Genome-wide Association Identifies the T Gene as a Novel Asthma Pharmacogenetic Locus

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Rationale: To date, most studies aimed at discovering genetic factors influencing treatment response in asthma have focused on biologic candidate genes. Genome-wide association studies (GWAS) can rapidly identify novel pharmacogenetic loci in asthma.

Objectives: To investigate if GWAS can identify novel pharmacogenetic loci in asthma.

Methods: Using phenotypic and GWAS genotype data available through the NHLBI-funded Single-nucleotide polymorphism Health Association-Asthma Resource Project, we analyzed differences in FEV1 in response to inhaled corticosteroids in 418 white subjects with asthma. Of the 444,088 single nucleotide polymorphisms (SNPs) genotyped, the lowest 50 SNPs by Pvalue were genotyped in an independent clinical trial population of 407 subjects with asthma.

Measurements and Main Results: The lowest P value for the GWAS analysis was 2.09 × 10⁻⁵. Of the 47 SNPs successfully genotyped in the replication population, three were associated under the same genetic model in the same direction, including two of the top four SNPs ranked by P value. Combined P values for these SNPs were 1.06 × 10⁻³ for rs3127412 and 6.13 × 10⁻⁴ for rs6456042. Although these two were not located within a gene, they were tightly correlated with three variants mapping to potentially functional regions within the T gene. After genotyping, each T gene variant was also associated with lung function response to inhaled corticosteroids in each of the trials associated with rs3127412 and rs6456042 in the initial GWAS analysis. On average, there was a twofold to threefold difference in FEV1 response for those subjects homozygous for the wild-type versus mutant alleles for each T gene SNP.

Conclusions: Genome-wide association has identified the T gene as a novel pharmacogenetic locus for inhaled corticosteroid response in asthma.

Keywords: polymorphism; genome; pharmacogenomics; glucocorticoid

Approximately 300 million individuals worldwide carry a diagnosis of asthma (1). Asthma is a genetic disease, known for more than three centuries to cluster in families. Based on twin studies, the broad sense heritability estimates (proportion of the total variance of a trait due to genetic causes) of an asthma diagnosis range from approximately 36–75%. For asthma control, the most widely prescribed medications are inhaled corticosteroids (ICS). Endogenous corticosteroid level and exogenous therapeutic

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response to corticosteroids are also strongly influenced by genetics, with broad sense heritability estimated between 0.40 and 0.56 (2–4). Moreover, the treatment response in asthma, including that of ICS, is characterized by large interindividual variability (5, 6) and high intra-individual repeatability (7). These facts strongly support the plausibility of a pharmacogenetic basis for corticosteroid responsiveness in asthma.

Investigations into the genetic basis for treatment response heterogeneity in asthma have largely focused on candidate genes (8–12). These candidate gene pharmacogenetic investigations have identified associations related to the variable response to corticosteroids as measured by differences in lung function (10, 13), airways responsiveness (11), bronchodilator response (14), and exacerbations (12). However, progress related to identifying sufficient numbers of variants to achieve the predictive medicine for any ICS treatment phenotype through the use of candidate genes has been slow. We hypothesized that a genome-wide association study (GWAS) analysis would identify novel loci associated with ICS response. GWAS provides the ability to rapidly identify novel pharmacogenetic variants by simultaneously interrogating genetic variants from across the genome. However, GWAS as applied to pharmacogenetics have been limited, largely because most pharmacogenetic studies are underpowered for GWAS (15). As part of the Single-Nucleotide Polymorphism Health Association-Asthma Resource Project (SHARP) funded by the NHLBI, genome-wide single-nucleotide polymorphism (SNP) data, along with clinical drug-treatment response data, were compiled from a large number of NHLBI-sponsored asthma clinical trials, thereby significantly increasing the sample size compared with studies focusing on any individual trial population alone. We conducted a GWAS of the SHARP data, focusing on those trials that documented response to ICS, as measured by the change in FEV₁.

METHODS
Detailed methods, including SHARP and replication clinical trial descriptions, are described in the online supplement.

Study Populations
SHARP included Childhood Asthma Management Program (CAMP) (16), Childhood Asthma Research and Education (CARE) network (6, 17), and Asthma Clinical Research Network (ACRN) (18–21) participants. CAMP, two of five CARE trials, and four of six ACRN trials enrolled subjects with ICS monotherapy treatment response and were included. Further information on SHARP can be found in dbGAP (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gap). Replication was performed in an 8-week clinical trial conducted by Forest pharmaceuticals, which has been detailed in other pharmacogenetic analyses (10, 13).

Phenotype
The primary outcome phenotype was prebronchodilator FEV₁ while on ICS minus prebronchodilator FEV₁ off ICS, typically the difference between a placebo run-in period to a point 6–12 weeks later after ICS therapy. Additionally, a “reversal group” of individuals with FEV₁ measured at the start and end of an ICS run-in were included. The mean (± SD) FEV₁% difference was nearly identical for all three groups (4 ± 7.9, 4.3 ± 10.9, and 4.1 ± 11.8 percent for standard, reverse, and mixed groups, respectively) (see Figure E1 in the online supplement). Of 792 subjects with ICS response data, 458 were white, forming the phenotypic analysis group. Overall, 444,088 SNPs and 4,113 genotyped white individuals passed QC. After merger with the phenotypic dataset, the final ICS analysis group was 418: 179 ACRN, 147 CAMP, and 92 CARE.

Replication genotyping was performed using Sequenom (San Diego, CA). SNP genotyping was performed using Taqman (Applied Biosystems, Foster City, CA). Because additional DNA was not available in CARE and ACRN, corresponding T genotypes were inferred using imputation with Markov Chain Haplotyping (22) based on HapMap Phase 2 Release 22 data (23). The ratio of empirically observed to expected (binomial) dosage variance for these SNPs was greater than 0.9, indicating good quality of imputation.

Statistical Analysis
GWAS analyses were performed using PLINK (24). Given similarities between CAMP and CARE phenotypic data, these two datasets were pooled. ACRN data were analyzed separately. The separate β-estimates and standard errors were then used to create combined β-estimates and P values, using a random-effects approach (25).

The ICS analysis comprised two phenotypic definitions: difference in raw FEV₁ scores (prefev on_ICS−prefev off_ICS) and difference in percent predicted FEV₁ scores (prefev% on_ICS−prefev% off_ICS). The primary outcome, raw differences, was adjusted for age, height, sex, study group, and six significant principal components. The percent predicted differences were adjusted for study group and six significant principal components. ACRN analyses were further adjusted for ICS therapy timing (i.e., standard, reverse, and mixed).

Generalized linear models evaluated the association between the selected SNPs and FEV₁ change in the replication population, adjusting for age, sex, and height using SAS (SAS Institute, Cary, NC). Analyses assumed an additive effect of the minor allele. A replication was defined by a nominal P less than 0.05.

RESULTS
Populations
A total of 418 SHARP subjects met phenotypic and genotypic inclusion criteria (Table 1). Of these, 239 pediatric CAMP and CARE subjects were pooled before analysis, whereas 179 adult ACRN subjects were analyzed separately. In addition to age, the adult SHARP subjects had a greater proportion of females and lower baseline FEV₁ as a percent of predicted before ICS therapy compared with the pediatric subjects. To limit potential for associations related to occult population stratification, we limited analysis to subjects self-designated as whites. Characteristics of our replication population, Forest, are also shown in Table 1. Overall, this population was similar to the SHARP ACRN subjects at baseline, with a more robust response to ICS therapy. This may be related, in part, to the low baseline FEV₁ associated with this population.

Initial GWAS Association
GWAS of the FEV₁ difference related to ICS therapy was conducted on 444,088 SNPs in CAMP/CARE pooled samples and separately in the ACRN subjects. Figure 1 demonstrates a Manhattan plot of the combined association P values. For internal consistency, we then checked for association of the SNPs with differences in FEV₁ as a percent predicted related to ICS therapy. The top 50 P values from the raw FEV₁ difference analysis that were also associated with differences in predicted FEV₁ in the combined analysis are shown in Table 2 and were taken forward for genotyping in the replication population. Four SNPs had P values less than 1 × 10⁻⁵, although this fell short of the threshold for genome-wide significance.

Replication Population Association
Of the 50 SNPs, 47 were successfully genotyped in the replication population. Three SNPs were associated with FEV₁ difference in
response to flunisolide, under the assumption of an additive model (Table 3) in the same direction as the original SHARP analysis. Table 3 also demonstrates the $P$ value for overall association, reflecting the combined association $P$ values for the ACRN, CAMP/CARE, and Forest populations, which was calculated using Stouffer $z$-transform test. Of note, two of the top four SHARP SNPs as ranked by $P$ value ($rs6456042$ and $rs3127412$) were among the three SNPs replicating in the Forest population. After replication, the lowest combined $P$ value was $6.13 \times 10^{-6}$, which is below the threshold for genome-wide significance ($1.13 \times 10^{-7}$).

**Mapping of Top SNPs**

$Rs6456042$ and $rs3127412$ are located on chromosome 6q27; both map to within 50 kb downstream of a transcription factor known as the $T$ gene (Entrez Gene: 6862). Using the CEU HapMap population, linkage disequilibrium (LD) (i.e., correlation between genotypes) measures were obtained for SNPs mapping to the $T$ gene with $rs6456042$ and $rs3127412$. For these two SNPs, which are in tight LD, three $T$ gene SNPs were correlated, with an $r^2$ correlation coefficient greater than or equal to 0.66 (see Figure E2). Furthermore, each of these three $T$ gene SNPs are located within potential functional regions of the gene: $rs3099266$ maps to the promoter region, $rs1134481$ to the 3’ untranslated region, and $rs2305089$ is a nonsynonymous variant coding for a glycine to aspartic acid amino acid change at position 177 within the gene. The SHARP genotype dataset did not contain any of these variants. Therefore, to elucidate whether the initial GWAS association actually represented a downstream correlation with a true association within the $T$ gene, we genotyped these three SNPs in the CAMP and Forest populations, and analyzed imputed genotypes for the SNPs in ACRN and CARE.

**T Gene Analysis**

Association analysis within CAMP and Forest demonstrated that each of the three $T$ SNPs were also associated with FEV$_1$ response to ICS therapy, with $P$ values for CAMP and Forest, respectively, as follows: $rs1134481$ (0.0009 and 0.01); $rs2305089$ (0.004 and 0.02); and $rs3099266$ (0.004 and 0.01) (Table 4). The strongest association noted was with $rs1134481$. For this variant, homozygous wild-type subjects had an improvement in FEV$_1$ as a percent of predicted of 2.8 ± 1.3 and 2.7 ± 1 versus 9.9 ± 2.1 and 5.3 ± 1.5 for those homozygous mutant for CAMP and Forest, respectively. Overall, after adjustment for age, sex, and height, each $T$ SNP demonstrated a twofold to threefold difference in FEV$_1$ response for those subjects homozygous for the wild-type versus mutant alleles in both populations (see Figure 3). Imputed analyses of the $T$ variants were also consistent with association through LD, with a significant association noted for ACRN. Although the imputed $T$ gene variants were not associated with ICS response in CARE, this is consistent with the primary SHARP GWAS results for the two SNPs in LD with the $T$ gene variants (see Table E1). Although CARE demonstrated the highest baseline level of FEV$_1$ (Table 1), adjustment for baseline FEV$_1$ did not significantly alter the associations with the $T$ gene SNPs (see Table E2).

**DISCUSSION**

GWAS holds tremendous promise because of the ability to discover new biology through simultaneous interrogation of variants across the entire genome. This is especially applicable to the field of drug response pharmacogenetics, where candidate gene studies have failed to explain enough of the genetic variation to allow predictive modeling. Through the SHARP project, we interrogated 444,088 SNP variants for their association with lung function response to ICS in a compilation of seven asthma clinical trials. We were able to replicate 3 of the top 50 GWAS variants.
TABLE 2. TOP 50 SHARP P VALUES FOR FEV1 CHANGE  

<table>
<thead>
<tr>
<th>SNP</th>
<th>Closest Gene*</th>
<th>ACRN P Value</th>
<th>CC P Value</th>
<th>SHARP P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10218533</td>
<td>RPS-1027O11.1</td>
<td>0.023</td>
<td>4.67 × 10⁻⁵</td>
<td>2.09 × 10⁻⁶</td>
</tr>
<tr>
<td>rs6456042</td>
<td>RPII-459F12</td>
<td>0.013</td>
<td>1.95 × 10⁻⁴</td>
<td>6.67 × 10⁻⁶</td>
</tr>
<tr>
<td>rs6431915</td>
<td>RPS-89H4H2.4</td>
<td>0.011</td>
<td>3.09 × 10⁻⁴</td>
<td>9.39 × 10⁻⁶</td>
</tr>
<tr>
<td>rs3127412</td>
<td>RPII-459F12</td>
<td>0.034</td>
<td>1.35 × 10⁻⁴</td>
<td>9.68 × 10⁻⁶</td>
</tr>
<tr>
<td>rs10218521</td>
<td>RPII-31J13.1</td>
<td>0.054</td>
<td>3.26 × 10⁻⁴</td>
<td>1.14 × 10⁻⁵</td>
</tr>
</tbody>
</table>

*Combined P value for additive genotypic effects for all populations. ACRN and combined ACRN/CARE P values are shown for their relative contribution to the overall P value.

Definition of abbreviations: ACRN = Asthma Clinical Research Network; CAMP = Childhood Asthma Management Program; CARE = Childhood Asthma Research and Education; CC = CAMP/CARE; SHARP = Single-Nucleotide Polymorphism Health Association-Asthma Resource Project; SNP = single-nucleotide polymorphism.

SNPs in an independent population, including two of the top four SNPs.

Although none of the replicated SNPs were located within a gene, the top two SNPs were in LD (correlated) with three known variants in the T gene. Using LD to identify disease genes takes advantage of the historical preservation of regions within the genome as they are passed down through generations (26, 27). Each of the three correlated T gene variants maps to a potentially functional region of the gene, with rs2305089 encoding for an amino acid change (Gly177Asp) within the T protein. Each was also significantly associated with ICS response, suggesting that the true biologic effect is likely mediated by this gene. There was a twofold to threefold difference in FEV1 response for those subjects homozygous for the wild-type versus mutant alleles for each T gene SNP in each of the genotyped populations (see Figure E3).

Despite the compilation of studies within SHARP, our study design remains underpowered for finding associations of genome-wide significance. However, our approach used the GWAS as an initial step to subsequently target variation within the T gene locus, resulting in an effect that was replicated in a larger replication cohort and the combined population. Each of the three interrogated T SNPs was associated with ICS response even after correction for multiple comparisons.

The T gene, which has not previously been implicated in either asthma or corticosteroid pathobiology, is a founding member of an ancient family of genes containing a common protein motif, the T locus (28). The T locus encodes a protein with DNA binding activity that likely plays a role in the development of all vertebrate organisms. The T gene itself (Brachyury homolog) encodes for a key mesodermal developmental transcription factor crucial to notochord development (29). However, the expression of T is sufficiently early in the developmental process that it has the potential to influence mesodermal development overall (30), including within the lung. Corticosteroids are also crucial to lung development; alterations in corticosteroid-responsive genes during development may thus influence later asthma susceptibility and treatment response. T is also expressed in normal adult lung tissue (31), although little is known about its transcriptional regulation of other genes. T and other T-box genes have been implicated in the function of bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) pathways (32). Specifically, decreased T expression inhibits chondrogenesis mediated by BMP2 and FGF3 (33). Notably, the BMP2 receptor and FGF3 have been independently implicated in corticosteroid resistance (34, 35), supporting a mechanistic basis for the T gene in ICS response in asthma.

To further explore the potential effects of the T gene on response to corticosteroids, we conducted a pathway analysis specifically focusing on potential T gene–NR3C1 (glucocorticoid receptor) interactions using the program GeneMania (http://genemania.org/) (see Figure E4). Although not conclusive, the results indicate that the T gene is coexpressed with three other genes (NRIPI, FOXA2, and TTPA) that directly interact or are predicted to interact with NR3C1.

Although variations in T have the potential to directly cause alterations in response to ICS therapy, the familial lineage of T also offers intriguing mechanistic possibilities. T is phylogenetically conserved with 50 known orthologs. Moreover, 16 other members of the T family are paralogous (i.e., the result of a prior gene duplication within the same ancestral chromosome).

| TABLE 3. ASSOCIATED REPLICATION SNPS |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SNP             | rs3127412       | rs6456042       | rs9955411       | Beta P Value    | Beta P Value    | Beta P Value    |
|                 | SHARP (overall) | SHARP (overall) | SHARP (overall) | Beta P Value    | Beta P Value    | Beta P Value    |
|                 | 0.078 9.68 × 10⁻⁶ | 0.079 6.67 × 10⁻⁶ | 0.080 0.00013 | 0.090 0.034 | 0.103 0.013 | 0.096 0.05 |
|                 | ACRN            | ACRN            | ACRN            | 0.076 0.00013 | 0.074 0.0002 | 0.076 0.0011 |
|                 | CAMP/CARE       | CAMP/CARE       | CAMP/CARE       | 0.071 0.02    | 0.072 0.02    | 0.092 0.02    |
|                 | Forest          | Forest          | Forest          | 1.06 × 10⁻⁵   | 6.13 × 10⁻⁵   | 4.64 × 10⁻⁵   |

*Definition of abbreviations: ACRN = Asthma Clinical Research Network; CAMP = Childhood Asthma Management Program; CARE = Childhood Asthma Research and Education; SHARP = Single-Nucleotide Polymorphism Health Association-Asthma Resource Project; SNP = single-nucleotide polymorphism.

*The combined row reflects the combined association P values for the ACRN, CAMP/CARE, and Forest populations using Stouffer z-transform test.
including genes encoding for a number of the T-box domain proteins, such as TBX21 (data from Ensembl [www.ensembl.org]). It is possible that there is sufficient overlap in the genomic structure of T and one of these paralogs that the mechanism of action is actually by the paralog; we have previously reported an independent ICS pharmacogenetic association with airways responsiveness in asthma resulting from variation in TBX21 (11). T is known to interact with other members of the T-box family (36), yielding an additional mechanism through which a T may affect ICS response. Additional work in defining the role of genetic variation within T and the role of T on asthma treatment response is warranted.

Our top GWAS hits from this analysis contrast with those that we have recently identified by a family-based screening algorithm (37). This is expected because the current analysis, given its larger sample size, ranked the SNPs by P value, whereas our prior study ranked SNPs by relative power for replication, which is only possible in a family-based study design. We also did not identify the GLCCI1 SNP from our prior study (37) as one of our most significant variants in this study; however, neither of the GLCCI1 variants associated in the previous study is incorporated on the genotyping chip used in this study. Specifically, because our GLCCI1 resequencing revealed only one other variant, rs37973, in tight LD with the original associated variant (rs37972) and because neither of these SNPs is present on the Affymetrix platform used for the current analysis, we did not a priori expect to replicate the GLCCI1 association.

There are several potential limitations to our study. As noted, we do not report any association exceeding the genome-wide significance threshold (Bonferroni adjusted). However, this adjustment may be overly conservative, because it does not account for LD within the genotyped populations. Additionally, our independent replication of the findings is reassuring of a true positive association. Moreover, the association of upstream variants within the T gene that were not in complete LD (r² = 0.66–0.82) with the initial association lends further credence to the validity of the association. Although we used a compilation of SNPs as an initial prioritization strategy, we do not report any association exceeding the genome-wide significance threshold (36), yielding an additional mechanism through which a T may affect ICS response. Additional work in defining the role of genetic variation within T and the role of T on asthma treatment response is warranted.

In conclusion, GWAS study of ICS response in SHARP has allowed the identification of a novel pharmacogenetic locus, the T gene. For variants within this gene, there was a twofold to threefold difference in FEV₁ response for those subjects homozygous for the wild-type versus mutant alleles. As genome-wide studies identify additional variants influencing drug treatment response in asthma and other diseases, we will continue to draw closer to the era of truly personalized medicine.

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