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Pharmacogenetics of inhaled long-acting beta2-agonists in asthma: a systematic review

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Abstract

Background Long-acting beta2-agonists (LABA) are recommended in asthma therapy, however, not all asthma patients respond well to LABA. We performed a systematic review on genetic variants associated with LABA response in patients with asthma.

Methods Articles published until April 2017 were searched by two authors using PubMed and EMBASE. Pharmacogenetic studies in patients with asthma and LABA response as an outcome were included.

Results In total, thirty-three studies were included in this systematic review, eight focused on children (n=6,051). Nineteen studies were clinical trials, while fourteen were observational studies. Studies used different outcomes to define LABA response, e.g. lung function measurements (FEV_1, PEF, MMEF, FVC), exacerbations, quality of life and asthma symptoms. Most studies (n=30) focussed on the *ADRB2* gene, encoding the beta2 adrenergic receptor. Thirty studies (n=14,874) addressed *ADRB2* rs1042713, 7 *ADRB2* rs1042714 (n=1,629) and 3 *ADRB2* rs1800888 (n=1,892). The association of *ADRB2* rs1042713 and rs180888 with LABA response heterogeneity was successfully replicated. Other variants were only studied in three studies but not replicated. One study focussed on the *ADCY9* gene. Five studies and a meta-analysis found increased risk of exacerbations in pediatrics using LABA carrying one or two A alleles (OR 1.52 [1.17; 1.99]). These results were not confirmed in adults.

Conclusions *ADRB2* rs1042713 variant is most consistently associated with response to LABA in children but not adults. To assess the clinical value of *ADRB2* rs1042713 in children with asthma using LABA, a randomized clinical trial with well-defined outcomes is needed.

Introduction

Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment in adults and children. For patients with moderate or severe asthma poorly controlled on low-dose ICS current treatment guidelines recommend increasing the ICS dosage or adding a long-acting beta-agonist (LABA) [1,2]. Both are effective therapies for managing asthma by controlling symptoms, improving lung function and/or reducing exacerbations in asthmatics[3-5].

Nevertheless, there is much variation in how patients respond to LABA. A post-hoc analysis of 85 patients from two randomized controlled trials (RCTs) showed a variability of ≥ 70% in changes in peak expiratory flow in patients receiving salmeterol[6]. Factors including suboptimal inhalation technique, poor adherence, comorbidities, psychosocial factors, and/or continued environmental exposure to allergens or air pollution can contribute to this variation[7].

Genetic variation can also play an important role in determining LABA response[8-12]. The contribution of genetic factors to observed differences in bronchodilator response is approximately 28.5% for short-acting beta-agonist (SABA)[13]. However, in clinical practice we cannot yet predict LABA response [14]. In 2016, a report by asthma experts commissioned by the FDA, warned for severe asthma exacerbations in patients treated with LABA, questioning the safety of LABA in asthmatic adults and

children [15-18]. A subset of 18% of the asthma patients treated with LABA had increased risk of worse asthma outcomes such as lung function decline, severe exacerbations and even death [19-25].

Variation in the *ADRB2* gene that codes for the beta2 adrenergic receptor (B2AR) is a usual suspect to predict LABA treatment outcomes, due to its central role in the working mechanism of LABA. It contains various single nucleotide polymorphisms (SNPs), a single base pair variation that occurs at a specific position. In 1992, nine genetic variants in the *ADRB2* gene were identified in patients with asthma, including rs1042713, rs1042714 and rs1800888[26]. Rs1042713 is known for amino acid change ("missense mutation") in wildtype Gly16Gly by the following variants: Arg16Arg and Arg16Gly. Rs1042714 leads to an amino acid change at position 27 and encodes for three genotypes: Glu27Gln, Gln27Gln and Glu27Glu. Both SNPsshowed functional relevance *in vitro* [13] and were further studied to search for associations with LABA response heterogeneity [11,27]. There is also some evidence for functional relevance of rs180888 at position 164 (Thr164Ile). These three SNPs reduce the degree of agonist-promoted downregulation of the B2AR expression and stimulate adenyl cyclase activity[13]. Adenyl cyclase, encoded by the *ADCY9* gene, catalyzes the formation of cyclic adenosine monophosphate from adenosine triphosphate and is stimulated by the B2AR, being responsible for the receptor's signal transduction. Figure 1 displays various SNPs within the *ADRB2* gene and the *ADCY9* gene.

Because of the large variety in LABA response in asthmatic patients and the suspected genetic component responsible for this heterogeneity, we systematically reviewed literature on LABA pharmacogenetics. Many papers studied the combined use of ICS and LABA. We will discuss the clinical potential of pharmacogenetics of the *ADRB2* and the *ADCY9* gene in asthma management.

Methods

This systematic review assessed studies on LABA pharmacogenetics in patients with asthma published until April 2017. The search was performed using prespecified keywords and Medical Subject Headings (MeSH) (table 1). Studies in adults and children were assessed separately. Conference abstracts, studies not conducted in humans and papers not written in English were excluded. Reporting of this systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [28].

Table 1. Search term

pharmacogenetics[MeSH Terms] OR pharmacogenomics[MeSH Terms] OR genetic polymorphism[MeSH Terms] OR genetic polymorphisms[MeSH Terms]) OR candidate genes[All fields] OR genome wide association studies[All fields]
AND
agonists, beta adrenergic[MeSH Terms] OR beta adrenergic agonists[MeSH Terms] OR agents, bronchodilator[MeSH Terms]) OR bronchodilators[MeSH Terms] AND long acting[All fields] AND (genetic[All fields] OR genes[All fields])

Abstract sections were screened to determine whether studies described the association of specific genetic polymorphisms with the response to LABA by two reviewers (EMAS and NMBO).

After the initial selection, full text papers were analysed and the following data was collected: name of the author, year of publication, involved genes and SNPs (rs-numbers), study population, design, number of included patients, medication (type, dosage, duration) used, parameters to define treatment response and study outcome. We screened review articles for additional research papers.

SNPedia [29], PharmGKB [30] and PubMed were used to find the dbSNP reference ID numbers ("rs numbers") when these were not reported. In case of disagreement concerning data extraction, consensus between authors was reached.

Quality assessment was performed using the STrengthening the REporting of Genetic Association studies (STREGA) checklist (see E2 in this article's Online Repository) [31,32].

Results

We identified 151 studies in PubMed and 72 in EMBASE (Figure 2). Three additional studies[33-35] were identified through review papers [13,36,37]. Of all studies, 33 were pharmacogenetics studies that studied LABA response heterogeneity in asthma. Fourteen were observational and 19 studies were clinical trials (Tables 2-3). Only 33.3% of the identified studies used a prospective genotype-stratified design. All studies were candidate gene studies; none were genome wide association studies (GWAS).

Study outcomes

Outcomes reported to define LABA response can be divided into three categories: 1) lung function measures such as differences in peak expiratory flow (PEF), differences in morning peak expiratory flow rate (a.m. PEFR), Forced Vital Capacity (FVC), maximum mid-expiratory flow (MMEF), Forced Expiratory Volume in one second (FEV1) and methacholine bronchial challenges [11,12,20,27,33,38-53], 2) exacerbations (such as asthma-related emergency department visits, oral corticosteroid prescriptions and school absence) [24,33,34,38,44,45,54-58], and 3) patient-centered outcomes (such as asthma control questionnaire (ACQ), asthma control test (ACT), night time awakenings, asthma symptoms, rescue use of short-acting bronchodilators and quality of life) [11,40-43,45,47,49,52,56].

Almost all studies (30 out of 33 studies) identified in our systematic review, investigated *ADRB2* rs1042713 (see reference E1 in this article's Online Repository). Seven of the 33 studies were conducted in children. Nineteen of the 33 studies were trials. Seven studies studied *ADRB2* rs1042714, whereas three studies investigated the *ADRB2* rs1800888 SNP (reference E1 and table 2). All studies included patients with wide age range. One study focused on *ADCY9* SNPs.

ADRB2

ADRB2 rs1042713 (Arg16Gly)

Adults

Lung function outcomes (sixteen studies)

Five observational studies (n=89 up to 604 participants) did not report an association between lung function measurements and different rs1042713 genotypes in patients treated with LABA [33,47,49,51,59].

One RCT and two retrospective analyses of RCTs (total n=446) showed that Arg16Arg patients have impaired therapeutic response to LABA when considering lung function as the treatment outcome [11,12,53]. A retrospective pharmacogenetics analysis of the Salmeterol Off CorticoSteroids (SOCS) trial, a RCT with 164 well-controlled patients, and the Salmeterol Inhaled Corticosteroids (SLIC) trial, a RCT with 175 uncontrolled patients, showed that addition of salmeterol was associated with 51.4 L/min lower a.m. PEF in Arg16Arg subjects compared with Gly16Gly patients (p=0.005) [11].

In Arg16Arg and Arg16Gly patients there was less protection against bronchoconstriction compared to Gly16Gly after the last dose of 1-2 weeks treatment with formoterol or salmeterol [53,60]. This was measured by methacholine and adenosine monophosphate bronchial challenges. Doses were up titrated with 5 minute intervals until a decrease in FEV1 exceeding 20% of baseline was achieved. The mean doubling dose/dilution difference between Arg16Arg/Arg16Gly patients and Gly16Gly patients in either methacholine or adenosine monophosphate provocative dose/dilution causing a 20% fall in FEV_1 from baseline (MCh PD_{20}/AMP PC_{20}) of 1.49 (95% CI 0.50-2.48) was shown in a retrospective analysis of six RCTs in patients treated with corticosteroids after the last dose of salmeterol or formoterol [53].

Furthermore, the LARGE trial - a RCT (n=87) with moderate asthma treated with albuterol and salmeterol - showed that methacholine responsiveness improved in Gly16Gly patients treated with salmeterol compared to Arg16Arg patients. There were no differences between the genotypes in PEFR improvement [12]. In another RCT no difference between genotypes in FEV1% predicted (4.2 (-10.8-19.3), p=0.565, n=64) was seen in Arg16Arg vs Arg16Gly patients [43].

Remarkably, two clinical trials (n=237) showed that Arg16Arg patients treated with ICS and LABA had more a.m. PEF improvement compared with ICS (LARGE trial, difference [Arg16Arg-Gly16Gly] -0.1 [-14.4, 14.2], p=0.99, n=87) [12] and improvement of FEV1% (p=0.023 after 8 weeks and p=0.032 after 16 weeks in 13 Arg16Arg vs. 30 Arg16Gly or Gly16Gly patients) [46].

Six RCTs (n=1001) of which four prospective did not show any interaction between LABA response and rs1042713 on lung function outcomes [20,27,40,42,52,53].

In a cross-over study 24 Gly16Gly patients received either placebo, leukotriene receptor antagonist (LTRA), or LABA as ICS add on treatment (follow-up 6 weeks) [61]. Primary outcome was provocative dose of methacholine: there was no significant difference in PD20: 1.5-fold [95%CI: 1.1 - 2.2] for LTRA vs 1.9-fold [95%CI: 1.2 - 2.9] for LABA, implicating that Gly16Gly patients respond equally to LABA and LTRA.

Exacerbations (three studies)

One observational study (n=108) focused on exacerbations and reported that Arg16Arg patients had more exacerbations during daily use of SABA, but not with LABA [33].

Two post hoc RCT analyses (n=2,107 patients) studied the influence on exacerbations despite LABA treatment (e.g. time to first severe exacerbation [34] and a worsening asthma event requiring oral or parenteral corticosteroids [55]). No significant associations were found.

Patient-centered outcomes (five studies)

Five studies addressed the effect of genotyping on patient-reported outcomes. One observational study (n=544) focused on patient-reported asthma outcomes during LABA treatment and reported no evidence of an effect of B2AR variation [49].

In a pharmacogenetic RCT, salmeterol was withdrawn in 25 (total n=67) of the included asthma patients with an Arg16Arg or Gly16Gly genotype after a six week run in period on fluticasone/salmeterol for all patients. Only in the Gly16Gly patients a significant decline of a.m. PEF

rate was observed (-14.4 L/s, p=0.06). Nevertheless, LABA discontinuation led to clinically meaningful asthma-related quality of life improvement in both groups [39].

Two additional studies (n=106 Arg16Arg and Gly16Gly patients) did not show an association between this genetic variation and variation in LABA response measured by symptom scores [40] and ACQ [42]. A post-hoc RCT analysis (n=183 patients receiving LABA and ICS) did not discover associations with night time awakenings [52].

Paediatric studies

Most studies used exacerbations and patient-centered outcomes, but not lung function as main outcome measures.

Exacerbations (six studies)

In total 2,666 children were studied in LABA pharmacogenetic studies with exacerbations as an outcome. Of these studies, five found increased risk of exacerbations [44,45,57,58] in Arg16Arg patients treated with LABA. Four were included in a meta-analysis [62].

The first paediatric studies showing an effect of Arg16 on LABA response heterogeneity were performed within BREATHE [45,57,58]. The association of carrying this genetic variant and LABA response was studied (n=546, 3 to 22 years) in patients treated with albuterol, salmeterol and ICS (37). An increased hazard of exacerbations over the previous six months was found in Arg16Arg patients treated with salmeterol compared with Gly16Gly patients (OR 3.40, 95%CI: 1.19-3.53, p=0.010).

Three years later a study (n=1,182) in BREATHE was published [57]. Compared to Gly16Gly patients, Arg16Arg patients had an OR for asthma exacerbation response of 2.70 [1.46-4.99], p=0.002.

An observational study was conducted (n=597) with reported regular use of asthma medication (double dose ICS or ICS and LABA) participating in the PACMAN cohort [56]. Increased risk of oral corticosteroid use was found in Arg16Arg patients treated with LABA and ICS compared to the ICS-only group: OR 14.9 [95%CI: 1.59-140.1].

In contrast, a population-based prospective cohort study (n=97 treated with fluticasone propionate and salmeterol), did not find effect of *ADRB2* genotype on LABA treatment outcomes [44]. There was no difference in risk of asthma exacerbations or lung function decline between Arg16Arg and Gly16Gly or Gly16Arg patients. Furthermore, a three-treatment, three-period crossover RCT (n=182, 6-17 years) showed that Arg16Gly did not predict probability of best response to ICS, ICS plus LABA or ICS plus montelukast defined by acute asthma exacerbations, number of asthma-control days and FEV1 (p=0.49) (5).

In 2016, a meta-analysis focusing on the association between *ADRB2* rs1042713 and LABA response was performed [54] in five childhood asthma cohorts (4,226 children and young adults) participating in the Pharmacogenomics in Childhood Asthma (PiCA) Consortium [63]: the previously published BREATHE (UK) [45,57,58] and PACMAN studies (NL) [56], as well as GALA II (USA), PAGES (UK) and PASS (UK). LABA use was associated with increased risk of asthma exacerbations carrying one or two Arg alleles at rs1042713: OR 1.52 per Arg allele [1.17; 1.99]. The risk was highest in GALA II and lowest in PASS. GALA II involved Latino Americans and PASS included Africans and Caucasians, but excluded Asians. There was no association between effect size for exacerbation risk and characteristics of the populations.

Patient centered outcomes (one study)

Arg16Arg children (n=62, 5-18 years) selected from BREATHE were randomized over ICS+montelukast or ICS+LABA+placebo in a RCT. Reported asthma-related school absences were reduced in children treated with montelukast compared with salmeterol (difference in score: -0.40 [95%CI: -0.22 to -0.58]) [45]. Salbutamol use was also reduced in the montelukast group compared to the salmeterol group (difference: -0.47 [95%CI: -0.16 to -0.79]).

ADRB2 Arg16 heterozygotes (three studies)

Only three studies described results for heterozygous patients. FEV1 decline was described for each Arg allele irrespective of ICS or LABA use (n=604 adults): 7.7±2.5 mL/year [47]. The earlier discussed paper (n=1,182, 3-22 years) showed increased risk of exacerbations in Arg16Gly compared to Gly16Gly patients (OR: 1.63 [95%CI: 1.02-2.60]) [57]. The previously mentioned meta-analysis showed an increased OR for exacerbations 1.52 [1.17-1.99] (p=0.0021) for each copy of the A allele in 637 children treated with ICS+LABA therapy, but no increased risk was seen in patients treated with ICS or ICS+LTRA or ICS+LTRA+LABA [54].

Overall, there is a difference between children and adults regarding the influence of the rs1042713 genotype on LABA response. Most studies in adults did not show a difference in risk of exacerbations and in LABA response, however, Arg16Arg and Arg16Gly children had less response to LABA add on treatment and more exacerbations compared to Gly16Gly patients. Furthermore, Arg16Arg children may have higher risk of exacerbations when treated with LABA compared to Gly16 children [45,54].

ADRB2 rs1042714 (Glu27Gln)

One study in BREATHE described tight linkage disequilibrium by not observing any individuals with the compound diplotype of Arg16Arg and Glu27Glu[58].

Adults (five studies)

A RCT (n=87) described that LABA response in the context of this genetic variant was age-dependent. Asthmatic 27Gln patients (≤50 years) had better response to LABA with low and moderate doses of ICS, while 27Glu patients (> 50 years) were more likely to respond to LABA and ICS combination therapy [41]. Younger 27Gln carriers responded better to ICS and LABA, this may be used in personalized asthma treatment. On the other hand, three RCTs comparing Gln27Glu, Gln27Gln and Glu27Glu (n=791) did not find any associations focusing on lung function outcomes such as FEV1, FEV1% predicted, FEV1/FEC ratio and a.m. PEF [43,49,52].

A post-hoc analysis of a RCT with 183 patients focused on exacerbations and did not find association between rs1042714 and LABA response [52]. Two studies, of which one prospective, did not find a pharmacogenetic effect of rs1042714 in LABA response heterogeneity (n=883) with asthma symptom scores as their main outcome [11,49,52].

Paediatrics (two studies)

Two studies (n= 643) focused on exacerbations and rs1042714 variance in LABA response heterogeneity, but did not find any differences [44,58].

To summarize results for *ADRB2* rs1042714, one adult study (n=87) showed an age-dependent effect. The other five other studies did not report any influence of variety in LABA response by rs1042714, but did not assess the effect of age.

ADRB2 rs1800888 (three studies)

One observational study reported that non-Hispanic white adults with asthma treated with LABA carrying Thr164Ile (n=18) needed more urgent outpatient health-care or emergency department visits for asthma exacerbations during the past year compared to Thr164Thr patients (n=18 Thr164Ile vs n=553 Thr164Thr; 2.6 [SD=3.5] vs. 1.1 [2.1] visits, p<0.0001). Thr164Ile was associated with reduced urgent visits in non-Hispanic, white patients not treated with LABA (nine Thr164Ile vs 216 Thr164 patients; 0.1 [0.2] vs 0.5 [1.6] visits, p=0.01). The replication cohort showed similar results in favor of these outcomes [64].

Two RCTs (a post-hoc and a genotype-stratified analysis, n=544 and 183 adults) studied lung function, but did not find any association between Thr164Ile and poor LABA response [49,52].

To conclude, only adults were studied. It remains inconclusive whether *ADRB2* rs180888 is associated with LABA response. During LABA treatment, the relationship between lung function and rs1800888 seems to be different compared to the relationship between rs1800888 and exacerbations. Future research on Thr164Ile and LABA response should include outcome measures in all previously described outcome categories.

Genetic association and candidate gene studies with other ADRB2 SNPs (three studies)

Various other SNPs within the *ADRB2* gene were investigated in adults (Tables 2-3), but there was no evidence for a pharmacogenetic effect on lung function response to salmeterol [49]. In contrast, a candidate-gene study showed that in 186 LABA-treated African Americans a 25 bp insertion-deletion at nucleotide -376 relative to the ATG start site (the -376 in –del variant) was associated with increased asthma-related hospital admissions: OR 13.43 [2.02-265.42], p=0.006 [64].

ADCY9 (one study)

The *ADCY9* gene has been positively associated with LABA response in a post-hoc analysis of a 12-week clinical trial (n=86 Korean adults) using lung function to measure LABA response. After a two-week 'run-in' period, patients received budesonide and formoterol. The following SNPs in *ADCY9* were studied: rs2230739 (Ile772Met), rs1045475, rs1045476, rs879619 and rs710893. Two were associated with LABA response. The *ADCY9* rs2230739 Ile772Met was described to be associated with improvement in predicted FEV1: 0.7 ± 9.6 after 8 weeks of treatment (p=0.03). The *ADCY9* rs879619 C/T polymorphism was associated with differences in percent change in MMEF: 7.5 ± 15 (p=0.016) after 8 weeks of treatment. Nevertheless, these changes are not clinically relevant and did not remain significant after a 12 week follow-up[46].

This study also described a gene-gene interaction of *ADCY9* Ile772Met and *ADRB2* Arg16Gly. There was significant FEV1% improvement (8.4 ± 7.5%) in the CT or CC Ile772Met – A/G Gly16Gly genotype combination compared to the TT Ile772Met – A/G or G/G genotype combination. This interaction showed additive effect on bronchodilator response to LABA in combination therapy [46]. These findings have not been further studied.

Quality of reporting

Results of the quality reporting assessment can be found in Supplementary information 1. 18 (54.5%) studies published according to the STREGA guidelines. Restriction of analysis to high quality papers did not change initial conclusions**.**

Discussion

This systematic review showed that most LABA pharmacogenetics studies focussed on variants in the *ADRB2* gene and that findings differ between adults and children. One variant within that gene and the rs1042713 variant is expected to be associated with LABA altered response in children, but not in adults. In children exacerbations were used as main outcome, whilst in adults mainly lung function. Only three studies measured exacerbations as an outcome in adults, but none found association between rs1042713 and LABA response.

Compared to adults, contribution of genetics to variability in LABA response is larger in children with asthma. This could be due to the different phenotype in children, which is often characterized by less airway wall rigidity, more atopy and shorter exposure to chronic airway inflammation [65]. Also shortened response and airway smooth muscle relaxation time in children and faster maximal bronchoconstriction post-exercise are characteristics that differ from adults [66]. These differences underline that children should not just be considered 'small adults' [67]. Another reason for not observing an effect in adults may be that studies with children have considerable higher numbers compared to the studies conducted in adults. The effect size of *ADRB2* SNP effects is small as is the sample size in adults.

In relation to LABA response, it is important to consider that LABA can cause desensitization or downregulation of the B2AR in human bronchial smooth muscle tissue over time. This may result in a decrease of receptors or an increase of receptor degradation [44]. Desensitization and downregulation result in reduced bronchoprotective effects of LABA [65]. Previous studies showed bronchoprotective subsensitivity for LABAs in Arg16 homozygous adults [44,47,53]. This effect is also seen in children, but has not been studied in relation to their genotypes [68]. ICS can play a role: ICS can reverse functional desensitization of B2ARs and increase receptor expression and density [66,69,70]. Loss of bronchoprotection due to regularly inhaled LABA seems to reverse only with high dose ICS [71,72]. Only two of the included studies mentioned this mechanism in their discussion as a possible reason for not measuring any effects [12,44]. The LARGE trial showed no differences between genotypes in PEFR response, but methacholine responsiveness improved in homozygous Gly27 patients treated with salmeterol compared to Arg16 homozygotes. This finding could highlight differences in desensitization of bronchoprotective effects between polymorphic variants [12].

Few studies focussed on the additive or synergic effect of multiple variants on LABA response. Only four studies (study populations 97 to 639 patients) focussed on *ADRB2* haplotypes [44,48,49,52]. This did not lead into new insights. Gene-gene interactions with the Arg16Gly were only shown in two studies. A gene-gene interaction was described in a Korean study: FEV1% improvement in the C/T or C/C Ile772Met and A/A genotype of the *ADRB2* Arg16Gly was found, but not in the T/T Ile772Met and A/G or G/G Arg16Gly genotype combination [46]. The construction of a genetic risk score might provide more information than focussing on a single SNP [73].

In Caucasian populations, promotor polymorphisms and rs1042714 Glu27Gln were in complete linkage disequilibrium with the Arg16 variant [58,74]. Glu27Gln haplotypes were confounded by tight disequilibrium with Arg16 variants, and therefore independent effects were difficult to assess. On the

contrary, a haplotype analysis study identified 12 haplotypes in the *ADRB2* gene and described that no individual SNP could be a surrogate marker for their haplotype findings. This may indicate that unique interactions of the SNPs within a haplotype will affect biologic and therapeutic phenotypes and that prediction with individual SNPs will be insufficient to use in pharmacogenetics.[74] Furthermore, other variants within the *ADRB2* gene do not show consistent associations with LABA response. Six other *ADRB2* SNPs have been studied, and their results did not show reproducible associations, however large scale and comprehensive analysis is required to clarify the full genetic architecture of this locus.

As can be expected based on the mechanism of action of SABA and LABA, there is overlap in genetic risk factors for poor SABA/LABA response in *ADRB2* [75] and *ADCY9* [76] genes. In contrast to LABA, SABA GWAS data are available. Genes only associated with SABA response heterogeneity in GWAS studies, but not found in LABA candidate gene studies are: *SLC24A4* [76], *SPATS2L* (replicated) [77], *SPATA13* and its associated antisense RNA [78], intronic SNPs in *COL22A1* and *CLOCK* genes [79], *ASB3* [75] and *FGF14* [80]. The SABA GWAS used change in lung function as outcome. These results are specific for SABA response heterogeneity associations, as patients were not treated with asthma co-medication. When studying LABA, interference with genes associated with ICS or LTRA response can occur and the effect of LABA can be influenced by ICS [81]. Genes associated with SABA response heterogeneity might influence LABA response heterogeneity as well.

It has to be mentioned that ethnic patient stratification within studies was not reported. The Arg16 and Gln27 are common, but vary within ethnicities. The reported allele frequency for the Arg16 allele is 0.39, 0.52 and 0.55 in European, African and East Asian healthy populations respectively [82]. Gln27 has an allele frequency of 0.59, 0.82 and 0.93 in European, African and East Asian healthy populations respectively, it is a very common allele [83]. On the contrary, Thr164Ile is very rare (minor

allele frequencies: 0.02, 0.00 and 0.00 in European, African and East Asian healthy controls, respectively) [84].

Furthermore, reporting ethnicity is important since unrecognized population stratification can lead to false positive or false negative associations [85]. For example, a rare insertion in African Americans treated with LABA leads to increased exacerbations [64]. We recommend new studies to report whether genetic associations with LABA were related to specific ethnicities. A lack of power can also lead to false negative association. More than half of the included studies did not perform power calculations. As effect sizes in LABA response are small, a large sample size to include enough cases and controls is necessary to create clinically translatable results.

Furthermore, studies focussing on LABA pharmacogenetics are difficult to compare due to variability in outcomes studied. In addition, different dimensions of response might be associated with different genetic profiles. Use of the ACQ for example, may result in different findings than exacerbations, as children with uncontrolled symptoms may not be the same as children with (sudden) severe exacerbations [86].

Most limitations of the included studies may be due to study design. Future research in this field should focus on prospective observational studies and RCTs focussing on genes shown to influence response heterogeneity and GWAS. These studies should focus on a variety of outcome measures, preferably including patient-centered outcomes as well as exacerbations and lung function measurements. It would even be more beneficial to standardize response definitions in pharmacogenetics or to define composite scores.

In order to move from association to implementation in clinical practice, clinical validity of identified genetic variants should be assessed. To provide indications about clinical validity, measures such as number needed to genotype, population attributable fraction (proportion of adverse events that can potentially be eliminated if patients carrying the genetic variant receive different treatments), positive and negative predictive value (probability of an adverse event when the genetic variant is present, and probability of no adverse events when the genetic variant is absent) and number needed to treat should be presented [87]. The identified pharmacogenetics studies only provide association measures such as OR and RR. This makes interpretation of the results for use in clinical practice difficult.

We used STREGA to assess the quality of the studies used in this systematic review. With this quality reporting checklist, we objectively assessed quality of reporting in genetic research reports. We recommend future researchers in this field to follow the latest quality guidelines to support validation and replication. Furthermore, in most studies, patients heterozygous for the associated variant were not included; making it more difficult to translate outcomes into treatment guidelines for all asthmatics.

In conclusion, there is considerable variability in pharmacogenetic LABA studies due to differences in study design and characteristics of included patients. Environmental conditions, such as socioeconomic factors (income, education), ethnic genetic variants, environmental allergen exposure, psychosocial stressors, behavioural risk factors (smoking, obesity), poor medication adherence, and lack of access to medicines or evidence-based care, can influence gene expression[88-90]. *ADRB2* rs1042713 has been shown to influence LABA response in children in observational studies. There is need for a RCT to evaluate the impact of *ADRB2* rs1042713 genotyping in children before starting a LABA [91]. To identify other SNPs involved in LABA response heterogeneity, larger studies

with well-defined, comparable outcomes and proper analyses (for example a GWAS including gene-gene and gene-environment interactions [92]) are needed.

Conflict of interest

EMAS, SJHV, AHM, GHK and MWP are conducting the PUFFIN trial that is supported by the Lung Foundation Netherlands, grant number 5.1.16.094.

Author contributions

AHM and SJHV designed the study. EMAS, ZZ and NMBO performed the literature search and quality assessment, EMAS performed the data-analysis under supervision of SJHV and AHM. CNAP, GHK and MWP provided advice regarding data interpretation. EMAS wrote the manuscript under supervision of SJHV and AHM. All authors provided critical feedback, revised the manuscript and helped shape the research, analysis and manuscript.

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Table 2. Included studies without the ADRB2 rs1042713 SNP

Study	Gene and SNPs	Study population	Design	Medication	Definition of the response	Study outcome
Ortega *et al.* 2014 [64]	*ADRB2* (5q32), 6 rare variants: rs148459047: (C/A), rs33973603: (A/G), rs1800888: (T/C) , rs3729943: (C/G), Leu342Pro: (T/C), (-376 in -del)	1165 African americans, age 30 (14); non-Hispanic whites, age 37 (16) and Puerto Rican asthma patients, age 38 (19)	candidate-gene study	LABA	Asthma related hopitsal admissions	Patients with the rare ADRB2 variant: Thr164Ile and (-376 in -del) had increased asthma-related hospital admissions compared to patients with the common allele.
Ambrose *et al.* 2012 [93]	*ADRB2* (5q32) poly-C genotype: all variation in the poly-C region (+1266 to +1278)	2250 Asthma patients (mean age 38 (17)); 73 Caucasian of European descent (n=1614), 7% Asian (n=156), 1% African (n=21) and 19% were of mixed or other race (n=434)	Randomised controlled trial	budesonide/formoterol or fluticasone/salmeterol	The relation between poly-C repeat polymorphism and number of severe asthma exacerbations and changes in pulmonary function measurements (FEV1 and a.m. PEF), total symptom scores, rescue medication use and night time awakenings	The extensive sequence diversity present in de poly-C repeat region of the *ADRB2* 3'UTR did not predict therapeutic response to ICS/LABA therapy

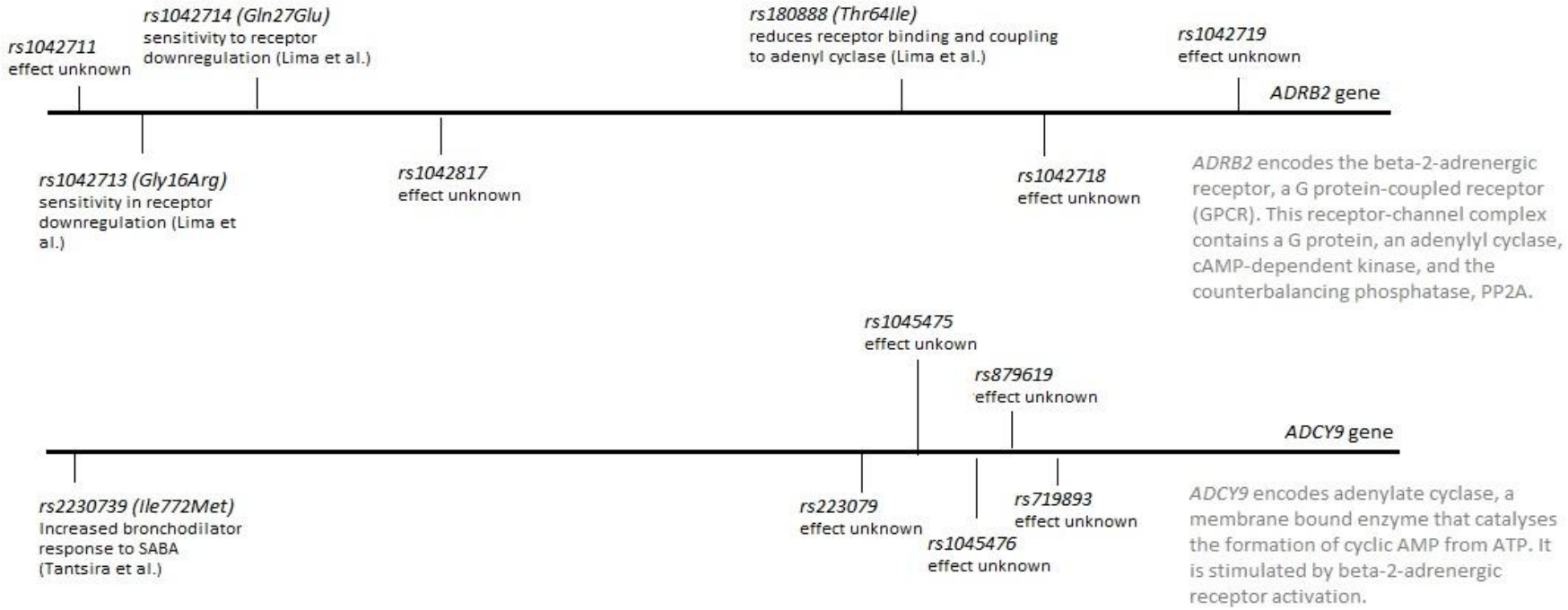

Figure 1. Schematic overview of the ADRB2 gene and the ADCY9 gene

and their most studied SNPs