Meta-analysis of genome-wide association studies of exacerbations in children using long-acting beta-agonists

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Keywords: childhood asthma, exacerbations, pharmacogenetics, genetic polymorphism, long-acting beta-agonist.

**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 associate with risk of exacerbations despite LABA use in children with asthma.

**Objective**

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 15,229,795 common SNPs (MAF ≥ 1%) with high imputation quality were meta-analysed. Eight independent variants were suggestively (p-value threshold ≤ 5x10-6) associated with exacerbations despite LABA use.

**Conclusion**

No strong effects of 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.

**Key messages**

- No strong effects of SNPs on exacerbations during LABA use were identified.

- Two identified loci (*TBX3*, *EPHA7)* were previously implicated in response to SABA.

**Summary**

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

**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 beta-agonist; MAF: minor allele frequency; meta-GWAS: meta-analysis of genome-wide association studies; LD: linkage disequilibrium; LTRA: leukotriene antagonist; 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 beta-agonist; SAGE: Study of African Americans, Asthma, Genes and Environments; SNP: single nucleotide polymorphism; *TBX3:* T-box transcription factor 3.

**INTRODUCTION**

Inhaled corticosteroids (ICS) are considered the cornerstone of asthma treatment in both adults and children. For patients with moderate or severe asthma poorly controlled on low-dose ICS, current treatment guidelines recommend increasing the dosage of ICS or adding a long-acting beta-agonist (LABA) as the next step1,2. Both are effective therapies for controlling asthma symptoms, improving lung function and/or reducing exacerbations3-5.

Nevertheless, there is a high interindividual variation in the responsiveness to step up options such as adding a LABA6. 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 pollution7.

Genetic variation has also been suggested to play an important role in determining the response to LABA8-12. The contribution of genetic factors to the observed differences in bronchodilator response is estimated to be 28.5% for short-acting beta-agonists (SABA)13. The role of genetic factors in the observed differences in the occurrence of exacerbations despite LABA use is thought to be similar or even more prominent14. However, in current clinical practice we cannot yet predict which patients will benefit from LABA use and which patients will still experience exacerbations15. In 2010, a report by asthma experts commissioned by the United States Food and Drug Administration, warned for severe asthma exacerbations in patients treated with LABA, questioning the safety of adding LABA to the pharmacological management of asthma in adults and children16-19. A subset of 18% of the included asthma patients treated with only LABA had an increased risk of worse asthma outcomes, such as a decline in lung function, severe exacerbations and even death20-26. Currently, according to the guidelines for the management of asthma, and as the FDA recommends27, 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 decades28-32. Variation in the *ADRB2* gene that encodes the beta2-adrenergic receptor (B2AR) is known to predict part of the LABA treatment response, due to its pivotal role in the pharmacological mechanism of LABA. This gene contains various single nucleotide polymorphisms (SNPs), a single base pair variation that occurs at a specific position. In 1992, nine SNPs in the ADRB2 gene were identified in patients with asthma with continuous use of asthma medication compared to healthy subjects33. Three of these SNPs have been replicated in candidate-gene studies28-32. 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 asthma34. 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 from different populations within the Pharmacogenetics in Childhood Asthma (PiCA) consortium35 and whether we could validate the association of previously reported SNPs in a candidate-gene study.

**METHODS**

**Study Populations**

This meta-GWAS included all the studies participating in the PiCA consortium35 with medication and genetic data available for at least 100 LABA users. Six independent studies were analysed: the Genes-environments & Admixture in Latino Americans Study (GALA II)36, the Study of African Americans, Asthma, Genes and Environments (SAGE)37, the Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects (PACMAN)38, Paediatric Asthma Gene Environment Study (PAGES)39, BREATHE32,40,41 and Singapore Cross Sectional Genetic Epidemiology Study (SCSGES)42. All studies were approved by the local institutional review boards and all patients and/or parents provided informed consent. A further description of these studies is presented in the Supplementary material S1. LABA use was reported via questionnaires or pharmacy data.

**Outcome definition**

The presence or absence of any asthma exacerbation during the last 6 or 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 the three following asthma related events within the past 6 or 12 months: 1) hospital admissions, 2) a short course of oral corticosteroid use and 3) emergency visits. The definition of exacerbations per cohort is described in Supplementary material S1.

**Genome-wide genotyping and imputation**

The genotyping platforms that were used are reported per study in Table 1. For further information regarding genotyping and quality control analyses, we refer to the descriptions of the (study design) papers of the cohorts36-42.

In all studies, imputation was carried out by means of the Michigan Imputation Server43 using the second release of the Haplotype Reference Consortium (HRC) (r1.1 2016) as reference panel44. Haplotype reconstruction and imputation were performed with SHAPEIT45 and Minimac246 for all studies, respectively. An exception was SCGES which used IMPUTE v. 2.0 to perform the imputation based on the 1000 genomes HapMap CHB and CHD samples. Our meta-GWAS included a total of common 15,299,795 SNPs (MAF ≥ 1%) with a high quality imputation score (Rsq ≥ 0.3) in all six populations. Due to the differences in imputation technology, the total number of overlapping SNPs in all six studies was 82,996.

**Association testing and meta-analysis**

GWAS analyses were carried out separately in all cohorts. Variants with minor allele frequency (MAF) <1% and also those with low imputation quality score (Rsq < 0.3) were filtered out.

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 the software PLINK 1.90b6 (BREATHE, PAGES, PACMAN, SCSGES) and EPACTS (GALAII 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 analysis47 using EIGENSOFT (GALAII and SAGE) and PLINK 1.90b6 (BREATHE, PAGES, PACMAN, SCGES).

The meta-analysis was conducted with GWAMA48. Heterogeneity was assessed using the I2 statistic and Cochrane’s Q test. Due to variety in ethnicities of the included patients, a random effect meta-analysis was performed. A genome-wide threshold of p-value ≤ 5x10-6 was applied to select variants suggestively associated with asthma exacerbations despite LABA use and a p-value threshold of ≤ 5x10-8 for genome-wide significant associations. R version 3.6.3 (R Core team, Vienna, Austria) was used to generate the Manhattan plot and quantile-quantile (QQ) plots.

**Functional evaluation of variants**

One independent variant per locus was defined after performing pairwise regressions conditioned on the most significant variant for each locus with more than one association signal (R2 < 0.3) with PLINK 1.90b6 using 1000G phase 349 as a reference. Based on data provided by the Encyclopedia of DNA Elements (ENCODE) project50, functional annotation, and a search for evidence for significant expression quantitative trait loci (eQTL) for the SNPs in high linkage disequilibrium (LD) (r2 > 0.8) was performed for the variants with at least suggestive association using HaploReg v4.151. To study relationships between identified genetic variations and gene expression, the Portal for the Genotype-Tissue Expression (GTEx)52 v 8.0 and Gene Expression Atlas53 were used.

**Validation of previously reported LABA genes**

Previous studies reported the association of three SNPs located within the *ADRB2* gene: rs1042713, rs1042714 and rs180088834 with asthma exacerbations despite LABA use. We attempted to validate the association of these available variants with asthma exacerbations despite LABA use using the results of the current meta-GWAS.

**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 any exacerbation as described above. The characteristics of PASS are described in Table 1 and Supplementary information 2.

**RESULTS**

**Study populations**

The characteristics of the meta-GWAS study populations consisting of 1,425 children treated with at least LABA and ICS and the number of SNPs analysed per study 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 Singapore Cross Sectional Genetic Epidemiology Study, and 332 from GALA II. In PACMAN, there were less female participants (34.9% *versus* > 42) and the mean age was lower compared to the other cohorts (10.3±3.5 *versus* > 11.4 years). The proportion of exacerbations defined as oral corticosteroids use was lower in the PACMAN and SCSGES studies (6.3% and 16.4% respectively) compared to the other studies. GALAII had the highest numbers of oral corticosteroids courses of the meta-GWAS (49.7%). The number of oral corticosteroids courses were even higher in the sub-analysis cohort (PASS, 53.5%).

**Genome-wide association meta-analysis**

The QQ-plots did not provide evidence for genomic inflation due to population stratification in each individual study (Figure S1A-S1E). In the meta-analysis, there were no associations with asthma exacerbations observed at genome-wide significance level (p-value ≤ 5 x 10-8). However, 22 variants were suggestively associated with exacerbations (p-value of ≤ 5 x 10-6) in our association meta-analysis of children and young adults with asthma (Table 2, Figure 1). The SNP rs7958534, which is located near the *TBX3* gene, had the strongest signal. The G allele of this SNP was associated with an increased risk of exacerbations with an odds ratio (OR) of 1.86 (95% confidence interval (CI) 1.47-2.35; p=1.15 x 10-7). Among the 22 identified SNPs, eight independent signals were identified. The forest plots of these SNPs are represented in Supplementary figure 3. Results of the sub-analysis of the independent SNPs in 359 children from the PASS study are represented in Table S3. None of the SNPs were associated with increased risk of exacerbations.

**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 not consistently associated with asthma exacerbations despite LABA use (Figure S2). 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, Supplementary information Table S1). A trend towards a similar association for rs1042713 with an odds ratio of 1.20 (95% CI 0.72-2.00) can be observed in the sensitivity analysis of LABA users without LTRA usage, albeit not statistically significant (Supplementary information Table S2).

**DISCUSSION**

To our knowledge, this study is the first meta-GWAS of asthma exacerbations in children and young adults treated with LABA. We combined six international studies with genomic data of children and young adults with asthma and identified eight independent variants that were at suggestively associated with asthma exacerbations despite LABA use. There were multiple SNPs identified near the genes *TBX3* and *EPHA7* in the initial GWAS; genes that were previously implicated in SABA response*.*

*TBX3* encodes T-box transcription factor 3. It acts as a transcriptional repressor with a role in vertebrate development, cell fate, cell differentiation and dell-cycle progression55. 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. In a whole-genome admixture mapping study, *TBX3* has been associated with differences in SABA response between 318 African American and 179 European adult patients with asthma56. In a GWAS of 38,199 European adults with asthma with FEV1 as the outcome, *TBX3* had the strongest signal in the initial GWAS (p-value: 2.50 x 10-12) and was replicated in a meta-GWAS with 54,550 European adults (p-value: 1.50 x 10-5)57. This association has also been assessed in a subset of 5,062 children with asthma (8-9 years of age) from the ALSPAC cohort, but *TBX3* was not in association (p-value: 3.17 x 10-1)57. The reported *TBX3* rs10850377 was in linkage equilibrium with our signal of *TBX3* rs795853458, 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 the potential role of the gene in relation to bronchodilators has not been studied extensively59. A GWAS investigating SABA responsiveness in 5,789 COPD patients with African American or Caucasian ethnicity found that *EPHA7* was genome-wide significant for an increased FEV1 post short-acting beta2-agonist response59. The reported *EPHA7* rs17575208 was in linkage equilibrium with our signal of *EPHA7* rs281813058, showing that these were independent SNPs. The other six identified independent SNPs and variants in linkage disequilibrium with these SNPs have not previously been related to short-acting beta2-agonist response.

We were unable find the association with exacerbations for the eight independent SNPs in PASS, but all point estimates in the different study populations 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 the results should be interpreted with caution. Some of the SNPs, including the SNPs near *TBX3* and *EPHA7,* were not genotyped on the arrays of the other meta-GWAS Caucasian cohorts BREATHE, PAGES and PACMAN. For these SNPs we thus cannot conclude whether the effect is a non-Caucasian effect or whether it is not shown due to the limited number of Caucasians having these SNPs genotyped. In both GWAS described above, Caucasian populations were included57,59.

In contrast to a previous meta-analysis of candidate gene studies in the PiCA consortium, this meta-GWAS did not identify a (suggestive) association between variation in the *ADRB2* gene and LABA response heterogeneity28,34 (Figure S2). Reasons for not confirming the association are 1) that the numbers of the populations that were included in the earlier published meta-analysis28 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 previously published 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 the results were more similar to the previously reported results28 (Table S1 and table S2).

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

We acknowledge the following limitations. First, despite this being the largest GWAS in children and young adults treated 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 the sub-analysis cohort 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. Second, ethnicities varied between included studies. There may be ethnic differences in response to LABA, making it more complex to discover SNPs that are associated with exacerbations despite LABA use. Third, although retrospective information of the outcome exacerbations is commonly used in genetic studies of children with asthma, we cannot ascertain the temporal relationship between LABA use and the timing of the exacerbation. This may have led to non-differential outcome misclassification, which usually dilutes the effect estimates towards the null value. Fourth, we did not have data regarding the adherence to the LABA therapy of all participants. It was therefore not possible to adjust for adherence and this may have influenced the outcome and phenotype within our study.

To conclude, no strong effects of SNPs on exacerbations during LABA use were identified. Our meta-GWAS of asthma exacerbations in children and young adults with asthma treated with LABA identified eight independent SNPs that were suggestively associated with exacerbations. Two of these independent SNPs were near genes that have previously been associated with bronchodilator responsiveness (*TBX3* and *EPHA7)* and these loci merit further investigation*.* This meta-GWAS contributes to the 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.

**Conflict of Interest**

E.M.A. Slob, S.J.H. Vijverberg, O. Ivanova, L.B. Richards, M.W. Pijnenburg, C. Longo, Y.Y. Sio, A.H. Neerincx, S.W. Turner, S. Mukhopadhyay, A. Jorgensen, D. Hawcutt and A. Andiappan have nothing to disclose. F.T. Chew and Y.Y. 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. E.G. 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. G.K. Koppelman reports grants from Lung Foundation of the Netherlands, TEVA the Netherlands, GSK, Vertex, Ubbo Emmius Foundation, TETRI Foundation, outside the submitted work; and he has served on advisory board meetings to GSK and PURE IMS. E.H.D. 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; .

**Financial support**

E.M.A. Slob, S.J.H. Vijverberg, A.H. Maitland-van der Zee, G.H. Koppelman and M.W. 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, R01HL141845, National Institute of Health and Environmental Health Sciences R01ES015794, 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, 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”.

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**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 and Puerto Rico | United Kingdom |
| Recent exacerbations:At least 1 exacerbationOCS use (%)Emergency asthma care (%)Hospitalizations (%)   | 9.06.34.7NA | 44.841.5NA15.7 |   64.245.653.0  12.1 | 32.3 16.220.3  1.3 |   73.249.759.9  16.9 | 86.953.5NA76.9 |
| Ethnicity:% Caucasian% Hispanic% African% Asian% other (including mixed)% Not answererd | 90.30.61.20.67.40.0 | 71.50.00.02.00.725.8 |  0.00.0100.00.00.00.0 |  0.00.00.099.80.20.0 |  0.0100.00.00.00.00.0 | 100.00.00.00.00.00.0 |
| 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 OmniExpressExome-8v1 BeadChip (Illumina) |
| Available variants after QC | 1.024.058 | 1.328.296 | 13.967.128 | 5.144.048 | 9.749.587 |  not applicable |

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

**Table 2. Summary of the meta-analysis for each locus (suggestively) associated with long-acting beta-agonist response**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nearest gene(s) or locations** | **SNP** | **Chr.a** | **Positionb** | **E/Rc** | **MAFd** | **OR (95%CI)** | **p-value** |
| *RMDN2* | **rs163085** | 2 | 38292519 | A/T | 0.346 | 0.59 (0.47-0.74) | 4.22 x 10-06 |
| *KLF7* | **rs9288377** | 2 | 207856365 | G/C | 0.366 | 0.59 (0.47-0.74) | 4.98 x 10-06 |
| *CLRN1* | **rs358959** | 3 | 150776600 | G/A | 0.257 | 0.63 (0.52-0.77) | 4.52 x 10-06 |
| *LOC10537-7766* | **rs4700987** | 5 | 180251561 | A/T | 0.262 | 2.80 (1.81-4.33) | 3.77 x 10-06 |
| *LINC00847* | rs4700988 | 5 | 180255963 | C/A | 0.262 | 2.83 (1.84-4.36) | 2.42 x 10-06 |
| *EPHA7* | rs1947048 | 6 | 93012151 | G/A | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
| rs12197506 | 6 | 93014723 | T/G | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
|  | rs1596491 | 6 | 93015896 | T/A | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
|  | rs1899806 | 6 | 93017419 | C/T | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
|  | rs1899807 | 6 | 93017512 | T/C | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
|  | rs2588041  | 6 | 93026285 | T/C | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
|  | rs2588042 | 6 | 93027959 | G/A | 0.166 | 2.50 (1.69-3.69) | 4.36 x 10-06 |
|  | **rs2818130** | 6 | 93034458 | A/G | 0.167 | 2.62 (1.75-3.91) | 2.61 x 10-06 |
|  | rs2818129 | 6 | 93035916 | A/G | 0.167 | 2.49 (1.69-3.66) | 4.18 x 10-06 |
| *BUB3* | **rs7918913** | 10 | 124928952 | C/T | 0.374 | 0.59 (0.47-0.74) | 4.96 x 10-06 |
| *TBX3*  | rs6489992 | 12 | 115352769 | A/G | 0.370 | 1.77 (1.40-2.23) | 4.96 x 10-06 |
|  | rs7972038 | 12 | 115352977 | T/C | 0.340 | 1.90 (1.50-2.40) | 1.43 x 10-06 |
|  | **rs7958534** | 12 | 115353100 | G/A | 0.336 | 1.86 (1.47-2.35) | 1.15 x 10-07 |
|  | rs10850402 | 12 | 115354123 | A/G | 0.342 | 1.88 (1.48-2.38) | 2.49 x 10-07 |
|  | rs7961916  | 12 | 115355126 | A/C | 0.318 | 1.83 (1.44-2.33) | 7.09 x 10-07 |
|  | rs7970471 | 12 | 115365549 | A/T | 0.288 | 1.80 (1.41-2.30) | 3.04 x 10-06 |
| *RAB22A* | **rs55950385** | 20 | 56559152 | G/A | 0.122 | 0.27 (0.16-0.45) | 8.98 x 10-07 |

**a** Chromosome; b Positions based on GRCh37/hg 19 build; c Effect allele / Reference allele; d Minor Allele Frequency; SNP: single nucleotide polymorphism; OR: odds ratio for effect alleles; CI: Confidence Interval. Independent SNPs of each gene are in boldface.

**Figure 1. Manhattan plot of meta-analysed association results of exacerbations in children using long-acting beta2-agonists.** Association results are shown as –log10 p-value on the y-axis per chromosome on the x-axis. The blue line represents the suggestive significance threshold (p ≤ 5 x 10-6).

**S1. Cohorts included in the meta-GWAS**

*BREATHE (n=226)*

The BREATHE cohort includes children and young adults (age 3-22 years) with asthma based on the diagnosis of the physician. The participants were recruited in primary and secondary care units in Scotland, United Kingdom. Clinical history, demographic and anthropometric information was obtained from all participants between 2004 and 200632,40,41. Exacerbations were defined as oral corticosteroids use and hospitalization six months prior to the baseline visit.

*GALA II (n=332)*

The Genes-environments & Admixture in Latino Americans study (GALA II) included Latino patients (age 8-21 years) with a physician diagnosis of asthma with active symptoms and asthma medication use during the last 2 years. Information about genetics, environment, exacerbations (defined by oral corticosteroids use, emergency room visits and hospitalizations in the past 12 months) and medication use was obtained36.

*PAGES (n=256)*

The Paediatric Asthma Gene Environment Study (PAGES) included children (age: 2-16 years) with a physician’s diagnosis of asthma in 15 hospitals in Scotland between 2008 and 2011. Genetic, exhaled NO and spirometry data and information about asthma symptoms, diet, medication, exacerbations, allergies and quality of life were obtained39. Exacerbations were defined as oral corticosteroids use and hospitalization six months prior to the baseline visit.

*PACMAN (n=194)*

The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) cohort is an observational cohort with children (age 4-13 years) with self-reported (regular) use of asthma medication. The children were recruited via Dutch community pharmacies. Asthma symptoms, exacerbations (defined by oral corticosteroids use and emergency room visits) and medication use were collected for 12 months between 2009 and 201238.

*SAGE (n=149)*

The Study of African Americans, Asthma, Genes and Environments (SAGE) included African American patients (age 8-21 years) with a physician diagnosis of asthma, active symptoms and asthma medication use during the last two years. Information about genetics, environment, exacerbations (defined by oral corticosteroids use, emergency room visits and hospitalisations in the past 12 months) and medication use was obtained37.

*Singapore Cross Sectional Genetic Epidemiology Study (n=463)*

The Singapore Cross Sectional Genetic Epidemiology Study included atopic patients (children and young adults) of Chinese ethnicity, living in Singapore. All subjects are not related to each other. Genetic and medication data, information about exacerbations (defined as a composite outcome of oral corticosteroids use, emergency room visits and hospitalisations in the past 12 months) and information about allergies was obtained42.

**S2. Cohort included in the sub-analysis**

*PASS (n=359)*

The Pharmacogenetics of Adrenal Suppression with Inhaled Steroids (PASS) study included Caucasian patients (age 5-18 years) with asthma using inhaled corticosteroids as part of their treatment across 25 sites in the United Kingdom*60.* Exacerbations were defined by oral corticosteroids use and hospitalizations in the past 6 months.These children were concerned to have adrenal suppression and thus required assessment of their adrenal function with a Low Dose Short Synacthen Test.

|  |  |  |  |
| --- | --- | --- | --- |
| **A** |  | **D** |  |
| **B** |  | **E** | **C:\Users\emslob\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Outlook\S6DJVPIV\PicaLABA_AnEvsNonAnE_qq.jpg** |
| **C** |  |  |  |

**Figure S1. Quantile-quantile plots of association results of asthma exacerbations despite LABA use among the six paediatric asthma cohorts.** Expected and observed association results are shown as –log10 *p*-value on the x-axis and y-axis, respectively. The QQ-plots of association results are shown per individual study: A) PACMAN (λGC = 1.005); B) BREATHE/PAGES (λGC = 0.890); C) GALAII (λGC = 0.932); D) SAGE (λGC = 0.872); E) Singapore (λGC = 1.025).

|  |  |
| --- | --- |
| **A** | **rs1042713** |
|  |  |
| **B** | **rs1042714** |
|  |  |
| **C** | **rs1800888** |
|  |  |

**Figure S2. Validation of previous *ADRB2* SNP association with exacerbations.**

**A. rs163085**

****

**B. rs9288377**

**C. rs358959**

**D. rs4700987**

**E. rs1947048**

**F. rs7918913**

**G. rs6489992**

**H. rs55950385**

**Figure S3. Forest plots the meta-analyses of the eight independent SNPs for the association with exacerbations**

**Table S1. Sensitivity analysis for LABA users without LTRA use in PACMAN (n=112)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nearest gene(s) or locations** | **SNP** | **Chr.a** | **Positionb** | **E/Rc** | **PACMAN****OR (95%CI)** | **p-value** |  **FDR** |
| *ADRB2* | **rs1042713** | 5 | 148206440 | A/G | 7.39 (1.95-28.01) | 0.0033 | 0.0065 |
| *ADRB2* | **rs1042714** | 5 | 148206473 | G/C | 0.49 (0.17-1.42) | 0.1862 | 0.1862 |

We included only LABA+ICS+SABA users without LTRA use.

**Table S2. Sensitivity analysis for LABA users without LTRA use in BREATHE & PAGES (n=140)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Nearest gene(s) or locations** | **SNP** | **Chr.a** | **Positionb** | **E/Rc** | **BREATHE/PAGES****OR (95%CI)** | **p-value** |  **FDR** |
| *ADRB2* | **rs1042713** | 5 | 148206440 | A/G | 1.20 (0.72-2.00) | 0.4894 | 1 |
| *ADRB2* | **rs1042714** | 5 | 148206473 | G/C | 0.90 (0.53-1.54) | 0.7096 | 1 |

We included only LABA+ICS+SABA users without LTRA use.

**Table S3. Results of the replication of the independent SNPs.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Nearest gene(s) or locations** | **SNP** | **Chr.a** | **Positionb** | **E/Rc** | **MAFd** | **OR (95%CI)** |
| *RMDN2* | rs163085 | 2 | 38292519 | A/T | 0.346 | NA |
| *KLF7* | rs9288377 | 2 | 207856365 | G/C | 0.262 | 0.72 (0.45-1.16) |
| *CLRN1* | rs358959 | 3 | 150776600 | G/A | 0.233 | 0.77 (0.47-1.26) |
| *LOC10537-7766* | rs4700987 | 5 | 180251561 | A/T | 0.165 | 0.99 (0.55-1.78) |
| *LINC00847* | rs4700988 | 5 | 180255963 | C/A | 0.165 | 0.99 (0.55-1.79) |
| *EPHA7* | rs1947048 | 6 | 93012151 | G/A | 0.155 | 0.95 (0.52-1.74) |
| rs12197506 | 6 | 93014723 | T/G | 0.155 | 0.95 (0.52-1.74) |
|  | rs1596491 | 6 | 93015896 | T/A | 0.155 | 0.95 (0.52-1.75) |
|  | rs1899806 | 6 | 93017419 | C/T | 0.155 | 0.95 (0.52-1.75) |
|  | rs1899807 | 6 | 93017512 | T/C | 0.155 | 0.95 (0.52-1.75) |
|  | rs2588041  | 6 | 93026285 | T/C | 0.155 | 0.95 (0.52-1.75) |
|  | rs2588042 | 6 | 93027959 | G/A | 0.155 | 0.96 (0.52-1.76) |
|  | rs2818130 | 6 | 93034458 | A/G | 0.157 | 1.03 (0.57-1.86) |
|  | rs2818129 | 6 | 93035916 | A/G | 0.159 | 1.08 (0.60-1.94) |
| *BUB3* | rs7918913 | 10 | 124928952 | C/T | 0.249 | 1.24 (0.78-1.99) |
| *TBX3*  | rs6489992 | 12 | 115352769 | A/G | 0.340 | 1.77 (1.40-2.23) |
|  | rs7972038 | 12 | 115352977 | T/C | 0.277 | 1.13 (0.69-1.86) |
|  | rs7958534 | 12 | 115353100 | G/A | 0.262 | 1.18 (0.71-1.96) |
|  | rs10850402 | 12 | 115354123 | A/G | 0.273 | 1.17 (0.71-1.94) |
|  | rs7961916  | 12 | 115355126 | A/C | 0.272 | 1.17 (0.70-1.93) |
|  | rs7970471 | 12 | 115365549 | A/T | 0.266 | 1.17 (0.71-1.93) |
| *RAB22A* | rs55950385 | 20 | 56559152 | G/A | 0.185 | 0.73 (0.43-1.24) |

**a** Chromosome; b Positions based on GRCh37/hg 19 build; c Effect allele / Reference allele; d Minor Allele Frequency; SNP: single nucleotide polymorphism; OR: odds ratio for effect alleles; CI: Confidence Interval.