

No evidence of large genetic effects on steroid response in asthma patients

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Background: Inhaled corticosteroids (ICSs) are considered the most effective anti-inflammatory therapy for asthma control and management; however, there is substantial treatment response variability.

Objective: We sought to identify genetic markers of ICS response by conducting the largest pharmacogenetic investigation to date in 2672 ICS-treated patients with asthma. **Methods:** Genotyping and imputation was performed in fluticasone furoate (FF) or fluticasone propionate-treated patients with asthma from 3 phase IIB and 4 phase IIIA randomized, double-blind, placebo-controlled, parallel group, multicenter studies. The primary end point analyzed was change in trough FEV₁ (Δ FEV₁) from baseline to 8 to 12 weeks of treatment.

Results: More than 9.8 million common genetic variants (minor allele frequency $\geq 1\%$) were analyzed to test for association with Δ FEV₁. No genetic variant met the prespecified threshold for statistical significance.

Conclusions: This study provides no evidence to confirm previously reported associations between candidate genetic variants and ICS response (Δ FEV₁) in patients with asthma. In addition, no variant satisfied the criterion for genome-wide significance in our study. Common genetic variants are therefore unlikely to prove useful as predictive biomarkers of ICS response in patients with asthma. (*J Allergy Clin Immunol* 2016;■■■■:■■■■-■■■■.)

Key words: Steroid response, asthma, fluticasone, genome-wide association studies, inhaled corticosteroids, pharmacogenetics

Asthma is a common, chronic inflammatory airway disease affecting more than 300 million adults and children worldwide.¹ It is characterized by variable, recurring symptoms including chronic inflammation, reversible airway obstruction, and increased bronchial hyperresponsiveness. It is a complex disease,

Abbreviations used

1000G: 1000 Genomes Project
FF: Fluticasone furoate
FP: Fluticasone propionate
GWAS: Genome-wide association study
ICS: Inhaled corticosteroid
MAF: Minor allele frequency
OEE: OmniExpressExome
PC: Principal component
SE: Standard error

with multiple genetic and environmental factors that increase risk, and it exhibits marked phenotypic heterogeneity. Heritability estimates for asthma, based on twin studies, range from 0.36 to 0.92.²

A wide range of therapies are available for the treatment of asthma, including inhaled corticosteroids (ICSs), beta-2 adrenergic receptor agonists, leukotriene modifiers, and anticholinergics. ICSs are considered the primary and most effective anti-inflammatory therapy for the control and management of asthma; however, there appears to be substantial variability in treatment response, with some patients not responding to ICS treatment at all, possibly due to their genetic background.³ Insight into individual ICS response would be valuable for patient management.

Over the past decade, a number of pharmacogenetic studies, including candidate gene as well as genome-wide association studies (GWASs), have provided preliminary evidence for the association of genetic variants with ICS response. For some ICS response studies, such as those implicating genetic variants in *STI1*,⁴ *TBX21*,^{5,6} and *WDR21A*,⁷ there are no published attempts to replicate the original observations. For those studies implicating *GLCC1*,⁸ *FBXL7*,⁹ *T*,¹⁰ *CRHR1*,¹¹ and *MAPT*,¹² attempts to demonstrate statistical associations through replication in independent samples have had mixed results.^{13,14} All associations were originally identified in relatively small sample sizes ranging from 118 to 781 patients and tended to have even smaller replication samples ranging from 64 to 407 patients.

Despite many publications in this area, genetic variants are not currently used to support clinical treatment of asthma. In an effort to better understand the role of genetics in ICS response and to potentially discover novel predictive variants, we have conducted the largest pharmacogenetic investigation reported to date, with 2672 fluticasone furoate (FF)- or fluticasone propionate (FP)-treated patients with asthma from 7 GSK-sponsored clinical studies. Using genotype imputation, 9.8 million genetic variants throughout the genome, including 98,000 rare protein-coding variants, were investigated for association with Δ FEV₁ from

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TABLE I. Study patient characteristics by study group

Characteristics	Study group			P value*
	Phase IIB studies	Phase IIIA studies	HZA106837	
Total patients	1125	679	868	—
FF patient numbers	895	424	868	—
FF doses (μ g)	25, 50, 100, 200, 300, 400, 600, 800	100, 200	100	—
FP patient numbers	230	255	0	—
FP doses (μ g)	50, 250, 500	250, 500	NA	—
Age (y)	42.1 $\pm$ 16.8	44.2 $\pm$ 15.6	42.7 $\pm$ 14.7	3.0 $\times 10^{-2}$
Age of asthma onset (y)	24.5 $\pm$ 16.8	29.2 $\pm$ 18.2	26.9 $\pm$ 17.7	5.1 $\times 10^{-7}$
Asthma duration (y)	17.7 $\pm$ 13.4	14.9 $\pm$ 13.0	15.7 $\pm$ 11.8	2.9 $\times 10^{-5}$
FEV ₁ at baseline (L)	2.3 $\pm$ 0.7	2.2 $\pm$ 0.8	2.2 $\pm$ 0.6	2.2 $\times 10^{-2}$
FEV ₁ at baseline (% predicted)	69.9 $\pm$ 10.1	69.2 $\pm$ 10.4	71.7 $\pm$ 11.8	5.1 $\times 10^{-5}$
FEV ₁ % reversibility at baseline	28.4 $\pm$ 16.8	29.0 $\pm$ 15.6	24.5 $\pm$ 14.7	3.4 $\times 10^{-10}$
Sex: % female	61.2	58.6	68.6	8.6 $\times 10^{-5}$
BMI (kg/m ²)	27.9 $\pm$ 6.7	27.9 $\pm$ 5.2	27.6 $\pm$ 5.9	5.5 $\times 10^{-1}$
Height (cm)	167.9 $\pm$ 13.4	165.9 $\pm$ 7.8	163.9 $\pm$ 8.8	1.1 $\times 10^{-13}$
FEV ₁ change (% predicted)	7.2 $\pm$ 13.4	6.9 $\pm$ 13.0	6.1 $\pm$ 11.8	1.0 $\times 10^{-1}$

Data represent mean and SD.

BMI, Body mass index; NA, not available.

*P values from 1-way ANOVA, testing for differences among study group means.

baseline after 8 to 12 weeks of ICS treatment. We found no evidence confirming the previously reported associations in candidate genes, and, furthermore, no variant satisfied the criterion for genome-wide significance. Our GWAS in 2672 FF- or FP-treated patients suggests that the contribution of any common variant, large genetic effect on steroid response in patients with asthma is unlikely, and also failed to identify significant contributions of rare coding variants.

METHODS

Patients

Patients who gave both consent and a sample for pharmacogenetic research were drawn from 7 randomized, double-blind, placebo-controlled, parallel group, multicenter clinical studies conducted in a total of 26 countries. Studies FFA109684 (NCT00603746), FFA109685 (NCT00603278), and FFA109687 (NCT00603382) were phase IIB studies designed to assess the dose response, efficacy, and safety of FF in patients with persistent uncontrolled asthma. Each study also included an FP treatment arm. Studies FFA112059 (NCT01159912), HZA106827 (NCT01165138), HZA106829 (NCT01134042), and HZA106837 (NCT01086384) were phase IIIA efficacy and safety studies that each included an FF monotherapy treatment arm; 2 of the studies, FFA112059 and HZA106829, also included an FP treatment arm. Approval by the appropriate institutional review boards and independent ethics committees was obtained for each study. The designs and patient characteristics of these studies were similar except that doses of FF or FP were not the same across studies (Table I), nor were the ICS maintenance therapies allowed or required before randomization into the study (see Table E1 in this article's Online Repository at www.jacionline.org). HZA106837 was unique in requiring patients to have experienced an asthma exacerbation during the year before enrollment, whereas the other studies required patients to have been exacerbation-free for a specified time period leading up to the study (see Table E1). All patients were 12 years and older. A total of 2672 FF- or FP-treated patients from these 7 studies comprised the pharmacogenetic analysis population.

Genetic variants

Genotyping was performed using the Human Omni1-Quad (Illumina, San Diego, Calif) beadchip for the phase IIB studies and the Human OmniExpressExome (OEE; Illumina) beadchip for the phase IIIA studies. Following

quality control, 3.7% of the Omni1-Quad variants and 0.7% of the OEE variants were excluded. Kompetitive Allele Specific PCR genotyping was also used for 37 variants of interest from candidate genes. Ninety-eight percent of patients were successfully genotyped on both genome-wide and Kompetitive Allele Specific PCR platforms.

Genotype imputation was performed using a prephasing approach,¹⁵ as implemented in the MaCH and Minimac software, and using a cosmopolitan reference panel of 2184 haplotypes from the 1000 Genomes Project (1000G).¹⁶ Imputation was conducted separately for phase IIB and phase IIIA studies because different genotyping platforms were used. Before imputation, variants with a call rate of less than 98%, variants not in Hardy-Weinberg proportions¹⁷ ($P < 1 \times 10^{-7}$ within either European or Asian ancestry subgroups), monomorphic variants, and variants that could not be mapped to the 1000G reference panel were excluded, leaving 914,586 and 761,515 variants for phase IIB and phase IIIA studies, respectively. For phase IIB studies, incorporating the imputed variants (imputation quality score [r^2] > 0.3 and minor allele frequency [MAF] $\geq 1\%$), there were 9,882,836 variants for analysis. For phase IIIA studies, incorporating the imputed variants (with $r^2 \geq 0.3$ and MAF $\geq 1\%$), there were 10,281,531 variants for analysis. Association with imputed variants was analyzed using posterior expected dosages, which accounts for uncertainty in the imputed genotypes and is asymptotically optimal.^{18,19} For the rare variant (MAF $< 1\%$) analysis, only directly genotyped OEE variants were used, hence restricting analyses to the 1547 patients from phase IIIA studies. A total of 98,517 protein-coding variants with an MAF of less than 1% mapping to 15,450 genes were identified for rare variant analysis. Most were nonsynonymous changes (96%), with smaller contributions from splice site (2.3%), nonsense (1.6%), and read-through (0.1%) mutations.

Each common genetic variant was assigned to 1 of 3 categories: (1) candidate genetic variants explicitly cited in previous publications (or studied previously by GSK) as potentially associated with ICS response or with asthma; (2) additional candidate genetic variants not explicitly cited in the literature but at genes harboring variants in the first category; and (3) other GWAS variants with an MAF of 1% or more not in the first 2 categories. Multiple test adjustment for the candidate gene variant categories took into account the number of variants and the linkage disequilibrium between them.²⁰ Prespecified per-variant significance levels were set for the first 2 variant categories using a Bonferroni correction at 0.0039 and 0.00096, respectively. We used $P \leq 1 \times 10^{-8}$ as appropriate for common variant GWAS analyses based on 1000G imputation reference data sets.²¹ The prespecified per-gene significance level was set as 3.2×10^{-6} for 15,450 gene-based rare variant tests (0.05/15,450).

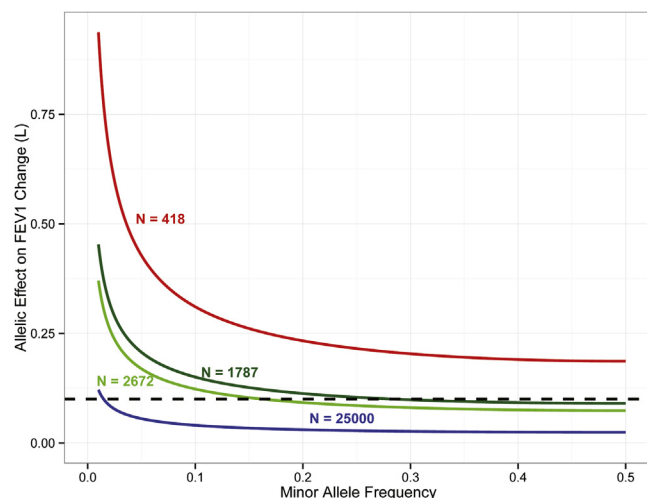


FIG 1. Power curves for selected sample sizes showing the smallest per allele effect size detectable with 80% power as a function of MAF. $N = 418$ is the size of the largest published GWAS discovery sample set of ICS response in asthma to date; $N = 1,787$ is the size of the European ancestry subset of this current data set; $N = 2,672$ is the size of the full current data set; $N = 25,000$ is a representative choice for a large sample size. The dotted line approximates the size of a clinically meaningful allelic effect on FEV₁ change (ie, 0.1 L for each copy of an allele, leading to a 0.2-L difference between homozygous genotypes).

Drug response

The primary end point for measuring ICS response was Δ FEV₁ at week 12 (study HZA106837) or week 8 (the other 6 studies) of treatment. *Baseline FEV₁* was defined as the predose value obtained on the first day of treatment, and *trough FEV₁* was defined as the predose measurement taken at the on-treatment clinic visit. For the 144 patients with asthma for whom FEV₁ change at week 8 or 12 was missing, the last observation carried forward from the most recent previous visit was used to impute its value, consistent with other analyses conducted for these clinical studies.

Statistical analysis

The study was well powered to detect an association between drug response and common genetic variants with clinically meaningful response differences between genotypes. Eighty percent power is achieved for (1) variants with an MAF of more than 16% assuming an allelic effect of 0.1 L on Δ FEV₁ (Fig 1); and (2) effects on Δ FEV₁ of at least 0.17 L for an MAF of 5% or more. The power to detect genetic associations with ICS response using rare variants (MAF < 1%) was estimated to be only 0.3% (see this article's Online Repository at www.jacionline.org).

Kinship coefficients were estimated using Kinship-based INference for GWAS²² to exclude patients so that no 2 were more related than second cousins. Genetic principal components (PCs) were calculated using Kinship-based INference for GWAS, and the first 10 PCs were used as covariates in all statistical models to control for confounding due to population stratification in these samples, which included patients from multiple race groups.

Baseline demographic and clinical variables were evaluated for univariate association with change in trough FEV₁, and those with independent associations with the end point (first 10 genetic PCs, baseline FEV₁ percent predicted, age, sex, body mass index, asthma duration, and country) were included as covariates in subsequent modeling.

To control for potential batch effects due to the use of different genotyping methodologies and study design features, association analyses were conducted separately for patients from the exacerbation study, HZA106837, phase IIB studies, and the remaining 3 phase IIIA studies. Patient numbers, dosages, baseline characteristics, and FEV₁ change percent predicted for the analysis

study groups are summarized in Table I, and differ between study groups. Association results derived within each of these 3 subgroups were meta-analyzed using a weighted z-score method²³ assuming a fixed-effect model.

For analysis of Δ FEV₁, linear regression was used. Relevant covariates were included in the models and the genotypes were coded to detect additive genetic effects. Gene-based analysis of the effect of rare variants on Δ FEV₁ was carried out using the sequence kernel association test (optimal test) method²⁴ in a subset of 1547 patients with asthma with OEE genotyping data (drawn from the phase IIIA studies only). An inverse normal transformation was applied to Δ FEV₁ to reduce the impact of outliers on test results.

Heritability of Δ FEV₁ attributable to common genetic variation was estimated using genome-wide complex trait analysis.²⁵

RESULTS

Our sample comprised 2672 patients with asthma drawn from 7 GSK clinical studies (Table I). All patients had mild-to-severe asthma with FEV₁ 40% to 90% predicted and were 12 years or older. Three of the 7 clinical studies were phase IIB, and 4 were phase IIIA. About 70% of the patients were of European ancestry, 15% were Hispanic, 10% were Asian, 5% were African, and less than 1% were Native American or of mixed ancestry. Most patients were treated with FF (82%), with the remainder (18%) being treated with FP. *Reversibility* (defined as 12% change in FEV₁ or at least 200 mL in response to a short-acting beta agonist) was required in all study patients. In only 1 of the 7 trials was history of acute exacerbations a requirement for entry, and active or heavy smokers (>10 pack-years) were excluded. All patients, apart from 382 patients in FFA109687, had been treated with ICS before the first visit of the trial. However, despite this, the ICS treatment response during the trial was clinically significant in all studies (least squares mean Δ FEV₁ [standard error (SE)] in the range 0.142 [0.040] to 0.367 [0.043]; see Table E1). Studies were placed into groups for analysis: the 3 phase IIB studies; the phase IIIA exacerbation study, HZA106837; and the remaining 3 phase IIIA studies. Many patients' baseline characteristics differed statistically significantly across these 3 study groups (Table I); for example, the proportion of females was higher in HZA106837 than in the other study groups. Patient characteristics displaying independent associations with Δ FEV₁ were included as covariates in subsequent statistical models. To specifically address the impact of height on our analyses, association *P* values were compared with and without height as a covariate; the correlation was 99%, suggesting that the inclusion of height would not affect these results. All patients within each study group were analyzed by combining dose groups because there was no significant association between dose and ICS response (see Table E1).

Analysis of Δ FEV₁ over 8 to 12 weeks

The analysis results for the association of Δ FEV₁ and candidate gene variants among 2672 patients are presented in Table E2 in this article's Online Repository at www.jacionline.org. Four variants within 1 candidate gene, *GLCCII*, were nominally associated ($P < .05$) with Δ FEV₁; however, no candidate genetic variant satisfied the prespecified, multiplicity-adjusted criteria for statistical significance. More than 9.8 million common variants were analyzed to test for additive association with Δ FEV₁; however, none met the prespecified genome-wide significance threshold of $P < 1 \times 10^{-8}$ (and indeed none met the classical $P < 5 \times 10^{-8}$ threshold). The genome-wide association results are summarized in a Manhattan plot (Fig 2) and a QQ-plot

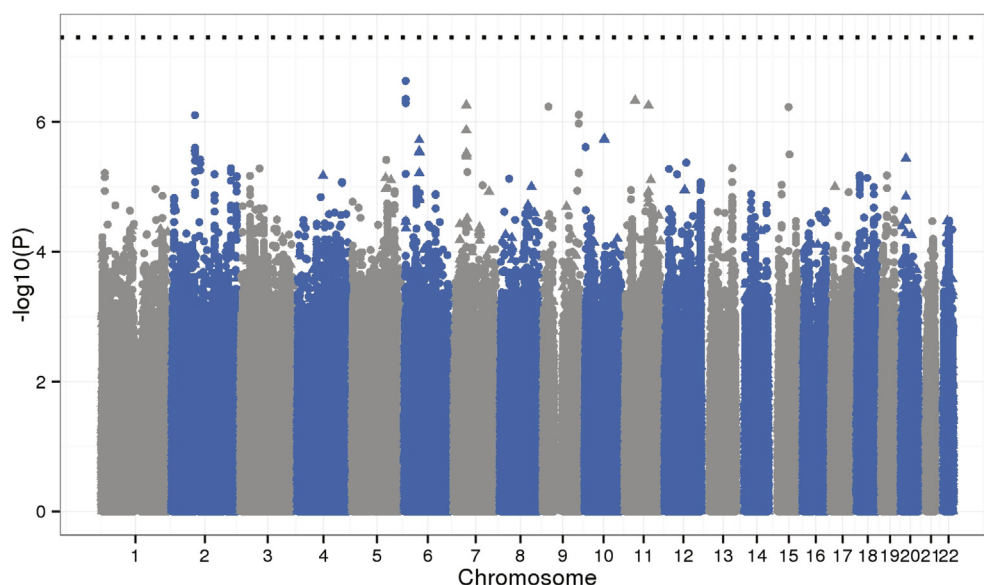


FIG 2. Manhattan plot summarizing association analyses between common ($MAF \geq 1\%$) genetic variants and ΔFEV_1 . ΔFEV_1 , Change from baseline in trough FEV_1 . The dotted black line indicates the prespecified criterion for statistically significant association ($P = 1 \times 10^{-8}$). Circular and triangular points represent imputed and directly genotyped variants, respectively.

(see Fig E1 in this article's Online Repository at www.jacionline.org). Similar nonsignificant results were obtained when analyzing the subset of 1787 patients with asthma of European ancestry (results not shown). The proportion of variation in ΔFEV_1 due to genome-wide common genetic variants ($MAF \geq 1\%$) was estimated at 10.0% (95% CI, 0% to 50%), which was not statistically significant ($P = .5$).

Rare variants ($MAF < 1\%$) with putatively functional protein-coding effects that mapped to 15,450 genes were identified. The cumulative effect of the rare variants within each gene was tested using SKAT-O. There was no evidence for the association of ΔFEV_1 with any gene in 1547 patients with asthma drawn from the phase IIIA studies, as shown in Figs E2 and E3 in this article's Online Repository at www.jacionline.org. Similar results were observed when evaluating the European subset ($n = 1109$) only (results not shown).

Comparative analysis with published reports

Our large pharmacogenetic study provided a unique opportunity to independently assess the effects of genetic variants reported previously to be associated with ICS response. Previously published data on ICS response in patients with asthma, from studies predominantly undertaken in populations of European ancestry, are summarized in Table E3 in this article's Online Repository at www.jacionline.org. Four of those studies provided sufficient information to allow for a direct comparison with our current results, albeit complicated by the fact that in many cases parameter estimates and SEs were not provided in the publications. We estimated the genetic effect and its SE on the basis of mean responses by genotype (details are provided in this article's Online Repository at www.jacionline.org). Because the previous reports were all conducted in patients of European ancestry, we performed this direct comparison with the European subset of our sample ($n = 1787$). For each comparison, the effect size in

European GSK study patients ($n = 1787$) is close to zero, and none was statistically significant at $P < .05$.

DISCUSSION

In this study, we report the largest analysis to date on the pharmacogenetics of ICS response in patients with asthma drawn from 7 clinical studies. Despite treatment with ICS before randomization into each study, a clinically relevant range of treatment responses was observed across all studies, all doses, and both medicines. We evaluated the role of common ($MAF \geq 1\%$) and rare ($MAF < 1\%$) genetic variation in up to 2672 patients with asthma treated with FF or FP for up to 12 weeks. Both candidate genetic variant and genome-wide approaches were incorporated into the analysis strategy, yielding different criteria for declaration of statistically significant results. However, none of the genetic variants met their particular prespecified criteria for statistically significant association with ΔFEV_1 in this large sample set.

This study was well powered to detect a 0.1 L per allele effect on ΔFEV_1 for genetic variants with an MAF of 16% or more in this sample set of 2672 (Fig 1) and to replicate the effects previously reported to be associated with ICS response. Our total sample set and the European subset were 6.4- and 4.3-fold larger, respectively, than the largest discovery sample set ($n = 418$) used in any of the previously published genome-wide studies (see Table E3), and 3.4- and 2.3-fold larger, respectively, than the largest discovery sample set ($n = 781$) used in any of the previously published candidate gene studies. Despite this, no statistically significant associations were observed following either candidate variant or genome-wide approaches. Also, we were unable to identify any statistically significant associations of rare coding variants at the gene level.

Many previously published reports, however, do highlight genetic associations with ICS response in patients with asthma. While acknowledging that the probability of replicating

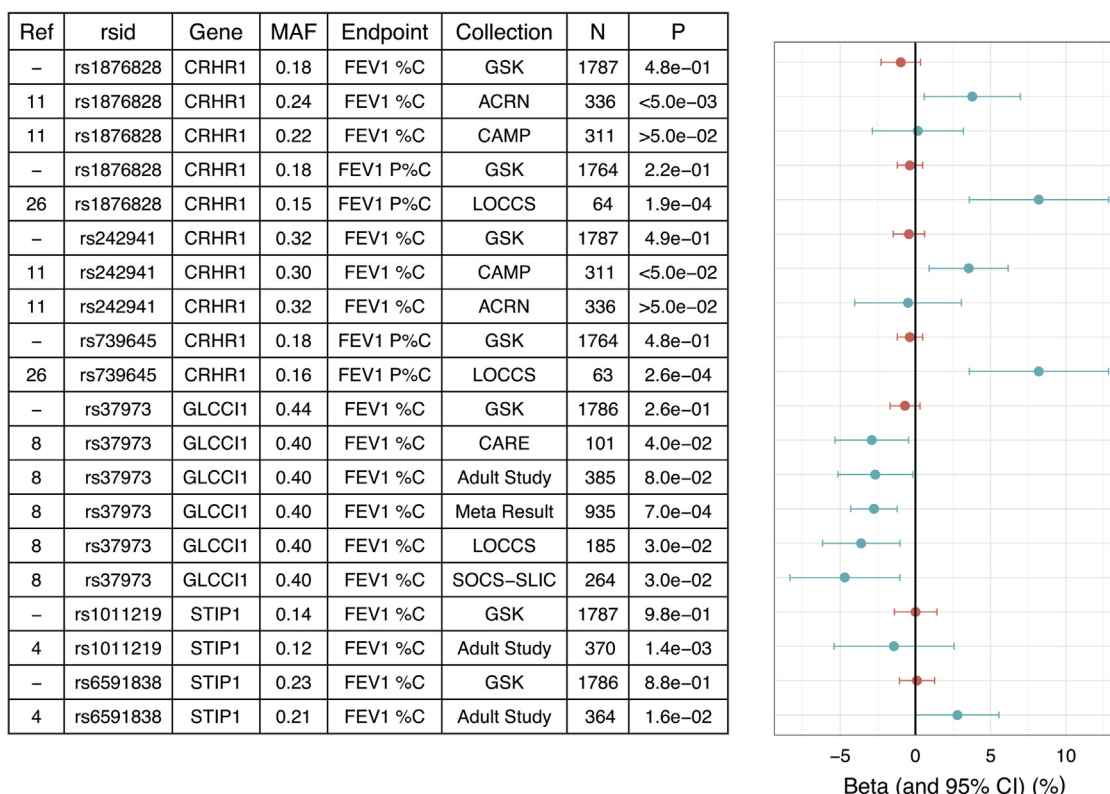


FIG 3. Comparison of 4 published ICS response association results (turquoise bars) with GSK results (red bars) in patients with asthma of European ancestry. ΔFEV_1 , Change from baseline in trough FEV₁; GSK, GlaxoSmithKline. Effect size (beta) and 95% CIs for non-GSK results were estimated from reported means and SEs of the 3 genotypes.

significant results may be smaller than expected²⁶ and that wide sample-to-sample *P*-value variability may occur,²⁷ our sample size was substantially larger than most of the original studies and was well powered to test for the effect sizes reported at marginal significance levels. Our inability to replicate previously associated loci provides strong evidence for our conclusion that there are unlikely to be common large genetic effects on steroid response.

Most of these published studies have been undertaken in small numbers (ranging from 118 to 781 in the discovery sample sets) of patients of European ancestry, comprising a combination of focused candidate gene studies and hypothesis-free genome-wide approaches. Some of these published associations have been the subject of attempted confirmation (see Table E3). For example, Park et al⁹ identified an association between rs10044254 in *FBXL7* and asthma symptom scores in a sample set of 124 from the childhood collection, childhood asthma management program. This result was confirmed in 77 patients drawn from the childhood asthma research and education collection, but not in the combined asthma clinical research network and leukotriene modifier or corticosteroid salmeterol collections of 110 patients. Five genetic variants of the *T* gene (*T* brachyury transcription factor) were associated with differences in FEV₁ raw scores (preFEV_{on} ICS – preFEV_{off} ICS) in 418 patients drawn from the asthma clinical research network, childhood asthma management program, and childhood asthma research and education collections¹⁰; however, none of these associations was confirmed in 407 individuals from the Forest

Pharmaceuticals collection. Genome-wide evaluation revealed association of rs37973, a *GLCCI1* variant, in 118 patients with FEV₁ response at 8 weeks; this was confirmed in 3 additional collections (salmeterol or corticosteroids/salmeterol and inhaled corticosteroid, Leukotriene Modifier or Corticosteroid or Corticosteroid-Salmeterol Trial and childhood asthma research and education) in the same publication.⁸ This association was not observed in patients with asthma of European ancestry in a previous study from our group using a larger sample size of 1916 European ancestry patients¹³ drawn from the same 7 GSK clinical studies reported here. We have not confirmed any of the previously published genetic associations with ΔFEV_1 listed in Table E3 in 2672 patients with asthma, nor in the subset of 1787 patients of European ancestry analyzed here. Ten of the 15 previously published variants (see Table E3) have MAFs of 16% or more in 1000G (combined ancestries), and 13 of the 15 variants have MAFs of 16% or more in the 1000G European ancestry population. This study was well powered ($\geq 80\%$) to detect associations between many of these previously published variants and ΔFEV_1 in our full sample set ($n = 2672$) and in our European ancestry subset ($n = 1787$); however, we did not observe any such associations. We explored this lack of confirmation further using 4 previously published genetic associations^{4,8,11,28} that provided sufficient information (appropriate end point, mean, and SD for each genotype) to enable us to perform a detailed comparison with our results (Fig 3). In every instance, the magnitude of the effect sizes we observed was smaller than those previously reported and

failed to achieve even a nominal level of statistical significance in our data (all $P \geq .05$).

We considered potential explanations for our failure to replicate any previous ICS response pharmacogenetic findings. Discrepancy between our results and those of the previous candidate gene studies may be due to differences in age of onset of the populations evaluated. Discovery analyses for some previous studies^{6,8-10} included children (age ≥ 5 years), whereas our study only included subjects 12 years and older. Because asthma appears to be mediated, at least in part, through different genetic loci by age (a robustly replicated variant associated with childhood-onset asthma, at 17q21, is not associated with asthma in subjects whose age of onset is >16 years of age),²⁹ it is possible that response to asthma treatment is also mediated by different genetic loci in subjects of differing age of onset.⁹ In this study, we evaluated response to both the propionate and furoate salts of fluticasone. Most subjects ($n = 2187$ [81.8%]) were treated with FF, whereas only 485 (18.2%) subjects were treated with FP. We did not perform pharmacogenetic analyses separately for these 2 treatment groups, consistent with the approach taken by many groups evaluating pharmacogenetics of ICS response.^{10,11} Chemical characteristics, however, do differ between these 2 fluticasone salts; receptor-binding capacity of FF is greater than that of FP. Nuclear occupancy is longer for FF, allowing for once-daily compared with twice-daily dosing.³⁰ It is also possible that downstream molecular effects may subsequently differ between these 2 salts, possibly resulting in differential regulation of steroid response target genes. For example, differences in open chromatin or the number of steroid receptor target sites may result from the longer nuclear occupancy of FF.^{31,32} These sort of studies are outside the scope of this article.

We observed statistically significant differences at baseline between the 3 study groups (phase IIB, phase IIIA, and HZA106837) across many characteristics (Table I). To adjust for this, the data were not pooled at an individual level, but the effect sizes observed within each study group were subsequently meta-analyzed. The most statistically significant difference between study groups was observed when evaluating height ($P = 1.1 \times 10^{-13}$). Covariates used in the previously published studies summarized in Fig 3 include height, along with age, sex, and baseline FEV₁, and differ from the covariates we used, which were the first 10 genetic PCs, baseline FEV₁ percent predicted, age, sex, body mass index, asthma duration, and country. We subsequently included height as a covariate and repeated the association analyses; the 2 sets of results were highly correlated, demonstrating that the inclusion of height as a covariate does not alter our results (data not shown). Our method for estimating the genetic effect and SE from previous publications led to 2 instances (observed in the leukotriene modifier or corticosteroid salmeterol study with very small sample sizes) in which the reported P values and CI ranges seemed to disagree (Fig 3). Apart from small sample size, this may be because variance estimates for previously published results did not include the effects of covariates that were reported in the original statistical models.

Given our lack of statistically significant genetic associations with ICS response, we believe that further genome-wide studies with larger sample sizes are unlikely to reveal clinically useful genetic biomarkers predictive of ICS response. Other approaches may be considered to support the identification of predictive biomarkers. Some have been successfully used for other therapeutic agents. The rate of clinically significant exacerbations in

subjects treated with mepolizumab varied according to eosinophil count³³ and serum periostin levels may influence anti-IL-13 therapy.³⁴ A gene expression signature developed for T_H2 cell status in airway epithelial cells could also provide a marker for steroid response.³⁵ Multivariate modeling of asthma traits is also being applied in the form of hierarchical clinical cluster analysis^{36,37} to define subgroups of patients with specific asthma subphenotypes, which may correlate with treatment response in future analyses.

In summary, this report presents the largest steroid response study in patients with asthma to date. It is adequately powered to detect associations between common genetic variants and clinically relevant Δ FEV₁. Given that no statistically significant genetic associations were identified, we conclude that response to ICS treatment in patients with asthma cannot be attributed to large contributions from a limited number of common genetic variants nor to rare variants with large effect sizes, and that genetic variants are therefore unlikely to prove useful as predictive biomarkers of ICS response in patients with asthma.

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Clinical implications: Variability in ICS treatment response in patients with asthma cannot be attributed to large contributions from common genetic variants, nor to rare genetic variants with large effect sizes.

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METHODS

Estimation of power for rare variant analysis

The SKAT R package version 1.0.1 (<http://cran.r-project.org/web/packages/SKAT/>) was used to estimate the power of the rare variant analysis. The input parameters used were as follows:

- Sample size: 1,547.
- Number of tests: 15,450, which corresponds to 15,450 genes with at least 1 rare variant (MAF < 1%).
- Gene region in length: 3 kb.
- Percentage of causal genetic variants among rare variants: 20%.
- Maximum effect size (for variant with MAF = 0.0001): 0.6.
- Alpha: 3.24×10^{-6} (0.05/15,450).
- BetaType = "Log": beta is a function of effect sizes—"Log" indicates that effect sizes of causal variants equal to $c|\log_{10}(\text{MAF})|$.
- Power will be computed for each of the 100 causal single nucleotide polymorphism/gene region sets, and then the average power will be obtained by taking an average of the computed powers.
- Percentage of coefficients of causal variants that are negative: 0.

Under these assumptions, there was 0.3% power to detect an association between drug response and gene-based rare genetic variants with clinically meaningful response differences between genotypes.

Method for comparing GSK association results with published results

A direct comparison of these association results with those from 4 previously published reports was complex because in many cases the parameter estimate associated with the genetic variant and its SE was not provided in the publication. To obtain an estimate of the effect of a genetic

variant, beta, and the SE of the effect, on the basis of the response summary statistics (mean and SE) by genotype, we derived the following formulas, which assume ordinary least squares regression and an additive genetic model, and that the reported response statistics are unadjusted for covariates. An estimate for the regression coefficient, beta, is given by Equation 1:

$$\hat{\beta} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^n (x_i - \bar{x})^2} = \frac{n_0(x_0 - \bar{x})(\bar{y}_0 - \bar{y}) + n_1(x_1 - \bar{x})(\bar{y}_1 - \bar{y}) + n_2(x_2 - \bar{x})(\bar{y}_2 - \bar{y})}{n_0(x_0 - \bar{x})^2 + n_1(x_1 - \bar{x})^2 + n_2(x_2 - \bar{x})^2} \quad (1)$$

where n_0 , n_1 , and n_2 are the number of patients of genotype 0, 1, and 2, $n = n_0 + n_1 + n_2$, $x_0 = 0$, $x_1 = 1$, and $x_2 = 2$, $\bar{x} = \frac{(n_0x_0 + n_1x_1 + n_2x_2)}{n}$ and $\bar{y} = \frac{(n_0\bar{y}_0 + n_1\bar{y}_1 + n_2\bar{y}_2)}{n}$, and $\bar{y}_0$, $\bar{y}_1$, and $\bar{y}_2$ are the phenotypic means of subjects with genotypes 0, 1, and 2, respectively.

Equation 2 was used to derive an estimate of the SE of beta:

$$s_{\hat{\beta}} = \sqrt{\frac{\frac{1}{n-2} \sum_{i=1}^n \hat{\epsilon}_i^2}{\sum_{i=1}^n (x_i - \bar{x})^2}} \quad (2)$$

where $\sum_{i=1}^n \hat{\epsilon}_i^2 = (n_0 - 1)n_0 \text{SE}_0^2 + n_0(\bar{y}_0 - a - \hat{\beta}x_0)^2 + (n_1 - 1)n_1 \text{SE}_1^2 + n_1(\bar{y}_1 - a - \hat{\beta}x_1)^2 + (n_2 - 1)n_2 \text{SE}_2^2 + n_2(\bar{y}_2 - a - \hat{\beta}x_2)^2$, SE_0 , SE_1 , and SE_2 are the SEs of the phenotypic means of subjects with genotypes 0, 1, and 2, respectively, and a is the intercept, which is $\bar{y}_0$.

The beta and SE of beta of all single nucleotide polymorphisms estimated from the literature are directly compared with the GSK results using 95% CIs around the estimate of beta. Because these 4 previously published studies were all performed in patients of European ancestry, we performed this direct comparison with the European subset of our sample ($n = 1787$).

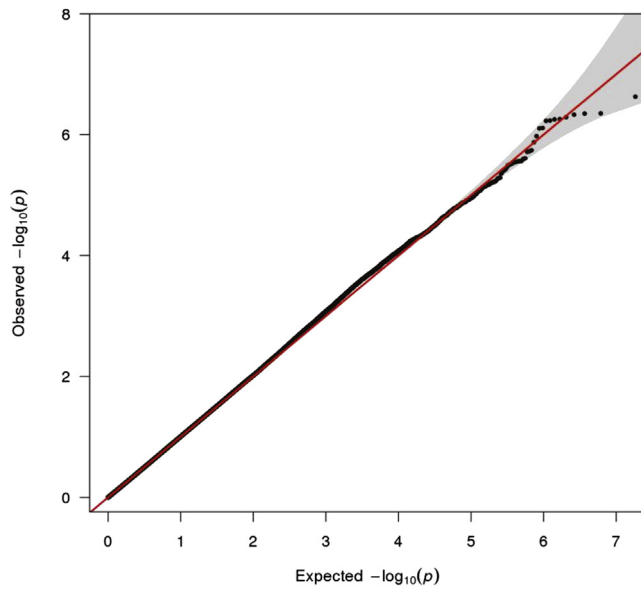


FIG E1. QQ plot for the association between ΔFEV_1 and common ($MAF \geq 1\%$) variants in 2672 patients with asthma. ΔFEV_1 , Change from baseline in trough FEV_1 .

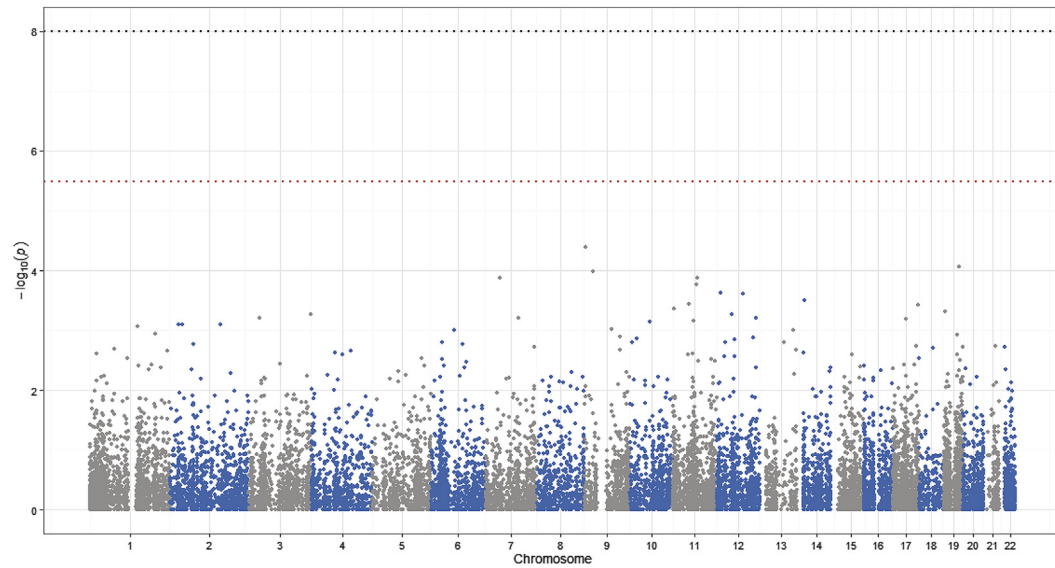


FIG E2. Manhattan plot summarizing association SKAT-O analysis between rare variants with ΔFEV_1 in 1547 patients with asthma. P value thresholds 1×10^{-8} (significance threshold for common variants) and 3.24×10^{-6} (significance threshold for rare variants) are indicated by the *black and red dashed lines*, respectively. ΔFEV_1 , Change from baseline in trough FEV_1 ; SKAT-O, sequence kernel association test.

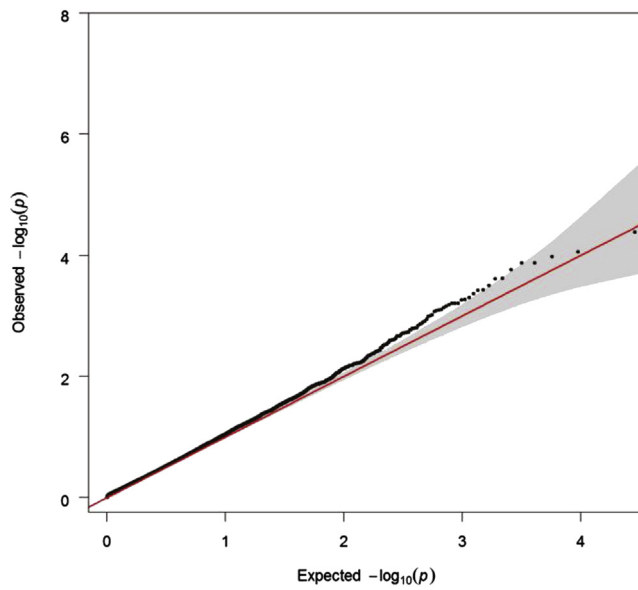


FIG E3. QQ plot for the association SKAT-O analysis between rare variants with ΔFEV_1 in 1547 patients with asthma. ΔFEV_1 , Change from baseline in trough FEV_1 ; SKAT-O, sequence kernel association test.

TABLE E1. Inclusion/exclusion criteria and ICS treatment response by study

Criterion	Clinical study						
	FFA109684	FFA109685	FFA109687	HZA106827	HZA106829	HZA106837	FFA112059
Inclusion							
Age (y)	≥12	≥12	≥12	≥12	≥12	≥12	≥12
Sex	M or eligible F	M or eligible F	M or eligible F	M or eligible F	M or eligible F	M or eligible F	M or eligible F
Asthma definition	NIH	NIH	NIH	NIH	NIH	NIH	NIH
Percent predicted FEV ₁	40%-90%	40%-90%	40%-90%	40%-90%	40%-90%	50%-90%	40%-90%
Asthma therapy before first visit	Using ICS for ≥8 wk before first visit and maintained a stable dose of ICS for 4 wk before visit 1 at various specified doses	Using ICS for ≥8 wk before first visit and maintained a stable dose of ICS for 4 wk before visit 1 at various specified doses	Noncorticosteroid controller or short-acting beta 2 agonist bronchodilators alone (with no ICS for ≥6 wk) for ≥3 mo before first visit	ICS for ≥12 wk before first visit	ICS for ≥12 wk before first visit	Currently using ICS therapy	ICS for ≥4 wk before first visit
Daily symptoms on 4+ of last 7 d before treatment initiation	1+ symptoms per day or rescue use	1+ symptoms per day or rescue use	1+ symptoms per day or rescue use	1+ symptoms per day or rescue use	3+ symptoms per day or rescue use	3+ days of rescue use	1+ symptoms per day or rescue use
Exacerbation before treatment initiation	NA	NA	NA	NA	NA	History of 1+ asthma exacerbations within the previous 12 mo	NA
Reversibility	12% and 200 mL reversibility of FEV ₁	12% and 200 mL reversibility of FEV ₁	12% and 200 mL reversibility of FEV ₁	12% and 200 mL reversibility of FEV ₁	12% and 200 mL reversibility of FEV ₁	12% and 200 mL reversibility of FEV ₁	12% and 200 mL reversibility of FEV ₁
Exclusion							
Life-threatening asthma episode requiring intubation or associated with hypercapnia, respiratory arrest, or hypoxic seizures	No specific time frame	No specific time frame	No specific time frame	Within the last 10 y	Within the last 10 y	Within the last 5 y	Within the last 10 y
Exacerbation before treatment initiation	OCS <3 mo or asthma hospitalization <6 mo	OCS <3 mo or asthma hospitalization <6 mo	OCS <3 mo or asthma hospitalization <6 mo	OCS <12 wk or asthma hospitalization <6 mo	OCS <12 wk or asthma hospitalization <6 mo	NA	OCS <6 mo or asthma hospitalization <6 mo
Smoking history	Active smoking <1 y or >10 pack-years	Active smoking <1 y or >10 pack-years	Active smoking <1 y or >10 pack-years	Active smoking <3 mo or >10 pack-years	Active smoking <3 mo or >10 pack-years	Active smoking <3 mo or >10 pack-years	Active smoking <3 mo or >10 pack-years
ICS treatment response							
Presented as LS mean ΔFEV ₁ (SE) in liters, by drug and dose	FF200 0.232 (0.037); FF400 0.229 (0.034); FF600 0.221 (0.0332); FF800 0.182 (0.0342); FP500 0.155 (0.0332)	FF100 0.142 (0.0403); FF200 0.173 (0.0404); FF300 0.228 (0.0402); FF400 0.215 (0.0414); FP250 0.160 (0.0409)	FF25 0.239 (0.0428); FF50 0.266 (0.0420); FF100 0.341 (0.0396); FF200 0.367 (0.0428); FP100 0.243 (0.0411)	FF100 0.332 (0.0302)	FF200 0.201 (0.0303); FP500 0.183 (0.03)	FF100 0.265 (0.014)	FF100 0.161 (0.040); FP250 0.159 (0.046)

ΔFEV₁, Change from baseline in trough FEV₁; F, female; LS, least squares; M, male; NA, not available; NIH, National Institutes of Health, 2007; OCS, oral corticosteroid.

TABLE E2. Analysis *P* value results for association of candidate gene variants with ΔFEV_1 in 2672 patients with asthma

RS no.	Gene	Reference allele	Nonreference allele	<i>P</i> value for ΔFEV_1
rs37972	<i>GLCCII</i>	C	T	.357
rs37973	<i>GLCCII</i>	A	G	.401
rs37976	<i>GLCCII</i>	T	C	.918
rs7792118	<i>GLCCII</i>	A	G	.737
rs715377	<i>GLCCII</i>	A	G	.610
rs12537880	<i>GLCCII</i>	A	G	.407
rs7800750	<i>GLCCII</i>	T	C	.542
rs37990	<i>GLCCII</i>	C	A	.186
rs38010	<i>GLCCII</i>	C	T	.424
rs2041362	<i>GLCCII</i>	G	T	.287
rs17141876	<i>GLCCII</i>	C	T	.747
rs1476823	<i>GLCCII</i>	T	C	.751
rs10229643	<i>GLCCII</i>	T	A	.452
rs13239966	<i>GLCCII</i>	C	T	.557
rs10243846	<i>GLCCII</i>	G	A	.447
rs13245462	<i>GLCCII</i>	C	T	.412
rs10435183	<i>GLCCII</i>	G	A	.866
rs7801924	<i>GLCCII</i>	G	A	.090
rs4725061	<i>GLCCII</i>	A	G	.441
rs10226865	<i>GLCCII</i>	A	G	.814
rs13222222	<i>GLCCII</i>	A	C	.346
rs6969971	<i>GLCCII</i>	T	C	.631
rs9648623	<i>GLCCII</i>	G	A	.255
rs13242904	<i>GLCCII</i>	C	T	.239
rs11978165	<i>GLCCII</i>	A	G	.262
rs6463755	<i>GLCCII</i>	T	C	.196
rs16872488	<i>GLCCII</i>	A	C	.824
rs2191318	<i>GLCCII</i>	T	C	.027
rs11976862	<i>GLCCII</i>	A	G	.062
rs4236406	<i>GLCCII</i>	T	C	.047
rs17142716	<i>GLCCII</i>	A	G	.679
rs17142727	<i>GLCCII</i>	A	G	.013
rs10253260	<i>GLCCII</i>	A	G	.122
rs10952084	<i>GLCCII</i>	C	A	.382
rs6964558	<i>GLCCII</i>	G	A	.308
rs6956039	<i>GLCCII</i>	T	G	.848
rs1468594	<i>GLCCII</i>	C	A	.155
rs10257285	<i>GLCCII</i>	T	C	.147
rs12668352	<i>GLCCII</i>	C	A	.340
rs929509	<i>GLCCII</i>	T	C	.124
rs4725068	<i>GLCCII</i>	G	A	.031
rs6967565	<i>GLCCII</i>	G	A	.510
rs3751123	<i>STIP1</i>	G	A	.593
rs3751122	<i>STIP1</i>	G	T	.341
rs2701528	<i>STIP1</i>	A	G	.428
rs4980524	<i>STIP1</i>	A	C	.356
rs6591838	<i>STIP1</i>	A	G	.320
rs2282490	<i>STIP1</i>	T	C	.833
rs11607165	<i>STIP1</i>	T	G	.843
rs2236647	<i>STIP1</i>	T	C	.701
rs2236648	<i>STIP1</i>	C	T	.384
rs2070232	<i>STIP1</i>	G	A	.714
rs1011219	<i>STIP1</i>	G	A	.426
rs894685	—	A	G	.248
rs9892359	<i>CRHR1</i>	A	G	.525
rs4792886	<i>CRHR1</i>	G	A	.208
rs4792887	<i>CRHR1</i>	C	T	.167
rs110402	<i>CRHR1</i>	A	G	.449
rs12103667	<i>CRHR1</i>	A	G	.423
rs3785877	<i>CRHR1</i>	G	A	.849

(Continued)

TABLE E2. (Continued)

RS no.	Gene	Reference allele	Nonreference allele	<i>P</i> value for ΔFEV_1
rs242941	<i>CRHR1</i>	C	A	.420
rs171440	<i>CRHR1</i>	A	G	.321
rs16940655	<i>CRHR1</i>	C	T	.062
rs242939	<i>CRHR1</i>	T	C	.142
rs12150390	<i>CRHR1</i>	T	C	.881
rs242936	<i>CRHR1</i>	G	A	.522
rs1912151	<i>CRHR1</i>	C	T	.881
rs1396862	<i>CRHR1</i>	G	A	.881
rs17763086	<i>CRHR1</i>	T	G	.895
rs75638861	<i>CRHR1</i>	G	A	.770
rs16940665	<i>CRHR1</i>	T	C	.895
rs17689966	<i>CRHR1</i>	A	G	.403
rs16940674	<i>CRHR1</i>	C	T	.895
rs1876828	<i>CRHR1</i>	C	T	.895
rs878886	<i>CRHR1</i>	C	G	.881
rs878887	<i>CRHR1</i>	C	T	.881
rs878888	<i>CRHR1</i>	A	G	.864
rs4525537	<i>CRHR1</i>	T	C	.881
rs4640231	<i>CRHR1</i>	G	C	.881
rs16940686	<i>CRHR1</i>	G	T	.883
rs1800547	<i>MAPT</i>	A	G	.916
rs9468	<i>MAPT</i>	T	C	.858
rs1052587	<i>MAPT</i>	T	C	.874
rs1528072	<i>KIAA1267</i>	C	A	.922
rs2532292	<i>KIAA1267</i>	A	T	.908
rs758391	—	G	A	.748

 ΔFEV_1 , Change from baseline in trough FEV₁; RS, reference single nucleotide polymorphism cluster ID.

TABLE E3. Previously reported genetic associations with ICS response in European patients

Gene	Summary of discovery results						Summary of subsequent results			
	Reference	Population	Number in analysis	Approach	Genetic variant	Association end point	Reference	Population	Number in analysis	Reported as confirmed
GLCCI1	8	CAMP	118	GWAS	rs37973	FEV ₁ response at 8 wk	8	SOCS/SLIC	264	Yes
							8	Adult	385	No
							8	LOCCS	185	Yes
							8	CARE	101	Yes
							13	Adult	1916	No
FBXL7	9	CAMP	124	GWAS	rs10044254	Symptom scores	9	CARE	77	Yes
							9	ACRN	110	No
							9	LOCCS	110	No
T	10	ACRN, CAMP/CARE	418	GWAS	rs3127412	Difference in FEV ₁ raw scores*	10	Forest	407	No
					rs6456042				407	No
					rs1134481				407	No
					rs2305089				407	No
					rs3099266				407	No
CRHR1	11	Adult, CAMP	781	Candidate gene	rs242941	Change in FEV ₁ at 6-8 wk	12	ACRN	336	No
					Haplotype containing rs1876828		26	LOCCS	64	No
					Change in FEV ₁ at 6-8 wk		12	ACRN	336	Yes†
					Change in FEV ₁ at 6-8 wk		26	LOCCS	64	Yes
MAPT	12	ACRN	224	Candidate gene	rs1800547	Change in FEV ₁ at 6-8 wk	12	CAMP	201	No
					rs9468		12	CAMP	201	No
					Change in FEV ₁ at 6-8 wk					
STIP1	4	Adult	382	Candidate gene	rs2236647	FEV ₁ response at 8 wk		No subsequent follow-up		
					rs1011219	FEV ₁ response at 8 wk		No subsequent follow-up		
TBX21	6	CAMP	194	Candidate gene	rs2240017	PC ₂₀ ‡		No subsequent follow-up		
TBX21	5	Adult	208	Candidate gene	rs9910408	FEV ₁ in nonsmoking and nonatopic subgroups		No subsequent follow-up		

ACRN, Asthma clinical research network; CAMP, childhood asthma management program; CARE, childhood asthma research and education; LOCCS, Leukotriene Modifier or Corticosteroid or Corticosteroid-Salmeterol Trial; SLIC, salmeterol and inhaled corticosteroid; SOCS, salmeterol or corticosteroids.

*preFEV_{on ICS} – preFEV_{off ICS}.

†Change in FEV₁ at 6 wk.

‡20% decrement in FEV₁.