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ORIGINAL ARTICLE

Genome-wide association study of leukotriene modifier response in asthma

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Heterogeneous therapeutic responses to leukotriene modifiers (LTMs) are likely due to variation in patient genetics. Although prior candidate gene studies implicated multiple pharmacogenetic loci, to date, no genome-wide association study (GWAS) of LTM response was reported. In this study, DNA and phenotypic information from two placebo-controlled trials (total $N = 526$) of zileuton response were interrogated. Using a gene–environment (G × E) GWAS model, we evaluated 12-week change in forced expiratory volume in 1 second (ΔFEV_1) following LTM treatment. The top 50 single-nucleotide polymorphism associations were replicated in an independent zileuton treatment cohort, and two additional cohorts of montelukast response. In a combined analysis (discovery +replication), rs12436663 in *MRPP3* achieved genome-wide significance ($P = 6.28 \times 10^{-08}$); homozygous rs12436663 carriers showed a significant reduction in mean ΔFEV_1 following zileuton treatment. In addition, rs517020 in *GLT1D1* was associated with worsening responses to both montelukast and zileuton (combined $P = 1.25 \times 10^{-07}$). These findings implicate previously unreported loci in determining therapeutic responsiveness to LTMs.

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INTRODUCTION

The leukotriene pathway is an important therapeutic target for asthma therapy. Leukotriene-modifying agents (LTMs) improve asthma by either selectively preventing leukotriene production from arachidonic acid via 5-lipoxygenase (5-LO) inhibition (e.g., zileuton), or by preventing leukotrienes from binding to the major cysteinyl leukotriene receptor, CysLT1 (e.g., montelukast, pranlukast and zarfirlukast).[1–3] LTMs are effective in improving lung function, asthma symptoms and quality of life in patients with asthma and allergic rhinitis by reducing airway hyperresponsiveness and eosinophilia.[4–6]

Despite their general efficacy, patient responses to LTMs are not consistent, with some individuals failing to respond to treatment.[7–9] The presence of significant inter-individual variability in the treatment response to LTMs suggests that variation in patient genetics may underlie this phenomenon.[7–11] Historically, variable response to leukotriene inhibition formed the basis for the first reported asthma pharmacogenetic study.[7] In an effort to characterize this pharmacogenetic relationship, researchers initially focused on candidate genes within pharmacological pathways for LTM response, including arachidonate 5-lipoxygenase (*ALOX5)*, the gene encoding 5-LO, in addition to ATP-binding cassette, subfamily C *(CFTR/MRP),* member 1 (*ABCC1)*,[9,11] cysteinyl leukotriene receptor 1 *(CYSLTR1)*,[9,11,12] cysteinyl leukotriene receptor 2 (*CYSLTR2)*,[9,11,12] leukotriene A4 hydrolase (*LTA4H)*,[9–12] leukotriene C4 synthase (*LTC4S)*[9–12] and others. Studies of *ALOX5* have identified multiple single-nucleotide polymorphism (SNP) associations with symptomatic improvement and changes in measures of lung function during LTM therapy.[7,9–12] Two studies of the *LTC4S* gene identified promoter and intronic SNPs associated with improved response to LTMs; in addition, regulatory variants in *CYSLT2* (but not *CYSLT1*) that were significantly associated with increased morning peak expiratory flow in patients taking montelukast were identified.[9–12] SNPs associated with LTM plasma levels and differential responsiveness to LTMs have also been reported for solute carrier organic anion transporter family, member 2B1 (*SLCO2B1)* and *ABCC1*.[9,10,13,14] Importantly, we have previously demonstrated that variants in multiple genes contribute to the response of both leukotriene inhibitors (e.g., zileuton) and leukotriene receptor antagonists (e.g., montelukast).[11]

Although the initial results of these candidate gene studies are promising, replication of identified associations has been problematic, making it difficult to estimate the degree of contribution of individual genes to LTM response. By necessity, candidate gene studies focus on characterizing the genotype–phenotype relationships within individual genes that are chosen *a priori* based on biological and clinical information, rather than an agnostic approach that considers data from the entire genome. Recent genome-wide association studies (GWASs) of symptomatic response to asthma medications have identified multiple genes associated with patient responsiveness to inhaled corticosteroids, adding to the growing evidence for a genetic basis for therapeutic efficacy in asthma patients.[15–17] Limited information from candidate gene studies of zileuton and montelukast response indicates that patient genetics has a role in LTM response; however, to date, no genome-wide investigation of LTM response has been reported. For these reasons, we performed a GWAS to identify novel genetic loci associated with leukotriene modifier response in asthmatic patients.

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MATERIALS AND METHODS

Overview of study populations

The discovery and replication cohorts evaluated in this study consisted of 526 adult patients with moderate, persistent asthma from two independent, placebo-controlled clinical trials (conducted by Abbott, Chicago, IL, USA)[18,19] evaluating the efficacy of a controlled-release (CR) formulation of zileuton. The primary GWAS cohort ('Abbott trial 1') included 160 patients randomized to receive zileuton and 144 patients randomized to receive placebo for 12 weeks (details of the trial are provided in Nelson *et al.*[18]). Replication of initial GWAS results was performed in a cohort of 149 patients randomized to receive zileuton and 73 patients receiving placebo for 12 weeks ('Abbott trial 2'), as adjunctive therapy to usual care (details of the trial are provided in Wenzel *et al.*[19]). To evaluate potential overlap between genetic predisposition to zileuton and montelukast responses, additional replications were performed using two clinical trials of montelukast response, which were previously described,[20,21] the American Lung Association Asthma Clinical Research Center (ALA-ACRC) trials: the Leukotriene Modifier Or Corticosteroid or Corticosteroid-Salmeterol trial (LOCCS) and Effectiveness of Low Dose Theophylline as Add On Therapy for the Treatment of Asthma (LODO). All studies were approved by the institutional review boards of their corresponding institutions, and all participants provided informed consent for genetic studies. Demographic characteristics of the cohorts evaluated in this study are shown in Table 1. Each cohort is described in detail below.

Zileuton populations (Abbott)

Full study design details of the two Abbott clinical trials evaluated in this study have been reported elsewhere.[18,19] In both cohorts, 'moderate asthma' was defined by the following criteria: (i) forced expiratory volume in 1 s (FEV_1) measurement of 40–75% of predicted FEV_1 when taken at least 48 h after the last theophylline use, at least 12 h after a long-acting β-agonist and at least 6 h after a short-acting β-agonist, (ii) $\geqslant$15% increase in FEV_1 at least 15 min after inhaled albuterol, and (iii) a history of 15% reversibility within the year before study enrollment.

The first Abbott cohort, termed 'Abbott trial 1' in this investigation, was a phase 3, randomized, placebo-controlled, multi-center study evaluating response to treatment with zileuton CR (1200 mg twice daily) vs zileuton immediate release (600 mg four times daily).[18] The study population was composed of 786 nonsmoking males and females, aged 12 years and older, who had mild to moderate asthma. Patients who had been hospitalized for asthma within 6 months, or who had taken any investigational drugs, excepting β-agonist inhaler usage, were excluded. The study population included both white (81.4%) and non-white (18.6%) individuals. Following a 2-week-placebo lead-in period, patients with an FEV_1 of 40–75% of predicted who used a β-agonist inhaler at least twice per day and showed an improvement of $\leqslant$10% over baseline FEV_1 were randomized to zileuton or placebo (1:1 ratio) and evaluated over a 12-week period. In this study, only data from the first 12 weeks following randomization to zileuton or placebo were evaluated. Although the clinical trial evaluated 786 patients, complete genome-wide genotype and phenotype data were only available from 160 patients who received zileuton, and 144 patients who received placebo; these patients represented the discovery GWAS population evaluated in this study.

The second Abbott cohort, termed 'Abbott trial 2' in this investigation, was a phase 3, double-blind, randomized, placebo-controlled, multi-center study to evaluate the long-term safety and efficacy of add-on zileuton CR (1200 mg twice daily), or placebo, in addition to usual care (that is, the usual treatment regimens these patients received while they were being treated by their primary care providers). For all patients, this could include treatment regimens including, but not limited to, theophylline, over 6 months.[19] The study population included 926 nonsmoking males and females, aged 12 years and older, who had not taken any investigational drugs and who demonstrated an FEV_1 of at least 40% of predicted when taken 48 h or more after the last theophylline administration and at least 12 h following long-acting β-agonist use and at least 6 h following short-acting β-agonist use, and demonstrated a 15% or greater increase in FEV_1 following inhaled albuterol, or reported a history of 15% reversibility within 1 year before entering into the study.[19] Patients were randomized to zileuton CR plus usual care, or placebo plus usual care, in a 2:1 ratio, and were evaluated over 6 months. Both white (85.7%) and non-white (14.3%) subjects were evaluated in this study. Although 926 subjects were included in the clinical population, for the GWAS, complete genome-wide genotype data were available from 149 patients randomized to receive zileuton and 73 patients receiving placebo. In this study, data from the first 12 weeks following randomization to zileuton or placebo were investigated. This population was used to replicate the results from the discovery GWAS.

Although both primary and replication cohorts contained comparable numbers of white subjects, and had patients of similar mean age, the discovery cohort included a greater proportion of females (48% for Abbott trial 1 vs 32.4% for Abbott trial 2). In addition, the discovery cohort patients showed a modestly greater mean % change in FEV_1 following 12-week zileuton administration (Table 1). Although the cohorts had high percentages of white subjects, the inclusion of non-white patients may have introduced population stratification. However, the genomic inflation factor values for these cohorts suggested that minimal stratification was present, and the quantile-quantile (QQ) plots of both GWAS were well behaved (Figure 1).

Montelukast populations (LOCCS and LODO)

The clinical outcomes and results have been reported elsewhere for the LOCCS and LODO trials.[20,21] LOCCS evaluated participants aged 15 years and older with physician-diagnosed asthma, who had been prescribed daily asthma medications for at least 1 year, had an FEV_1 of 50% or more of predicted values and had poor asthma control as defined by a score of 1.5 or greater on the Asthma Control Questionnaire.[20,21] One hundred and sixty-six patients taking montelukast (5 or 10 mg daily) were evaluated over 16 weeks, with time to treatment failure as the primary phenotype outcome. Genotype and phenotype data from 210 patients, of which 69 patients were taking montelukast, were available for additional replication of the zileuton GWAS findings.

The LODO trial evaluated patients aged 6 years and older with physician-diagnosed asthma, FEV_1 of 60% or greater than predicted, evidence of airway reversibility (defined as 12% or greater β-agonist reversibility using up to four puffs of albuterol within 2 years before enrollment or PC_{20} FEV_1 methacholine (provocative concentration of methacholine producing a 20% fall in FEV_1) of 8 mg ml^{-1} or less within 2 years of enrollment) and poor asthma control as defined by a score of 1.5 or greater on the Asthma Control Questionnaire.[20,21] The data evaluated from LODO included complete genotype and phenotype information from 122 patients, of which 64 patients taking montelukast were available for interrogation as an additional replication population.

This study evaluated data from LOCCS and LODO for the first 12 weeks following randomization to montelukast. Both study populations were used to replicate the results of the zileuton GWAS. The characteristics of

Table 1. Characteristics of study populations evaluated by GWAS of LTM response in asthmatics

	Abbott trial 1	*Abbott trial 2*	*LOCCS*	*LODO*
Total sample size available	304	222	210	122
Number of patients taking zileuton (Abbott) or montelukast (LOCCS, LODO), *N* (%)	160 (52.6)	149 (67.1)	69 (32.9)	64 (52.4)
Age, mean (s.d.) (yrs.)	34.3 (12.6)	35.8 (13.6)	35.2 (14.9)	40 (15.0)
Male, *N* (%)	146 (48.0)	72 (32.4)	94 (44.8)	43 (35.2)
Caucasian, *N* (%)	259 (85.2)	187 (84.2)	162 (77.1)	108 (88.5)
FEV_1 change, mean (s.d.) (%)	13.5 (25.9)	12.5 (45.0)	13.9 (13.2)	10.1 (12.4)

Abbreviations: GWAS, genome-wide association study; FEV_1, forced expiratory volume in 1 s; LOCCS, Leukotriene Modifier Or Corticosteroid or Corticosteroid-Salmeterol trial; LODO, Effectiveness of Low Dose Theophylline as Add On Therapy for the Treatment of Asthma; *N*, sample size.

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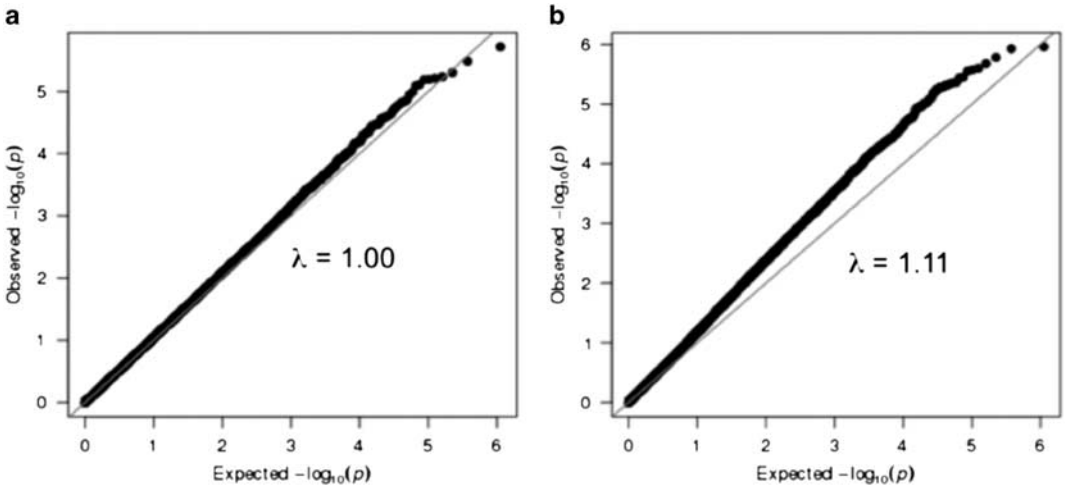

Figure 1. Quantile–quantile (Q–Q) plots of Abbott populations. Q–Q plots show results for Abbott trial 1 (discovery) (**a**) and Abbott trial 2 (replication) (**b**). Lambda (λ) indicates the genomic inflation factor values.

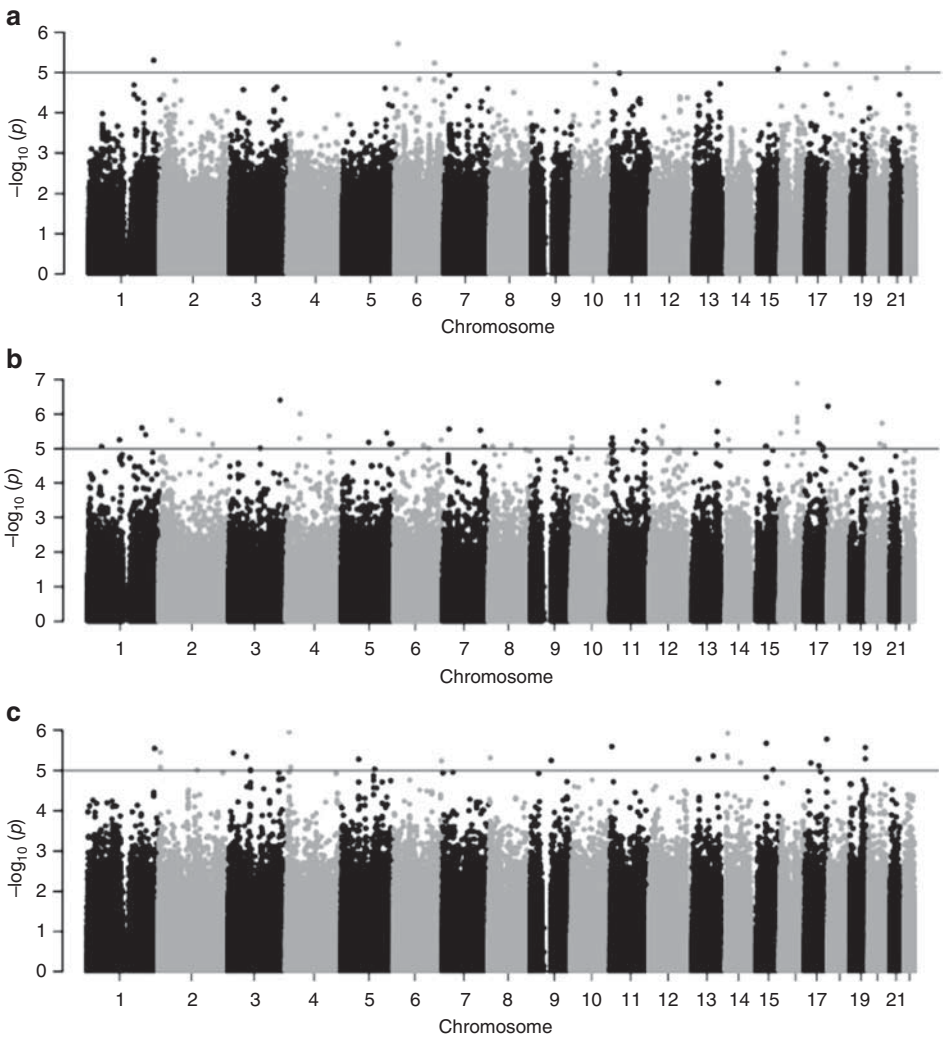

Figure 2. Results of the GWAS of zileuton response. Manhattan plots show GWAS results for the discovery cohort (Abbott trial 1) for additive (**a**) dominant (**b**) and recessive (**c**) genetic models. For each plot, the *y* axis represents $-\log_{10}$(*P*-values) for each SNP (gray and black dots), grouped by chromosomes 1–22 (*x* axis). Horizontal lines in the plots indicate the threshold for suggestive genome-wide significance ($P = 1 \times 10^{-05}$).

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Table 2. Replicated SNP associations from GWAS of zileuton response

SNP	*Chr.*	*SNP position*	*Gene symbol*	*Genetic model*	*Abbott trial 1*		*Abbott trial 2*		*Combined P-value*[a]
					β (L)	*P-value*	*β (L)*	*P-value*	
rs3812141	6	84290863	*PRSS35*	A	−0.50	1.47X10^{-05}	−0.28	2.82X10^{-02}	8.19X10^{-07}
rs517020	12	128028656	*GLT1D1*	A	−0.43	4.25X10^{-05}	−0.32	1.00X10^{-02}	7.03X10^{-07}
rs1872085	17	69039675	*SDK2*	R	0.69	4.64X10^{-05}	0.37	3.02X10^{-02}	2.46X10^{-06}
rs6929584	6	70439591		D	0.66	6.55X10^{-05}	0.40	4.61X10^{-02}	5.41X10^{-06}
rs6929584	6	70439591		A	0.59	7.23X10^{-05}	0.40	4.61X10^{-02}	5.90X10^{-06}
rs281508	2	46264711	*PRKCE*	R	0.77	7.28X10^{-05}	0.52	1.07X10^{-02}	1.24X10^{-06}
rs2193412	2	50990306	*NRXN1*	R	0.60	7.94X10^{-05}	0.36	3.07X10^{-02}	4.07X10^{-06}
rs2720499	8	17436739	*SLC7A2*	R	−2.42	8.29X10^{-05}	−1.41	2.07X10^{-03}	2.94X10^{-07}
rs11812286	10	60743620		R	−2.27	9.47X10^{-05}	−1.28	1.74X10^{-02}	2.62X10^{-06}
rs3093009	6	167469467	*CCR6*	A	−0.38	9.85X10^{-05}	−0.35	2.51X10^{-03}	4.17X10^{-07}
rs3812141	6	84290863	*PRSS35*	D	−0.47	1.03X10^{-04}	−0.34	1.77X10^{-02}	2.87X10^{-06}
rs6790709	3	29022448		A	−0.36	1.05X10^{-04}	−0.23	2.05X10^{-02}	3.41X10^{-06}
rs7976838	12	58372473	*SLC16A7*	R	−2.32	1.56X10^{-04}	−1.83	1.23X10^{-03}	3.53X10^{-07}
rs7777754	7	50633527	*GRB10*	R	−0.76	1.91X10^{-04}	−0.58	2.23X10^{-02}	6.47X10^{-06}
rs16862610	3	151535497		D	−0.56	1.98X10^{-04}	−0.37	3.82X10^{-02}	1.18X10^{-05}
rs281499	2	46272110		R	0.54	1.99X10^{-04}	0.47	3.37X10^{-03}	1.10X10^{-06}
rs12991650	2	211549073		A	−0.41	2.29X10^{-04}	−0.29	7.97X10^{-03}	2.81X10^{-06}
rs7543083	1	46803927	*MKNK1*	R	0.61	2.31X10^{-04}	0.42	4.30X10^{-02}	1.54X10^{-05}
rs2971886	2	54704804	*SPTBN1*	D	0.46	2.45X10^{-04}	0.31	3.65X10^{-02}	1.36X10^{-05}
rs2899832	14	34810692	*MRPP3*	R	−1.47	2.61X10^{-04}	−0.91	1.45X10^{-02}	5.63X10^{-06}
rs1910505	11	11065815		A	0.30	2.86X10^{-04}	0.29	8.70X10^{-03}	3.78X10^{-06}
rs4689355	4	6228036	*JAKMIP1*	R	0.61	2.96X10^{-04}	0.54	7.67X10^{-04}	4.64X10^{-07}
rs9952762	18	71530638		D	0.64	3.31X10^{-04}	1.00	7.58X10^{-04}	5.17X10^{-07}
rs8053211	16	83011254	*ATP2C2*	A	−0.30	3.34X10^{-04}	−0.18	3.77X10^{-02}	1.87X10^{-05}
rs4689318	4	6069792		R	−0.74	3.68X10^{-04}	−0.67	1.24X10^{-02}	6.78X10^{-06}
rs7532095	1	232796266		R	1.02	3.93X10^{-04}	0.56	4.63X10^{-02}	2.70X10^{-05}
rs517020	12	128028656	*GLT1D1*	D	−0.43	3.94X10^{-04}	−0.32	2.65X10^{-02}	1.52X10^{-05}
rs1419391	7	126711989		D	0.43	4.75X10^{-04}	0.35	1.89X10^{-02}	1.30X10^{-05}
rs14138	2	46267950	*PRKCE*	R	1.15	4.80X10^{-04}	0.77	6.80X10^{-03}	5.03X10^{-06}
rs9842582	3	5402679		R	−2.14	4.83X10^{-04}	−1.41	1.40X10^{-02}	9.86X10^{-06}
rs208546	7	25587086		D	0.40	4.87X10^{-04}	0.27	4.23X10^{-02}	2.98X10^{-05}
rs12436663	**14**	**34708871**	***MRPP3***	**R**	**−1.54**	**4.87X10^{-04}**	**−2.09**	**2.85X10^{-05}**	**6.28X10^{-08}**
rs9462164	6	36311688		A	−0.33	4.93X10^{-04}	−0.25	2.22X10^{-02}	1.57X10^{-05}
rs917063	14	70717825		D	−0.42	4.98X10^{-04}	−0.41	3.33X10^{-03}	2.77X10^{-06}
rs9952762	18	71530638		A	0.60	5.20X10^{-04}	1.00	7.58X10^{-04}	8.39X10^{-07}
rs2722964	7	82896763	*SEMA3E*	D	−0.40	5.42X10^{-04}	−0.32	2.01X10^{-02}	1.56X10^{-05}
rs2247921	7	82899314	*SEMA3E*	D	−0.40	5.42X10^{-04}	−0.30	2.94X10^{-02}	2.27X10^{-05}
rs7741397	6	166169058		D	−0.40	5.90X10^{-04}	−0.33	1.30X10^{-02}	1.12X10^{-05}
rs2832438	21	30059808	*GRIK1*	R	−1.18	5.98X10^{-04}	−1.18	3.92X10^{-02}	3.33X10^{-05}
rs2237807	7	126654886	*GRM8*	A	−0.30	5.99X10^{-04}	−0.19	4.96X10^{-02}	4.25X10^{-05}
rs13008940	2	196082797		R	−1.00	6.48X10^{-04}	−0.56	3.25X10^{-02}	2.96X10^{-05}
rs917063	14	70717825		A	−0.34	6.76X10^{-04}	−0.39	1.05X10^{-03}	1.45X10^{-06}
rs11654033	17	8256374		D	0.41	7.13X10^{-04}	0.35	1.85X10^{-02}	1.87X10^{-05}
rs2741335	8	27393708	*CHRNA2*	A	−0.28	7.23X10^{-04}	−0.27	7.29X10^{-03}	8.07X10^{-06}
rs243224	14	89023803	*FOXN3*	D	−0.40	7.25X10^{-04}	−0.30	2.78X10^{-02}	2.82X10^{-05}
rs7696471	4	90345932		R	−1.91	7.50X10^{-04}	−0.67	4.66X10^{-02}	4.89X10^{-05}
rs1432145	18	37698512		D	−0.43	7.80X10^{-04}	−0.35	2.16X10^{-02}	2.37X10^{-05}
rs10928465	2	134353582		D	−0.40	8.09X10^{-04}	−0.30	3.50X10^{-02}	3.94X10^{-05}
rs10935952	3	155007998		A	0.28	8.22X10^{-04}	0.22	2.63X10^{-02}	3.02X10^{-05}

Abbreviations: Chr, chromosome; Chr pos, physical position (base pairs); SNP, single-nucleotide polymorphism. Genetic model refers to genetic association model, where A = additive, D = dominant and R = recessive. *β* refers to effect size (L). Bolded text indicates the SNP with the lowest combined *P*-value.
[a]Combined *P*-values for Abbott trial 1 and Abbott trial 2.

LOCCS and LODO were comparable to each other, and to Abbott, although LODO demonstrated a lower % mean change in FEV_1 as compared with the other cohorts (Table 1).

Phenotyping

For the zileuton discovery and replication GWAS, we evaluated the difference between the FEV_1 during the placebo run-in period at baseline (randomization) and the FEV_1 at 12 weeks on zileuton, as the primary outcome phenotype.[18,19] Similarly, for the LOCCS and LODO studies, we also evaluated 12-week ΔFEV_1 from baseline (randomization) relative to montelukast administration. For both zileuton cohorts, the mean (± s.d.) FEV_1% difference was similar (13.5 ±25.9 for Abbott trial 1 and 12.5 ± 45.0 for Abbott trial 2) (Table 1).

Genotyping

Genome-wide genotyping of the Abbott patients was conducted using the Illumina HumanHap550 chip (Illumina, San Diego, CA, USA). LOCCS and LODO patients were genotyped using the Infinium HD Human610-Quad BeadChip (Illumina). The software PLINK v.1.07 [ref. 22] was used for quality control (QC) and statistical analysis of genotype data. For QC, SNPs with a study-wise missing data proportion above 0.05 were removed from the analysis. SNPs failing to meet Hardy–Weinberg equilibrium ($P < 0.0001$), in addition to SNPs with >10% missing genotypes were also dropped from the analysis. A total of 542 562 SNPs from the Abbott trials passed QC and were included in the analysis. For LOCCS and LODO, a total of 545 934 SNPs passed QC. To assess population stratification in the primary analysis, the *P*-values were adjusted for genomic control using PLINK.

Statistical analysis

The zileuton GWAS evaluated 542 562 SNPs in a discovery cohort of 304 patients, of which 160 patients received zileuton and 144 patients received placebo (Abbott trial 1). The association of SNP genotypes with changes in FEV_1 related to zileuton or montelukast administration was determined using linear regression (PLINK v.1.0.7). Regression models were adjusted for baseline FEV_1, age, race and gender. To enrich for SNP associations related

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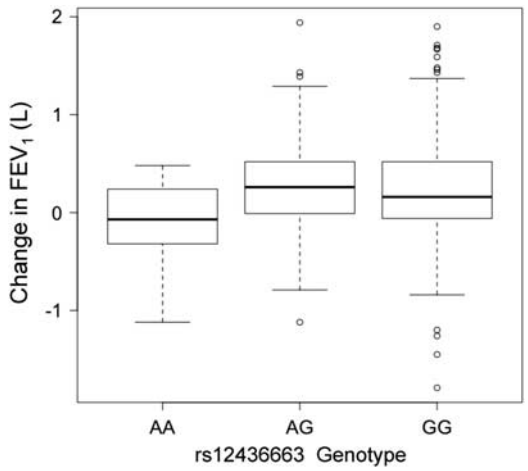

Figure 3. rs12436663 genotype-dependent change in lung function. The boxplots show ΔFEV_1 (L) grouped by rs12436663 genotype: variant genotype ('AA' ($N = 11$ patients), left), heterozygous genotype ('AG' ($N = 151$ patients), center) and reference genotype ('GG' ($N = 362$ patients), right).

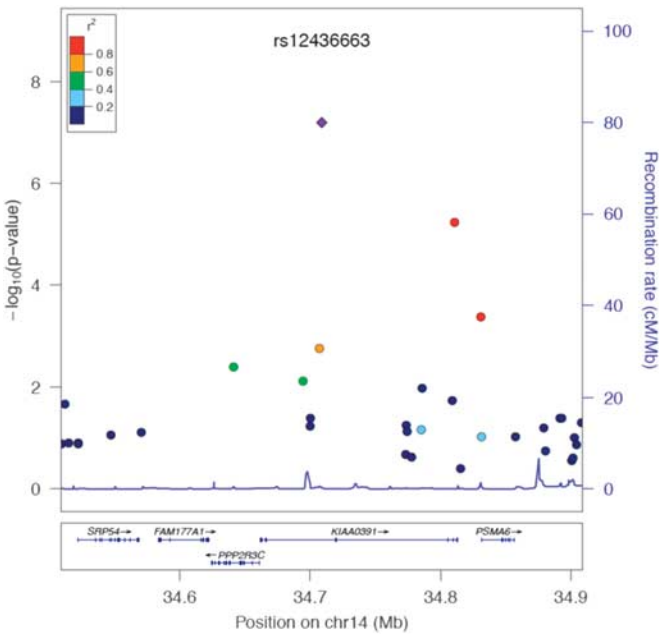

Figure 4. Regional association (LD) plot for rs12436663. The regional association plot was generated from the GWAS association data (discovery+replication) with Locus Zoom (http://csg.sph.umich.edu/locuszoom/),[33] specifying a ±200-kb region from rs12436663 (diamond symbol) for all GWAS SNPs (circle symbols), and using the HapMap Phase II CEU version 18 (no LD) as the reference genome build.

specifically to drug treatment response (i.e., represented true pharmacogenetic (drug x genotype) associations), we performed a primary GWAS that evaluated a gene–environment (G × E) interaction model to jointly evaluate SNP genotype by treatment group (zileuton or placebo) in all individuals in the cohort. Additive, dominant and recessive genetic models were evaluated using PLINK. SNP associations were considered significant if they met criteria for genome-wide significance ($P < 10^{-08}$). In addition, the top 50 SNPs, based on *P*-values, were carried forward for replication. For replication, SNPs that also met a threshold of $P < 0.05$ in the replication populations, and possessed a shared direction of effect (as indicated by the signs of the β coefficient) in the discovery and replication cohorts, were included in the combined analyses (discovery+replication). Combined *P*-values were then calculated from the one-sided *P*-values of the replication populations using R version 2.15 software.[23]

RESULTS

Figure 2 shows the discovery GWAS results. Although none of the SNPs in the discovery cohort achieved genome-wide significance, 10 of the 50 SNPs carried forward for replication approached genome-wide significance and were also at least nominally significant in the replication cohort; the replicated SNPs are shown in Table 2.

In the combined analysis of the discovery and replication cohorts, one SNP, rs12436663, achieved genome-wide significance and was associated with worsening response to zileuton (that is, significant reduction in mean ΔFEV_1) in both the discovery and replication cohorts (combined $\beta = -1.85$ L; combined $P = 6.28 \times 10^{-08}$, recessive model; Table 2). Relative to the patients who carried at least one reference allele, patients who were homozygous for rs12436663 demonstrated a mean ΔFEV_1 of −0.123 L for the homozygous rs12436663 'AA' genotype vs mean ΔFEV_1 of 0.233 L for individuals with either the 'AG' or 'GG' genotypes ($P = 0.03$) (Figure 3). rs12436663 resides in an intronic region within *KIAA0391*, also called *MRPP3*, a gene that encodes a mitochondrial RNase P protein involved in post-translational modification of transfer RNA molecules (Figure 4).

To identify shared SNP associations for treatment responses related to leukotriene inhibition and leukotriene receptor antagonism, the replicated zileuton GWAS results were carried forward for an additional replication in the LOCCS and LODO patients who received montelukast. From this analysis, we identified one SNP, rs517020, that replicated in LOCCS and was associated with reduced responses to both zileuton and montelukast (Table 3). rs517020 is found in an intron of the gene glycosyltransferase 1 domain containing 1 (*GLT1D1*).

DISCUSSION

This study is the first genome-wide analysis of the clinical response to leukotriene modifiers, and also provides additional evidence of shared pharmacogenetic loci for zileuton and montelukast. We conducted a discovery GWAS evaluating lung function following zileuton treatment, focusing on the interaction of zileuton treatment and genotype (G × E). We performed the G × E GWAS to enrich for associations that represented true pharmacogenetic effects, instead of more general associations with asthma. Although none of the SNPs achieved genome-wide significance in the discovery cohort, when the replicated SNP *P*-values from discovery and replication analyses were combined, we identified rs12436663, which met genome-wide significance. Furthermore, through replication of the zileuton GWAS SNP associations in the two montelukast treatment arms of the LOCCS and LODO trials, we identified an additional SNP, rs517020, associated with both zileuton treatment response and montelukast treatment response (in LOCCS).

rs12436663 resides in an intronic region of *MRPP3*, a.k.a. *KIAA0391*, a gene that has a crucial role in transfer RNA processing and maturation. Complex diseases associated with this gene include myocardial infarction, coronary artery disease, juvenile rheumatoid arthritis and psoriasis, all of which, like asthma, feature dysregulation in multiple immunological genes and pathways as causal mechanisms.[24–27] Multiple SNPs within the same chromosomal 14q13 region occupied by *MRPP3* are also associated with cardiovascular disorders, and the region contains microsattelites that are implicated in autoimmune diseases.[25] Although there is no direct evidence to suggest that *MRPP3* is involved in asthma, variation within the *MRPP3*-encoding locus may potentially affect

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Table 3. Replicated GWAS SNP association for zileuton and montelukast treatment responses

SNP	Gene Symbol	Abbott trial 1 (Discovery)		Abbott trial 2 (Replication)		LOCCS (Montelukast)		LODO (Montelukast)		Combined P-value[a]
		β (L)[b]	P-value[b]	β (L)[b]	P-value[b,c]	β (L)[b]	P-value[b,c]	β (L)[b]	P-value[b,c]	
rs517020	*GLT1D1*	−0.435	4.25×10^{-05}	−0.316	1.00×10^{-02}	−0.152	3.76×10^{-02}	−0.132	0.046	1.25×10^{-07}

Abbreviations: β, effect size (L); G × E, gene–environment interaction GWAS; LOCCS, Leukotriene Modifier Or Corticosteroid or Corticosteroid-Salmeterol trial; LODO, Effectiveness of Low Dose Theophylline as Add On Therapy for the Treatment of Asthma. [a]Combined *P*-values for Abbott, LOCCS and LODO. [b]Additive genetic model. [c]*P*-value threshold for replication = 0.05.

leukotriene synthesis and responses in asthma, presumably through altering post-transcriptional modification of transfer RNAs in leukotriene-producing cells. Leukotriene- and histamine-mediated allergic responses are induced through antigen binding to immunoglobulin E receptors on mast cells and basophils. Furthermore, the 14q region contains a cluster of genes (including *MRPP3*) that encode members of the ubiquitin–proteasome pathway, and markers within these genes are implicated in immunoglobulin E phenotypes and variation within immune response cascades. Consistent with this information, our results suggest that *MRPP3* could represent a novel candidate gene for leukotriene modifier responses in asthma.

We also evaluated whether the SNPs that replicated in Abbott also replicated in LOCCS and LODO, that is, represented associations that were shared between the zileuton-treated and montelukast-treated cohorts, and identified a SNP that replicated in Abbott cohorts and LOCCS. This SNP, rs517020, was associated with worsening responses to both medications. rs517020 was present within an intronic region of *GLT1D1*, whose gene product transfers glycosyl groups such as galactose, *N*-acetylglucosamine and sialic acid to the HIV-1 protein, gp120.[28–31] Although *GLT1D1* has unknown roles in asthma and asthma treatment response, as it was present within a predicted QTL region for allergic/atopic asthma it may have a role in the development or severity of asthma phenotypes, including reversible airflow obstruction.[32] In addition, *GLT1D1* may also contribute to LTM pharmacological response through its glycosyltransferase activity. For example, a wide range of bioactive lipids in the leukotriene pathway is modulated by glycosylation, and the activity of glycosyltransferases on these lipids could potentially affect multiple diverse functions throughout the body.

Although our study presents novel findings, these results should be interpreted in the context of some important limitations. First, the zileuton replication study population included individuals taking zileuton as 'add on' therapy to usual care, whereas the primary zileuton population included individuals on zileuton monotherapy. Although this might bias towards the null, it does not detract from the associations noted. Second, although these associations are generalizable to the overall treatment pathway, it is difficult to discern whether any loci in the zileuton-treated populations that did not replicate in the montelukast-treated populations represent false positives in the initial population, or are actually specific to zileuton response. Achieving replication in the montelukast populations boosts confidence in the generalizability of these findings towards LTM responses. Third, we did not use imputed GWAS data in our initial GWAS, which may have identified additional associations or strengthened existing ones. Fourth, because of the small sample size, replication in a larger cohort would be helpful to validate these findings. Finally, although multiple loci related to LTM response were identified through candidate gene studies (in particular, seven SNPs within *ALOX5, ABCC1* and *LTC4S*[11]), we were unable to replicate these SNPs in either primary or discovery GWAS cohorts. Five of the seven SNPs were not genotyped in these populations (due to differences in genotyping platforms used), and the remaining two did not achieve nominally significant associations with ΔFEV_1 (data not shown). Additional studies are necessary to ascertain the involvement of *MLLT3* and *GLT1D1* in asthma, and leukotriene modifier responses.

Evidence from an increasing number of genetic association studies implicates genes within leukotriene production and metabolism pathways as involved in clinical response to zileuton. Our results provide insight into both unique and shared clinical effects of LTMs in treatment of asthma, and represent a potential mechanism for the responsiveness to leukotriene antagonists in asthma patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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