



Pharmacogenetics of asthma: toward precision medicine

Elin T.G. Kersten and Gerard H. Koppelman

Purpose of review

Although currently available drugs to treat asthma are effective in most patients, a proportion of patients do not respond or experience side-effects; which is partly genetically determined. Pharmacogenetics is the study of how genetic variations influence drug response. In this review, we summarize prior results and recent studies in pharmacogenetics to determine if we can use genetic profiles for personalized treatment of asthma.

Recent findings

The field of pharmacogenetics has moved from candidate gene studies in single populations toward genome-wide association studies and meta-analysis of multiple studies. New technologies have been used to enrich results, and an expanding number of genetic loci have been associated with therapeutic responses to asthma drugs. Prospective, genotype-stratified treatment studies have been conducted for β 2-agonists, showing attenuated response in children carrying the Arg16 variant in the β 2-adrenoreceptor gene.

Summary

Although there has been much progress, many findings have not been replicated and currently known genetic loci only account for a fraction of variability in drug response. More research is necessary to translate into clinical practice. A polygenic predictive approach integrated in complex networks with other 'omics' technologies could aid to achieve this goal. Finally, to change clinical practice, studies that compare precision medicine with traditional medicine are needed.

Keywords

asthma, pharmacogenetics, precision medicine

INTRODUCTION

Asthma is a chronic inflammatory airway disease that affects more than 300 million people worldwide. It is a heterogeneous disease, with considerable variation in age at onset, clinical expression, and severity, which may reflect heterogeneity in causal pathways. Therefore, asthma is considered by some authors to be a syndrome rather than one disease [1^{*}]. The three main classes of drugs currently prescribed to patients with asthma are β 2-agonists, both short-acting β 2-agonist (SABA) and long-acting β 2-agonist (LABA), inhaled corticosteroids (ICSs), and leukotriene modifiers (LTMs). In addition, in the past years biologicals, such as anti-IgE, soluble IL-4 receptor, anti-thymic stromal lymphopoietin (TSLP), anti-IL-5, and anti-IL-13 have been investigated in patients with severe asthma, specifically targeting cytokines relevant to specific causal pathways in asthma [1^{*}]. The response to asthma treatment is heterogeneous; and there is great interindividual variation in response to

asthma drugs. This variation in treatment response is partly genetically determined. The discovery of genes important in asthma development and treatment response now opens up the opportunity to tailor asthma treatment to the right patient, and make it more personal.

Current international treatment guidelines mostly use a 'one size fits all' approach, where choice of asthma drugs is based on a stepwise treatment protocol for all patients [2]. This protocol is

Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen; GRIAC Research Institute, Groningen, The Netherlands

Correspondence to Elin T.G. Kersten, Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen; GRIAC Research Institute, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel: +31 0 50 361 4213; fax: +31 0 50 361 4235; e-mail: e.t.g.kersten@umcg.nl

Curr Opin Pulm Med 2017, 23:12–20

DOI:10.1097/MCP.0000000000000335

KEY POINTS

- Precision medicine is the tailoring of healthcare to the individual through the identification of clinical, biological, or genetic markers.
- Evidence on the adverse effects of LABAs in children homozygous for the Arg16 allele of the rs1042713 polymorphism is increasing.
- Although a growing number of genetic loci have been associated with therapeutic responses to glucocorticoids, the individual effect of each SNP is limited.
- The first GWASs on treatment response to LTM have identified novel genetic loci associated with lung function response to LTM.
- Future studies should use a polygenic predictive approach integrated in complex networks of multiple 'omics'.

very similar for any subtype of asthma: childhood and adult onset, allergic and nonallergic, or occupational asthma. This results in a trial and error approach to find an effective treatment strategy for the individual patient. Can we do better?

Personalized or precision medicine is the tailoring of healthcare to the individual through the identification of clinical markers, biomarkers, or genetic features. We prefer to use the term precision medicine, as we expect that evidence on treatment efficacy will mostly depend on groups of patients rather than individual patients. In 2015, the Precision Medicine Initiative was launched in the United States, boosted by the availability of large-scale databases (such as the human genome sequence), powerful methods for characterizing patients (such as proteomics, metabolomics, and genomics), as well as computational tools for analyzing large sets of data [3²²]. Whereas any genetic, gene expression, or biomarker can be used to make asthma treatment more precise (Table 1), much attention has been given to the role of genetic variation in asthma treatment (pharmacogenetics).

Pharmacogenetics, the study of how inherited genetic variations influence drug response, will be the main topic of this review. All genes harbor variants, most of which are single-DNA base pair substitutions, called single-nucleotide polymorphisms (SNPs). Two different study designs can be used. In a candidate gene study, genes are selected that are potentially relevant to the pharmacokinetic or pharmacodynamic properties of a drug. In a genome-wide association study (GWAS), the whole genome is investigated by genotyping hundreds of thousands of SNPs that cover most of the genetic

variation to identify novel gene variants that influence therapeutic responsiveness.

In this review, we summarize prior results, highlight important studies over the past 18 months in asthma pharmacogenetics and explore future directions. The methods used for this review are outlined in Table 2.

β2-AGONISTS

Inhaled β2-agonists are widely used bronchodilators that are available in three classes: SABA, LABA, and ultra-long acting β2-agonist. β2-agonists bind to the β2-adrenoreceptor (β2AR), a G protein-coupled receptor present mainly on airway smooth muscle cells. Stimulation of the β2AR induces a signal transduction pathway, resulting in increased intracellular cyclic-3',5'-adenosine monophosphate and activation of protein kinase A. Cyclic-3',5'-adenosine monophosphate increases intracellular calcium retention and protein kinase A phosphorylates key regulatory proteins; both resulting in airway smooth muscle relaxation.

Pharmacogenetic studies of β2-agonists have mainly focused on the gene encoding the β2AR (*ADRB2*). The most extensively studied SNP in the *ADRB2* gene is glycine for arginine at codon 16 (rs1042713), which is present in its homozygous form in about 16% of the Caucasian population, and has been related to enhanced receptor downregulation *in vitro*. The homozygous Arg16 variant has been associated with a decline in lung function and increase in exacerbations after regular SABA therapy in adolescent and adult asthmatic patients. One of the very first prospective pharmacogenetic trials in medicine was performed in asthmatic adults stratified on rs1042713 genotype [4]. This Beta Adrenergic Response by Genotype (BARGE) trial investigated the response to 16-weeks regular albuterol (salbutamol) four times per day compared with placebo. Arg16 homozygotes had lower morning peak flow and demonstrated a deterioration of symptom control during albuterol treatment, in contrast to Gly16 homozygotes. This finding is consistent with the in-vitro observations indicating that continuous stimulation of the β2AR would lead to enhanced receptor downregulation in Arg16 homozygotes, with relative protection against downregulation in Gly16 homozygotes. However, the clinical relevance of this finding is limited, as current guidelines advice to use albuterol as needed, and not on a regular basis.

Studies searching for the effect of rs1042713 on the response to treatment with LABAs have shown conflicting results, and the adverse effects appear less evident in adults than in children [5,6²²,7,8²²,9].

Table 1. 'Omics' tools that could make asthma treatment more precise

'Omics'	Description	Example
Genomics	Any DNA variation	SNP in the β 2-adrenoreceptor
Epigenomics	Epigenetic variations	DNA methylation mark, micro RNA
Transcriptomics	Gene expression signature	Th2-high asthma gene expression in epithelial cells
Proteomics	Protein markers	Serum periostin as marker of Th2 asthma
Microbiomics	Variation in commensal and pathogenic microorganisms	Mycoplasma pneumonia in unstable asthma
Phenomics	Phenotypic characteristics	Eosinophilia in blood or sputum
Exposomics	Exposure characteristics	An asthma patient who smokes cigarettes
Humanomics	Personal preference	Inhaler preference

Th2-high patients have a gene expression profile in airway epithelial cells induced by Th2 cytokines such as IL-13.
 SNP, single nucleotide polymorphism; Th2, T helper 2.

In asthmatic adults, the prospective, genotype-stratified, crossover Long-acting β 2-Adrenergic Response by Genotype (LARGE) trial investigated the response to 18-week twice daily add-on salmeterol compared with placebo [5]. Lung function improved in both Arg16 and Gly16 homozygotes during LABA treatment, but only Gly16 homozygotes were protected against bronchial hyperresponsiveness provoked by metacholine [5]. In a recent publication, Wechsler *et al.* [6[■]] reported no negative associations between the rs1042713 polymorphism and the effects of 18-week add-on LABA compared with tiotropium on time to asthma exacerbation, quality of life, Asthma Control Questionnaire score, and forced expiratory volume in 1 s (FEV₁) in 1070 black asthmatic adults using ICSs.

In children, an increased risk for exacerbations was found in Arg16 homozygotes in a cohort of 1182 patients (aged 3–22 years) on daily salmeterol [7]. Recently, data from 4226 children from five independent populations of white Northern European and Latino origin were meta-analyzed, showing an increased odds ratio (OR) for asthma exacerbations of 1.52 ($P=0.0021$) for each copy of the Arg16 allele among children treated with ICSs plus LABA [8[■]]. Lipworth *et al.* [9] showed in a prospective randomized trial that in patients aged 5–18 years with the Arg16 homozygous genotype on ICSs add-on montelukast compared with salmeterol significantly

improved asthma symptoms, asthma-related school absence, and quality of life. Thus, in contrast to the adult data, evidence on the adverse effects of LABAs in children carrying the Arg16 allele is increasing, and future studies should address if prospective genotyping of this SNP could improve medication advices to children not controlled on ICSs.

Several GWASs on bronchodilator response to SABAs have been performed, identifying SNPs associated with increased bronchodilator response to albuterol, such as rs295137 in the promotor region of *SPATS2L* (a gene affecting β 2AR expression) [10], and polymorphisms in *SPATA13-AS1* (a gene involved in smooth muscle contraction) [11]. Although some of these findings were replicated in independent cohorts, none met the threshold of genome-wide significance. Recently, Israel *et al.* [12[■]] published the results of a GWAS in which they studied 444 088 SNPs in 724 white adults and children with mild-to-moderate asthma. They identified four SNPs that were associated with decreased FEV₁ response to albuterol. These findings were replicated in an independent cohort of 439 adults with moderate-to-severe asthma. The combined P value for these SNPs reached statistical genome-wide significance in a meta-analysis of the discovery and replication cohorts together. All four SNPs map to a genetic region on chromosome 2 near *ASB3*, a gene associated with smooth muscle proliferation.

Table 2. Methods for this review

We searched MEDLINE, Web of Science, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL) and Clinicaltrials.gov to identify English language articles published between January 2015 and July 2016. We used the search terms 'asthma' in combination with 'pharmacogenetics' or 'pharmacogenomics' or 'personalized medicine', 'precision medicine' or 'individualized medicine'. Reference types were randomized controlled trials, reviews, and editorials. The articles were reviewed by the authors and those deemed most relevant were used in this review. We also searched the reference lists of articles identified by our search strategy and selected those we judged relevant. Additionally, we selected some references based on our previous knowledge of the subject. No exclusion criteria were used. In reporting the published evidence, priority was given to recent studies, genetic findings that were genome wide significant and/or replicated, or studies with a high-quality study design, such as prospective randomized controlled studies.

Many other candidate genes have been associated with an increased or decreased bronchodilator response to SABAs, as reviewed by Blake and Lima in 2015 [13[¶]], and summarized in Table 3. These studies suggest that variants in the β 2-agonist signaling pathway [14,20,23] and variants in the nitric oxide (an endogenous bronchodilator) biosynthetic pathway [16–18] can influence the acute bronchodilator response to SABAs, but few have studied the effect on treatment response to LABAs. Future prospective genotype-stratified studies are necessary to confirm these associations and study the effect on LABA responsiveness.

GLUCOCORTICOIDS

ICSs are the most potent and commonly used first-line anti-inflammatory agents in the treatment of asthma. Glucocorticoids bind to glucocorticoid receptors in the cytoplasm and are then translocated into the nucleus. There, the activated glucocorticoid receptors bind to glucocorticoid response elements in glucocorticoid-responsive genes either encoding anti-inflammatory proteins or repressing pro-inflammatory proteins.

Many pharmacogenetic studies of glucocorticoids focus on candidate genes encoding proteins involved in the glucocorticoid pathway, such as corticotropin-releasing hormone receptor 1 (encoded by *CRHR1*), proteins that form the glucocorticoid receptor heterocomplex and factors responsible for the assembly of this heterocomplex. Hawkins *et al.* [25] identified four SNPs in the *STIP1* gene encoding heat-shock organizing protein that correlated with improvement in lung function after 4 and 8 weeks of ICS treatment in white asthmatic adults. Vijverberg *et al.* [26[¶]] reported that the *ST13* SNPs rs138335 and rs138337 were associated with an increased risk of asthma-related hospital visits in northern European children and adolescents on ICSs. *ST13* encodes a cochaperone protein (Hsp70 interacting protein) of the glucocorticoid receptor heterocomplex. Alterations in the expression or folding of cochaperones like heat-shock organizing protein and Hsp70 interacting protein may alter binding of glucocorticoids to the receptor and thereby influence ICS responsiveness.

In a small prospective study, Keskin *et al.* [27[¶]] were the first to evaluate the pharmacogenetics of the acute response to a single high dose of ICS during an asthma exacerbation. They studied the effect of eight SNPs that were previously identified to influence the long-term response to ICS, and found only one (rs41423247 in the *NR3C1* gene encoding the glucocorticoid receptor) to be associated with a higher acute improvement in FEV₁. This

study suggests that the genetic variants that influence the variability in response to maintenance treatment may differ from those underlying acute treatment responses.

New candidate genes that might influence the response to ICSs were identified through GWAS. Tantisira *et al.* [28] identified a SNP (rs37972) in the promoter region of the glucocorticoid-induced transcript 1 gene (*GLCCI1*) that was associated with an impaired lung function response to ICS treatment in four independent populations comprising 935 white adults and children. This SNP was in complete linkage disequilibrium with rs37973, a functional SNP that downregulates *GLCCI1* expression and could cause a decreased response to exogenous glucocorticoids. However, two subsequent studies in non-Hispanic white adults [29], and in northern European white children and adolescents [30], were unable to find an association of this variant with FEV₁ response, risk of exacerbations, or uncontrolled symptoms.

Dahlin *et al.* [31[¶]] found a novel SNP, rs2395627, in the *CMTR1* gene, associated with an increased risk of asthma exacerbations despite ICS treatment in a GWAS study conducted in 806 Caucasian asthmatic adults and children. *CMTR1* encodes a methyltransferase that catalyzes methylation of RNA transcripts and participates in the cellular defense against viral infections. They hypothesized that this gene may play a role in the pathogenesis of asthma exacerbations because of viral infections despite ICS treatment and reported upregulated *CMTR1* mRNA expression in nasal lavage samples from 18 asthmatic children during acute exacerbations, compared with 1–2 weeks following an exacerbation.

Wang *et al.* [32^{¶¶}] described a novel approach to GWAS by using a pharmacodynamic model to study dose–response relationships based on repeated measures of drug response at multiple dosages. They identified five SNPs with significant effects on response curves of corticosteroids for asthma. Four of these SNPs were associated with lung function response to ICSs in a replication in three independent clinical trials. These SNPs were located in the vicinity of candidate genes associated with oxidized nicotinamide adenine dinucleotide (NAD⁺) metabolism (such as *TNKS*), oxidative damage repair (such as *MSRA*), and genes related to nicotine dependence (such as *MYO1E*).

In the past decade, several other genes have been associated with either increased or decreased responsiveness to maintenance treatment with ICSs, as summarized in Table 4. However, many of these findings have not been replicated. This could be because of the use of different endpoints, heterogeneity in study groups (based on ethnicity, age, sex,

Table 3. Candidate genes and loci for pharmacogenetic studies of β_2 -agonists

Drug class	Gene	Associated variant	Minor allele frequency ^a	Outcome [Reference]
SABA	ADRB2	rs1042713	0.37	lower morning peakflow and deterioration of symptom control [4]
	ADCY9	rs2230739	0.30	increased FEV ₁ bronchodilator response [10,14]
	CRHR2	rs7793837	0.26	increased FEV ₁ bronchodilator response [10,15]
	ARG1	rs2781659	0.34	increased FEV ₁ bronchodilator response [10,16–18]
		rs2781667	0.31	
	ARG2	rs7140310	0.11	increased FEV ₁ bronchodilator response [17]
		rs10483801	0.16	
	SPATS2L	rs295137	0.41	increased FEV ₁ bronchodilator response [10]
	THRB	rs892940	0.42	increased FEV ₁ bronchodilator response [19]
	SLC24A4	rs77441274	0.0017	increased FEV ₁ bronchodilator response [20]
	SLC22A15	rs1281748	0.0026	increased FEV ₁ bronchodilator response [20]
		rs1281743		
	intergenic	rs11252394	0.08	increased FEV ₁ bronchodilator response [21]
	COLA22A1	rs6988229	0.20	increased FEV ₁ bronchodilator response [21]
	CLOCK	rs1522113	0.05	increased FEV ₁ bronchodilator response [21]
	SPATA13-AS1	rs9507294 rs912142	n/r	increased FEV ₁ bronchodilator response [11]
		rs2248119 rs9551086 rs9553225		
	ASB3	rs350729 rs1840321 rs1384918 rs1319797	n/r	increased FEV ₁ bronchodilator response [12 [■]]
LABA	ADRB2	rs1042713	0.16	- in adults: no effect on lung function [6 [■]], increased bronchoprotection [5] - in children: increased risk for exacerbations [7,8 [■]]
	ADRB2	rs1800888	0.02	increased risk for exacerbations [22]
	ADCY9	rs2230739	0.30	increased FEV ₁ response to LABA/ICS combination [23]
	NOS3	rs1799983	n/r	increased FEV ₁ response to LABA/ICS combination [24]

FEV₁, forced expiratory volume in 1 s; ICS; inhaled corticosteroid; LABA; long-acting β_2 -agonist, SABA; short-acting β_2 -agonist, n/r; not reported.

^aAs reported in cited reference; in case allele frequency was specified in different ethnic populations, the allele frequency in Caucasians is reported.

and asthma phenotype) and in treatment regimens. Furthermore, the response to glucocorticoids involves numerous proteins, therefore, the individual effect of one SNP is very limited and it is not possible to predict the clinical response to ICSs based on these SNPs. For future studies of drug response to ICSs a polygenic approach would be more appropriate, preferably integrated in complex networks using systems biology and multiple omics. For example, SNPs could be integrated with epigenetic changes and gene expression patterns that predict treatment response, thereby improving prediction.

LEUKOTRIENE MODIFIERS

LTM block the actions of cysteinyl leukotrienes (CysLT); a group of lipid mediators derived from arachnidonic acid by the enzyme 5-lipoxygenase

(5-LO). CysLT promote bronchoconstriction, plasma exudation, and mucus secretion, mainly through their effect on the CysLT receptor 1. LTMs exert their effect either by inhibiting 5-LO or antagonizing the CysLT receptor 1.

Pharmacogenetic studies of LTMs have mainly focused on candidate genes encoding enzymes involved in the synthesis and transport of CysLT and the CysLT receptors. In the first pharmacogenetic study looking at LTM responsiveness, Drazen *et al.* [45] reported a decreased FEV₁ response to 2-week treatment with 5-LO inhibitor ABT-761 in patients with a tandem repeat polymorphism in the promotor region of the *ALOX5* gene coding for 5-LO. However, the allele frequency of the *ALOX5* repeat polymorphism is too low (less than 2% in Caucasians) to fully explain the variability in response to LTM. Early studies indicated a

Table 4. Candidate genes and loci for pharmacogenetic studies of glucocorticoids

Drug class	Gene	Associated variant	Minor allele frequency ^a	Outcome [Reference]
ICS	<i>CRHR1</i>	rs242941	0.30	increased FEV ₁ response [33]
		rs1876828	n/r	
	<i>ORMDL3</i>	rs2872507	0.38	increased FEV ₁ response [34]
	<i>KRT25</i>	rs72821893	0.035	reduced FEV ₁ response [35]
	<i>TBX21</i>	rs2240017	0.045	increased bronchoprotection against Mch [36]
	<i>FCER2</i>	rs28364072	0.26	increased risk of exacerbations [37,38]
	<i>STIP1</i>	rs4980524	0.41	increased FEV ₁ response [25]
		rs6591838	0.21	
		rs2236647	0.43	
	<i>ST13</i>	rs138335	n/r	decreased risk of exacerbations [26 [■]]
		rs138337	n/r	increased risk of exacerbations [26 [■]]
	<i>NR3C1</i>	rs41423247	0.2	decreased acute FEV ₁ response to single dose [27 [■]]
	<i>HDAC1</i>	rs1741981	0.47	decreased FEV ₁ response [39]
	<i>CYP3A4</i>	rs35599367	0.04	improved asthma control on questionnaire [40]
	<i>GLCCI1</i>	rs37972	0.40	decreased FEV ₁ response [28]
	<i>T gene</i>	rs3127412	n/r	increased FEV ₁ response [41]
		rs6456042	n/r	
	<i>FBXL7</i>	rs10044254	n/r	increased asthma symptom scores despite ICS treatment [42]
	<i>TAAR6</i>	rs7772821	0.23	increased FEV ₁ response [43]
	<i>CA10</i>	rs967676	0.38	decreased FEV ₁ response in atopic patients [44]
	<i>CTNNA3</i>	rs1786929	0.33	decreased FEV ₁ response [44]
	<i>CMTR1</i>	rs2395627	n/r	increased risk of exacerbations [31 [■]]
	<i>intergenic</i>	rs6924808	0.48	decreased FEV ₁ response [32 ^{■■}]
	<i>TNKS</i>	rs10481450	0.31	increased FEV ₁ response [32 ^{■■}]
	<i>intergenic</i>	rs1353649	0.32	increased FEV ₁ response [32 ^{■■}]
	<i>MYO1E</i>	rs12438740	0.35	increased FEV ₁ response [32 ^{■■}]

FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; Mch, metacholine; n/r, not reported.

^aAs reported in cited reference; in case allele frequency was specified in different ethnic populations, the allele frequency in Caucasians is reported.

polymorphism in *LTC4S*, encoding leukotriene C4 synthase that converts LTRA4 to LTRC4, is associated with an increased FEV₁ response to LTM [46]. Subsequent larger studies confirmed the influence of the *ALOX5* promotor variant and the protective effect of *LTC4S* SNP rs730012 [47], on exacerbation rates during treatment with montelukast. Further studies identified additional SNPs associated with FEV₁ response to montelukast in the *ALOX5* gene [47–49], in *MRP1* (a gene coding for a transmembrane protein that transports LTC4 to the extracellular space) [47,49], and in *CYSLTR2* [48], as summarized in Table 5. However, most of these results have not been replicated.

Recently, Dahlin *et al.* [50[■]] published the results of the first GWAS on the response to LTM. First, they performed a GWAS of the FEV₁ response to 12-week treatment with zileuton in a cohort of 304 adult asthmatics. Then, they tested the replicability of the top 50 SNPs in a cohort of 222 asthmatics aged

≥ 12 years treated with zileuton for 12 weeks. In the combined analysis, one SNP (rs12436663) in *MRPP3*, a gene that encodes a mitochondrial RNase P protein, reached genome-wide significance. Homozygous carriers of the minor allele had a significantly worse FEV₁ response to treatment. *MRPP3* has previously been linked to cardiovascular and autoimmune diseases, and variations within its coding region may affect leukotriene synthesis or processing, but its exact role remains to be unraveled. The authors then tested the replicability in two independent cohorts of asthmatic patients receiving 12 weeks of treatment with montelukast. This analysis identified one SNP (rs517020), in the intron of the gene glycosyltransferase 1 domain containing 1 (*GLT1D1*), associated with reduced responses to both zileuton and montelukast. This suggests that some genes may have a class effect on all LTM, whereas others may only influence the response to either 5-LO inhibitors or leukotriene receptor antagonists.

Table 5. Candidate genes and loci for pharmacogenetic studies of leukotriene modifiers

Drug class	Gene	Associated variant	Minor allele frequency ^a	Outcome [Reference]
5-LO inhibitor	<i>ALOX5</i>	promotor repeat	3 repeats 0.038 4 repeats 0.172	decreased FEV ₁ response [45]
		rs2115819	0.47	increased FEV ₁ response [49]
		rs892690	0.42	decreased FEV ₁ response [49]
		rs2029253	0.43	decreased FEV ₁ response [49]
	<i>LTC4S</i>	rs730012	0.27	increased FEV ₁ response [46]
		rs272431	0.003	increased FEV ₁ response [49]
	<i>MRP1</i>	rs215066	0.05	increased FEV ₁ response [49]
		rs119774	0.07	increased FEV ₁ response [49]
	<i>MRPP3</i>	rs12436663	n/r	decreased FEV ₁ response [50 [■]]
LTRA	<i>ALOX5</i>	promotor repeat	3 repeats 0.006 4 repeats 0.17	reduced risk of exacerbations [47] reduced risk of exacerbations [47]
		rs2115819	0.47	increased FEV ₁ response [47]
		rs4987105	0.19	increased PEF response [48]
		rs4986832	0.19	increased PEF response [48]
	<i>LTC4S</i>	rs730012	0.30	reduced risk of exacerbations [47]
	<i>MRP1</i>	rs119774	0.07	increased FEV ₁ response [47]
	<i>LTA4H</i>	rs2660845	0.30	increased risk of exacerbations [47]
	<i>CYSLTR2</i>	rs912277	0.07	increased PEF response [48]
		rs912278	0.41	increased PEF response [48]
	<i>GLT1D1</i>	rs517020	n/r	decreased FEV ₁ response [50 [■]]
	<i>MLLT3</i>	rs6475448	n/r	increased FEV ₁ response [51 [■]]

5-LO, 5-lipoxygenase; FEV₁, forced expiratory volume in 1 s; LTRA, leukotriene receptor antagonist; n/r, not reported.

^aAs reported in cited reference; in case allele frequency was specified in different ethnic populations, the allele frequency in Caucasians is reported.

In a second GWAS, the same study group performed a GWAS of the FEV₁ response to 8-week treatment with montelukast in two cohorts comprising 133 adult asthmatics [51[■]]. Then, they tested the replicability of the top 200 SNPs in two independent cohorts comprising 184 asthmatics aged ≥ 6 years. Four SNPs were replicated in all four cohorts, of which one variant, rs6475448, achieved genome-wide significance. Homozygous carriers of this SNP showed an increased FEV₁ response to montelukast. rs6475448 is present within *MLLT3*, a gene proposed to regulate cell fates for megakaryocytes and erythroid cells. Red blood cell precursors are capable of transforming arachidonate and LTA4 to bioactive eicosanoids, and could play a role in the leukotriene pathway. Additional studies, including prospective genotype stratified studies, are necessary to confirm the association of *MRPP3*, *GLT1D1*, and *MLLT3* with LTM responses.

NOVEL THERAPIES

In the past years, novel biologic therapies, such as anti-IgE, soluble IL-4 receptor, anti-TSLP, anti-IL-5, and anti-IL-13 have been investigated in patients

with severe asthma, specifically targeting cytokines of biologic pathways in asthma. Pharmacogenetic biomarkers could aid to identify subgroups of patients responsive to these drugs. For example, in a dose ranging study of pitrakinra, an IL-4/IL-13 pathway antagonist that blocks the IL-4α receptor subunit encoded by *IL4RA*, the frequency of exacerbations was significantly reduced in study participants with a specific *IL4RA* variant; the GG genotype in the SNP rs8832 [52]. Other candidate genes that might influence response to novel biologicals are those encoding Th2-cytokines and their receptor, such as a variant of the IL-13 gene that was recently associated with increased FEV₁ response to 2 years of ICS treatment [53].

CONCLUSION

In the past years, there has been much progress in the field of pharmacogenetics both by candidate gene studies and GWAS. New technologies, such as admixture mapping, the use of expression Quantitative trait loci and RNAseq are used to enrich GWAS results. An expanding number of genetic loci have been associated with therapeutic responses.

Wu *et al.* [54[■]] have calculated that pharmacogenomics testing to predict response to ICSs is potentially cost-saving and noninferior in improving health. However, we are still far away from applicability in clinical practice.

The only polymorphism that has currently been studied in prospective, genotype stratified treatment studies has been the glycine for arginine substitution at codon 16 in the *ADRB2* gene (rs1042713), with conflicting outcomes for SABAs and LABAs in adults [4,5,6[■]]. However, evidence on the adverse effects of LABAs in children homozygous for Arg16 allele is increasing [8[■]]. Future studies should address if prospective genotyping of this SNP could improve medication advices to children not controlled on ICSs.

For other asthma drugs, especially ICSs, we are further away from clinical practice. Researchers have struggled to replicate many of the previously described associations, which could be because of underpowered sample sizes, heterogeneous populations, different asthma phenotypes or treatment regimens, and different endpoints used to assess treatment response. As numerous different proteins are involved in the treatment response to medications, the individual effect of one SNP is limited. Furthermore, epigenetic changes (such as DNA methylation, histone modification, and RNA-associated silencing), gene–gene interactions, and gene–environment interactions (including interactions of drug combinations) could all affect pharmacogenetic associations. To achieve personalized treatment, we need a polygenetic approach, in which a panel of variants predictive of therapeutic responsiveness is used, preferably integrated in complex networks of multiple omics.

Acknowledgements

G.H.K. has received a grant from the Dutch lung foundation (Longfonds AF 5.1.16.094).

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Chung KF. Asthma phenotyping: a necessity for improved therapeutic precision and new targeted therapies. *J Intern Med* 2016; 279:192–204. Review with focus on asthma phenotypes and biomarkers; and new therapies that specifically target cytokines relevant to particular causal pathways of asthma.
2. Reddel HK, Bateman ED, Becker A, *et al.* A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015; 46:622–639.
3. Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med* 2015; 372:793–795. Opinion article that outlines the Precision Medicine Initiative, giving a clear explanation on the concept and potential of precision medicine.
4. Israel E, Chinchilli VM, Ford JG, *et al.* Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet* 2004; 364:1505–1512.
5. Wechsler ME, Kunselman SJ, Chinchilli VM, *et al.* Effect of beta2-adrenergic receptor polymorphism on response to longacting beta2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, cross-over trial. *Lancet* 2009; 374:1754–1764.
6. Wechsler ME, Yawn BP, Fuhlbrigge AL, *et al.* Anticholinergic vs long-acting beta-agonist in combination with inhaled corticosteroids in black adults with asthma: the BELT randomized clinical trial. *JAMA* 2015; 314:1720–1730. Prospective, randomized, genotype stratified study to compare treatment outcomes of 18 weeks add-on LABA to tiotropium in patients with the rs1042713 polymorphism using ICSs. Arg16Gly *ADRB2* genotype was not associated with differences in treatment effects.
7. Basu K, Palmer CN, Tavendale R, *et al.* Adrenergic beta (2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. *J All Clin Immunol* 2009; 124:1188–1194.
8. Turner S, Francis B, Vijverberg S, *et al.* Childhood asthma exacerbations and the Arg16 beta2-receptor polymorphism: a meta-analysis stratified by treatment. *J All Clin Immunol* 2016; 138:107–113. Meta-analysis of data from 4226 children from five independent populations of white Northern European and Latino origin, showing an increased OR for asthma exacerbations of 1.52 ($P=0.0021$) for each copy of the Arg16 allele among children treated with ICSs plus LABA. This study shows how a large meta-analysis has established the need for further prospective genotype-stratified clinical trials in pediatric patients.
9. Lipworth BJ, Basu K, Donald HP, *et al.* Tailored second-line therapy in asthmatic children with the Arg (16) genotype. *Clin Sci (Lond)* 2013; 124:521–528.
10. Himes BE, Jiang X, Hu R, *et al.* Genome-wide association analysis in asthma subjects identifies SPATS2L as a novel bronchodilator response gene. *PLoS Genet* 2012; 8:e1002824.
11. Padhukasahasram B, Yang JJ, Levin AM, *et al.* Gene-based association identifies SPATA13-AS1 as a pharmacogenomic predictor of inhaled short-acting beta-agonist response in multiple population groups. *Pharmacogenomics J* 2014; 14:365–371.
12. Israel E, Lasky-Su J, Markezich A, *et al.* Genome-wide association study of short-acting beta2-agonists. A novel genome-wide significant locus on chromosome 2 near *ASB3*. *Am J Respir Crit Care Med* 2015; 191:530–537. GWAS that identified four novel SNPs associated with decreased acute bronchodilator response to albuterol, that map to a genetic region on chromosome 2 near the *ASB3* gene, a gene associated with smooth muscle proliferation. The combined P value for these SNPs reached statistical genome-wide significance in a meta-analysis of the discovery and replication cohorts together.
13. Blake K, Lima J. Pharmacogenomics of long-acting β_2 -agonists. *Expert Opin Drug Metab Toxicol* 2015; 11:1733–1751. Review summarizing previous results from pharmacogenomic studies on β_2 -agonists.
14. Tantisira KG, Small KM, Litonjua AA, *et al.* Molecular properties and pharmacogenetics of a polymorphism of adenylyl cyclase type 9 in asthma: interaction between beta-agonist and corticosteroid pathways. *Hum Mol Genet* 2005; 14:1671–1677.
15. Poon AH, Tantisira KG, Litonjua AA, *et al.* Association of corticotropin-releasing hormone receptor-2 genetic variants with acute bronchodilator response in asthma. *Pharmacogenet Genomics* 2008; 18:373–382.
16. Litonjua AA, Lasky-Su J, Schneider K, *et al.* ARG1 is a novel bronchodilator response gene: screening and replication in four asthma cohorts. *Am J Respir Crit Care Med* 2008; 178:688–694.
17. Vonk JM, Postma DS, Maarsingh H, *et al.* Arginase 1 and arginase 2 variations associate with asthma, asthma severity and beta2 agonist and steroid response. *Pharmacogenet Genomics* 2010; 20:179–186.
18. Duan QL, Gaume BR, Hawkins GA, *et al.* Regulatory haplotypes in ARG1 are associated with altered bronchodilator response. *Am J Respir Crit Care Med* 2011; 183:449–454.
19. Duan QL, Du R, Lasky-Su J, *et al.* A polymorphism in the thyroid hormone receptor gene is associated with bronchodilator response in asthmatics. *Pharmacogenomics J* 2013; 13:130–136.
20. Drake KA, Torgerson DG, Gignoux CR, *et al.* A genome-wide association study of bronchodilator response in Latinos implicates rare variants. *J Allergy Clin Immunol* 2014; 133:370–378.
21. Duan QL, Lasky-Su J, Himes BE, *et al.* A genome-wide association study of bronchodilator response in asthmatics. *Pharmacogenomics J* 2014; 14:41–47.
22. Ortega VE, Hawkins GA, Moore WC, *et al.* Effect of rare variants in *ADRB2* on risk of severe exacerbations and symptom control during longacting beta agonist treatment in a multiethnic asthma population: a genetic study. *Lancet Resp Med* 2014; 2:204–213.

23. Kim SH, Ye YM, Lee HY, *et al.* Combined pharmacogenetic effect of ADCY9 and ADRB2 gene polymorphisms on the bronchodilator response to inhaled combination therapy. *J Clin Pharm Ther* 2011; 36:399–405.
 24. Iordanidou M, Paraskakis E, Tavidou A, *et al.* G894T polymorphism of eNOS gene is a predictor of response to combination of inhaled corticosteroids with long-lasting beta2-agonists in asthmatic children. *Pharmacogenomics* 2012; 13:1363–1372.
 25. Hawkins GA, Lazarus R, Smith RS, *et al.* The glucocorticoid receptor heterocomplex gene ST1P1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. *J Allergy Clin Immunol* 2009; 123:1376–1383.
 26. Vijverberg SJ, Koster ES, Tavendale R, *et al.* ST13 polymorphisms and their effect on exacerbations in steroid-treated asthmatic children and young adults. *Clin Exp Allergy* 2015; 45:1051–1059.
- Candidate gene study in which two SNPs in *ST13* were nominally associated with an increased risk of exacerbations in asthmatic children and adolescents using ICSs. In a meta-analysis with three replication cohorts, *ST13* rs138335 remained associated with an increased risk of asthma-related hospital visits and oral corticosteroid use in the previous year; OR=1.22 ($P=0.013$).
27. Keskin O, Uluca U, Birben E, *et al.* Genetic associations of the response to inhaled corticosteroids in children during an asthma exacerbation. *Pediatr Allergy Immunol* 2016; 27:507–513.
- First publication on the pharmacogenomics of the acute response to a single high dose of ICS during an asthma exacerbation. Candidate gene study in 82 children that studied the effect of SNPs that were previously associated with long-term ICS response, and found only one (rs41423247 in the *NR3C1* gene encoding the glucocorticoid receptor) to be associated with a higher improvement in FEV1 4 h after a single high dose of fluticasone.
28. Tantisira KG, Lasky-Su J, Harada M, *et al.* Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. *N Engl J Med* 2011; 365:1173–1183.
 29. Hosking L, Bleecker E, Ghosh S, *et al.* GLCCI1 rs37973 does not influence treatment response to inhaled corticosteroids in white subjects with asthma. *J Allergy Clin Immunol* 2014; 133:587–589.
 30. Vijverberg SJ, Tavendale R, Leusink M, *et al.* Pharmacogenetic analysis of GLCCI1 in three north European pediatric asthma populations with a reported use of inhaled corticosteroids. *Pharmacogenomics* 2014; 15:799–806.
 31. Dahlin A, Denny J, Roden DM, *et al.* CMTR1 is associated with increased asthma exacerbations in patients taking inhaled corticosteroids. *Immun Inflamm Dis* 2015; 3:350–359.
- GWAS that identified six novel SNPs associated with differential risk of asthma exacerbations in adults and children on ICSs; the top result being rs2395627, in the *CMTR1* gene. *CMTR1* encodes a methyltransferase that participates in the cellular defense against viral infections. *CMTR1* was differentially expressed in paired nasal lavage samples obtained from asthmatic children during and 1–2 weeks following an acute exacerbation. This may indicate that this gene plays a role in the pathogenesis of asthma exacerbations because of viral infections.
32. Wang Y, Tong C, Wang Z, *et al.* Pharmacodynamic genome-wide association study identifies new responsive loci for glucocorticoid intervention in asthma. *Pharmacogenomics J* 2015; 15:422–429.
- Novel approach to GWAS by integrating pharmacodynamic properties of drug reactions to study dose–response to glucocorticoids. They identified five loci, many of which map to metabolic genes related to lung function and asthma risk, that were genome-wide significantly associated with dose-dependent response to ICSs. Four of these SNPs were associated with lung function response to ICSs in a replication study in three independent clinical trials.
33. Tantisira KG, Lake S, Silverman ES, *et al.* Corticosteroid pharmacogenetics: association of sequence variants in *CRHR1* with improved lung function in asthmatics treated with inhaled corticosteroids. *Hum Mol Genet* 2004; 13:1353–1359.
 34. Berce V, Kozmus CE, Potocnik U. Association among *ORMDL3* gene expression, 17q21 polymorphism and response to treatment with inhaled corticosteroids in children with asthma. *Pharmacogenomics J* 2013; 13:523–529.
 35. Leusink M, Vijverberg SJ, Koenderman L, *et al.* Genetic variation in uncontrolled childhood asthma despite ICS treatment. *Pharmacogenomics J* 2016; 16:158–163.
 36. Tantisira KG, Hwang ES, Raby BA, *et al.* TBX21: a functional variant predicts improvement in asthma with the use of inhaled corticosteroids. *Proc Natl Acad Sci U S A* 2004; 101:18099–18104.
 37. Tantisira KG, Silverman ES, Mariani TJ, *et al.* FCER2: a pharmacogenetic basis for severe exacerbations in children with asthma. *J Allergy Clin Immunol* 2007; 120:1285–1291.
 38. Koster ES, Maitland-van der Zee AH, Tavendale R, *et al.* FCER2 T2206C variant associated with chronic symptoms and exacerbations in steroid-treated asthmatic children. *Allergy* 2011; 66:1546–1552.
 39. Kim MH, Kim SH, Kim YK, *et al.* A polymorphism in the histone deacetylase 1 gene is associated with the response to corticosteroids in asthmatics. *Korean J Intern Med* 2013; 28:708–714.
 40. Stockmann C, Fassel B, Gaedigk R, *et al.* Fluticasone propionate pharmacogenetics: CYP3A4*22 polymorphism and pediatric asthma control. *J Pediatr* 2013; 162:1222–1227.
 41. Tantisira KG, Damask A, Szefer SJ, *et al.* Genome-wide association identifies the T gene as a novel asthma pharmacogenetic locus. *Am J Respir Crit Care Med* 2012; 185:1286–1291.
 42. Park HW, Dahlin A, Tse S, *et al.* Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids. *J Allergy Clin Immunol* 2014; 133:664–669.
 43. Chang HS, Heo JS, Shin SW, *et al.* Association between TAAR6 polymorphisms and airway responsiveness to inhaled corticosteroids in asthmatic patients. *Pharmacogenet Genomics* 2015; 25:334–342.
 44. Perin P, Potocnik U. Polymorphisms in recent GWA identified asthma genes CA10, SGK493, and CTNNA3 are associated with disease severity and treatment response in childhood asthma. *Immunogenetics* 2014; 66:143–151.
 45. Drazen JM, Yandava CN, Dube L, *et al.* Pharmacogenetic association between ALOX5 promoter genotype and the response to antiasthma treatment. *Nat Genet* 1999; 22:168–170.
 46. Asano K, Shiomi T, Hasegawa N, *et al.* Leukotriene C4 synthase gene A (–444)C polymorphism and clinical response to a CYS-LT (1) antagonist, pranlukast, in Japanese patients with moderate asthma. *Pharmacogenetics* 2002; 12:565–570.
 47. Lima JJ, Zhang S, Grant A, *et al.* Influence of leukotriene pathway polymorphisms on response to montelukast in asthma. *Am J Respir Crit Care Med* 2006; 173:379–385.
 48. Klotzman M, York TP, Pillai SG, *et al.* Pharmacogenetics of the 5-lipoxygenase biosynthetic pathway and variable clinical response to montelukast. *Pharmacogenet Genomics* 2007; 17:189–196.
 49. Tantisira KG, Lima J, Sylvia J, *et al.* 5-lipoxygenase pharmacogenetics in asthma: overlap with Cys-leukotriene receptor antagonist loci. *Pharmacogenet Genomics* 2009; 19:244–247.
 50. Dahlin A, Litonjua A, Irvin CG, *et al.* Genome-wide association study of leukotriene modifier response in asthma. *Pharmacogenomics J* 2016; 16:151–157.
- First GWAS of lung function response to LTM. In the combined analysis of the discovery and replication cohorts of asthmatic patients on zileuton, one SNP (rs12436663) in *MRPP3*, a gene that encodes a mitochondrial RNase P protein, was associated with decreased FEV1 response, with genome wide significance. The replicability was then tested in two independent cohorts of asthmatic patients treated with montelukast. This analysis identified one SNP (rs517020), in the intron of *GLT1D1*, associated with reduced responses to both zileuton and montelukast.
51. Dahlin A, Litonjua A, Lima JJ, *et al.* Genome-wide association study identifies novel pharmacogenomic loci for therapeutic response to montelukast in asthma. *PLoS One* 2015; 10:e0129385.
- GWAS of response to montelukast. Four SNPs were replicated in all four discovery and replication cohorts, of which one variant, rs6475448 within *MLLT*, was associated with an increased FEV1 response to montelukast with genome-wide significance.
52. Slager RE, Otulana BA, Hawkins GA, *et al.* IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti-IL-4 receptor alpha antagonist. *J Allergy Clin Immunol* 2012; 130:516–522.
 53. Nakamura Y, Suzuki R, Mizuno T, *et al.* Therapeutic implication of genetic variants of IL13 and STAT4 in airway remodelling with bronchial asthma. *Clin Exp Allergy* 2016; 46:1152–1161.
 54. Wu AC, Gay C, Rett MD, *et al.* Pharmacogenomic test that predicts response to inhaled corticosteroids in adults with asthma likely to be cost-saving. *Pharmacogenomics* 2015; 16:591–600.
- Study to identify clinical and economic circumstances under which a pharmacogenomic test for ICS responsiveness may be cost-saving.