing.

To validate our findings, we then studied the three genes (and only these genes) in the second independent population, CAMP. *CRHR1* showed positive association with significantly improved lung function after 8 weeks of inhaled corticosteroid therapy in this study as well. Specifically, rs242941 (minor allele frequency ~30%) was associated with positive treatment response in both the Adult Study and CAMP ($P = 0.025$ and 0.006, respectively) (Fig. 2A). In the Adult Study, the mean percent change in FEV_1 for those homozygous for the minor allele was 13.28 ± 3.11, compared with 5.49 ± 1.40 for those homozygous for the wild-type allele. Similarly, in CAMP, the percent change was 17.80 ± 6.77 versus 7.57 ± 1.50 for the variant and wild-type homozygotes, respectively. In CAMP, evaluation of the placebo arm revealed no association of rs242941 or any of the other genotyped SNPs with change in lung function. Moreover, while inhaled corticosteroid usage was associated with improved FEV_1 at 8 weeks ($P < 0.001$), variation in rs242941 significantly enhanced the improvement in lung function associated with this form of therapy (interaction $P = 0.02$).

Haplotypic associations

Since rs242941 is intronic and unlikely to affect function of *CRHR1*, we sought to capture more of the information from across the gene by testing multi-SNP haplotypes in the *CRHR1* gene. Owing to linkage disequilibrium (LD) and/or limited haplotype diversity, haplotypes may be distinguished using a subset of SNPs, termed 'haplotype-tag SNPs' (htSNPs) (9). We found that the htSNPs rs1876828, rs242939 and rs242941 distinguished all four haplotypes imputed with at least 2.5% frequency in both the Adult Study and CAMP populations. Genotypes for these SNPs were in Hardy–Weinberg equilibrium in all study cohorts. Utilizing the htSNPs, the average haplotypic frequencies for the four haplotypes analyzed in the two populations were 0.46, 0.27, 0.21 and 0.05, respectively. One common haplotype (frequency 27%), termed GAT, was associated with a significantly enhanced response to inhaled corticosteroids in both the Adult Study and CAMP ($P = 0.02$ and 0.01, respectively). The estimated 8 week improvement in FEV_1 for those subjects imputed to have the homozygous GAT/GAT haplotype was more than twice that for those homozygous for non-GAT haplotypes in the Adult Study (13.73 ± 3.80 versus 5.54 ± 1.29%, respectively), and nearly three times that in CAMP (21.83 ± 8.07 versus 7.35 ± 1.41%, respectively) (Fig. 3). Improvement in those heterozygous for the GAT haplotype was intermediate between the two groups, suggesting an additive effect.

Secondary replication

To further verify our findings, subsequently we evaluated the *CRHR1* gene in the third clinical trial population, ACRN, by genotyping only the three htSNPs (rs1876828, rs242939 and rs242941). Although neither the rs242941 SNP ($P = 0.29$) nor the GAT haplotype ($P = 0.59$) was significantly associated with lung function response in this population, the second of the three SNPs, rs1876828, was strongly associated with improved FEV_1 over the 6 week study period ($P = 0.006$) (Fig. 2B). Homozygotes for the minor allele had an average increase in their FEV_1 of 23.72 ± 9.75 compared with 5.14 ± 1.31% for homozygotes for the common allele. We did not observe any haplotypic association stronger than this SNP in ACRN.

DISCUSSION

Our results identify genetic variants associated with the therapeutic response to corticosteroids. Specifically, we have demonstrated that genetic variation in *CRHR1* is associated with an enhanced pulmonary function response to inhaled corticosteroids in all three of our asthmatic populations. Our data suggest that this pharmacogenetic effect related to the use of inhaled corticosteroids is robust. In the evaluation of three *CRHR1* htSNPs, one SNP and one specific haplotype were associated with a salutary therapeutic response at 8 weeks in both an adult and a pediatric population. The strong association of a second htSNP with response to inhaled corticosteroids in a third population and the significant interaction of *CRHR1* variation with inhaled steroid usage, resulting in enhanced improvement in lung function in the pediatric population, lend additional credence to the pharmacogenetic role of this gene.

While the association with a different SNP distinguishes the third study from the first two, the finding of associations in

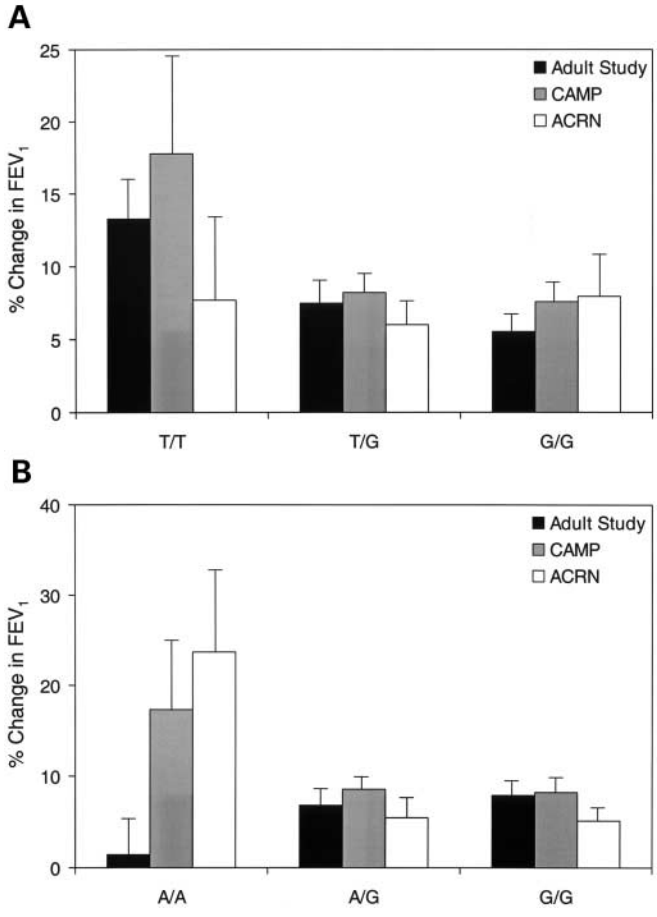

Figure 2. Association of *CRHR1* SNPs with longitudinal response to inhaled corticosteroids in asthmatics, adjusted for age, sex, height and baseline FEV_1. (**A**) rs242941 is associated with the response over 8 weeks in two populations (Adult Study and CAMP). Individuals with the variant TT genotype demonstrated at least a doubling of the improvement in lung function with corticosteroid use compared with those with the wild-type CC genotype. This SNP was not associated with response in the ACRN population. (**B**) rs1876828 is associated with the response over 6 weeks in the ACRN population. Individuals with the variant AA genotype demonstrated a quadrupling of improvement in lung function with corticosteroid use compared with those with the wild-type GG genotype. The AA genotype in the CAMP children was also associated with a doubling of lung function, but this was not statistically significant. Mean values ± SEM are shown.

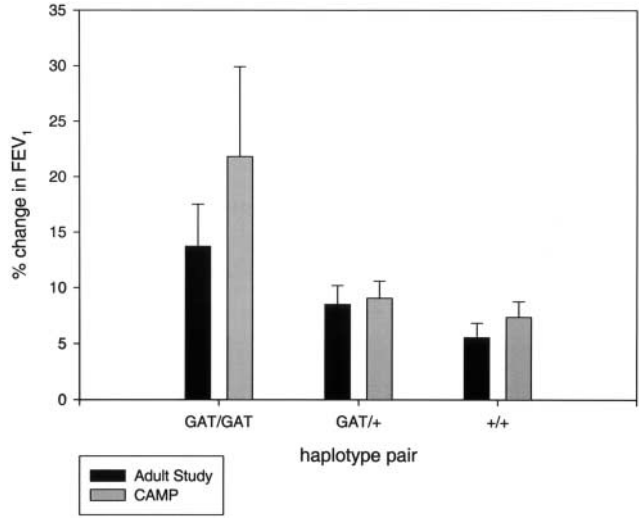

Figure 3. Eight week response to inhaled corticosteroids, stratified by *CRHR1* GAT haplotype status in the Adult Study and CAMP. Utilizing the htSNPs rs1876828, rs242939 and rs242941, the mean FEV_1 improvement in those adults imputed with the GAT/GAT homozygous haplotype was 13.7%, while in those homozygous for two non-GAT haplotypes it was 5.5%. In CAMP, those imputed for the GAT/GAT haplotype demonstrated a 21.8% improvement in FEV_1 versus 7.4% for those with no GAT haplotype. Improvement in those heterozygous for the GAT haplotype was intermediate between the two groups, suggesting an additive effect. Mean values ± SEM are shown.

all three populations is nonetheless significant. Given the variability among the three populations studied, the varying sample sizes and the fact that the three htSNPs are all non-coding, the likely explanation for the difference is that the actual causal variant in *CRHR1* remains to be discovered and that the three SNPs studied are imperfectly correlated markers in LD with that variant. Systematic analysis of the haplotype structure and sequence variation of the *CRHR1* gene will be required to identify the actual casual variants, which might lie in the structural gene or in regulatory sequences controlling alternative splicing, transcription or translation (10–14).

Corticotropin-releasing hormone (CRH) is a well-recognized neuroendocrine mediator of the immune system response to stress. A relationship of CRH to the pathogenesis of asthma (15) has been postulated. CRHR1 is the predominant CRH receptor in the pituitary gland, mediating the release of adrenocorticotropic hormone (ACTH) (16,17) and the catecholaminergic response to CRH (18,19). Peripherally, CRH may bind to mast cells via CRHR1 (20). Alterations of any of these CRH effects, as mediated by the *CRHR1* gene, have the potential to influence the pathogenesis of asthma. For example, decreased expression or function of CRHR1, imposed by genetic variation, would be expected to diminish the capacity to secrete cortisol in response to inflammation, owing to decreased ACTH release. Therefore, asthmatic patients with alterations in this gene would be more likely to respond following the administration of an exogenous corticosteroid. Our data support this hypothesis—improvement in lung function was associated consistently with the variant allele in the associations found in each of our three populations.

Potential limitations of this study included lack of complete sequence information prior to genotyping. Our sequencing efforts focused on the exons of candidate genes, limiting our knowledge of the full LD pattern of the gene. Therefore, we cannot fully exclude a gene if no association was noted in our initial analyses. Candidate genes of great interest, such as *CRH* and the glucocorticoid receptor, may fall into this category. A second potential limitation is multiple comparisons. To compensate for spurious statistical associations owing to multiple comparisons, we carefully designated a limited number of corticosteroid response measures, all related to a single phenotype, longitudinal change in FEV_1. Moreover, our study relies on the replication of effects in a second and a third, very different, populations prior to relevance being attributed to a gene.

In summary, our findings of an association of *CRHR1* genetic variants with the enhanced response to inhaled

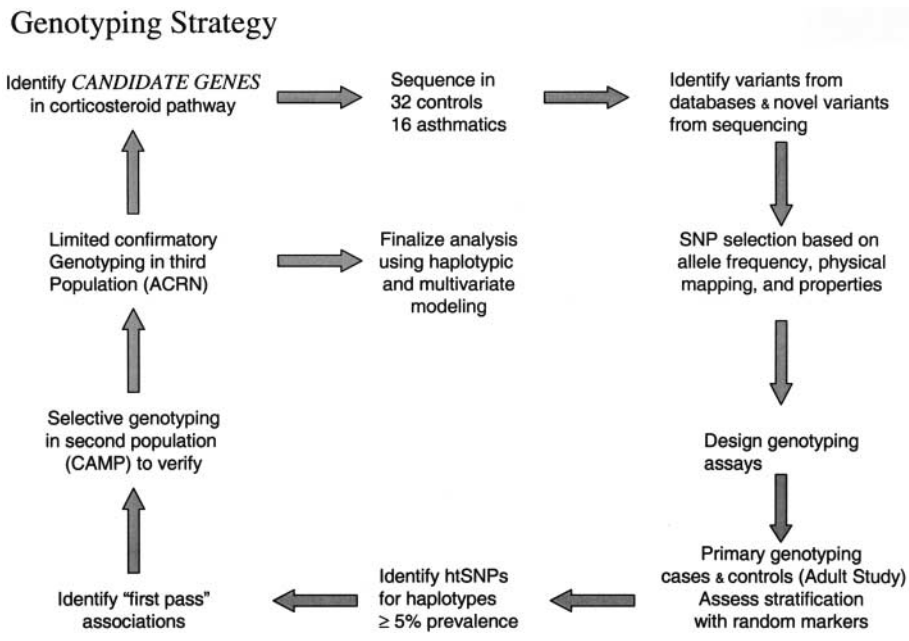

Figure 4. General methodologic approach. After identifying candidate genes of interest, variants were identified via DNA sequencing and public databases. SNPs were selected with preference for known functional variants, allele frequencies >10% and no less than every 10 kb apart. Genotyping was performed initially on our Adult Study and htSNPs were identified. Any gene with single allelic or haplotypic effects with significant ($P < 0.05$) effects were then genotyped in our pediatric population (CAMP). Replicated findings were re-tested in our second adult population (ACRN) before our final, multivariate analysis.

corticosteroids in three diverse asthmatic populations provide novel insights into the therapy of asthma. Animal model studies of this pathway support our findings by implicating CRH in the inflammatory response in asthma (E.S. Silverman, personal communication). Genetic association with a therapeutic response to this class of commonly used medications is an important step in the development of individualized therapy for asthma, providing a potential mechanism to decrease both morbidity and cost. Moreover, since the proportion of non-responders to treatment with corticosteroids is similar between asthma and other diseases, these findings may be relevant to the myriad of other diseases whose therapeutic approaches include the utilization of corticosteroids.

MATERIALS AND METHODS

A graphical summary of the approach utilized for genotyping and analyzing candidate genes for the pharmacogenetic response to inhaled corticosteroids is shown in Figure 4.

Study populations

We utilized DNA samples from three clinical trials. All patients or their legal guardians consented to the study protocol and ancillary genetic testing. The Adult Study was a multicenter 8 week randomized clinical trial comparing the effect of once-daily high-dose inhaled flunisolide versus standard inhaled corticosteroid therapy; 470 moderate to severe adult asthmatics participated. Since the change in the FEV_1 in both treatment groups was the same ($P = 0.30$), we utilized the combined study cohort in our analyses. Inclusion criteria were a history of asthma, ≥12% improvement in FEV_1 with albuterol and using inhaled steroids at randomization. Exclusion criteria were non-asthma pulmonary disease, smoking (≥10 pack-years) and recent asthma exacerbations requiring systemic steroids. Subjects were phoned weekly and had spirometry at 4 and 8 weeks.

CAMP is a multicenter, randomized, double-blinded clinical trial testing the safety and efficacy of inhaled budesonide versus nedocromil versus placebo over a mean of 4.3 years. Trial design and methodology have been published (21,22). CAMP enrolled 1041 children aged 5–12 years with mild to moderate asthma. Entry criteria included asthma symptoms and/or medication use for ≥6 months in the previous year and airway responsiveness with provocative concentration of methacholine causing a 20% reduction in FEV_1 (PC_{20}) ≤ 12.5 mg/ml. Follow-up visits with spirometry occurred at 2 and 4 months and every 4 months thereafter. The replication sample subjects were the 311 Caucasian CAMP children randomized to the corticosteroid group, evaluated at their 2 month follow-up visit.

Two completed trials conducted by the ACRN, the salmeterol or corticosteroids (23) and salmeterol ± inhaled corticosteroids (24) trials, had a common initial 6 week run-in period utilizing four inhalations twice-daily of triamcinolone prior to separate randomization to one of the two trials. Details regarding the entry criteria, run-in period and randomization have been published with the primary trial results (23,24). All patients met the American Thoracic Society definition of asthma and criteria for treatment with inhaled corticosteroids. Of the 339 subjects eligible for randomization, 336 had DNA available, forming the basis of our second replication sample.

Genotyping

In 14 candidate genes involved in innate glucocorticoid synthesis and metabolism, cellular receptors, and transcriptional regulators 131 SNPs were genotyped (Supplementary Material). The genes were selected carefully by experts in the fields of endocrinology and steroid biology as being those biological candidates most likely influencing drug-treatment response. SNPs were selected utilizing two sources, public databases and cDNA sequencing performed at the Whitehead Institute. We over-sampled exonic regions and attempted coverage of at least one SNP every 10 kb. Replicate genotyping was performed in CAMP on the three candidate genes with a measurable effect in the Adult Study and ACRN on the three htSNPs of the single gene with associations in both the Adult Study and CAMP.

SNPs were genotyped via a SEQUENOM MassARRAY MALDI-TOF mass spectrometer (Sequenom, San Diego, CA, USA) for analysis of unlabeled single-base extension minisequencing reactions with a semiautomated primer design program (SpectroDESIGNER, Sequenom). Our protocol implemented the very short extension method (25), whereby sequencing products are extended by only one base for three of the four nucleotides and by several additional bases for the fourth nucleotide (representing one of the alleles for a given SNP), permitting clearly delineated mass separation of the two allelic variants at a given locus.

Statistical methodology

The FEV_1 phenotypic measures in our populations reflect similar outcomes over similar time frames. The percent change in FEV_1 was defined as the FEV_1 at the end of the period minus the FEV_1 at the beginning of the trial divided by the FEV_1 at the beginning of the trial multiplied by 100. In the Adult Study, we tested associations between individual SNPs and asthma phenotypes using generalized linear models under the assumption of an additive model. Genes with significant associations ($P < 0.05$) were genotyped in CAMP and tested for associations. In CAMP an additional analysis, incorporating in interaction term testing for additive genotype with inhaled steroid usage, was performed for the SNP that replicated in both populations. Single SNP analyses were performed using SAS, version 8 (Cary, NC, USA).

For the 14 SNPs spanning ~27 kb of the *CRHR1* gene, which were successfully genotyped in both the Adult Study and CAMP, we inferred haplotypes using the program *Phase* (26). Four common haplotypes comprised 90 and 94% of the total haplotypic substructure for the Adult and CAMP Caucasians, respectively. Subsequently, we used our haplotype-tag approach (27) to identify htSNPs for haplotypes with ≥5% frequency. We chose a minimal subset of htSNPs that was identical for both Adult Study and CAMP, noting that the common haplotypes, although differing in frequency, were represented in both populations, allowing us to compare haplotype-specific effects across the two populations. These SNPs were tested for haplotype association using the *Haplo.score* program (28), where score tests, derived from generalized linear models, are used for global tests of association, as well as haplotype-specific tests. Linkage phase ambiguity (inherent in methods that infer haplotypes from unphased marker data) is addressed by computing the weighted conditional distribution of haplotypes given the observed genetic data for all study subjects. We modified the method to include data from individuals with partially missing marker information. *Haplo.score* permits analysis of continuous and categorical phenotypes, with and without covariate adjustment. Given replication in two asthmatic populations, the htSNPs were tested in the ACRN population. Multivariable individual SNP and haplotypic analyses adjusting for age, sex and baseline FEV_1 were performed for any significant, unadjusted association and are reported throughout the text and figures. Height was also incorporated into the multivariable models involving the CAMP and ACRN populations. In a separate analysis of a random panel of 59 SNPs across the genome in each of our three populations, we found no evidence of population stratification ($P > 0.05$ for dichotomizations of each study into highest and lowest quartiles).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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