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 FEV_1 (ΔFEV_1) from baseline to 8 to 12 weeks of treatment.
Results: More than 9.8 million common genetic variants (minor allele frequency ≥ 1%) were analyzed to test for association with ΔFEV_1. 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 (ΔFEV_1) 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 2017;139:797-803.)

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

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

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, 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 *STIP1*,[4] *TBX21*,[5,6] and *WDR21A*,[7] there are no published attempts to replicate the original observations. For those studies implicating *GLCCI1*,[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 ΔFEV_1 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 (μg)	25, 50, 100, 200, 300, 400, 600, 800	100, 200	100	—
FP patient numbers	230	255	0	—
FP doses (μg)	50, 250, 500	250, 500	NA	—
Age (y)	42.1 ± 16.8	44.2 ± 15.6	42.7 ± 14.7	3.0×10^{-2}
Age of asthma onset (y)	24.5 ± 16.8	29.2 ± 18.2	26.9 ± 17.7	5.1×10^{-7}
Asthma duration (y)	17.7 ± 13.4	14.9 ± 13.0	15.7 ± 11.8	2.9×10^{-5}
FEV_1 at baseline (L)	2.3 ± 0.7	2.2 ± 0.8	2.2 ± 0.6	2.2×10^{-2}
FEV_1 at baseline (% predicted)	69.9 ± 10.1	69.2 ± 10.4	71.7 ± 11.8	5.1×10^{-5}
FEV_1 % reversibility at baseline	28.4 ± 16.8	29.0 ± 15.6	24.5 ± 14.7	3.4×10^{-10}
Sex: % female	61.2	58.6	68.6	8.6×10^{-5}
BMI (kg/m^2)	27.9 ± 6.7	27.9 ± 5.2	27.6 ± 5.9	5.5×10^{-1}
Height (cm)	167.9 ± 13.4	165.9 ± 7.8	163.9 ± 8.8	1.1×10^{-13}
FEV_1 change (% predicted)	7.2 ± 13.4	6.9 ± 13.0	6.1 ± 11.8	1.0×10^{-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 proportions[17] ($P < 1 \times 10^{-7}$ 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 $[\hat{r}^2] > 0.3$ and minor allele frequency [MAF] ≥ 1%), there were 9,882,836 variants for analysis. For phase IIIA studies, incorporating the imputed variants (with $\hat{r}^2 \geq 0.3$ and MAF ≥ 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).