

SYSTEMATIC REVIEWS AND META-ANALYSIS

Pharmacogenomics of inhaled corticosteroids and leukotriene modifiers: a systematic review

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Clinical
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Allergy

Summary

Background Pharmacogenetics studies of anti-inflammatory medication of asthma have expanded rapidly in recent decades, but the clinical value of their findings remains limited.

Objective To perform a systematic review of pharmacogenomics and pharmacogenetics of inhaled corticosteroids (ICS) and leukotriene modifiers (LTMs) in patients with asthma.

Methods Articles published between 1999 and June 2015 were searched using PubMed and EMBASE. Pharmacogenomics/genetics studies of patients with asthma using ICS or LTMs were included if ≥ 1 of the following outcomes were studied: lung function, exacerbation rates or asthma symptoms. The studies of Single Nucleotide Polymorphisms (SNPs) that had been replicated at least once were assessed in more detail.

Results In total, 59 publications were included in the systematic review: 26 addressed LTMs (including two genomewide Genome-Wide association studies [GWAS]) and 33 addressed ICS (including four GWAS). None of the GWAS reported similar results. Furthermore, none of the SNPs assessed in candidate gene studies were identified in a GWAS. No consistent reports were found for candidate gene studies of LTMs. In candidate gene studies of ICS, the most consistent results were found for rs28364072 in FCER2. This SNP was associated with all three outcomes of poor response, and the largest effect was reported with the risk of exacerbations (hazard ratio, 3.95; 95% CI, 1.64–9.51).

Conclusion and Clinical Relevance There is a lack of replication of genetic variants associated with poor ICS or LTM response. The most consistent results were found for the FCER2 gene [encoding for a low-affinity IgE receptor (CD23)] and poor ICS response. Larger studies with well-phenotyped patients are needed to assess the clinical applicability of ICS and LTM pharmacogenomics/genetics.

Keywords asthma, inhaled corticosteroids, leukotriene modifiers, pharmacogenetics, pharmacogenomics

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Introduction

Asthma is a chronic inflammatory disease that affects approximately 1–18% of the world's population [1]. A wide variability in patient response to asthma control medications, such as inhaled corticosteroids (ICS) and leukotriene modifiers (LTM), has been reported [2–4]. Approximately 35–40% of the patients receiving ICS for 8–12 weeks do not show an improvement in lung function [2, 3]. Moreover, approximately 10% of the patients with asthma receiving ICS experience severe

asthma symptoms despite regular use of this medication [5]. In the United States, the mean annual proportion of adult patients with asthma using ICS has increased from 39.9% (1998–1999) to 51.2% (2008–2009) and the proportion of LTM users has increased from 12.4% to 20.4% [6].

Inhaled corticosteroids are the preferred first-line treatment for persistent asthma (GINA step 2) [1]. Glucocorticoids (GCs) suppress inflammation in the airways by binding to the glucocorticoid receptor (GR). Subsequently, the receptor complex translocates to nucleus

and represses the activation of inflammatory genes, for example by the recruitment of histone deacetylase-2 (HDAC2) and reversing histone acetylation [7]. GCs also modulate the activity of genes encoding proteins involved in airway epithelial barrier and airway remodelling [8] and enhance the transcription of several anti-inflammatory genes [9].

Leukotriene modifiers are recommended for patients with moderate to severe persistent asthma, as one of the possible add-on therapies to ICS in the step 3 of the GINA guideline [4, 10]. These drugs have anti-inflammatory effects and reduce bronchoconstriction as well. LTMs consist of two groups of medications: cysLT1 receptor antagonists (i.e. montelukast, pranlukast and zafirlukast) and inhibitors of the 5-lipoxygenase pathway (i.e. zileuton, ABT-761) [11]. It has previously been reported in various studies that 35–78% of patients do not respond to LTMs with regard to lung function improvements [2, 3, 12].

Continued environmental exposures, poor adherence to medication, and misdiagnosis influence treatment response in patients with asthma [13, 14]. However, genetic variation might also be an important factor [4, 10]. Pharmacogenetics assesses the relation between variations in single genes and treatment response. Pharmacogenomics was defined by the National Institutes of Health (NIH) in 2016 as ‘the influence of genetic variation on the individual’s response to medication to design and develop safer treatment options with higher efficacy and less adverse effects based on the genetic profile of the patients’ (<https://ghr.nlm.nih.gov/primer/genomicresearch/pharmacogenomics>). Pharmacogenomics and pharmacogenetics are often used interchangeably in literature. In this paper, we will use the term pharmacogenomics.

In the last decade, advanced genotyping technologies have yielded a large number of pharmacogenomics studies. Nevertheless, the potential clinical value of the findings from these studies remains unclear. In this systematic review, we provide an up-to-date assessment of the scientific evidence regarding the use of genetic testing before starting ICS or LTMs by comparing the study designs, outcome definitions, study populations and reported findings in this field.

Methods

Articles published from 1999 until the end of June 2015 were searched in PubMed and EMBASE using specified search terms (Tables S1 and S2). Publications were included based upon keywords in their titles and abstracts that indicated that they had investigated genetic effects on the response to ICS or LTM based on lung function tests [i.e. forced expiratory volume in one-second (FEV₁), either as absolute values or as a

percentage of predicted (FEV₁%pred) and airway hyper-responsiveness to methacholine], exacerbation rates and asthma symptoms [i.e. Asthma Control Test (ACT), and Asthma Control Questionnaire (ACQ)]. Conference abstracts, articles assessing adverse drug effects and articles that had not published in English were excluded. We also screened review articles for possible missed publications. From all the publications selected, the following data were extracted by author FN: year of publication, journal name, authors, ICS and LTM type, genes and single nucleotide polymorphisms (SNPs), outcome, study design, ethnic backgrounds of patients, sample size, age range and duration of the study. We assessed the outcomes, population structure and the effect measures of the studies investigating the SNPs that had been replicated at least once. This systematic review is reported according to PRISMA guidance [15].

Pharmacogenomics of ICS

In the primary search, 146 studies assessing ICS pharmacogenomics were identified in PubMed and 315 studies were identified in EMBASE. Of these studies, 33 met the inclusion criteria (Fig. 1), with 29 being candidate gene studies (Table S3) [16–43] and four being GWAS [44–47].

GWAS studies of ICS

Three of the four GWAS [44–47] were conducted by the same research group. In all three publications [44–46], different numbers of Caucasian patients from the Single-Nucleotide Polymorphism Health Association-Asthma Resource Project (SHARP) were included. SHARP included patients from three studies: The Childhood Asthma Management Program (CAMP) study [48], Childhood Asthma Research and Education (CARE) and Asthma Clinical Research Network (ACRN). Despite similarities in the study populations, there were differences in the study designs, outcome measurements, genotyping platforms and sample sizes (Table 1). The overlap in the populations included in the three GWAS was not reported. Different genes and SNPs were identified to be associated with response to ICS in these three studies. Changes in the lung function measurements were assessed as the outcome in two of the three studies [44, 45]. The first of the GWAS of pharmacogenomics of ICS was published in 2011. In this study, 118 asthmatic child–parent trios from the CAMP study were studied and a family-based association analysis was conducted [44]. A SNP (rs37972 C>T) located in the *GLCCI1* (glucocorticoid-induced transcript 1 protein) gene was associated with changes in FEV₁% predicted in children treated with budesonide. Subsequently, this SNP was genotyped in four separate populations (935 adult

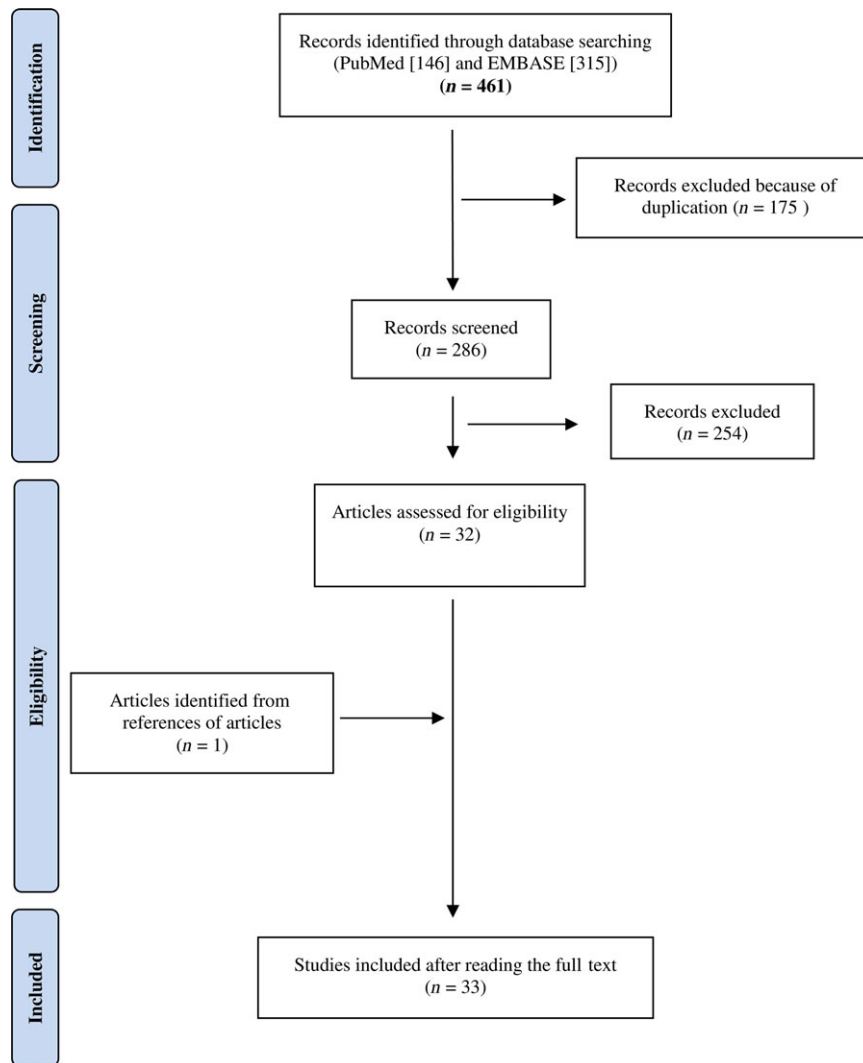


Fig. 1. Inclusion flowchart for pharmacogenomics studies of ICS.

patients) and in two of them, patients homozygous for the wild-type allele (C) had an approximately 12% increase in FEV1% predicted as compared to the patients homozygous for the mutant allele (T) with an approximately 4% increase (pooled P -value = 7×10^{-4}) [44]. This SNP (rs37972) is in complete linkage disequilibrium (LD) with rs37973 SNP in the same gene, which was found to influence *GLCCI1* gene expression [44], yet the function of *GLCCI1* remains largely unknown.

The second study by the same research group appeared in 2012. In it, researchers assessed the response to ICS by changes in FEV1% predicted in patients from the SHARP project (in total, 418 Caucasian children and adults) [45]. In the GWAS analysis of the 418 patients, none of the SNPs reached a genome-wide threshold. Subsequently, 47 SNPs identified in the GWAS with the lowest P -values were genotyped in a replication population of asthmatic adults ($n = 407$).

Two SNPs, rs6456042 and rs3127412, had the lowest P -values from this analysis ($P = 1.06 \times 10^{-5}$ and $P = 6.13 \times 10^{-6}$, respectively). These SNPs were found to be in high LD with three SNPs within functional regions of the *T* gene (rs3099266, rs1134481 and rs2305089). The most significant result was found for rs1134481 with wild-type homozygotes improving >10% in FEV1% at the end of week 8 compared to an improvement of only 4% in FEV1% in variant allele homozygotes ($P = 1.57 \times 10^{-5}$). The *GLCCI1* gene, which was identified in the previous study, was not found in the GWAS analysis. Differences in the study designs and genotyping platforms could have played a role here.

In the third GWAS [46], in 2014, three SNPs [rs2388639 in the gene *LOC728792*, rs10044254 in the gene *FBXL7* (F-box and leucine-rich repeat protein 7) and rs1558726 in the gene *RMST* (rhabdomyosarcoma 2-associated transcript)] were associated with self-

Table 1. GWAS analysis of response to ICS and LTMs

Study	Genotyping platform and number of SNPs after pruning	Discovery phase population	Study design and duration	Number of asthmatic patients	Medication in the discovery phase	Replication population	Definition of response	SNPs chosen for replication (gene, chromosome position)	MAF cut-off and MAF	Study outcome
<i>GWAS analysis of response to ICS</i>										
Tantisira <i>et al.</i> (2011)	HumanHap550v3 Bead Chip (Illumina) 534 290 SNPs	Caucasian children (CAMP)	Clinical trial, 16 months	118 child-parent trios	Budesonide 200 µg twice daily	Caucasian patients ($n = 935$) Children: (CARE trial, $n = 101$, fluticasone propionate 100 µg twice daily) Adults: (Adult study, $n = 385$, 1000 µg daily (increased up to 2000 µg if necessary)) (LOCCS, $n = 185$, fluticasone 100 µg twice daily) (SOCS)/SLIC, $n = 264$, triamcinolone 400 µg twice daily)	Changes in FEV1 from baseline	rs37972 (<i>GLCCI1</i> , 7p21.3)	Cut-off not mentioned T = 0.4	In two replication populations patients homozygous for the wild-type allele (C) had approximately 12% improvements in FEV1%pred compared with the 4% increase in TT carriers after 4–8 weeks of treatment with ICS (combined) P value = 7×10^{-4} . Patients homozygous for the wild-type allele of all three SNPs had a two to three-fold increase in FEV1%pred compared to homozygotes for the mutant allele. Combined P values of study populations for the rs1134481, rs2305089 and rs3099266 were 1.57×10^{-5} , 2.3×10^{-4} and 1.1×10^{-4} respectively
Tantisira <i>et al.</i> (2012)	Affymetrix (Santa Clara, CA) using a Human SNP array 6.0 444 088 SNPs	Caucasians children: (CAMP and CARE trials) ($n = 239$) Adults: (ACRN trial) ($n = 179$)	Clinical trial, 6–8 weeks	418	CAMP: budesonide 200 µg twice daily CARE: fluticasone propionate 100 µg twice daily ACRN: Triamcinolone 400 µg twice daily	Adults ($n = 407$) Flutisolid 1000 µg once daily (increased to 2000 µg if necessary)	Changes in FEV1% pred from baseline	rs3099266, rs1134481 and rs2305089 (<i>T gene</i> , 6q27)	Not mentioned	Patients homozygous for the wild-type allele of all three SNPs had a two to three-fold increase in FEV1%pred compared to homozygotes for the mutant allele. Combined P values of study populations for the rs1134481, rs2305089 and rs3099266 were 1.57×10^{-5} , 2.3×10^{-4} and 1.1×10^{-4} respectively
H. Park <i>et al.</i> (2014)	HumanHap550v3 Bead Chip or Infinium HD Human610-Quad Bead Chip (Illumina, San Diego, CA) 440 862 SNPs	Caucasian children (CAMP)	Clinical trial, 8 weeks	124	Budesonide 200 µg twice daily	Caucasian Children: (CARE, $n = 77$, fluticasone propionate 1000 µg daily (increased up to 2000 µg if necessary)) Adults: (LOCCS, $n = 110$, fluticasone 100 µg twice daily) (ACRN, $n = 110$, Triamcinolone 400 µg twice daily)	Self-reported asthma symptoms based on diary cards. Scores ranged from 0 (absent) to 3 (severe)	rs1558726 (<i>RMT12q21</i>), rs2388639 (<i>LOC728792</i>) and rs10044254 (<i>FBXL7</i> , 5p15.1)	Cut-off ≥ 0.05 rs1558726 A = 0.09 rs2388639 A = 0.19 rs10044254 G = 0.18	The combined P values of rs2388639, rs10044254 and rs1558726 SNPs for the pediatric CAMP and CARE subjects were 8.56×10^{-9} , 9.12×10^{-8} and 1.02×10^{-5} respectively. Homozygotes for the mutant allele for

Table 1 (continued)

Study	Genotyping platform and number of SNPs after pruning	Discovery phase population	Study design and duration	Number of asthmatic patients	Medication in the discovery phase	Replication population	Definition of response	SNPs chosen for replication (gene, chromosome position)	MAF cut-off and MAF	Study outcome
T. Park <i>et al.</i> (2014)	Illumina Human660W BeadChip (Illumina, San Diego, USA) 430 487 SNPs	Korean adults	Clinical trial, 4 weeks	189	1000 µg of fluticasone propionate daily	Same population with the discovery phase	Changes in FEV1%	14 SNPs within <i>ALLC</i> (from GWAS) and 11 additional SNPs in <i>ALLC</i> (2q35)	Cut-off ≥ 0.05 MAF of the SNPs ranged between 0.056 and 0.362	rs10044254 had significantly poorer responses to treatment compared to the patients homozygous or heterozygous for the wild-type allele (increase of 1.14 (as median score) in homozygotes for the mutant allele vs -0.28 in homozygotes for the reference allele). rs17017879, rs7558370, rs1123610, rs6754459, rs17445240 and rs13418767 were significantly associated with change in FEV1% (P value $< 1.0 \times 10^{-5}$).
<i>GWAS analysis of response to LTM</i>										
Dahlin <i>et al.</i> (2015)	Illumina HumanHap550 chip (Illumina, San Diego, CA, USA) 542 562 SNPs	African-American and Caucasians (> 12 years age)	Clinical trial, 12 weeks	160	Zileuton CR 1200 mg twice daily	African-American and Caucasians: Abbott 2 ($n = 149$, zileuton CR 1200 mg) LOCCS ($n = 69$) and LODO ($n = 64$) montelukast 5 or 10 mg daily	Changes in FEV1	rs12436663 (<i>MRPP3</i> , 14q13.2) was associated with response to zileuton. rs517020 (<i>GLT1D1</i> , 12q24.33) was associated with response to montelukast and zileuton	Not mentioned	Patients carrying two copies of the A allele in rs12436663 had a mean change of -123 mL compared to patients carrying at least one G allele 283 mL ($\beta = -1.85$ L) (combined P value from discovery and replication phase = 6.28×10^{-8}). In the zileuton trial, a mean decrease of 435 mL in FEV1 and in the montelukast trial (LOCCS) a mean decrease of 152 mL in FEV1 in rs517020 carriers was estimated (combined P value = 1.25×10^{-7})

Table 1 (continued)

Study	Genotyping platform and number of SNPs after pruning	Discovery phase population	Study design and duration	Number of asthmatic patients	Medication in the discovery phase	Replication population	Definition of response	SNPs chosen for replication (gene, chromosome position)	MAF cut-off and MAF	Study outcome
Dahlin et al. (2015)	Illumina HumanHap550 chip (San Diego, CA, USA), 532 264 SNPs	African-American and Caucasian adolescents and adults (LOCCS and LODO)	Clinical trial, 8 weeks	133	Montelukast 5 or 10 mg daily	184 African-American and Caucasian children (CLIC, 5–10 mg montelukast and PACT, 5 mg montelukast)	Changes in FEV1	rs6475448 (MLL73, 9p22)	Cut-off ≥ 0.05	Patients homozygous for the A allele had a higher increase of FEV1 from baseline in LOCCS (changes in FEV1 of 344 mL compared to -4.66 mL for homozygotes for G allele) ($\beta = 1.87$) (combined P value = 1.97×10^{-9}).

reported asthma symptoms [symptom scores ranged from 0 to 3 (severe)] in children treated with ICS from discovery and replication phases (combined P -values were 8.56×10^{-9} for rs2388639, 9.12×10^{-8} for rs10044254 and 1.02×10^{-5} for rs1558726). These associations were not replicated in adult patients [46]. Alterations in the *FBXL7* gene expression were shown in the presence of rs10044254 SNP in immortalized B cells [46]. This gene encodes a member of the F-box protein family, which plays a role in phosphorylation-dependent ubiquitination of proteins [49]. Homozygotes for the mutant allele for rs10044254 had significantly poorer responses compared with heterozygotes or homozygotes for the wild-type allele (median score of 1.14 in homozygotes for mutant allele vs -0.28 in homozygotes for the reference allele).

In the most recent GWAS of ICS in 2014, the changes in FEV1% from baseline measure was assessed in 189 Korean patients with asthma (15–75 years) [47]. A SNP (rs11123610) located within the *ALLC* (allantoicase) gene was associated with lung function improvement ($P = 3.6 \times 10^{-7}$). Subsequently, 25 additional SNPs within *ALLC* were selected for genotyping. Among the selected SNPs, six (rs17017879, rs7558370, rs11123610, rs6754459, rs17445240 and rs13418767) were statistically significantly associated with the change in FEV1% in response to ICS treatment (P -value $< 1.0 \times 10^{-5}$). The largest effect was found for rs11123610 with the differences in mean FEV1% higher than 20.3% among its genotypes. *ALLC* is located in chromosome 2q35 and encodes for the allantoicase enzyme, which is involved in the uric acid degradation pathway. However, this enzyme does not exist in mammals and its function is unclear [50].

Candidate gene studies of ICS

In the 29 candidate gene studies on pharmacogenomics of ICS, more than 500 SNPs in 120 different genes were studied. The most commonly studied genes were *CRHR1* (corticotropin-releasing hormone receptor 1), which was studied five times in a total of 843 patients; *FCER2* [Fc fragment of IgE, low-affinity II, receptor for (CD23)] was studied five times in a total of 2275 patients; *NR3C1* (nuclear receptor subfamily 3, group C, member 1) was studied four times in a total of 953 patients; *GLCCI1* was studied three times in a total of 3931 patients; *TBX21* (T-box 21) was studied three times in a total of 400 patients; and *STIP1* (stress induced phosphoprotein 1) was studied three times in a total of 671 patients. Only four SNPs within three genes (*CRHR1*: rs242941, rs1876828; *GLCCI1*: rs37973 and *FCER2*: rs28364072) were positively replicated at least once (Table 2). Response to medication was assessed using different outcomes: lung function measurements such as changes in FEV1 over time [16–29], annual decline

Table 2. Replicated genes of ICS studies

Study	Gene (Chromosome position) and SNPs	Study population	Design	MAF in the study population (minor allele)	Medication	Definition of response	Study outcome
Hosking <i>et al.</i> (2014)	<i>GLCCI1</i> (7p21.3) rs37973 (A/G)	White adolescents and adults (<i>n</i> = 1916)	Pooled data of 6- to 8-week trials and one 12-week clinical trial	0.44 (G)	Various doses of Fluticasone furoate ranged between 25 and 800 µg daily, Fluticasone propionate 100–500 µg twice daily	Changes in FEV1 from baseline after 8 weeks in 6 studies and at week 12 in one study.	There was no significant association between changes in FEV1 and rs37973 genotypes.
Vijverberg <i>et al.</i> (2014)	<i>GLCCI1</i> (7p21.3) rs37972 (T/C)	North European children BREATHE (<i>n</i> = 1037) PACMAN (<i>n</i> = 431) PAGES (<i>n</i> = 323)	Meta-analysis of three pediatric asthma cohorts	BREATHE: 0.45 (T) PACMAN: 0.44 (T) PAGES: 0.53 (T)	ICS maintenance dose varied between patients*	Exacerbations: Hospital visits OCS use Poor asthma symptoms: ACT scores ≤ 19 ACQ-scores ≥ 1.5	There was no significant association between increased risk of asthma exacerbations, asthma symptoms and rs37972 genotypes.
Izuhara <i>et al.</i> (2014)	<i>GLCCI1</i> (7p21.3) rs37973 (A/G)	Adult Japanese (<i>n</i> = 224)	Asthma cohort	0.44 (G)	ICS maintenance dose varied between patients*	Annual decline in FEV1	rs37973 GG was associated with a decline in FEV1 of 30 mL/year or more (estimated effect: 1.10; 0.02–2.18, <i>P</i> = 0.047) There was no association between rs37973 genotypes and the outcome, when decline in FEV1 was analyzed as a continuous variable.
Tantisira <i>et al.</i> (2004)	<i>CRHR1</i> (17q21.31) rs242941 rs1876828	Caucasian children and adults: Adult study (Adults, <i>n</i> = 415) CAMP (Children, <i>n</i> = 201) ACRN (Adults, <i>n</i> = 224)	Three independent 6–8 week clinical trials	rs242941 0.3 (T) rs1876828 (T)	Flunisolide 1000 µg once daily (increased to 2000 µg if necessary) Budesonide 200 µg twice daily Tramcinolone acetonide 400 µg twice daily	Changes in FEV1% from baseline	rs242941: for the minor allele homozygotes, the percent change was 13.28 ± 3.11 in Adult study and 17.80 ± 6.77 in CAMP compared

Table 2 (continued)

Study	Gene (Chromosome position) and SNPs	Study population	Design	MAF in the study population (minor allele)	Medication	Definition of response	Study outcome
Dijkstra <i>et al.</i> (2008)	<i>CRHR1</i> (17q21.31) rs242941 rs1876828	Adults (<i>n</i> = 98)	Asthma cohort	rs242941 0.4 (T) rs1876828 0.2 (T)	The dose of ICS was calculated to an equivalent daily dose of beclomethasone. Mean daily dose of ICS ($\mu\text{g}/\text{d}$): 749 (426–1152) Budesonide 200 μg twice daily	Changes in FEV1 from baseline (immediate effect) Rate of decline in FEV1 annually (long-term effect)	with 5.49 ± 1.40 and 7.57 ± 1.50 for those homozygous for the wild-type allele ($P = 0.025$ and $P = 0.006$, respectively). rs1876828: Homozygotes for the minor T allele had an average increase in their FEV1 of 23.72 ± 9.75 compared with $5.14 \pm 1.31\%$ for homozygotes for the common allele in patients from ACRN.
Rogers <i>et al.</i> (2009)	<i>CRHR1</i> (17q21.31) rs242941	African-American and Caucasian children (CAMP, <i>n</i> = 311)	4-year clinical trial	0.3 (T)		Exacerbations: - Emergency Department visits - Hospitalizations - Oral prednisone burst. Lung function measurements: change in FEV1%pred: $\leq 7.5\%$ considered as poor responders	The minor allele (T) showed a significant association with poor response based on lung function (OR: 1.6, CI 95% 1–2.7, $P = 0.05$)
Mougey <i>et al.</i> (2013)			16-week Clinical trial	rs242941 0.3 (T)	Fluticasone 100 μg twice daily	Change FEV1% pred Asthma symptoms: Slopes	Minor allele of rs1876828 was

Table 2 (continued)

Study	Gene (Chromosome position) and SNPs	Study population	Design	MAF in the study population (minor allele)	Medication	Definition of response	Study outcome
	<i>CRHR1</i> (17q21.31) rs1876828 rs242941	Caucasian children, adolescence and adults (<i>n</i> = 65)		rs1876828 0.15 (T)		of plots of ACQ scores versus time	associated with improvements in FEV1% pred ($P = 1.89 \times 10^{-4}$). Major allele of rs242941 was associated with improvements in FEV1% pred ($P = 2.07 \times 10^{-3}$). There was significant association between rs28364072 variant and severe exacerbations in both white and African-American children (hazard ratio, 3.95; 95% CI, 1.64–9.51; and hazard ratio, 3.08; 95% CI, 1.00–9.47). The minor allele of rs28364072 in <i>FCER2</i> was associated with recurrent exacerbations in white subjects (OR: 1.9 for minor allele, $P < 0.05$) but it did not reach significance in multivariate analysis (OR: 1.4, $P = 0.18$). The minor allele was also associated with poor response
Tantisira <i>et al.</i> (2007)	<i>FCER2</i> (19p13.3) rs28364072 (A/G)	African-American and Caucasian children (CAMP, <i>n</i> = 311)	4-year clinical trial	0.26 (G) in Caucasians and 0.44 (G) in African-Americans	Budesonide 200 µg twice daily	Severe exacerbations: Emergency department visits and/or hospitalizations.	
Rogers <i>et al.</i> (2009)		African-American and Caucasian children (CAMP, <i>n</i> = 311)	4-year clinical trial	0.26 (G) in Caucasians and 0.44 (G) in African-Americans	Budesonide 200 µg twice daily	Exacerbations: - Emergency department visit - Hospitalization - Oral prednisone burst. Lung function measurements: change in FEV1% pred: $\leq 7.5\%$ considered as poor responders	

Table 2 (continued)

Study	Gene (Chromosome position) and SNPs	Study population	Design	MAF in the study population (minor allele)	Medication	Definition of response	Study outcome
Koster <i>et al.</i> (2011)		Caucasian children PACMAN (<i>n</i> = 386) BREATHE (<i>n</i> = 939) CAMP (<i>n</i> = 311)	PACMAN & BREATHE: asthma cohorts CAMP: 4-year clinical trial	PACMAN 0.27 (C) BREATHE 0.26 (C)	For BREATHE and PACMAN, ICS maintenance dose varied between patients* CAMP: Budesonide 200 µg twice daily	Exacerbations: - Emergency department visits - Hospitalization Asthma symptoms: - ACQ- scores - Respiratory symptoms (wheeze, shortness of breath and cough) - Asthma-related sleep disturbances - Asthma-related limitations in daily activities - Additional (airway) medication use during the preceding 12 months	considering lung function changes (OR: 2.1, 95%CI: 1.2–3.5, <i>P</i> = 0.006). The rs28364072 variant was associated with increased risk of asthma-related hospital visits in the meta-analysis (OR: 2.38, 95%CI: 1.47–3.85, <i>P</i> = 0.0004). The variant was associated with increased risk of uncontrolled asthma measured by ACQ scores (OR: 2.64, 95%CI: 1.00–6.98) and was associated with increased daily steroid dose (OR: 2.46, 95%CI: 1.38–4.39).

in FEV1 [30–32], bronchial hyperresponsiveness [BHR] to methacholine [26, 29, 33, 34], asthma symptoms based on questionnaires [25, 35–40] and exacerbations despite treatment [37, 38, 41–43].

ICS Pharmacogenomics: replicated genes and SNPs

The replication of SNPs previously associated with ICS response remains complicated. Three candidate gene studies of *GLCCI1* have tried to replicate the results of the GWAS analysis for rs37972 conducted by Tantisira et al. [30], but only one study could replicate the results. In the successful replication study, a decline of over 30 mL/year in FEV1 was used as the cut-off marker for poor response in 224 adult Japanese patients with asthma. Patients with GG genotypes had a decrease in FEV1 by 1.10 units (95% CI 0.02–2.18, P -value <0.05) (1 unit = 30 mL/year) [30]. The two other replication studies (including Caucasian patients) could not replicate the results of the GWAS study [17, 37]. In one study (n = 1916 adult patients with asthma), the same outcome of interest as the GWAS (changes in FEV1 from baseline) was measured in a 8- to 12-week trial [17]. The second unsuccessful replication study was a meta-analysis of three paediatric asthma cohorts (n = 1791 patients), and the risk of asthma exacerbations despite ICS use was assessed as the outcome [37]. In total, the association between *GLCCI1* and ICS response has been studied in 3916 patients, and in 3692 patients no statistically significant associations were found [17, 37]. Differences in outcome measures and ethnic backgrounds could play a role in the lack of replication.

Another gene that has been associated with response to ICS treatment in various studies is *CRHR1*. This gene encodes a receptor that binds to corticotropin-releasing hormone (CRH), which is a major regulator of the hypothalamic–pituitary–adrenal pathway. This protein is needed for the activation of signal transduction pathways that regulate various physiological processes, including stress and immune responses [51, 52]. It has been hypothesized that the change in the level of the endogenous corticosteroids secretion due to variations within *CRHR1*, might change the response to the exogenous corticosteroids [53]. In total, 20 SNPs within this gene have been studied in four different studies [39, 42, 53, 54]. Two SNPs have been replicated (rs1876828 and rs242941).

Rs1876828 has been studied in three studies [39, 46, 47] in five different populations (a total of 1003 patients). In two populations, a statistically significant association with lung function was found in adult and paediatric patients with asthma (289 patient in total) [39, 53]. In the study by Tantisira et al., patients homozygous for the minor allele (A) had a fourfold greater improvement in their FEV1% (23.72 ± 9.75)

compared to homozygotes for the common G allele ($5.14 \pm 1.31\%$) [53]. A comparable association was found in a later study by Mougey et al., in which AA genotype carriers had an eightfold greater improvement in FEV1% predicted as compared to the GG carriers ($24.2 \pm 9.10\%$ vs $2.98 \pm 1.88\%$) [39]. However, in three other populations (including more than 700 adult and paediatric patients in total) there was no interaction between this SNP and response to ICS [53, 54].

The other replicated SNP within *CRHR1* is rs242941. Interaction between rs242941 and ICS response (based on lung function measurements) has been studied in four studies (a total of 1314 patients) [39, 42, 53, 54]; however, there was a discrepancy in the findings of these studies. In two separate populations (616 Caucasian paediatric and adult patients with asthma in total) within one study, the variant (T allele) was significantly associated with improvements in FEV1% in 6–8 weeks of treatment with ICS [53]. Patients from the CAMP study were included in one of the populations. In both populations, patients homozygous for the minor allele had a higher improvement in mean FEV1% ($13.3 \pm 3.1\%$ and $17.8 \pm 6.8\%$) than homozygotes for the wild-type allele ($5.5 \pm 1.4\%$ and $7.6 \pm 1.5\%$) [53]. However, in two other studies [39, 42] (including a total of 375 paediatric and adult asthmatics with African American and Caucasian backgrounds), the minor allele was significantly associated with poor lung function improvement. Mougey et al. [39] found a mean decrease of 10 ± 3.2 in FEV1% predicted after 16 weeks of treatment with ICS (P = 0.003) in 65 patients with minor allele. On the other hand, Rogers et al. [42] categorized children of the CAMP trial to non-responders (improvement of FEV1% predicted <7.5% from baseline) and responders (improvement of $\geq 7.5\%$). Children carrying the T allele of rs242941 had a higher risk of a poor response to ICS (OR: 1.9, P = 0.02). Inconsistencies in the findings of these studies might be the result of differences in the definitions of outcome measures and the duration of the studies. Moreover, in two other populations (322 patients in total) there was no statistically significant interaction between *CRHR1* rs242941 and lung function measurement [53, 54]. Definition of the outcome measure and the methods used for patient inclusion were also different in these studies. No statistically significant association was found between rs242941 and other response outcomes [39, 42].

The *FCER2* gene encodes for a low-affinity IgE receptor (CD23) [55]. In total, 25 SNPs within this gene have been studied, and one (rs28364072) has been successfully replicated in three studies [38, 42, 43]. The association between this SNP and ICS response was initially identified in Caucasian and African American children from the CAMP study [43]. The SNP was

associated with a risk of severe exacerbations despite ICS use [hazard ratio (HR): 3.95; 95% CI: 1.64–9.51 in White children; and HR: 3.08; 95% CI: 1.00–9.47 in African Americans] [43]. A second study found statistically significant associations with ICS response when categorizing the patients to responders (change in FEV1% >7.5%) and non-responders (change in FEV1% <7.5%) (OR: 2.1, 95% CI: 1.2–3.5) [42]. A meta-analysis of three paediatric asthma cohorts also found a statistically significant association with the risk of asthma-related hospital visits (OR: 2.38, 95% CI: 1.47–3.85) [38]. It has been shown that variation in rs28364072 is associated with an altered *FCER2* expression [43]. Consistent results across these studies demonstrate a considerable amount of evidence for a gene–drug

interaction. Genetic variants in the *TBX21* [25, 26, 33] and 17q21 loci [24, 29] have also been found to influence ICS response in several studies; however, as the associations were found using different outcome measures, it is not further discussed in the manuscript.

Pharmacogenomics of leukotriene modifiers

In total, 250 studies on LTM pharmacogenomics were identified. Twenty-six studies met the inclusion criteria (Fig. 2). Of these studies, 24 were candidate gene studies (Table S4) [21, 39, 56–76] and two were GWAS [77, 78]. None of the SNPs that had been associated with treatment response in the candidate gene studies were identified in GWAS studies of LTMs.

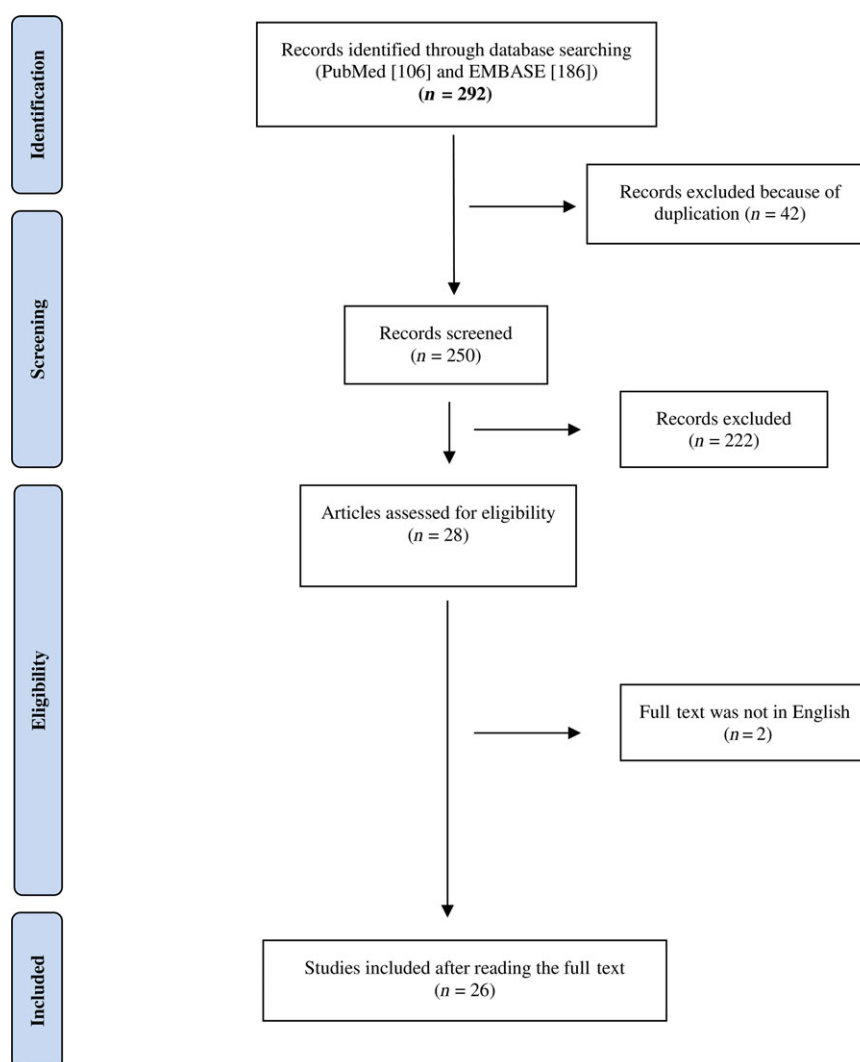


Fig. 2. Inclusion flowchart for pharmacogenomics studies of LTMs.

GWAS studies of LTMs

Two GWAS have been published recently by one research group (Table 1) [77, 78]. Both studies assessed the changes in FEV1 from baseline to the end of trial (8–12 weeks) in White and non-White patients with asthma. The first, a GWAS analysis of LTMs by Dahlin et al. [77] included 160 patients >12 years age receiving zileuton. This study found that after combining the analyses of discovery and replication cohorts, one SNP (rs12436663) within the *MRPP3* gene (also known as *KIAA0391*) was significantly associated with the changes in FEV1 from baseline after 12 weeks of treatment (in 309 patients). The mean change of FEV1 for patients carrying AA genotype was –123 mL, and the mean change for patients carrying at least one G allele was 283 mL. This gene encodes for MRPP3 protein that functions in the maturation of mitochondrial tRNA [79] and has previously been found to be associated with autoimmune diseases such as Crohn's disease and psoriasis [80]. In this first GWAS, additional replication was also performed in two other clinical trials consisting of 133 adolescent/adult patients with asthma treated with montelukast to find the SNP associations shared between zileuton and montelukast treated patients. Rs517020 SNP within the *GLT1D1* (glycosyltransferase 1 domain containing 1) gene was associated with a poorer response to both montelukast and zileuton (combined P -value 1.25×10^{-7}). A mean decrease of 152 mL in FEV1 for montelukast trial and 435 mL for zileuton trial was estimated in the additive model. The product of *GLT1D1* has glycosyl transferase activity [81], but its role in asthma is still unknown. In the second GWAS [78], the discovery cohorts included 133 adolescents/adults with asthma from two clinical trials of montelukast (used for replication analysis in the first GWAS [77]). The replication cohort included two other montelukast clinical trials (including a total of 184 children with asthma). The change in FEV1 from baseline to 8 weeks of LTM treatment was measured in all studies. Rs6475448 within *MLLT3* (myeloid/lymphoid or mixed-lineage leukaemia; translocated to, 3) exceeded genomewide significance ($P = 1.97 \times 10^{-9}$) after combining the P -values of the four trials. This gene encodes for a protein that is a part of a complex that increases the catalytic rate of RNA polymerase II transcription [82]. Patients homozygous for the variant allele (A) had a mean increase in FEV1 of 344 mL compared to –4.66 mL for patients with GG genotype. The SNPs identified in the first GWAS were not found to be associated with response to montelukast in the second GWAS. Moreover, SNPs identified in LTM candidate gene studies were not identified in these GWAS.

Candidate gene studies of LTMs

More than 250 SNPs in 30 different genes were studied in candidate gene studies of LTMs. The majority of the candidate gene studies of response to LTMs (22 of 24) assessed the response to CysLT1 receptor antagonists, with only two assessing the response to 5-lipoxygenase inhibitors. Arachidonate 5-lipoxygenase (*ALOX5*), *ALOX5*-activating protein (*ALOX5AP*), leukotriene A4 hydrolase (*LTA4H*), LTC4 synthase (*LTC4S*), the ATP-binding cassette family member *ABCC1*/multidrug resistance protein 1 (*MRP1*) and cysteinyl leukotriene receptor 1 and 2 (*CysLTR1* and *CysLTR2*) are the genes that encode the main proteins in LT pathway (Fig. 3). Among these genes, *LTC4s* (investigated in 12 studies, 1345 patients in total), *ALOX5* (eight studies, 1277 patients in total), *LTA4H* (five studies, 1030 patients in total), *CysLTR1* (five studies, 991 patients in total) and *MRP1* (three studies, 360 patients in total) were studied most often. However, only three variants in two genes (*ALOX5*, Sp1 repeats in the core promotor and rs2115819, and *MRP1* rs119774) were found to be statistically significantly associated with response to LTMs and positively replicated for the same outcome at least once (Table 3). In 21 of the 24 studies, lung function measurements were considered as the primary outcome. However, the definition of this outcome was different among the various studies having been defined as follows: change in FEV1% from baseline over time [21, 56–62], change in FEV1 after using short-acting β_2 -agonists [SABA] [63, 64], changes in PEF [60, 62, 63, 65] and BHR to adenosine monophosphate (AMP), methacholine or aspirin [in patients with aspirin-intolerant asthma (AIA)] [66–68]. Furthermore, an exercise challenge test was performed in study patients with exercise-induced bronchoconstriction (EIB) as the primary outcome parameter [69–73]. Asthma-related symptoms were measured in four studies [21, 39, 65, 74], and asthma exacerbation rates were studied in two studies [56, 57]. The definition and assessment method of uncontrolled asthma symptoms and exacerbations rates varied between the studies.

LTM pharmacogenomics: replicated genes and SNPs

Two genes in the leukotriene pathway have frequently been studied and successfully replicated: *ALOX5* and *MRP1*. The products of these genes play important roles in the production and transport of cysteinyl leukotrienes (LTC4, LTD4 and LTE4) that cause inflammation and constriction in the airways [83, 84]. Two genetic variants in *ALOX5* (Sp1 repeats, and rs2115819) and one genetic variant in *MRP1* (rs119774) have been frequently studied and successfully replicated. Five studies

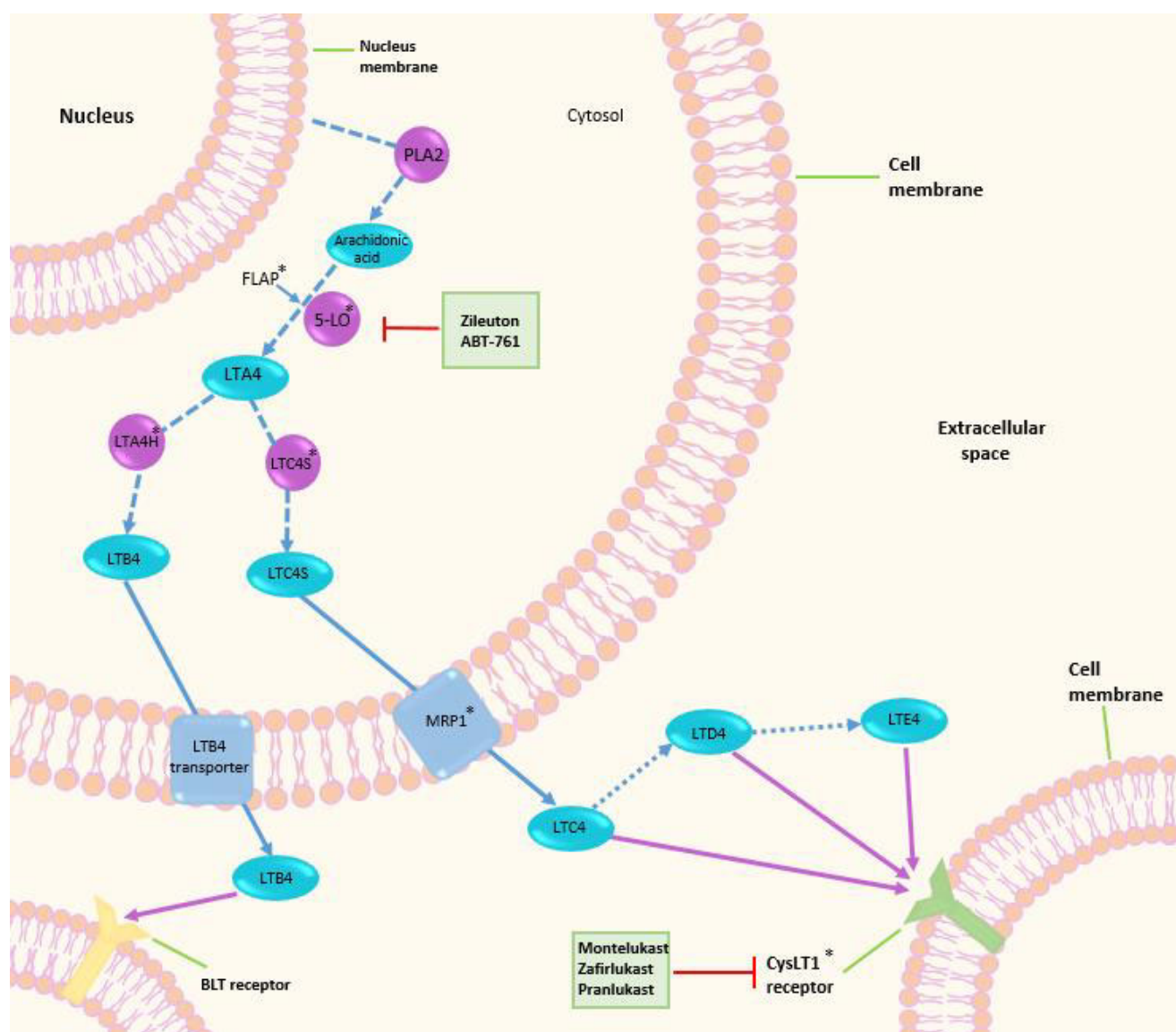


Fig. 3. Leukotriene synthesis pathway. PLA2, Phospholipase A2; 5-LO, 5-lipoxygenase; FLAP, 5-lipoxygenase-activating protein; LT; Leukotriene; LTA4H, Leukotriene A4 hydrolase; LTC4S, Leukotriene C4 Synthase; MRP1, multidrug resistance protein 1; CysLT1, Cysteinyl leukotriene receptor 1; BLT receptor, Leukotriene B4 receptor. *proteins and enzymes encoded by genes studied in pharmacogenomics studies of LTMs.

have investigated the association between response to LTMs and genetic variation in the core promoter SNP of *ALOX5* [56–58, 60, 66]. Normally, this locus contains a transcription factor binding region with five binding sites (Sp1 repeats); however, deletion and addition of binding sites results in the genetic variation [85]. Changes in FEV1 were assessed in four separate clinical trials, including 402 patients with asthma in total, to investigate the interaction between *ALOX5* Sp1 repeats and response to LTMs [56–58, 60]. Two studies (with a total of 175 patients) found statistically significant improvements in FEV1% [58] and increase in FEV1% pred [57] in patients carrying at least one wild-type allele as compared to patients homozygous for the

mutant allele while they were on treatment with LTMs. In the first pharmacogenomics study of asthma medication, published in 1999, Drazen et al. reported a change of $18.8 \pm 4\%$ in FEV1 in patients homozygous for the wild-type allele (five tandem repeats) compared with $-1.2 \pm 3\%$ in patients homozygous for the mutant genotype (tandem repeats other than 5) [58]. In a later study, patients with at least five tandem repeats had an increase of $8.6\% \pm 8$ compared with $-1.4\% \pm 8.04$ in patients homozygous for the mutant allele [57]. These two confirmative studies both used a different LTM in their studies (ABT-761 and montelukast). Two other studies (with a total of 218 patients) did not report any significant association between Sp1 repeats and

Table 3. Candidate gene studies of response to LTMIs

Study	Gene and SNP	Study population	Design	MAF	Medication	Definition of response	Study outcome
Drazen <i>et al.</i> (1999)	<i>ALOX5</i> (10q11.2) tandem repeats of the Sp1-binding domain	Adults ($n = 114$)	12-week Clinical trial	0.23 (tandem repeats other than 5)	ABT-761, 300 mg daily	Changes in FEV ₁ 10% from baseline	The mean change in FEV ₁ 10% from baseline was $18.8 \pm 3.6\%$ in wild-type (5/5) patients and $23.3 \pm 6.0\%$ in heterozygous (5/X) patients compared to the patients with the mutant genotype (X/X) with the change in FEV ₁ 10% of $-1.2 \pm 2.9\%$ ($P < 0.0001$ and $P < 0.0006$). There was no association between different genotypes and the outcomes.
Fowler SJ <i>et al.</i> (2002)	<i>ALOX5</i> (10q11.2) tandem repeats of the Sp1-binding domain	Adult atopic asthmatics ($n = 52$)	Retrospective analysis of four placebo-controlled trials	0.12 (tandem repeats other than 5)	Montelukast Zafirlukast	Bronchial hyper-responsiveness (BHR) to adenosine monophosphate (AMP), changes in FEV ₁ (l), FEF ₂₅₋₇₅ (l/s) and PEF _R (l/min)	There was no association between different genotypes and the outcomes.
Lima <i>et al.</i> (2006)	<i>ALOX5</i> (10q11.2) tandem repeats of the Sp1-binding domain	Caucasian adults ($n = 61$)	6-month Clinical trial	0.2 (tandem repeats other than 5)	Montelukast 10 mg daily	Changes in FEV ₁ 10% pred from baseline Asthma exacerbation rate. One or more of the following: a more than 30% decrease in peak expiratory flow rate for two consecutive days, a course of oral steroids, an unscheduled visit to the clinic, the emergency room, or hospital or an increase of four puffs of rescue inhaler use in one day	Homozygotes for the mutant allele for tandem repeats (repeats other than 5) had a 73% decrease in the risk of exacerbations ($P = 0.04$). No association was found between this SNP and changes in FEV ₁ 10% pred.
Klotsman <i>et al.</i> (2007)	<i>ALOX5</i> (10q11.2) tandem repeats	Caucasian, Hispanic and African-American	12-week Clinical trial	0.24 (tandem repeats other than 5)	Montelukast 10 mg daily	Changes in FEV ₁ 10% pred from baseline	No associations were found between this SNP and changes in FEV ₁ 10% pred.

Table 3 (continued)

Study	Gene and SNP	Study population	Design	MAF	Medication	Definition of response	Study outcome
Telleria <i>et al.</i> (2008)	of the Sp1-binding domain ALOX5 (10q11.2) tandem repeats of the Sp1-binding domain	adolescents and adults (<i>n</i> = 166) Adolescent and adult atopic asthmatics (<i>n</i> = 61)	6-month clinical trial	0.4 (four tandem repeats)	Montelukast	Changes in FEV1% pred from baseline Number of exacerbations: A decrease in peak expiratory flow rate in the morning of at least 25% compared with baseline.	Homozygotes for the mutant allele (4/4) had 1.88 ± 0.92 rate of asthma exacerbations, with a mean reduction of 1.33 ± 1.22 and patients homozygous for the common allele (5/5) and heterozygotes (4/5) had an exacerbation rate of 0.4 ± 0.21 with a reduction of 4.41 ± 2.76 ($P = 0.001$). Homozygotes for the mutant allele had a mean decrease of $1.4\% \pm 8.04$ in FEV1% pred compared to the homozygotes for the common allele and heterozygotes with a mean increase of $8.6\% \pm 7.6$ in FEV1% pred ($P = 0.0006$).
Lima <i>et al.</i> (2006)	ALOX5 (10q11.2) rs2115819 (A/G)	Caucasian adults (<i>n</i> = 61)	6-month clinical trial	0.47 (G)	Montelukast 10 mg daily	Changes in FEV1% pred from baseline Asthma exacerbation rate. One or more of the following: a more than 30% decrease in peak expiratory flow rate for two consecutive days, a course of oral steroids, an unscheduled visit to the clinic, the emergency room, or hospital or an increase of four	There was a significant association between rs2115819 and changes in FEV1% pred ($P < 0.05$): Patients homozygous for the G allele had 30% improvements in the FEV1%pred (95% CI = -0.017 to 1.21) compared to 4.4% (95% CI, -0.025 to 0.66) and 2.0% (95% CI, 0.013 – 0.075) improvements in the AA and AG genotype groups, respectively. No association was found between this SNP and exacerbations.

Table 3 (continued)

Study	Gene and SNP	Study population	Design	MAF	Medication	Definition of response	Study outcome
Tantisira <i>et al.</i> (2009)	<i>ALOX5</i> (10q11.2) rs2115819 (A/G)	Caucasian children, adolescence and adults (<i>n</i> = 239)	12-week clinical trial	(G)	Zileuton CR, 1200 mg twice daily or immediate-release (IR), 600 mg 4 times daily	puffs of rescue inhaler use in one day Changes in FEV1% pred from baseline	Significant association was found between rs2115819 changes in FEV1% from baseline (<i>P</i> = 0.01). G allele carriers had higher improvements in the FEV1% pred at the end of the 12 weeks and improvement in FEV1% pred from baseline was more than 20% in GG carriers compared with the AA carriers with mean improvement of 15% from the baseline.
Mougey <i>et al.</i> (2013)	<i>ALOX5</i> (10q11.2) rs2115819 (A/G)	Caucasian children, adolescence and adults (<i>n</i> = 65)	16-week clinical trial	0.4 (G)	Montelukast 5 or 10 mg daily	Changes in FEV1% pred Asthma symptoms: Slopes of plots of ACO scores versus time	No association was found between rs2115819 and the outcomes.
Kotani <i>et al.</i> (2012)	<i>ALOX5</i> (10q11.2) rs2115819 (A/G)	Japanese adults (<i>n</i> = 21)	4–8 week clinical trial	0.2 (A)	Montelukast 10 mg once daily	Self-measured changes in PEF after SABA	No significant association was found between the rs2115819 and changes in PEF and FEV1.
Lima <i>et al.</i> (2006)	<i>MRP1</i> (16p13.1) rs119774 (C/T)	Caucasian adults (<i>n</i> = 61)	6-month clinical trial	0.07 (T)	Montelukast 10 mg daily	Changes in FEV1% pred from baseline Asthma exacerbation rate. One or more of the following: a more than 30% decrease in peak expiratory flow rate for two consecutive days, a course of oral steroids, an unscheduled visit to the clinic, the emergency room, or hospital or an increase	There was a significant association between rs119774 and changes in FEV1% pred (<i>P</i> < 0.05). Heterozygotes for the T allele had 24% improvement (95% CI: −0.105 to 0.577) in the FEV1% pred compared with the patients homozygous for the C allele with 2.2% improvements in the

Table 3 (continued)

Study	Gene and SNP	Study population	Design	MAF	Medication	Definition of response	Study outcome
Tantisira <i>et al.</i> (2009)	<i>MRP1</i> (16p13.1) rs119774 (C/T)	Caucasian children, adolescence and adults (<i>n</i> = 239)	12-week clinical trial	Not reported	Zileuton CR, 1200 mg twice daily or immediate-release (IR), 600 mg 4 times daily	of four puffs of rescue inhaler use in one day. Changes in FEV1% pred from baseline	FEV1%pred (95% CI: -0.005 to 0.049). Significant association was found between rs119774 and changes in FEV1% from baseline (<i>P</i> = 0.05).
Mougey <i>et al.</i> (2013)	<i>MRP1</i> (16p13.1) rs119774 (C/T)	Caucasian children, adolescence and adults (<i>n</i> = 65)	16-week clinical trial	0.06 (T)	Montelukast 5 or 10 mg daily	Changes in FEV1% pred Asthma symptoms: Slopes of plots of ACQ scores versus time	No association was found between rs119774 and the outcomes

response to montelukast when considering changes in FEV1% predicted as the outcome [56, 60]. There are inconclusive results regarding the effect of *ALOX5* Sp1 repeats on the occurrence of exacerbations [56, 57]. In a study by Lima *et al.* [56], patients homozygous for the tandem repeats other than five showed a 73% lower risk of exacerbations. However, in a study by Telleria *et al.* patients homozygous for the wild-type allele had a higher reduction in the amount of exacerbations compared to patients homozygous for the mutant allele receiving 6 months of treatment with montelukast (reduction in exacerbation rate: -4.4 and -1.3, respectively) [57]. Asthma exacerbation was defined as a fall in PEF rate in the morning of at least 25% compared with the baseline [57]. In contrast, Lima *et al.* defined asthma exacerbation according to the presence of one or more of the following items: an increase in the use of a rescue inhaler with up to four puffs in one day, a decrease in PEF rate of more than 30% for two consecutive days, or an unscheduled visit to emergency room/clinic/hospital or an OCS course [56]. In the fifth study, BHR to AMP was considered as the treatment response in a retrospective analysis of four placebo-controlled trials including 52 adult patients with asthma treated with montelukast or zafirlukast. Researchers did not find a statistically significant difference between heterozygotes and wild-type homozygotes in terms of response to LTMs and in this study there was no homozygote for the mutant allele [66].

The association of *ALOX5* rs2115819 A>G and *MRP1* rs119774 C>T with response to LTMs has been studied in four [39, 56, 59, 63] and three studies [39, 56, 59], respectively. Two earlier studies by one research group investigating the association of SNPs within five candidate genes of LT pathway (*MRP1*, *ALOX5*, *LTA4H*, *LTC4S* and *CysLTR1*) with changes in FEV1 reported statistically significant associations for both SNPs in Caucasian patients treated with montelukast and zileuton. Different study populations (300 children and adults with asthma in total) were included in these studies [56, 59]. In the first study, patients with the GG genotype for rs2115819 showed greater improvements in FEV1 with 6 months of treatment with montelukast [FEV1 improvement: 30% (95% CI: -0.017 to 1.21)], compared to the patients with AA and AG genotypes [FEV1 improvement 4.4% (95% CI: 0.025-0.66) and 2% (95% CI: 0.013-0.075), respectively]. Likewise, patients homozygous for the C allele at rs119774 had greater improvements in FEV1 24% (95% CI: -0.105 to 0.577), compared to patients heterozygous for this allele, who showed improvements of 2.2% (95% CI: -0.005 to 0.049); there were no patients homozygous for the T allele. In the second study, these findings were replicated in patients treated with zileuton for 12 weeks. Patients carrying G allele of rs2115819 had more than

20% improvement in the FEV1% predicted compared with the AA carriers that had approximately 15% improvement in the FEV1% at the end of the trial ($P = 0.01$) [59]. Improvements of FEV1% were not shown for the rs119774 in the study. However, no associations were found for these SNPs with the changes in FEV1 and ACQ scores in a later study by this research group in which 60 asthmatic adults and children with Caucasian ethnicity who had been treated with montelukast for 4 months were studied [39]. Furthermore, rs2115819 was not associated with the changes in morning PEF in 81 patients treated with montelukast [63] and exacerbation rates [56].

Discussion

Since 1999, over fifty studies have investigated if and how genetic variation can explain why response to asthma maintenance therapy varies between individuals with asthma. However, despite the number of studies, the clinical applicability of these pharmacogenomics findings remains unclear. Few variations have been replicated and effect estimates are relatively low. The most consistent results were found for a SNP (rs28364072) within *FCER2* in relation to ICS response. All three studies assessing the association between this SNP and response to ICS, reported significant associations between the SNP and different outcomes (exacerbations, lung function measurements and asthma symptoms) in children with asthma treated with ICS [38, 42, 43]. More evidence was provided in the *in vitro* study of *FCER2* that confirmed altered gene expression in patients carrying the variant allele [43]. There have been other successful replications in the pharmacogenomics of ICS (i.e. *CRHR1* and *GLCCI1*) and pharmacogenomics of LTM (i.e. *MRP1* and *ALOX5*), but the results were not consistent. Differences in study design and reported outcomes made it impossible to perform meta-analyses. Interestingly, GWAS and candidate gene studies delivered different results. Some of these SNPs might not have been genotyped or imputed in GWAS studies. Another reason that none of the SNPs associated with treatment response in candidate gene studies were identified in GWAS could be the relatively small sample sizes in GWAS (<500). One of the important measures of quality in genetic association studies is the sample size [86]. It has been shown that to detect a relative risk between 1.7 and 2.0, a sample size of 1000 cases and 1000 controls in GWAS analysis is required [87, 88]. As effect sizes in complex diseases such as asthma are even smaller (1.1–2.0), larger numbers of cases and controls are needed. Various candidate gene studies on LTM pharmacogenomics were performed in small study populations (<100 patients), and this might have resulted in a low power to detect genetic variations with a low or

moderate effect influencing treatment response. There is also a large heterogeneity in the patient populations studied within asthma pharmacogenomics. In our review, we did not find remarkable differences between the different ethnic groups studied and associations between genetic variants and response to medication. Differences in response to medication among different ethnicities have been previously shown [89–91]. The majority of the study populations in our review included only adults or a combination of adults and children. The biological factors influencing the response to treatment in children might differ from adults, and disease phenotypes vary between children and adults with asthma [92]. This could lead to failed replication when combining adult and paediatric data. For example, a genetic variation influencing *FBXL7* expression has been found to be associated with an improvement in asthma symptoms in response to ICS in two independent paediatric asthma cohorts, but failed to be replicated in an adult asthmatic population [46]. In addition, rs28364072 within *FCER2* was found to be associated with response to ICS in paediatric patients [33, 38, 43], but as this SNP has only been studied in paediatric populations, its influence on response to ICS in adult patients remains unclear. These examples imply that findings from the studies in paediatrics cannot easily be generalized to the adult asthma population and vice versa. The comparability of results is further complicated by the heterogeneity in outcome definitions. Different outcome phenotypes might reflect different aspects of the disease [26, 42]. Even within one dimension of the disease, such as exacerbations, there was no consistency in how this disease aspect was defined (i.e. emergency room visits, hospitalizations, need for treatment with oral corticosteroids, and a decrease in peak expiratory flow rate in the morning of at least 25%) [56, 57]. The most often used outcome parameters were lung function measurements. However, exacerbations cause stress and high expenses to both the patients and the healthcare system [93] and, therefore, considering these parameters as response phenotypes is important. Patient-centred outcomes, such as asthma symptoms, were not frequently studied. In summary, the consideration of different outcome phenotypes on the pharmacogenomics analysis of asthma is crucial; yet, there is also a need to standardize the definitions of response phenotypes.

In conclusion, there is still not enough evidence to implement genetic testing in clinics before starting a patient on medication. To provide a larger body of evidence, large sample sizes of well-phenotyped asthmatic patients and different standardized outcome phenotypes should be taken into account in pharmacogenomics studies. Collaborations and large meta-analysis might show more evidence of the influence of genetic markers on the response of asthma to anti-inflammatory medication.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. PubMed search terms for LTMs.

Table S2. PubMed search terms for ICS.

Table S3. Candidate gene studies of response to ICS.

Table S4. Candidate gene studies of response to LTMs.

Table S1. PubMed search terms for LTMs.

"pharmacogenetics"[MeSH Terms] OR "pharmacogenetics"[All Fields] OR "pharmacogenomics"[All Fields] OR "polymorphism, genetic"[MeSH Terms] OR "polymorphism"[All Fields] AND "genetic"[All Fields] OR "genetic polymorphism"[All Fields] OR "polymorphism"[All Fields] OR "polymorphism, genetic"[MeSH Terms]
AND
("leukotriene antagonists"[All Fields] OR "leukotriene modifiers"[All Fields] OR ("leukotriene antagonists"[Pharmacological Action] OR "leukotriene antagonists"[MeSH Terms] OR ("leukotriene"[All Fields] AND "antagonists"[All Fields]) OR "leukotriene antagonists"[All Fields] OR "antileukotrienes"[All Fields]))

Table S2. PubMed search terms for ICS

"pharmacogenetics"[MeSH Terms] OR "pharmacogenetics"[All Fields] OR "pharmacogenomics"[All Fields] OR "polymorphism, genetic"[MeSH Terms] OR "polymorphism"[All Fields] AND "genetic"[All Fields] OR "genetic polymorphism"[All Fields] OR "polymorphism"[All Fields] OR "polymorphism, genetic"[MeSH Terms]
AND
"inhaled corticosteroid"[All Fields] OR "inhaled glucocorticoids"[All Fields] OR "inhaled glucocorticosteroid"[All Fields]

Table S3. Candidate gene studies of response to ICS.

Gene and SNPs	Study population	Design	Medication	Ethnicity/country	Definition of response	Study outcome	Study
<i>TBX21</i> (chr11) rs2240017	Pediatric population (n=139)	4-year clinical trial	Budesonide 200 µg twice daily	Caucasian	Change in FEV1% AHR to methacholine (PC20)	The estimated mean increase in log-transformed PC20 was 3.5 fold more in patients carrying mutant allele (MAF (G) = 0.04) compared to wild-type homozygotes (P=0.0001). There was no association found with changes in FEV1%.	Tantisira <i>et al.</i> 2004
131 SNPs in 14 genes Ref [53]: <i>ALOX15</i> (17p13.3) <i>CRH</i> (8q13) <i>CRHBP</i> (5q13.3) <i>CRHR1</i> (17q21.31) <i>FCER2</i> (19p13.3) <i>GATA3</i> (10p15) <i>HSD11B1</i> (1q32-q41) <i>IL18BP</i> (11q13) <i>MAPK8</i> (10q11.22) <i>NFATC4</i> (14q11.2) <i>NR3C1</i> (5q31.3) <i>POMC</i> (2p23.3) <i>STAT3</i> (17q21.31) <i>STAT5A</i> (17q11.2)	children and adults from: Adult study (Adults, n=415) CAMP (Children, n=201) ACRN (Adults, n=224)	Three independent 6-8 week Clinical trials	Flunisolide 1000µg daily (increased up to 2000µg if necessary) Budesonide 200 µg twice daily Triamcinolone 400 µg twice daily	Caucasian	Changes in FEV1% from baseline	The FEV1 % change was 13.28 ± 3.11 and 17.80 ± 6.77 for the minor allele of rs242941 (MAF (T) = 0.3) homozygous compared with 5.49 ± 1.40 and 7.57 ± 1.50 for those homozygous for the wild-type allele in adult study (P=0.02) and CAMP (P=0.006), respectively. Homozygotes for the minor T allele of rs1876828 had an average increase in their FEV1 of 23.72 ± 9.75 compared with 5.14 ± 1.31% for homozygotes for the common allele in patients from ACRN.	Tantisira <i>et al.</i> 2004
<i>IL-13</i> (5q31) rs1800925 rs20541 rs848 rs2243204 rs1881457	Pediatric population (n=503)	CAMP: Clinical trial Costa Rica study: asthma cohort	Budesonide 200 µg twice daily	Caucasian	ED visits and Hospitalizations (at least once during the first 4 years of the trial)	Rs1800925 (MAF (T) = 0.2) was significantly associated with increased risk of asthma exacerbations in white (non-Hispanic) children on ICS (P=0.02).	Hunninghake <i>et al.</i> 2007
<i>FCER2</i> (19p13.3) rs28364072	Children from CAMP (n=311)	4-year clinical trial	Budesonide 200 µg twice daily	African-American and Caucasian	Severe exacerbations: -Emergency department visits and/or hospitalizations.	Significant associations found between rs28364072 and severe exacerbations in both white and African-American children (hazard ratio, 3.95; 95% CI, 1.64-9.51; and hazard ratio, 3.08; 95% CI, 1.00-9.47).	Tantisira <i>et al.</i> 2007

<i>NR3C1</i> (5q31.3) rs6190 rs41423247 rs6195 rs10052957	Pediatric population (n=113)	Observational study	Budesonide and fluticasone	Caucasian	Asthma control -Assessment of the increased demand for ICS.	There was no association between these SNPs and response to ICS.	Szczepaniewicz <i>et al.</i> 2008
<i>CRHR1</i> (17q21.31) rs1876828 rs242941 rs242939	Adults (n=98)	Observational study	The dose of ICS was calculated to an equivalent daily dose of beclomethasone.	Not mentioned	Changes in FEV1 from baseline (immediate effect) Rate of decline in FEV1 annually (long-term effect)	No associations were found between SNPs and the outcomes.	Dijkstra <i>et al.</i> 2008
<i>IPO13</i> (1p34.1) rs6671164 rs4448553 rs1990150 rs2240447 rs2486014 rs2301993 rs2301992 rs1636879 rs7412307 rs2428953	Pediatric population (CAMP children, n=654)	8-month Clinical trial	Budesonide 200 µg twice daily	Caucasian	changes in methacholine airway hyper-responsiveness (AHR-PC20).	There was no association between these SNPs and change in methacholine PC20 in patients treated with budesonide.	Raby <i>et al.</i> 2009
<i>TBX21</i> (chr11) rs2240017 C>G <i>NK2R</i> (10q22.1) G231E G>A <i>ADRB2</i> (5q31-q32): 46 A>G <i>ADCY9</i> (16p13.3) 1772M T>C	Adults (n=53)	12-week clinical trial	Budesonide 400 µg daily	Korean	Asthma symptoms control based on GINA guidelines Change in FEV1% predicted Changes in PEF% predicted Changes in AQLQ	Among mutant allele carriers for rs2240017 (MAF (G) = 0.12) and G231E G>A (MAF (A) = 0.4) higher proportion of patients had not well-controlled asthma compared to wild-type carriers (P= 0.006 and 0.041, respectively). There was a significant association between G231E G>A and changes in FEV1%pred. Patients carrying G allele had 19.53% improvement in FEV1% pred compared to A allele carriers with 15.75% improvement in FEV1% pred (P= 0.03). There was no statistically significant association between changes in FEV1%pred and rs2240017. None of the other SNPs showed any statistically significant associations with the outcomes.	Y-M Ye <i>et al.</i> 2009
<i>CRHR1</i> (17q21.31) rs242941	Children from CAMP (n=311)	4-year clinical trial	Budesonide 200 µg twice daily	African-American	Exacerbations: - Emergency Department	The minor allele (MAF (T)=0.3) of rs242941 showed a significant	Rogers <i>et al.</i>

<i>FCER2</i> (19p13.3) rs28364072				and Caucasian	visit - Hospitalization - Oral prednisone burst. Lung function measurements: change in FEV1% predicted: ≤7.5% considered as poor responders	association with poor response based on lung function (OR: 1.6, CI 95% 1–2.7, P=0.05). The minor allele of rs28364072 in <i>FCER2</i> was associated with recurrent exacerbations in patients with Caucasian ethnicity (OR: 1.9 for minor allele, P < 0.05) but it did not reach significance in multivariate analysis (OR: 1.4, p = 0.18). The minor allele of rs28364072 was also associated with poor lung function (OR 2.1 1.2–3.5, P= 0.006).	2009
<i>HSPCB</i> (6p12) <i>HSPCA</i> (14q32.33) <i>HSPA8</i> (11q24.1) <i>DNAJB</i> (19p13.2) <i>PTGES3</i> (12q13.3) <i>FKBP5</i> (6p21.31) <i>FKBP4</i> (12p13.33) <i>STIP1</i> (11q13)	Adults (n=382)	4-8 week Clinical trial	Beclomethasone Flunisolide triamcinolone fluticasone propionate	Caucasian	Changes FEV1% from baseline	FEV1% change at 4 weeks for rs6591838 (MAF (G) = 0.2) (18.41±26.15 for GG vs 4.57±16.82 for AA) and rs2236647 (MAF (T) = 0.4) (12.05±23.99 for TT vs 5.23±17.52 for CC) within <i>STIP1</i> were significantly associated with the response to ICS (P= 0.02 and 0.04, respectively). rs6591838 (MAF (G) = 0.2) (20.7 ±28.29 for GG vs 5.5±17.63 for AA) and rs1011219 (MAF (A) = 0.12) (6.92±18.74 for GG vs -36.85±18.59 for AA) were associated with FEV1% change at 8 weeks (P= 0.01 and 0.005, respectively).	Hawkins <i>et al.</i> 2009
<i>DUSP1</i> (5q34) rs881152	Adults (n=165)	6-week clinical trial	Beclomethasone 160 µg twice daily	African-American	Change in FEV1% from baseline. Changes in self-reported asthma control (ACT)	There was no association between rs881152 (MAF (A) = 0.14) and changes in FEV1% but the SNP was significantly associated with ACT score changes over 6 weeks of treatment with ICS. Mean change for each genotype: GG: 2.81±6.22, AG: 1.38±7.36 and AA: -3.5±9.13 (P= 0.01)	Jin <i>et al</i> 2010
<i>CTLA4</i> (2q33) rs3087243	Pediatric population (n=102)	4-week clinical trial	fluticasone 200 µg daily for children <12 and 400 µg daily for children >12.	Slovenian	Changes in %FEV1 pred AHR to Methacholine PC20 and logPC20 FEV1/FVC ratio.	Atopic asthmatics homozygous for the A allele of rs3087243 (MAF (A) = 0.4), had 21.7% increase in FEV1%pred compared with an 8.6% increase in heterozygotes and a 5.8% increase in G allele homozygotes (P <0.01).	Berce <i>et al.</i> 2010

<i>ARG1</i> (6q23) rs2781667 rs2781668 rs17599586 <i>ARG2</i> (14q24.1) rs2145467 rs2295643 rs17249437 rs17249444 rs12896052 rs7140310 rs3742879 rs10483801	Adults (n=200)	Observational study	Not mentioned	The Netherlands	Annual FEV1 decline changes in FEV1 upon bronchodilator.	Annual FEV1 decline was significantly higher in homozygotes for the C allele of the <i>ARG1</i> rs2781667 (MAF (T)= 0.28) compared to the T allele carriers. (19.9ml/year, 95% CI: 4.8–35.0) (P <0.01). Patients carrying mutant allele for rs7140310 (MAF (G) = 0.13) and rs10483801 (MAF (A) = 0.16) within <i>ARG2</i> had higher bronchodilator response (P=0.007 and P=0.03, respectively) The other investigated SNPs in <i>ARG1</i> and <i>ARG2</i> did not show a significant association with the FEV1 decline.	Vonk <i>et al.</i> 2010
<i>WDR21A</i> (14q24.3) rs7142086 rs3213729 rs7160796 rs12892806 rs2302588 rs12885397 rs3742832 rs2240981 rs2806039 rs7143683 rs7144738	adolescents and adults (n=230)	4-week clinical trial	Fluticasone propionate 1000 µg daily	Korean	Changes in FEV1% pred from baseline	Patients homozygous for variant allele of rs7160796 (MAF (C) = 0.24) had a greater change in FEV1 (58.04%) compared to homozygotes for wild-type (24.51%) or heterozygotes (24.51%) (P= 0.04).	Cho <i>et al.</i> 2011
<i>CDH1</i> (16q22.1) rs2902185 rs11075699 rs1125557 rs1212597188 rs7199991 rs7186053 rs10431924 rs4783573 rs7188750 rs8056633 rs4783689 rs16958383 rs2276330 rs1801552	Two separate study populations including 57 and 146 adults using ICS	Observational study	All doses were recalculated as beclomethasone equivalents	Northern region of the Netherlands	Annual decline in FEV1 post bronchodilator FEV1/VC ratio	The minor alleles of rs8056633 (G), rs16958383 (A), rs7203904 (C) and rs17690554 (G) SNPs were significantly associated with less FEV1 decline. Rs7188750 (A), rs4783573(A), rs3785078 (C), rs8056633 (G), rs16958383 (A), rs7203904 (C) and rs17690554 (G) SNPs were associated with higher post bronchodilator FEV1/VC in the presence of ICS in population 2. Rs7203904 had a statistically significant association with post bronchodilator FEV1/VC in population 1. The minor alleles of rs1125557 (G),	Ierodiakonou <i>et al.</i> 2011 [ref 31]

rs3785078 rs7203904 rs17690554						rs7199991 (C) and rs7186053 (A) were significantly associated with accelerated FEV1 decline in population 2.	
<i>SERPINE1</i> (7q22.1) rs1799889: 4G/4G 4G/5G 5G/5G	Adults (n=281)	Observational study	Not mentioned	The Netherlands	Changes in FEV1 from baseline	ICS treatment showed an immediate improvement in FEV1 in asthmatics carrying the 5G allele (4G/5G or 5G/5G) (increase in mean FEV1: 189.9 mL, 95% CI 30.5–349.2 mL) this improvement was absent in 4G/4G (-63.1 mL, 95% CI -298.3–172.1 mL) (P= 0.04).	Dijkstra <i>et al.</i> 2011
<i>CD14</i> (5q31.1) rs2569190 (-159C/T)	Pediatric population (n=247)	4-week clinical trial	Fluticasone propionate	Slovenian	Changes in FEV1% pred from baseline.	No association between the SNP and the response to medication.	Perin <i>et al.</i> 2011
<i>FCER2</i> (19p13.3) rs28364072	Children and adolescents from three study populations: PACMAN (n=386) BREATHE (n=939) CAMP (n=311)	PACMAN & BREATHE: asthma cohorts CAMP: 4-year clinical trial	Not mentioned	Caucasian	Exacerbations: - Emergency department visits - Hospitalization Asthma symptoms: - ACQ- scores - Respiratory symptoms (wheeze, shortness of breath and cough) - Asthma-related sleep disturbances - Asthma-related limitations in daily activities - Additional (airway) medication use during the preceding 12 months.	In the meta-analysis, the rs28364072 variant was associated with increased risk of asthma-related hospital visits (OR: 2.38, 95%CI: 1.47–3.85, P= 0.0004). The <i>FCER2</i> variant was associated with increased risk of uncontrolled asthma measured by ACQ scores (OR: 2.64, 95%CI: 1.00–6.98) and was associated with increased daily steroid dose (OR: 2.46, 95%CI: 1.38–4.39).	Koster <i>et al.</i> 2011
<i>VEGFA</i> (6p12) rs2146323 rs833058	Pediatric population (n=131)	12-month clinical trial	Fluticasone propionate 250 µg daily children < 7 and children >7 400 µg daily	Slovenia	Changes in FEV1% pred Changes in FEV1/FVC ACT (scores <20 defined as uncontrolled)	Patients with AA genotype for rs2146323 (MAF (A)= 0.3) showed a greater improvement in the FEV1% pred (9% increase) than patients with AC or CC (5% increase) after 12 months (P= 0.03). No association was found with ACT and FEV1/FVC.	Balantic <i>et al.</i> 2012
<i>HDAC1</i> (1p34) rs1741981	Pediatric population(n=70)	8-week clinical trial	Not mentioned	Korean	Changes in FEV1% from baseline	Subjects with the CC genotype of rs1741981	M. Kim <i>et al.</i>

<i>HDAC2</i> (6q21) rs58677352						(MAF (C) = 0.47) showed significantly lower FEV1% (14.1% ± 5.9%) increases in response to ICS compared with the CT or TT genotype carriers (19.4% ± 8.9%) (P = 0.03). There was no association between rs58677352 and lung function.	2013
<i>CYP3A4</i> (7q21.1) rs35599367 rs2246709 rs4646437 <i>CYP3A5</i> (7q21.1) rs776746 rs10264272 rs15524 <i>CYP3A7</i> (7q22.1) rs2687133 rs2257401 rs2740565	Pediatric population (n=268)	observational study	Fluticasone Propionate	Hispanics and non-Hispanics	Asthma control scores ranged from 0 (well-controlled) to 15 (poorly-controlled).	Among children with rs35599367 (<i>CYP3A4</i> *22) variant allele (T, frequency=0.04), average asthma control scores were significantly lower (2.9 ± 2.2) compared with the CC carriers (5.0 ± 3.7) (P=0.02).	Stockmann <i>et al</i> 2013
<i>ORMDL3</i> (17q12) rs2872507	Pediatric population (n=311)	Observational study	fluticasone 200 µg daily for children <12 and 400 µg daily for children >12.	Caucasian	Change in FEV1% pred AHR to methacholine (PC20) Changes in FEV1/FVC	Patients with AA genotype of rs2872507 (MAF (G) = 0.38) showed statistically significant improvements in FEV1% pred (13.3%) compared to GG carriers (4.9%) (P= 0.02).	Berce <i>et al.</i> 2013
<i>TBX21</i> (chr11) rs9910408	Adults (n=208)	Observational study	Not mentioned	White asthmatics	Change in FEV1% pred AHR to methacholine (PD20) Changes in ACT and AQLQ scores	Patients with AA genotype of rs9910408 (MAF (G) = 0.4) had a greater decrease of AHR compared to GG genotype (P= 0.049, OR:2.74, 95%CI 1.06-7.06). Patients with AA genotype had a greater improvement of FEV1 compared to GG carriers (P= 0.01, OR:5.78, 95%CL 1.49-22.37) Non-atopic patients with AA had higher improvements in AQLQ scores compared to GG (P= 0.04, OR:23.82, 95% CI: 1.05-542.70). In total study population (atopic + non-atopic), no	Lopert <i>et al.</i> 2013

						association was found between rs9910408 and ACT/AQLQ scores.	
<i>GLCCI1</i> (7p21.3) rs37973	adolescents and adults (n=1916)	Seven 8 -12 week clinical trials	Various doses of Fluticasone furoate (ranged between 25-800 µg daily) or Fluticasone propionate (100-500 µg twice daily)	Caucasian	Changes in FEV1 from baseline	There was no significant association between changes in FEV1 and rs37973 genotypes.	Hosking <i>et al.</i> 2014
<i>GLCCI1</i> (7p21.3) rs37973 <i>STIP1</i> (11q13) rs1011219 rs6591838 <i>T gene</i> (6q27) rs3127412	Adults (n=224)	Observational study	ICS maintenance dose varied between patients	Japanese	Annual decline in FEV1	Rs37973 GG was associated with a decline in FEV1 of 30ml/year or more (estimated effect: 1.10, 0.02 to 2.18 0.047) There was no association between rs37973 genotypes and the outcome, when decline in FEV1 was analyzed as a continuous variable. There was no association between annual decline in FEV1 and <i>STIP1</i> and <i>T gene</i> SNPs.	Izuhara <i>et al.</i> 2014
<i>GLCCI1</i> (7p21.3) rs37972	Children and adolescents from three study populations: BREATHE (n=1037) PACMAN (n=431) PAGES (n=323)	Meta-analysis of three pediatric asthma cohorts	Budesonide 200 µg twice daily in CAMP	Caucasian	Exacerbations: - Hospital visits - OCS use Asthma symptoms: - ACT scores ≤ 19 (not well controlled) - ACQ-scores ≥ 1.5 (not well controlled)	There was no significant association between increased risk of OCS use, increased risk of asthma exacerbations and rs37972 genotypes	Vijverberg <i>et al.</i> 2014
<i>IL-10</i> (1q31-q32) rs1800871 rs1800896	Adolescents and adults (n=419)	2 months Case-Control	Not mentioned	South India	Changes in FVC (mL) from baseline. Changes in FEV1 (mL) from baseline.	No statistically significant difference among SNPs variants and lung function measurements. P < 0.016 was considered as statistically significant by applying Bonferroni correction.	Raeiszadeh Jahromi <i>et al.</i> 2015

<i>IL-17F</i> (6p12) rs1889570							
<i>TAAR6</i> (6q23.2) rs11961899 rs9402427 rs9493381 rs9402428 rs9373024 rs9389007 rs9373026 rs8192624 rs8192625 rs7772821 rs11154685 rs4305746 rs6912930 rs17802869 rs6903874	Adolescents and adults (n=246)	4-week Prospective controlled trial	Fluticasone propionate 1000µg daily	Korean	Changes in %FEV1 from baseline	The changes in FEV1% was higher for the GG carriers of rs7772821 (MAF (G) = 0.22) (60.77%) than the patients with the T/G or T/T genotypes (21.32 and 31.60%, respectively) (P=0.002 in the co-dominant model).	Chang <i>et al.</i> 2015
<i>NR3C1</i> (5q31.3): rs4607376 rs4912912 rs7701443 rs9324924 rs2963155 rs4912905 rs6865292 rs17209258 rs6196 <i>FKBP4</i> (12p13.33): rs1981655 rs11833878 <i>ST13</i> (22q13.2): rs138335 rs138337 <i>SERPINA6</i> (14q32.1): rs1956179 rs7158343 rs10498639 rs1998056 rs2281518 rs2281519 rs2281520	children and adolescents: PACMAN (n=357) BREATHE (n=820) PAGES (n=391) CAMP (n=172) PASS (n=172) GALA 2 (n=745)	A meta-analysis of 6 asthmatic populations	Budesonide 200 in CAMP	Caucasians and Hispanics	Asthma exacerbations: Asthma-related hospital visits OCS use as a rescue medication	Major allele (G) of the rs138335 SNP within the <i>ST13</i> gene was associated with increased risk of asthma-related hospital visits OR = 1.22 (P = 0.013) and OCS use OR = 1.22 (P = 0.0017), in the past 12 months.	

rs11629171 <i>CREBBP</i> (16p13.3): rs130021 rs11076787 rs886528 rs2526689 <i>TBP</i> (6q27): rs2235506 rs3800235 <i>NCOA3</i> (10q11.2): rs2425941 rs6066394 rs2143491 rs6018600 rs11700063 <i>SMAD3</i> (15q22.33): rs744910 <i>ARG1</i> (6q23): rs2781667 <i>17q21 locus</i> : rs7216389 <i>IL2RB</i> (22q13.1): rs2284033 <i>PDE4D</i> (5q12): rs1544791 rs1588265							
<i>17q12-21 locus</i> 47 SNPs ref [28]	Pediatric population (n=110)	Clinical trial	Fluticasone (100 µg to 200 µg according to the severity of the disease)	Caucasian	Changes in FEV1%pred and AHR to Mch (PD20) Poor treatment response: Decrease in FEV1%pred and low PD20 despite high levels of treatment during the trial despite high levels of treatment.	rs72821893 (MAF (T) = 0.03) in <i>KRT25</i> was significantly associated with FEV1%pred ($P = 3.75 \times 10^{-5}$), Mch PD20 ($P = 0.00095$) and poor treatment response based on Mch PD20 ($P = 0.006$).	Leusink <i>et al.</i> 2015

ACQ, Asthma Control Questionnaire; ACRN, Asthma Clinical Research Network; ACT, Asthma Control Test; AHR, airway hyper-responsiveness; AQLQ, Asthma quality of life questionnaire; CAMP, Childhood Asthma Management Programme; ED, emergency department; FEV1, forced expiratory volume in 1 second; FEV1% pred, percentage of predicted FEV1; FVC, Forced vital capacity; ICS, inhaled corticosteroids; OCS, oral corticosteroid; OR, odds ratio; PACMAN, Pharmacogenetics of Asthma Medication in Children: Medication with Anti-Inflammatory effects; PC20, provocation concentration causing a 20% fall in FEV1; PD20, provocation dose causing a 20% decline in FEV1.

Table S4. Candidate gene studies of response to LTMs.

Gene and SNPs	Study population	Design	Medication	Ethnicity /country	Definition of response	Study outcome	Study
<i>ALOX5</i> (10q11.2) tandem repeats of the Sp1-binding domain	adult asthmatics (n=114)	12-week Clinical trial	ABT-761, 300 mg daily	Not mentioned	Changes in FEV1% from baseline	The mean change in FEV1 from baseline was 18.8±3.6% in wild-type (5/5) patients and 23.3±6.0% in heterozygous (5/X) patients compared to the patients with the mutant genotype (X/X) with the change in FEV1 of -1.2±2.9% ($p < 0.0001$ and $p < 0.0006$) (MAF (X) = 0.23).	Drazen <i>et al.</i> 1999
<i>LTC4S</i> (5q35) Rs730012	Adults (n=23)	2-week clinical trial	Zafirlukast 20 mg twice daily	Not mentioned	FEV1% change from baseline FVC % change from baseline PEF % change from baseline.	No significant association found	Sampson <i>et al.</i> 2000
<i>ALOX5</i> (10q11.2) tandem repeats of the Sp1-binding domain	adult atopic asthmatics (n=52)	Retrospective analysis of 4 placebo-controlled trials	Montelukast/ zafirlukast	Scotland	AHR to AMP	There were no statistically significant differences between heterozygotes and homozygotes for the wild-type allele (MAF (X) = 0.11).	Fowler <i>et al.</i> 2002
<i>LTC4S</i> (5q35) Rs730012	Adults with EIB (n=50)	4-week clinical trial	Pranlukast 225 twice daily	Japanese	Changes in maximal % fall in FEV1 after exercise challenge test from baseline. Responders were defined as children showing 10% post-treatment improvement.	C allele carriers responded better to the treatment compared with the A allele homozygotes ($P < 0.01$).	Asano <i>et al.</i> 2002
<i>LTC4S</i> (5q35) Rs730012	Adults (n=26)	8-week clinical trial	Montelukast 10 mg daily	Not mentioned	Responders we defined as: Increase in PEF ≥7% Improvement of daytime and nocturnal symptoms by a score of ≥0.5 Decrease in the use of b2-agonists by ≥50%, Improvement in the Quality of Life Questionnaire (QLQ) by ≥1	No significant association found.	Mastalerz <i>et al.</i> 2002
<i>LTC4S</i> (5q35) Rs730012	Children/ad adolescences (n=12)	3-week clinical trial	Montelukast 5 mg daily for patients < 15 10 mg for patients ≥ 15 years of age.	Caucasian and African-American	Changes in FENO from baseline Changes in FEV1% pred from baseline	No statistically significant results	Whelan <i>et al.</i> 2003
<i>LTC4S</i> (5q35) Rs730012	(n=78) Not mentioned	retrospective analysis of 8 randomized,	Montelukast Zafirlukast	Not mentioned	AHR to AMP and Methacholine.	No association significant found	Currie <i>et al.</i> 2003

		placebo- controlled trials					
<i>ALOX5</i> (10q11.2) rs2115819 rs2029253 rs745986 rs892690 rs892691 core promoter tandem repeats <i>LTA4H</i> (12q22) rs2660845 rs2241136 rs2247570 <i>LTC4S</i> (5q35) rs272431 rs272440 rs730012 <i>MRP1</i> (16p13.1) rs119774 rs129081 rs152033 rs1967120 rs212081 rs215066 rs2239330 rs2239996 rs246221 rs35587 rs3887893 rs3902401 rs4148356 <i>CYSLTR1</i> (Xq13.2- q21.1): rs2412222 rs320995 rs321029 rs321092	adult asthmatics (n=61)	6-month Clinical trial	Montelukast 10 mg daily	Caucasian	Changes in FEV1% pred from baseline Asthma exacerbation rate - One or more of the following: a more than 30% decrease in peak expiratory flow rate for two consecutive days, a course of oral steroids, an unscheduled visit to the clinic, the emergency room, or hospital or an increase of four puffs of rescue inhaler use in one day	Significant associations found between rs2115819 (MAF (G) = 0.47) in <i>ALOX5</i> and rs119774 (MAF (T) = 0.07) in <i>MRP1</i> and the changes in FEV1% pred p<0.05. Significant association found between rs730012 (MAF (C) = 0.3) (<i>LTC4S</i>), rs2660845 (MAF (G) = 0.29) (<i>LTA4H</i>) and exacerbation rates (p values 0.023 and <0.001 respectively) Patients homozygous for the mutant allele of tandem repeats (MAF (X) = 0.2) in <i>ALOX5</i> had significant reduction in exacerbation rates (P=0.04).	Lima <i>et al.</i> 2006
<i>LTC4S</i> (5q35) rs730012	Asthmatic children with EIB	8-week clinical trial	Montelukast 5 mg daily	Korean	Changes in maximal % fall in FEV1 after exercise challenge test from baseline.	No significant association found	S-Y Lee <i>et al.</i> 2007

<i>CYSLTR1</i> (Xq13.2-q21.1): rs320995	(n=100)				Responders were defined as children showing 10% post-treatment improvement		
<i>PLA2G4A</i> (1q31.1): rs3736741 rs2307200 <i>LTC4S</i> (5q35): rs730012 <i>CYSLTR1</i> (Xq13.2-q21.1): rs320995 <i>CYSLTR2</i> (13q14.2): rs912278 rs912277 <i>ALOX5AP</i> (13q12): rs3803278 rs12721458 rs3803277 <i>CYP3A4</i> (7q21.1): rs1057910 rs2740574 <i>CYP2C9</i> (10q21): rs1799853 <i>ADRB2</i> (5q31-q32): rs1042713 rs1042714 rs1042711 <i>NR3C1</i> (5q31.3): rs6188 rs6196 rs6190 rs6195 <i>ALOX5</i> (10q11.2): rs4987105 rs4986832 rs2229136 rs2228064 tandem repeats of the core promoter	Adolescents and adults (n=166)	12-week Clinical trial	Montelukast 10 mg daily	Caucasian, Hispanic and African-American	Changes in morning PEF Changes in FEV1% pred from baseline	Significant associations found between change in morning PEF and rs912277 (MAF (G) 0.07) (P=0.02) and rs912278 (MAF (G) = 0.41) within <i>CYSLTR2</i> (P=0.02), rs4987105 (MAF (T) = 0.19) (P=0.01), rs4986832 (MAF (A) = 0.19) (P=0.01) and tandem repeats of the core promoter (MAF (X) = 0.24) (P=0.05) within <i>ALOX5</i> . Significant associations found between the changes in FEV1% pred and rs6188 (MAF (A) = 0.29) (P=0.01) and rs6196 (MAF (G) = 0.14) (P=0.02) within <i>NR3C1</i> , rs1042714 (MAF (G) = 0.39) (P= 0.02) and rs1042711 (MAF (C) = 0.39) (P=0.02) within <i>ADRB2</i> .	Klotsman <i>et al.</i> 2007
<i>ALOX5</i> (10q11.2) tandem repeats of the Sp1-binding domain	Adolescent and adult atopic	6-month clinical trial	Montelukast	Spain	Changes in FEV1% pred from baseline Number of exacerbations:	Homozygotes for the mutant allele had a rate of asthma exacerbations of 1.88±0.92, with a mean reduction of	Telleria <i>et al.</i> 2008

	asthmatics (n=61)				-A decrease in peak expiratory flow rate in the morning of at least 25% compared with baseline.	1.33±1.22 and patients homozygous for the common allele and heterozygotes had an exacerbation rate of 0.4±0.21 with a reduction of 4.41±2.76 (P= 0.001) (MAF (4 tandem repeats) = 0.4) Homozygotes for the common allele and heterozygotes had a mean increase of 8.6%±7.6 in FEV1%pred compared to the homozygotes for the mutant allele with a mean decrease of 1.4%±8.04 in FEV1% pred (P=0.0006).	
<i>IL-13</i> (5q31) – 1512A/C – 1112C/T + 2044G/A	Asthmatic children with EIB (n=80)	8-week clinical trial	Montelukast 5 mg daily	Korean	Changes in maximal % fall in FEV1 after exercise challenge test from baseline. Responders were defined as children showing 20% post-treatment improvement.	Significant association found between -112C/T (MAF (T) = 0.2) and the outcome (P=0.02).	Kang M-J <i>et al.</i> 2008
<i>TBXA2R</i> (19p13.3) +795T>C +924T>C	Asthmatic children with EIB (n=100)	8-week clinical trial	Montelukast 5 mg daily	Korean	Changes in maximal % fall in FEV1 after exercise challenge test from baseline. Responders were defined as children showing 10% post-treatment improvement.	No significant associations were found between any of the SNPs and the outcome. In combination of 2 SNP, patients with the +924T>C TT (MAF (C) = 0.2) homozygote and +795T>C (MAF (C) = 0.36) hetero- or homozygote (CT or CC) had a 3.67-fold poorer response compared with the patients with the common alleles (OR: 3.67; 95% CI: 1.15–11.15).	Kim J-H <i>et al.</i> 2008
<i>LTC4S</i> (5q35) Rs730012	Adults (n=50)	4-weeks clinical trial	Pranlukast 225 mg daily	Japanese	Changes in %FEV1 from baseline	Significant association found between increases in FEV1% and C allele carriers (MAF (C) = 0.34) (P = 0.03)	Asano <i>et al.</i> 2009
<i>ALOX5</i> (10q11.2): rs2115819 rs2029253 rs745986 rs892690 <i>CysLTR1</i> (Xq13.2-q21.1): rs2412222 rs320995 rs321029	Children, adolescence and adults (n=239)	12-week Clinical trial	Zileuton (CR, 1,200 mg twice daily or immediate-release (IR), 600 mg 4 times daily)	Caucasian	Changes in FEV1% pred from baseline	There were significant associations with the outcome for several SNPs: <i>ALOX5</i> (rs2115819) (P=0.01), (rs892690) (P=0.01), (rs2029253) (P=0.03). <i>MRP1</i> (rs119774, rs215066) (P=0.05). <i>LTC4S</i> (rs272431) (P=0.005). None of these findings met the Bonferroni correction adjusted P values.	Tantisira <i>et al.</i> 2009

rs321092 <i>LTA4H</i> (12q22): rs2660845 rs2241136 rs2247570 <i>LTC4S</i> (5q35): rs272431 rs272440 <i>MRP1</i> (16p13.1): rs119774 rs129081 rs152033 rs1967120 rs212081 rs215066 rs2239330 rs2239996 rs246221 rs35587 rs3887893 rs3902401 rs4148356							
<i>ALOX5AP</i> (13q12) rs10507391 rs9551963 <i>LTA4H</i> (12q22) rs17525488 rs2540493 rs2540491 rs2540487	Children and adolescents (n=649)	Clinical trial	Not specified	Hispanic (Puerto Ricans and Mexicans)	Change in FEV1% upon SABA (albuterol)	Significant associations were found for two SNPs within the <i>LTA4H</i> gene (rs2540491 (MAF (A) = 0.37) (P=0.008) and rs2540487 (MAF (A) = 0.2) (P=0.03)) and the outcome. After stratifying by ethnicity, these associations were found only for Puerto Ricans. No significant association between SNPs within <i>ALOX5AP</i> and other SNPs of <i>LTA4H</i> and the outcome.	Tcheurek djan <i>et al.</i> 2010
<i>LTC4S</i> (5q35) rs730012 <i>PTGDR</i> (14q22.1) rs803010 rs8004654	Asthmatic children with EIB (n=92)	8-week clinical trial	Montelukast 5 mg daily	Korean	Changes in maximal % fall in FEV1 after exercise challenge test from baseline. Responders were defined as children showing 10% post-treatment improvement.	Statistically significant association with rs803010 (PTGDR, MAF (C)= 0.25)) (P=0.04). Higher number of non-responders were heterozygous and homozygous for the C allele. Two SNPs within PTGDR are in high LD (D'=0.978). No association found for rs730012	Kang <i>et al.</i> 2011

						(LTC4S, (MAF(C)=0.2).	
AGT (1q42.2) 38 SNPs Ref [69]	Adults with Aspirin Induced Asthmatics (AIA) (n=56)	12-week Clinical trial	Montelukast 10 mg daily	Korean	Aspirin induced rate of FEV1% decline (>15% non-responders, <15% responders)	Significant associations found between 2 SNPs (+2401C>G (rs2478544, MAF (G)= 0.25) (P=0.0009, after correction 0.02) and +2476C>T (rs2478545, MAF (T) = 0.29) (P= 0.002 after correction 0.03) with aspirin induced rate of FEV1% decline.	Pasaje <i>et al.</i> 2011
VEGFA (6p12) rs2146323 rs833058	Two pediatric populations (n=91)	12-month clinical trial	Montelukast 47 children received montelukast regularly (12> years, 5mg/day and >12 years, 10 mg/day) 44 children received montelukast episodically at onset of symptoms (minimum 7 days or until symptoms had disappeared for 48 hours up to maximum 20 days)	Slovenia	Changes in %FEV1 pred from baseline Changes in FEV1/FVC ACT scores (<20 uncontrolled)	Among regular montelukast users, rs2146323 AA genotype was associated with uncontrolled asthma (OR 0.07, 95% CI: 0.009-0.57 (P=0.02)) and for patients using montelukast episodically, AA genotype was associated with reduced FEV1/FVC ratio (P=0.04). Homozygotes for the T allele of rs833058 had improvements in FEV1% pred. However, CC or CT carriers had no improvement in FEV1% pred (P = 0.03)	Balantic <i>et al.</i> 2012
ALOX5 (10q11.2) rs2115819 LTA4H (12q22) rs2660845	Adult (n=21)	4-8 week Clinical trial	Montelukast 10 mg daily	Japanese	Self-measured changes in PEF (L/min) Changes in FEV1 (L) after SABA	Significant associations were found between genotypes of the rs2660845 (MAF(A)=0.42) and changes in PEF and FEV1 (P < 0.05). In AA genotype carriers, PEF significantly improved (more than 50 L/min) compared with G allele carriers with a very low improvement in PEF (P < 0.05). G allele carriers had no improvements in FEV1 compared with AA carriers that showed more than 0.2 L improvements (P<0.05) No significant association between the rs2115819 and the outcomes were reported.	Kotani <i>et al.</i> 2012
169 SNPs within 25 genes: ABCC1 (16p13.1)	Children, adolescents and adult	16-week Clinical trial	Montelukast	Caucasian	Changes in FEV1% pred from baseline Slopes of plots of ACQ scores	Homozygotes for the major alleles of rs739645, rs1876831 (MAF (T) = 0.2), rs1876829 (MAF (C)= 0.2) and	Mougey <i>et al.</i> 2013

<i>ABCC6</i> (16p13.1) <i>ADRB2</i> (5q31-q32) <i>ALOX5</i> (10q11.2) <i>ALOX5AP</i> (13q12) <i>CHRM2</i> (7q31-q35) <i>COL2A1</i> (12q13.11) <i>CPAMD8</i> (19p13.11) <i>CREM</i> (10p11.21) <i>CRHR1</i> (17q21.31) <i>CYSLTR1</i> (Xq13.2-q21.1) <i>F2RL3</i> (19p12) <i>FBN2</i> (5q23.3) <i>HAL</i> (12q23.1) <i>HDAC2</i> (6q21) <i>HSPA8</i> (11q24.1) <i>IMP5</i> (13q32.2) <i>LTA4H</i> (12q22) <i>LTC4S</i> (5q35) <i>MGAT4B</i> (5q35) <i>NR3C1</i> (5q31.3) <i>STIP1</i> (11q13) <i>TFCP2</i> (12q13) <i>TNF</i> (6p21.3) <i>URP2</i> (11q13.1)	(n=60)				versus time	rs1876828 (MAF (T) =0.2) within <i>CRHR1</i> had improvements in FEV1% pred (4.6 ±1.8%) compared to homozygotes for the minor alleles with no improvements in FEV1% pred from baseline ($P=8.6 \times 10^{-3}$). Rs242950 (MAF (T) = 0.09) within <i>CRHR1</i> was significantly associated with ACQ slope. Heterozygotes had reduced asthma symptoms whereas homozygotes for the major allele had an increase in symptoms ($P=3.57 \times 10^{-3}$). Homozygotes for the minor allele and heterozygotes had significant improvements in FEV1% pred from baseline compared to homozygotes for the major allele of rs3757016 (MAF (T) = 0.4) within <i>HDAC2</i>. ($P=8.63 \times 10^{-3}$).	
<i>SLCO2B1</i> (11q13) Rs12422149 rs2306168	20 African-American, 55 Caucasian and 5 Hispanic adult asthmatics (in total 80 patients)	Clinical trial	Montelukast 10 mg	African-American, Caucasian and Hispanic	Montelukast plasma concentration Asthma symptoms utility index (ASUI) was used to measure response to montelukast	Individuals with GG genotype for rs12422149 had significant increases in ASUI after one and 6 months of therapy compared to heterozygotes ($P<0.0001$). There was no homozygous for A (minor allele) in the study population (MAF (A) ranged between 0.08 – 0.2 for different ethnicities) Heterozygotes for rs12422149 had significantly lower plasma concentration (3±1 ng/ml) compared to GG (7±0.9 ng/ml) carriers ($P=0.02$). No significant associations found between rs2306168 and plasma concentrations of montelukast nor asthma symptoms.	Mougey <i>et al.</i> 2009
<i>SLCO2B1</i> (11q13)	Healthy	Clinical trial	Montelukast 10 mg	Finland	Montelukast plasma	The rs12422149 genotype had no	Tapanine

Rs12422149	individuals (n=33)		(single dose)		concentration	significant effect on the plasma concentrations of montelukast.	n <i>et al.</i> 2013
<i>SLCO2B1</i> (11q13) Rs12422149 c.1175C>T c.1457C>T c.43C>T c.601G>A c.644A>T g.-282G>A	Healthy individuals (n=24)	Clinical trial	Montelukast 10 mg (single dose)	korean	Montelukast plasma concentration	No association found between these SNPs and plasma concentrations of montelukast.	K. Kim <i>et al.</i> 2013

ACT, Asthma Control Test; AHR, airway hyper-responsiveness; AMP, adenosine monophosphate; EIB, Exercise-induced bronchoconstriction; FEV1, forced expiratory volume in 1 second; FEV1% pred, percentage of predicted FEV1; FVC, forced vital capacity; OR, odds ratio; PEF, forced expiratory flow; SABA, short acting beta agonist; SP1, specificity protein 1.