Received: 6 January 2021 | Revised: 1 March 2021 | Accepted: 3 March 2021

DOI: 10.1111/pai.13494

ORIGINAL ARTICLE

WILEY

Genome-wide association studies of exacerbations in children using long-acting beta2-agonists

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Abbreviations: B2AR, beta2-adrenergic receptor;; CI, confidence interval; ENCODE, Encyclopedia of DNA Elements; *EPHA7*, ephrin-type A receptor 7 gene; GALA II, Genes-environments & Admixture in Latino Americans Study; GTEx, Genotype-Tissue Expression; GWAS, genome-wide association study; HRC, Haplotype Reference Consortium; ICS, inhaled corticosteroids; LABA, long-acting beta2-agonist; LD, linkage disequilibrium; LTRA, leukotriene antagonist; MAF, minor allele frequency; meta-GWAS, meta-analysis of genome-wide association studies; OR, odds ratio; PACMAN, Pharmacogenetics of Asthma medications in Children: Medication with Anti-inflammatory effects; PAGES, Paediatric Asthma Gene Environment Study; PASS, Pharmacogenetics of Adrenal Suppression Study; PCA, principal component analysis; PiCA, Pharmacogenetics in Childhood Asthma consortium; SABA, short-acting beta2-agonist; SAGE, Study of African Americans, Asthma, Genes and Environments; SCSGES, Singapore Cross Sectional Genetic Epidemiology Study; SNP, single nucleotide polymorphism; TBX3, T-box transcription factor 3.

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Funding information
EMA Slob, SJH Vijverberg, AH Maitland-van der Zee, GH Koppelman and MW Pijnenburg are conducting the PUFFIN trial that is supported by the Lung Foundation Netherlands, grant number 5.1.16.094. The PACMAN cohort study was funded by a strategic alliance between GlaxoSmithKline and Utrecht Institute for Pharmaceutical Sciences. The Genes-environments and Admixture in Latino Americans (GALA II) Study and the Study of African Americans, Asthma, Genes and Environments (SAGE) were supported in part by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, Harry Wm. and Diana V. Hind Distinguished Professor in Pharmaceutical Sciences II, the National Heart, Lung and Blood Institute of the National Institutes of Health R01HL117004, R01HL128439, R01HL135156, X01HL134589, R01HL141992 and R01HL141845, National Institute of Health and Environmental Health Sciences R01ES015794 and R21ES24844, the National Institute on Minority Health and Health Disparities P60MD006902, RL5GM118984, R01MD010443 and R56MD013312, the Tobacco-Related Disease Research Program under Award Number 24RT-0025 and 27IR-0030 and the National Human Genome Research Institute U01HG009080. MP-Y was funded by the Ramón y Cajal Program by the Spanish Ministry of Science, Innovation and Universities (MICIU) (RYC-2015-17205) and by a grant by MICIU, the State Research Agency and the European Regional Development Fund from the European Union (MINECO/AEI/FEDER, UE, SAF2017-83417R). Esther Herrera-Luis was supported by a MICIU fellowship (PRE2018-083837). Natalia Hernandez-Pacheco was supported by a fellowship (FI16/00136) from ISCIII and co-funded by the European Social Fund from the European Union (ESF) "ESF invests in your future."

Editor: Ömer Kalaycı

Abstract

Background: Some children with asthma experience exacerbations despite long-acting beta2-agonist (LABA) treatment. While this variability is partly caused by genetic variation, no genome-wide study until now has investigated which genetic factors associated with risk of exacerbations despite LABA use in children with asthma. We aimed to assess whether genetic variation was associated with exacerbations in children treated with LABA from a global consortium.

Methods: A meta-analysis of genome-wide association studies (meta-GWAS) was performed in 1,425 children and young adults with asthma (age 6-21 years) with reported regular use of LABA from six studies within the PiCA consortium using a random effects model. The primary outcome of each study was defined as any exacerbation within the past 6 or 12 months, including at least one of the following: 1) hospital admissions for asthma, 2) a course of oral corticosteroids or 3) emergency room visits because of asthma.

Results: Genome-wide association results for a total of 82 996 common single nucleotide polymorphisms (SNPs, MAF ≥1%) with high imputation quality were meta-analysed. Eight independent variants were suggestively (*P*-value threshold $\leq 5 \times 10^{-6}$) associated with exacerbations despite LABA use.

Conclusion: No strong effects of single nucleotide polymorphisms (SNPs) on exacerbations during LABA use were identified. We identified two loci (*TBX3* and *EPHA7)* that were previously implicated in the response to short-acting beta2-agonists (SABA). These loci merit further investigation in response to LABA and SABA use.

KEYWORDS
childhood asthma, exacerbations, genetic polymorphism, long-acting beta2-agonist, pharmacogenetics

Key Message

No strong effects of single nucleotide polymorphisms (SNPs) on exacerbations during long-acting beta2-agonists use were identified. We identified two loci (*TBX3* and *EPHA7*) that were previously implicated in response to short-acting beta2-agonists.

1 | INTRODUCTION

Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment. For patients poorly controlled on low-dose ICS, current guidelines recommend increasing the ICS dose or adding a long-acting beta2-agonist (LABA).[1,2] Both are effective for asthma control, improving lung function and/or reducing exacerbations.[3-5]

Nevertheless, there is high variation in responsiveness to step up options such as adding LABA.[6] Various factors contribute to this variation including suboptimal inhalation technique, poor adherence to treatment, comorbidities, psychosocial factors and/or continued environmental exposure to allergens or air pollution.[7]

Genetic variation has also been suggested to play an important role in determining response to LABA.[8-12] Contribution of genetic

factors to observed differences in bronchodilator response is estimated to be 28.5% for short-acting beta2-agonists (SABA).[13] The role of genetics in observed differences in occurrence of exacerbations despite LABA use is thought to be similar or even more prominent.[14] However, in current clinical practice we cannot yet predict which patients benefit from LABA and which patients still exacerbate.[15] In 2010, a report commissioned by the United States Food and Drug Administration (FDA), warned for severe asthma exacerbations in patients treated with LABA, questioning safety of adding LABA to the treatment of asthma in adults and children.[16-19] 18% of the included asthma patients treated with only LABA had increased risk of worse outcomes, such as decline in lung function, severe exacerbations and even death.[20-26] Currently, according to the guidelines for asthma management, and as the FDA recommends,[27] LABA is always prescribed in combination with ICS to decrease these risks.

Several candidate gene studies in children and young adults with asthma investigating LABA pharmacogenetics were performed during the last decades.[28-32] Variation in the *ADRB2* gene encoding the beta2-adrenergic receptor is known to predict part of LABA response, due to its pivotal role in the pharmacological mechanism of LABA. In 1992, nine single nucleotide polymorphisms (SNPs) in *ADRB2* were identified in patients with asthma with use of asthma medication compared to healthy subjects.[33] Three of these SNPs have been replicated in candidate gene studies.[28-32] Nonetheless, these studies may only have evaluated a small portion of the genomic variation estimated to be involved in LABA response heterogeneity.

To the best of our knowledge, no genome-wide association study (GWAS) of exacerbations despite LABA use has yet been conducted in children and young adults with asthma.[34] Given the large variation in LABA response among asthmatic patients and the suspected genetic component responsible for this heterogeneity, we aimed to assess whether genome-wide genetic variation is associated with exacerbations in children treated with LABA within the Pharmacogenetics in Childhood Asthma (PiCA) consortium[35] and whether we could validate the association of previously reported SNPs in a candidate gene study.

2 | METHODS

2.1 | Study populations

This meta-GWAS included all studies participating in PiCA[35] with medication and genetic data available for at least 100 LABA users. Six independent studies were analysed: Genes-environments & Admixture in Latino Americans Study (GALA II),[36] Study of African Americans, Asthma, Genes and Environments (SAGE),[37] Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN),[38] Paediatric Asthma Gene Environment Study (PAGES),[39] BREATHE[32,40,41] and Singapore Cross Sectional Genetic Epidemiology Study (SCSGES).[42] All studies were approved by local institutional review boards and all patients and/or parents provided informed consent. Further description of these studies is presented in Supplementary material S1. LABA use was reported via questionnaires or pharmacy data.

2.2 | Outcome definition

The presence or absence of any asthma exacerbation during 6-12 months in patients treated with LABA was considered as the outcome for LABA response. Exacerbations were evaluated as a binary outcome measure and were defined as any of these three asthma events within the past 6 or 12 months: (a) hospital admissions, (b) a short course of oral corticosteroid use and (c) emergency visits. The control group of each study consisted of patients with absence of any exacerbations during 6-12 months. The definition of exacerbations per study is described in Supplementary Material S1.

2.3 | Genome-wide genotyping and imputation

We reported the genotyping platforms of each study in Table 1. For further information regarding genotyping and quality control analyses, we refer to the papers of the studies.[36-42]

In all studies, imputation was carried out by means of the Michigan Imputation Server[43] using the second release of the Haplotype Reference Consortium (HRC) (r1.1 2016) as reference panel.[44] Haplotype reconstruction and imputation were performed with SHAPEIT[45] and Minimac2[46] for all studies, respectively. An exception was SCGES which used IMPUTE v2.0 to perform imputation based on 1000 genomes HapMap CHB and CHD samples. Our meta-GWAS included a total of common 15,229,795 SNPs (MAF ≥1%) with a high-quality imputation score (Rsq ≥0.3) in all six populations. Due to differences in genotyping platforms listed in Table 1, the total number of overlapping of genotyped or imputed SNPs in all six studies was 82 996.

2.4 | Association testing and meta-analysis

GWAS was carried out separately in all cohorts. Logistic regression was used to evaluate the association of genetic variants with LABA response heterogeneity in all studies by means of the binary Wald test implemented in PLINK 1.90b6 (BREATHE, PAGES, PACMAN and SCSGES) and EPACTS (GALA II and SAGE). The logistic regression models were adjusted for age (in years), sex and study-specific principal component (PC) scores of genetic ancestry to correct for potential bias due to population stratification. These were estimated using a PC analysis[47] using EIGENSOFT (GALA II and SAGE) and PLINK 1.90b6 (BREATHE, PAGES, PACMAN, and SCGES).

The meta-analysis was conducted with GWAMA.[48] Heterogeneity was assessed using the I^2 statistic and Cochrane's Q test.[49] Due to variety in ethnicities of the included patients, a random effect meta-analysis was performed. A genome-wide threshold (P-value $\leq 5 \times 10^{-6}$) was applied to select variants suggestively

TABLE 1 Characteristics of the children and adolescents with asthma treated with LABA included in all studies

	PACMAN (n = 175)	BREATHE/ PAGES (n = 306)	SAGE (n = 149)	SCSGES (n = 463)	GALA II (n = 332)	PASS (n = 359)
Gender (% female)	35	42	47	42	45	44.0
Mean age, (SD) years	10.3 (3.5)	11.4 (3.1)	14.3 ± 3.3	14.7 ± 6.2	13.1 ± 3.3	11.2 ± 3.7
Recruitment country	The Netherlands	United Kingdom	United States of America	Singapore	United States of America	United Kingdom
Recent exacerbations						
At least 1 exacerbation	9.0	44.8	64.2	32.3	73.2	86.9
OCS use (%)	6.3	41.5	45.6	16.2	49.7	53.5
Emergency asthma care (%)	4.7	NA	53.0	20.3	59.9	NA
Hospitalizations (%)	NA	15.7	12.1	1.3	16.9	76.9
Ethnicity						
% European	90.3	71.5	0.0	0.0	0.0	100.0
% Hispanic	0.6	0.0	0.0	0.0	100.0	0.0
% African	1.2	0.0	100.0	0.0	0.0	0.0
% Asian	0.6	2.0	0.0	99.8	0.0	0.0
% other (including mixed)	7.4	0.7	0.0	0.2	0.0	0.0
% Not answered	0.0	25.8	0.0	0.0	0.0	0.0
Proportion of LTRA users (%)	21.1%	52.4%	26.8%	Unknown	42.8%	64.7%
Genotyping platform	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Illumina Infinium CoreExome-24 BeadChip (Illumina)	Axiom LATI array (Affymetrix Inc)	Illumina HumanHap 550 k BeadChip version 3 (Illumina)	Axiom LATI array (Affymetrix Inc)	Illumina Human OmniExpress Exome-8v1 BeadChip (Illumina)
Available variants after QC	1.024.058	1.328.296	13.967.128	5.144.048	9.749.587	Not applicable

Abbreviations: NA: not available; OCS, oral corticosteroids; SD, standard deviation;

associated with exacerbations despite LABA use and a threshold of $\leq 5 \times 10^{-8}$ for genome-wide significant associations. R version 3.6.3 (R Core team, Vienna) was used to generate the Manhattan plot and quantile-quantile (QQ) plots.

2.5 | Functional evaluation of variants

One independent variant per locus was defined after pairwise regressions conditioned on the most significant variant for each locus with more than one association signal (R^2 <0.3) using SNPsnap[50] with 1000G phase 3 as reference. Based on data provided by Encyclopedia of DNA Elements (ENCODE),[51] functional annotation and a search for evidence for significant expression quantitative trait loci (eQTL) for SNPs in high linkage disequilibrium (LD) (r^2 >0.8) were performed for variants with at least suggestive association using HaploReg v4.1.[52] To study relationships between identified genetic variations and gene expression, the Portal for Genotype-Tissue Expression (GTEx)[53] v8.0 was used.

2.6 | Validation of previously reported genes

Previous studies reported the association of three SNPs located within *ADRB2*: rs1042713, rs1042714 and rs1800888[34] with exacerbations despite LABA use. We attempted to validate the association of these available variants with exacerbations despite LABA use using results of the current meta-GWAS.

2.7 | Sub-analysis in PASS

The independent SNPs in the meta-GWAS were further investigated in a subset of LABA users from the Pharmacogenetics of Adrenal Suppression study (PASS)[54] using the same definition for exacerbations as described above. The characteristics of PASS are described in Table 1 and Supplementary Information 2. PASS is a unique cohort with children concerned to have adrenal suppression and was in need of assessment of their adrenal function with a low-dose short

TABLE 2 Summary of the meta-analysis for each locus (suggestively) associated with exacerbations despite long-acting beta2-agonist use

Nearest gene(s) or locations	SNP	Chr.[a]	Position[b]	E/R[c]	MAF[d]	OR (95% CI)	*P*-value	Cochran's Q statistic	Cochran's Q *P*-value	I^2 (95% CI)
RMDN2	**rs163085**	2	38292519	A/T	0.346	0.59 (0.47-0.74)	4.22×10^{-6}	1.54	6.73×10^{-1}	0.0 (0.0-70.2)
KLF7	**rs9288377**	2	207856365	G/C	0.366	0.59 (0.47-0.74)	4.98×10^{-6}	1.52	4.67×10^{-1}	0.0 (0.0-86.3)
CLRN1	**rs358959**	3	150776600	G/A	0.257	0.63 (0.52-0.77)	4.52×10^{-6}	3.80	4.34×10^{-1}	0.0 (0.0-78.1)
LOC10537-7766	**rs4700987**	5	180251561	A/T	0.262	2.80 (1.81-4.33)	3.77×10^{-6}	0.65	4.19×10^{-1}	0.0[e]
LINC00847	rs4700988	5	180255963	C/A	0.262	2.83 (1.84-4.36)	2.42×10^{-6}	0.15	6.99×10^{-1}	0.0[e]
EPHA7	**rs1947048**	6	93012151	G/A	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs12197506	6	93014723	T/G	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs1596491	6	93015896	T/A	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs1899806	6	93017419	C/T	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs1899807	6	93017512	T/C	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs2588041	6	93026285	T/C	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs2588042	6	93027959	G/A	0.166	2.50 (1.69-3.69)	4.36×10^{-6}	0.33	8.48×10^{-1}	0.0 (0.0-37.0)
	rs2818130	6	93034458	A/G	0.167	2.62 (1.75-3.91)	2.61×10^{-6}	0.53	7.67×10^{-1}	0.0 (0.0-60.7)
	rs2818129	6	93035916	A/G	0.167	2.49 (1.69-3.66)	4.18×10^{-6}	0.60	7.41×10^{-1}	0.0 (0.0-65.3)
BUB3	**rs7918913**	10	124928952	C/T	0.374	0.59 (0.47-0.74)	4.96×10^{-6}	0.26	8.77×10^{-1}	0.0 (0.0-20.9)
TBX3	**rs6489992**	12	115352769	A/G	0.370	1.77 (1.40-2.23)	4.96×10^{-6}	1.64	4.40×10^{-1}	0.0 (0.0-87.3)
	rs7972038	12	115352977	T/C	0.340	1.90 (1.50-2.40)	1.43×10^{-6}	0.83	6.60×10^{-1}	0.0 (0.0-75.0)
	rs7958534	12	115353100	G/A	0.336	1.86 (1.47-2.35)	1.15×10^{-7}	1.20	5.48×10^{-1}	0.0 (0.0-82.7)
	rs10850402	12	115354123	A/G	0.342	1.88 (1.48-2.38)	2.49×10^{-7}	0.69	7.10×10^{-1}	0.0 (0.0-69.7)
	rs7961916	12	115355126	A/C	0.318	1.83 (1.44-2.33)	7.09×10^{-7}	0.38	8.27×10^{-1}	0.0 (0.0-45.3)
	rs7970471	12	115365549	A/T	0.288	1.80 (1.41-2.30)	3.04×10^{-6}	1.36	5.06×10^{-1}	0.0 (0.0-84.7)
RAB22A	**rs55950385**	20	56559152	G/A	0.122	0.27 (0.16-0.45)	8.98×10^{-7}	0.66	4.16×10^{-1}	0.0[e]

Note: Independent SNPs of each gene are in boldface.

Abbreviations: CI, confidence interval; OR, odds ratio for effect alleles; SNP, single nucleotide polymorphism.

[a]Chromosome

[b] positions based on GRCh37/hg 19 build

[c] effect allele / reference allele

[d] minor allele frequency

[e] confidence intervals cannot be computed due to the limited amount of studies.

Synacthen test. Therefore, we decided to perform a sub-analysis instead of inclusion to the initial meta-GWAS.

3 | RESULTS

3.1 | Study populations

The characteristics of the study populations consisting of 1,425 children treated with at least LABA and ICS are shown in Table 1. Analyses were performed in a subset of 175 patients with LABA use from PACMAN, 306 from BREATHE and PAGES, 149 from SAGE II, 463 from SCSGES and 332 from GALA II. The proportion of exacerbations defined as oral corticosteroids (OCS) use was lower in PACMAN and SCSGES (6.3% and 16.2%, respectively) compared to other studies. GALA II had the highest numbers of OCS courses of the meta-GWAS (49.7%). The number of OCS courses was even higher in PASS (53.5%).

3.1.1 | Genome-wide association meta-analysis

The Q-Q plots did not provide evidence for genomic inflation due to population stratification in each study (Figure S1A-S1E). In the meta-analysis, no associations with asthma exacerbations were genome-wide significant (*P*-value $\leq 5 \times 10^{-8}$). However, 22 variants were suggestively associated with exacerbations (*P*-value $\leq 5 \times 10^{-6}$) in our meta-analysis of children and young adults with asthma (Table 2, Figure 1). The SNP rs7958534, located near *TBX3*, had the strongest signal. The G allele of this SNP was associated with increased risk of exacerbations (odds ratio (OR) 1.86 (95% confidence interval (CI) 1.47-2.35; $P = 1.15 \times 10^{-7}$). Among the 22 identified SNPs, eight independent signals were identified. The forest plots of these SNPs are represented in Supplementary Figure S4. Results of the sub-analysis of the independent SNPs in 359 children from PASS are represented in Table S1. None of the SNPs were associated with increased risk of exacerbations.

3.2 | Functional evaluation of variants

Next, the eight independent SNPs resulting from the meta-GWAS were further investigated in GTEX.[52] Here, the independent SNP rs4700987 (nearest gene: *LOC105377766)* has been described as a lung eQTL for *zinc finger protein 62 (ZFP62)*[53] (Figure S2).

3.3 | Validation of previous reported LABA associations from candidate gene studies

Of the three previously reported SNPs, two were available in all cohorts of the current meta-GWAS dataset. All three variants were

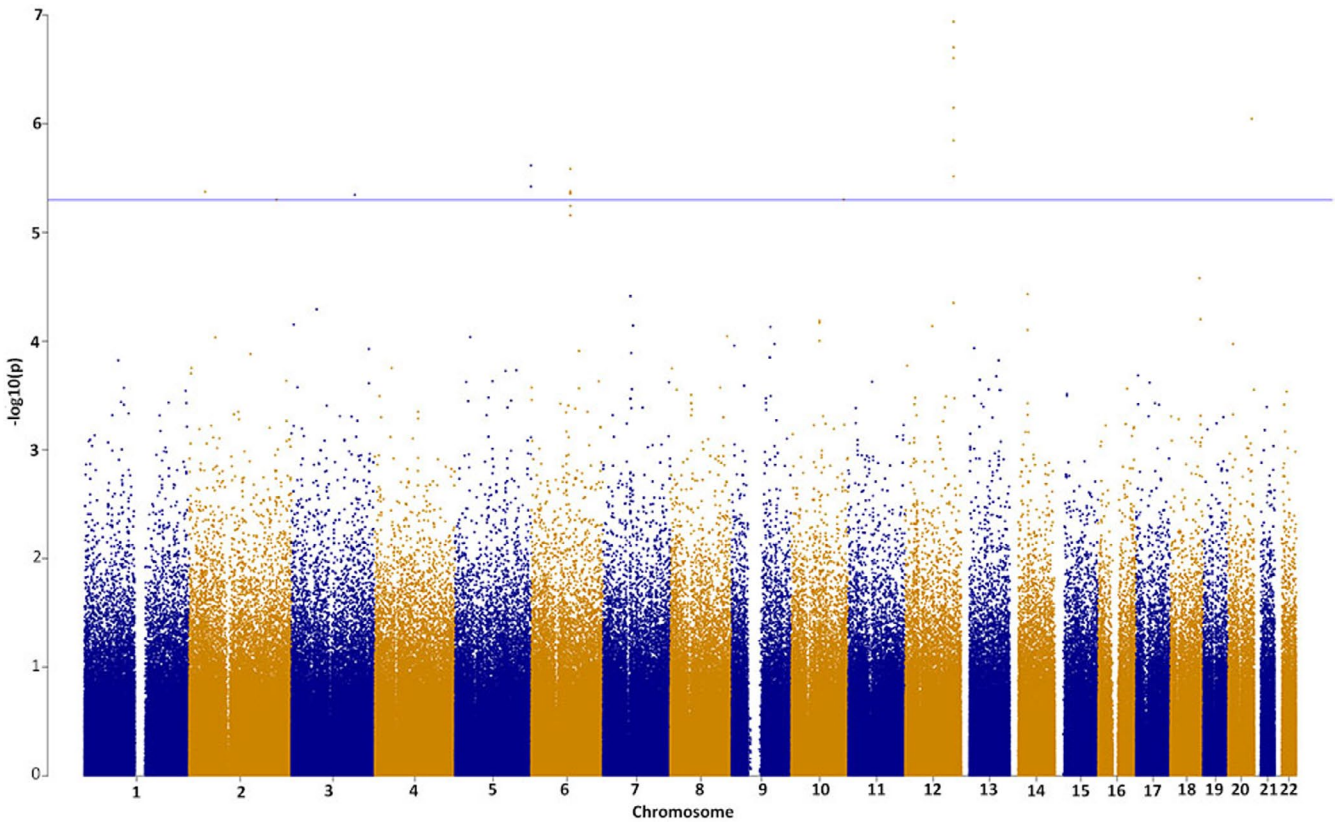

FIGURE 1 Manhattan plot of meta-analysed association results of exacerbations in children using long-acting beta2-agonists. Association results are shown as $-\log_{10}$*P*-value on the y-axis per chromosome on the x-axis. The blue line represents the suggestive significance threshold ($P \leq 5 \times 10^{-6}$)

not consistently associated with exacerbations despite LABA use (Figure S3). However, a sensitivity analysis in PACMAN in which we stratified for LABA users without leukotriene antagonist (LTRA) use shows a significant association for *ADRB2* rs1042713, the A allele increased the risk of exacerbations: OR 7.39 (95% CI 1.95-28.01, Table S2). A trend towards a similar association for rs1042713 (OR 1.20 (95% CI 0.72-2.00)) can be observed in the sensitivity analysis of LABA users without LTRA use, albeit not statistically significant (Table S3).

4 | DISCUSSION

To our knowledge, this study is the first meta-GWAS of asthma exacerbations in children and young adults treated with LABA, all GWAS included in this meta-GWAS have not been published before. We combined six international studies with genomic data of children and young adults with asthma and identified eight independent variants suggestively associated with exacerbations despite LABA. The effect size of the suggestive significant independent SNPs ranges from 0.27 to 2.80, which is common in GWAS in the field of asthma exacerbations in children.[55] There were multiple SNPs identified near *TBX3* and *EPHA7* in the initial GWAS; genes previously implicated in SABA response.

TBX3 encodes T-box transcription factor 3. It acts as a transcriptional repressor with in vertebrate development, cell fate, cell differentiation and dell-cycle progression.[56] This gene could possibly play a role in asthma, since variants located near *TBX3* have been identified in genetic and epigenetic studies focusing on asthma. However, little is known about the mechanism behind the association. In a whole-genome admixture mapping study, *TBX3* was associated with differences in SABA response between 318 African-American and 179 European adult patients with asthma.[57] This study combined data from the Severe Asthma Research Program (SARP1-2) and the Collaborative Study on the Genetics of Asthma (CSGA). Patients included in SARP1-2 had severe asthma and were non-smokers or had mild-to-moderate asthma with a prebronchodilator FEV_1 ≥80% predicted and treatment with either no or low-to-moderate dose ICS (<880 μg fluticasone or equivalent). CSGA included families with an asthmatic sibling pair from three ethnicities (Caucasian, African-American and Hispanic-American). In a GWAS of 38 199 European adults with asthma with FEV_1 as the outcome, *TBX3* had the strongest signal (*P*-value: 2.50×10^{-12}) and was replicated in a meta-GWAS with 54 550 European adults (*P*-value: 1.50×10^{-5}).[58] The largest study in the initial GWAS was Generation Scotland: the Scottish Family Health Study (GS:SFHS), including volunteers across Scotland aged 18-98 years. The majority of individuals in the replication study was from the UK BiLEVE study, which selected adults from the middle and extremes of the FEV1 distribution among both heavy smokers (mean 35 pack-years) and never smokers. This association has also been assessed in a subset of 5,062 children with asthma (8-9 years) from Avon Longitudinal Study of Parents and Children (ALSPAC), but *TBX3* was not in association (*P*-value: 3.17×10^{-1}).[59] ALSPAC included any newborn child with an estimated birth date between 1 April 1991 and 31 December 1992 from mothers in the old administrative county of Avon. The reported *TBX3* rs10850377 was in linkage equilibrium with our signal of *TBX3* rs6489992,[60] a regulatory region variant, showing that these were independent SNPs.

The ephrin-type A receptor 7 gene, *EPHA7*, has previously been found to be expressed in resected non-small cell lung cancer human specimens, but its role in relation to bronchodilators in asthma and COPD has not been studied extensively, and therefore, little is known about its role in the current observed association.[61] A GWAS investigating SABA responsiveness in 5,789 moderate-to-severe COPD patients (GOLD stage 2 or greater and all former smokers) with African-American or European ethnicity found that *EPHA7* was genome-wide significant for an increased FEV_1 post-SABA response[619]. Subjects that experienced a recent COPD exacerbation were excluded. The reported *EPHA7* rs17575208 was in linkage equilibrium with our signal of *EPHA7* rs1947048,[60] an intergenic variant, showing that these were independent SNPs. The other six identified independent SNPs and variants in linkage disequilibrium with these SNPs have not previously been identified, little information exists about their pharmacogenetic role in the association with exacerbations despite LABA use.

In GTEX, rs4700987 (nearest gene: *LOC105377766)* has been described as a lung eQTL for *ZFP62*. Studies in humans investigating the function of *ZFP62* are lacking, due to a previous murine study[61] it is hypothesized that this gene may be involved in muscular cell differentiation.

We were unable find the association with exacerbations for the eight independent SNPs in PASS, but all point estimates were in the same direction. The PASS study includes a specific study population. These children were concerned to have adrenal suppression and required assessment of their adrenal function with a low-dose short Synacthen test. Therefore, a reliable comparison of these children with the children included in the meta-GWAS cannot be made and results should be interpreted with caution. Some of the SNPs, including SNPs near *TBX3* and *EPHA7*, were not genotyped on arrays of the other meta-GWAS European cohorts BREATHE, PAGES and PACMAN. For these SNPs, we thus cannot conclude whether the effect is non-European or whether it is not shown due to limited Europeans having these SNPs genotyped. In both GWAS described above, European populations were included.[58,60]

In contrast to a previous meta-analysis of candidate gene studies in PiCA, this meta-GWAS did not identify an association between variation in *ADRB2* and LABA response heterogeneity[28,34](Figure S3). Reasons for not confirming the association are 1) that the numbers of populations that were included in the earlier published meta-analysis[28] differ due to quality control measures and a larger part of the study population was examined, 2) our GWAS was based on LABA users with or without leukotriene antagonist (LTRA) use, while the previous meta-analysis only included LABA users without LTRA, and 3) we also added other cohorts in this meta-GWAS compared to the previous meta-analysis leading to regression to the

mean. Nonetheless, a sensitivity analysis in PACMAN, BREATHE and PAGES for LABA users without LTRA showed that results were more similar to the previous results[28] (Tables S2,S3).

Our study has strengths and limitations. First, we combined six paediatric asthma cohorts with different ethnicities, enlarging the sample size of the LABA meta-GWAS in children and young adults with asthma. Second, we identified novel loci possibly helping to increase knowledge of genes that can identify which child with asthma would benefit from LABA. Two of these locations were near genes earlier reported in relation to bronchodilator responsiveness, increasing the validity of our results.

We acknowledge the following limitations. First, despite being the largest GWAS in children and young adults with LABA, the inability to reach genome-wide significance for some potentially important SNPs with respect to the odds of exacerbation may have been due to lack of power. Only a small number of children were treated with LABA in PASS, and this may have impacted association finding. PASS participants were suspected to have adrenal suppression. The selection of participants in this study may have also led to our inability to identify similar associations for the eight independent SNPs. We also included a proportion of patients who used LTRA besides the LABA use in the GWAS (see for proportions Table 1). The proportions differed per study, and this heterogeneity may have influenced the inability to reach genome-wide significant results. Second, ethnicities varied between included studies. There may be ethnic differences in response to LABA, making it more complex to discover SNPs associated with exacerbations despite LABA use. Accordingly, the wide confidence intervals demonstrated for I^2 potentially indicate the presence of a reasonable amount of heterogeneity, which is presumably due to the limited number of studies included in the meta-analyses.[63] Moreover, previous analysis of SAGE and GALA II genotyped with an array optimized for those admixed populations has revealed previously unknown SNPs for asthma exacerbations[55] despite ICS use. It could also be that in addition to ethnic variation of LABA response or even in the absence of ethnic variation of LABA response relevant SNPs were only genotyped in some of the samples thus decreasing power. Given the limited sample size in each study, we chose to ensure that the variants analysed were based on the largest sample size available, being shared by all the studies. This resulted in a limited number of total variants, but ensured the largest statistical power to detect associations. Therefore, future studies should provide a higher genetic coverage, analysing a larger amount of SNPS. Third, although retrospective information of exacerbations is commonly used in genetic studies of children with asthma, we cannot ascertain the temporal relationship between LABA use and timing of the exacerbation. This may have led to non-differential outcome misclassification, which usually dilutes effect estimates towards null. Fourth, as we did not have data regarding adherence to LABA of all participants, it was not possible to adjust for adherence and this may have influenced the outcome.

To conclude, no strong effects of SNPs on exacerbations despite LABA use were identified. Eight independent SNPs suggestively associated with exacerbations were identified. Two of these independent SNPs were near genes previously associated with bronchodilator responsiveness (*TBX3* and *EPHA7)*, and these merit further investigation. This meta-GWAS contributes to knowledge of pharmacogenetic markers that can determine whether children experience exacerbations despite LABA use, potentially leading to further understanding of which patients would benefit from LABA treatment. Further investigation in future studies including data in children with exacerbations despite LABA use (such as the PACT trial[64] and the PUFFIN trial[65] once these are genome-wide genotyped) could potentially lead to more concise meta-GWAS results.

CONFLICT OF INTEREST

EMA Slob, SJH Vijverberg, LB Richards, MW Pijnenburg, C. Longo, YY Sio, AH Neerincx, SW Turner, S. Mukhopadhyay, A. Jorgensen, D. Hawcutt and A. Andiappan have nothing to disclose. FT Chew and YY Sio report grants from Singapore Ministry of Education Academic Research Fund, Singapore Immunology Network, National Medical Research Council (NMRC) (Singapore), Biomedical Research Council (BMRC) (Singapore) and the Agency for Science Technology and Research (A*STAR) (Singapore) during the conduct of the study; and consulting fees from Sime Darby Technology Centre; First Resources Ltd; Genting Plantation, and Olam International, outside the submitted work. M. Pino-Yanes reports grants from the Spanish Ministry of Science, Innovation, and Universities, the State Research Agency and the European Regional Development Fund from the European Union (MICIU/AEI/FEDER, UE). E. Herrera-Luis reports a fellowship from MICIU. N. Hernandez-Pacheco declares funding from Instituto de Salud Carlos III (ISCIII) and the European Social Fund. EG Burchard reports grants from the National Institutes of Health, the Tobacco-Related Disease Research Program, the Sandler Family Foundation, the American Asthma Foundation, the Amos Medical Faculty Development Program from the Robert Wood Johnson Foundation and from the Harry Wm. and Diana V. Hind Distinguished Professorship in Pharmaceutical Sciences II. GK Koppelman reports grants from Lung Foundation of the Netherlands, TEVA the Netherlands, GSK, Vertex, Ubbo Emmius Foundation and TETRI Foundation, outside the submitted work; and he has served on advisory board meetings to GSK and PURE IMS. EHD Bel reports grants and personal fees from GlaxoSmithKline, AstraZeneca, Novartis and Teva, personal fees from Sanofi/Regeneron, Sterna and Chiesi and grants from Roche, outside the submitted work. AH Maitland-van der Zee has received a research grant from ERACOSYSMED for this work, and she received research grants outside the submitted work from GSK, Boehringer Íngelheim and Vertex, she is the PI of a P4O2 (Precision Medicine for more Oxygen) public-private partnership sponsored by Health Holland involving many private partners that contribute in cash and/or in kind (Boehringer Ingelheim, Breathomix, Fluidda, Ortec Logiqcare, Philips, Quantib-U, Smartfish, SODAQ, Thirona,

TopMD and Novartis), and she has served in advisory boards for AstraZeneca, GSK and Boehringer Ingelheim with money paid to her institution.

AUTHOR CONTRIBUTIONS

Elise M. Slob: Conceptualization (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing-original draft (lead). **Levi B. Richards:** Conceptualization (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing-original draft (lead). **Susanne J. H. Vijverberg:** Resources (equal); Writing-review & editing (equal). **Cristina Longo:** Conceptualization (supporting); Formal analysis (supporting); Investigation (supporting); Writing-review & editing (equal). **Gerard H. Koppelman:** Formal analysis (supporting); Investigation (supporting); Writing-review & editing (equal). **Mariëlle W. H. Pijnenburg:** Writing-review & editing (equal). **Elisabeth H. D. Bel:** Writing-review & editing (equal). **Anne H. Neerincx:** Writing-review & editing (equal). **Esther Herrera-Luis:** Formal analysis (supporting); Investigation (supporting); Writing-review & editing (equal). **Javier Perez-Garcia:** Formal analysis (supporting); Investigation (supporting); Writing-review & editing (equal). **Fook Tim Chew:** Resources (equal); Writing-review & editing (equal). **Yang Yie Sio:** Resources (equal); Writing-review & editing (equal). **Anand K. Andiappan:** Resources (equal); Writing-review & editing (equal). **Steve Turner:** Resources (equal); Writing-review & editing (equal). **Somnath Mukhopadhyay :** Writing-review & editing (equal). **Colin N. A. Palmer:** Resources (equal); Writing-review & editing (equal). **Daniel Hawcutt:** Resources (equal); Writing-review & editing (equal). **Andrea L. Jorgensen:** Formal analysis (supporting); Investigation (supporting); Writing-review & editing (equal). **Esteban G. Burchard:** Resources (equal); Writing-review & editing (equal). **Natalia Hernandez-Pacheco:** Formal analysis (supporting); Investigation (supporting); Writing-review & editing (equal). **Maria Pino-Yanes:** Formal analysis (supporting); Investigation (supporting); Resources (equal); Writing-review & editing (equal). **Anke-H. Maitland - van der Zee:** Conceptualization (equal); Formal analysis (equal); Investigation (equal); Project administration (lead); Resources (equal); Writing-review & editing (equal).

ETHICAL APPROVAL OF INCLUDED STUDIES

PACMAN was approved by the Medical Ethics Committee of the University Medical Centre Utrecht (Utrecht, the Netherlands; protocol number: 08/023). The Human Research Protection Program Institutional Review Board of the University of California, San Francisco (San Francisco, United States) approved GALA II and SAGE (ethics approval numbers: 10-00889 and 10-02877, respectively). BREATHE was approved by the Tayside Committee on Medical Research Ethics (Dundee, United Kingdom). PAGES was approved by the Cornwall and Plymouth Research Ethics Committee (Plymouth, United Kingdom). SCSGES was approved by the Institutional Review Board at the National University of Singapore (Singapore) (ethics approval number: B-14-150, 07–023, 09–256, 10–445, and 13–075). The Liverpool Paediatric Research Ethics Committee (Liverpool, United Kingdom) (reference number: 08/H1002/56) approved PASS.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Slob EMA, Richards LB, Vijverberg SJH, et al. Genome-wide association studies of exacerbations in children using long-acting beta2-agonists. *Pediatr Allergy Immunol*. 2021;32:1197–1207. https://doi.org/10.1111/pai.13494