***ADRB2* Haplotypes and Asthma Exacerbations in Children and Young Adults: A Meta-Analysis**

**Running Title:** *ADRB2* Haplotypes and Asthma Exacerbations

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**ABSTRACT**

**Background:** The polymorphism Arg16 in β2-adrenergic receptor (*ADRB2*) gene has been associated with an increased risk of exacerbations in asthmatic children treated with long-acting β2-agonists (LABA). However, it remains unclear whether this increased risk is mainly attributed to this single variant or the combined effect of the haplotypes of polymorphisms at codons 16 and 27.

**Objective:** We assessed whether the haplotype analysis could explain the association between the polymorphisms at codons 16 (Arg16Gly) and 27 (Glu27Gln) in *ADRB2* and risk of asthma exacerbations in patients treated with LABA and inhaled corticosteroids (ICS)

**Methods:** The study was undertaken using data from 10 cohorts (n=5,903) of the multi-ethnic Pharmacogenomics in Childhood Asthma (PiCA) consortium. Asthma exacerbations were defined as asthma-related use of oral corticosteroids or hospitalization/emergency department visits in the past 6 or 12 months prior to the study visit/enrolment. The association between the haplotypes and the risk of asthma exacerbations was performed per cohort using haplo.stats package adjusted for age and sex. Results were meta-analyzed using the inverse variance weighting method assuming random-effects.

**Results:** In subjects treated with LABA and ICS (n=832, age:3-21 years), Arg16/Gln27 vs. Gly16/Glu27 (OR:1.40, 95%CI:1.05-1.87, I2=0.00%) and Arg16/Gln27 vs. Gly16/Gln27 (OR:1.43, 95%CI:1.05-1.94, I2=0.00%), but not Gly16/Gln27 vs. Gly16/Glu27 (OR:0.99, 95%CI:0.71-1.39, I2=0.00%), were significantly associated with an increased risk of asthma exacerbations. The sensitivity analyses indicated no significant association between the ADRB2 haplotypes and asthma exacerbations in the other treatment categories i.e., Saba alone (n=973), ICS alone (n=2,623), ICS plus leukotriene receptor antagonist (LTRA; n=338), or ICS plus LABA plus LTRA (n=686).

**Conclusion and clinical relevance:** The *ADRB2* Arg16 haplotype, presumably mainly driven by the Arg16, increased the risk of exacerbations in asthmatic patients treated with LABA and ICS. This finding could be beneficial in *ADRB2* genotype-guided treatment in asthmatic patients and will improve patient outcomes.

**Keywords:** asthma exacerbations; long-acting β2-agonists; inhaled corticosteroids; *ADRB2*; haplotypes

**INTRODUCTION**

Asthma is a common, heterogeneous, and chronic respiratory disease. Despite treatment, patients might experience exacerbations that can be life-threating. The combination therapy of inhaled corticosteroids (ICS) and long-acting β2-agonists (LABA) is one of the recommended treatments for the control of asthma in children.1 However, response to treatment with LABA varies inter-individually and this might be partly mediated by genetic variation.2

The β2-adrenergic receptor is a member of the G protein-coupled transmembrane receptors broadly located on airway smooth muscle cells.3 The β2-adrenergic receptor (*ADRB2*) gene, a small intron-less gene on chromosome 5q31.32, encodes the receptor and contains different single nucleotide polymorphisms (SNPs). Of these SNPs, the coding non-synonymous variants rs1042713 (Arg16Gly), a Glycine-to-Arginine amino acid substitution at codon 16, and rs1042714 (Gln27Glu), a Glutamine-to-Glutamic acid amino acid substitution at codon 27, that are in linkage disequilibrium, have been found to be associated with asthma and asthma phenotypes.4-6

Although various studies have investigated the association between the *ADRB2* polymorphisms and response to LABA, the results are conflicting and inconclusive.7-11 A recent meta-analysis in the Pharmacogenomics in Childhood Asthma12 (PiCA) consortium showed that asthmatic children carrying 1 or 2 Arg allele(s) at rs1042713 and treated with ICS plus LABA have an increased risk of exacerbations.10 Results of previous studies showed that the Gln allele at rs1042714 was a risk factor for asthma and also associated with a less effective response to treatment with inhaled β2-agonists during an acute asthma exacerbation.6,13 Furthermore, most studies, as well as the recent meta-analysis in the PiCA consortium,10 evaluated the effect of each variant independently but not the combined effect of their haplotypes that might yield additional insight into the association between the *ADRB2* variants and asthma exacerbations. Therefore, it is still unclear whether the combined effect of the *ADRB2* polymorphisms at codons 16 and 27 is associated with an increased risk of asthma exacerbations or whether the association is driven by just the single polymorphism at codon 16.

Therefore, we aimed to assess whether the haplotype analysis could explain the association between the polymorphisms at codons 16 and 27 of *ADRB2* and asthma exacerbations in patients treated with LABA and ICS.

**METHODS**

**Study population**

Data from ten studies participating in the Pharmacogenomics in Childhood Asthma (PiCA) consortium12 were analyzed. All studies have been approved by their local medical ethics committees/institutional review boards and parents or participants provided written consent.

BREATHE is an observational study that includes children and young adults (age: 3-22 years)14 with physician-diagnosed asthma recruited from primary and secondary care units in Tayside, Scotland, and Brighton, United Kingdom. The Effectiveness and Safety of Treatment with Asthma Therapy in children (ESTATe) is a study that includes children and young adults (4-19 years) with physician-diagnosed asthma recruited from primary care units in the Netherlands. The followMAGICS study is the follow-up study of the observational Multicenter Asthma Genetics in Childhood Study (MAGICS), which includes physician-diagnosed asthmatic children and young adults (age: 7-25 years)15 recruited from secondary and tertiary centers in Germany and Austria. The Genes-Environment and Admixture in Latino Americans (GALA II) and the Study of African Americans, Asthma, Genes, and Environments (SAGE) studies are two independent case-control asthma cohorts (age: 8-21 years) that focus on two different racial/ethnic groups based on the self-identified ethnicity of the four grandparents of each subject: Hispanics/Latinos (GALA II) and African Americans (SAGE) in the United States and Puerto Rico.16,17 The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study in the Netherlands,18 is an observational cohort study that included children (age 4-12 years) with self-reported regular use of asthma medication recruited through community pharmacies. Children were selected from community pharmacies in the Netherlands that belonged to the Utrecht Pharmacy Practice Network for Education and Research (UPPER).19 The Pediatric Asthma Gene Environment Study (PAGES) is a cross sectional observational study designed to relate asthma outcomes to environmental and genetic factors. Children (age: 5-16 years) with physician-diagnosed asthma were recruited from primary and secondary care centers across Scotland.20 The Pharmacogenetics of Adrenal Suppression Study (PASS) in the United Kingdom (age: 5-18 years) is a multicenter cohort of asthmatic children. The study initially aimed to explore the association between use of corticosteroids and adrenal suppression, and how genetic factors influence this association.21,22 The Singapore Cross Sectional Genetic Epidemiology Study (SCSGES)23 (age: 6-31 years) is an ongoing cross-sectional genetic epidemiology study on allergic diseases among Singapore Chinese individuals. The ethnicity of subjects was self-reported Chinese and confirmed by principal component analysis. Asthma was defined by having a physician-diagnosis of symptoms prior to recruitment.23,24 The SLOVENIA study is a case-control cohort (age: 5-18) and includes asthmatic children and young adults recruited from tertiary health centers from Murska Sobota, Slovenia.25 Further details on the study population are described in the in the Supporting Information.

**Medication data**

Data on asthma treatment was collected either from pharmacy records, parent/patient-reported medication use, or completed study questionnaires (PACMAN, followMAGICS, BREATHE, GALA II, PAGES, SAGE, and SCSGES) or physician prescriptions and pharmacy records (ESTATe, PASS, and SLOVENIA). Asthma treatment was categorized as follows: (1) as-required short-acting β2-agonists (SABA) (2) inhaled corticosteroids (ICS) monotherapy, (3) ICS in combination with LABA, (4) ICS in combination with leukotriene receptor antagonists (LTRA), and (5) ICS in combination with LABA and LTRA. All children in categories 2-5 used as-required SABA.

**Main outcome**

The outcome of interest was asthma exacerbation which t was defined based on the American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines as worsening of asthma symptoms (asthma-related) which requires a short course (3-5 days) oral systemic corticosteroids (OCS) use, hospitalization or emergency department (ED) visits.26 Cases were determined if subjects had at least one asthma exacerbation (described above) in the past 6 or 12 months prior to the study visit or enrolment.

Data on asthma exacerbations, asthma-related OCS use or hospitalization/ED visits, were reported by the parent/child at the study visit or based on study questionnaires or physician records: 1) BREATHE, and PASS: hospitalization or OCS use in the past six months preceding the study visit; 2) PACMAN: ED visits or OCS use in the past 12 months preceding the study visit; 3) GALA II, SLOVENIA, ESTATe, SAGE, PAGES, and SCSGES hospitalization/ED visits or OCS use in the past 12 months preceding the study visit. In followMAGICS, only data on asthma-related hospitalization or ED visits were available in the past 12 months preceding the study visit.12

**Genotyping**

In BREATH and PAGES, genotypes were determined by using Taqman-based allelic discrimination assays on an ABI 7,700 sequence detection system (Applied Biosystems, Foster City, Calif).4,27 In followMAGICS, samples were genotyped using Illumina Sentrix HumanHap300 BeadChip array (Illumina, Inc.).15 In both GALA II and SAGE, samples were genotyped using the Axiom® LAT1 array (Affymetrix Inc.), and quality control (QC) procedures were performed as described previously.28,29 In PACMAN and ESTATe, samples were genotyped using the Illumina Infinium CoreExome-24 BeadChip (Illumina, Inc.).30 In PASS, genotyping was performed using the Illumina Omni Express 8v1 array (Illumina, Inc.). QC procedures and imputation are described elsewhere.22 In SCSGES, genotyping was conducted using Kompetitive Allele Specific PCR (KASP) genotyping platform (LGC, Inc). QC was performed based on the quality of clustering. No imputation was performed.23 In the SLOVENIA study, genotyping of 336 samples was performed with the Illumina Global Screening Array-24 v1.0 BeadChip (Illumina). QC procedures and imputation described elsewhere.30

**Functional annotation of variants and expression quantitative trait loci (eQTL) analysis**

We used HaploRegv4.1 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php)31 to retrieve all proxy SNPs in strong linkage disequilibrium (LD) (r2 threshold > 0.8, limit distance 100 kb, and population panel CEU using 1000 Genomes project) with rs1042713 and rs1042714 in *ADRB2* and to assess the predicted functions of the variants including protein structure, effects on gene regulation, and splicing. We also checked the correlation of the SNPs and their proxies with the expression level of *ADRB2* in whole blood using expression quantitative trait loci (eQTL) data from Genenetwork.32

**Statistical analyses**

Descriptive statistics were used to calculate means and standard deviations for continuous variables and percentages for categorical variables. Hardy-Weinberg equilibrium (HWE) was assessed for each SNP using a web program (http://www.oege.org/software/hwe-mr-calc.shtml) which uses the Pearson chi-squared test for HWE testing.33 In our main analysis, we analyzed the association between haplotype combinations of polymorphisms at codons 16 and 27 of the *ADRB2* gene and asthma exacerbations in the category of children treated with LABA plus ICS. We used the haplo.stats package (version 1.7.7)34 in R adjusting for age and sex in each study separately, and the resulting odds ratio (ORs) were meta-analyzed. The statistical methods of the haplo.stats package assume that all subjects are unrelated and linkage phase of the genetic markers is unknown.34 To address potential heterogeneity between studies, we used the inverse variance weighting method assuming random-effects. We also reported I2 and Cochran’s Q-test of the meta-analysis.35 Forest plots were made using the ‘metafor’ package in R (version 3.3.3).36

Data on asthma-related OCS use were not available in followMAGICS. Therefore, in a sensitivity analysis, we repeated the haplotype analysis (as described above) separately for asthma-related hospitalization/ED visits outcome as well as for asthma-related OCS use outcome. Furthermore, to test the robustness of our result in the treatment category of ICS plus LABA, we repeated the haplotype analysis (as described above) in the other treatment categories as follows; SABA as required, ICS monotherapy, ICS plus LTRA, and ICS plus LTRA plus LABA. Since we investigated the association of haplotype combinations of two polymorphisms and asthma exacerbation, we considered a P-value less than 0.025 (0.05/2) for our main meta-analysis to be statistically significant.

**RESULTS**

**Study characteristics**

The characteristics of the study populations are presented in Table 1. Data on age, sex, and treatment were available for 5,903 children and young adults. Out of these 5,903 subjects, data on asthma exacerbation were available in 5,726 subjects.

Asthma exacerbation occurred in 2,494 patients (43%) and the proportion of asthma exacerbation ranged from 9.7% (PACMAN) to 86% (PASS) across the studies. The mean age (SD) of the patients ranged between 8.7 (2.3) years for PACMAN and 17.1 (3.0) years for followMAGICS, and in all studies, the majority of patients were male. The percentage of subjects treated with ICS plus LABA differed across studies and ranged from 10.2% in GALA II to 50.3% in followMAGICS. In addition, all patients in SLOVENIA and SCSGES were treated with ICS monotherapy.

Table 2 shows the *ADRB2* genotyping and haplotype data. The risk allele (Arg) frequency for rs1042713 was highest in African-Americans, SAGE, (0.51), followed by SCSGES, (0.45). The risk allele (Arg) frequency for rs1072713 ranged between (0.34) for ESTATe and (0.41) for PACMAN across the European studies. The risk allele (Gln) frequency for rs1042714 was highest in SCSGES (0.92) followed by African-Americans, SAGE, (0.82). The risk allele (Gln) frequency for rs1042714 was similar across the European studies and ranged between (0.54) for PASS and (0.60) for ESTATe and SLOVENIA. Both SNPs were in HWE in all studies and they showed a complete LD (D' ~ 1) with r2 thatranged from 0.10 in SCSGES to 0.50 in PASS.

Three haplotypes were determined at positions 16 and 27, and haplotype frequencies were as following: Gly16/Glu27 (ranged from 0.07 to 0.46), Arg16/Gln27 (ranged from 0.34 to 0.56) and Gly16/Gln27 (ranged from 0.17 to 0.37) (Table 2).

**Risk of asthma exacerbations in children treated with ICS plus LABA**

Data on the outcome (asthma-related OCS use or hospitalization/ED visits), haplotypes, and ICS plus LABA treatment were available in seven studies (n = 832 , age = 3-21 years). The meta-analysis indicated that Arg16/Gln27 vs. Gly16/Glu27 (OR:1.40, 95% CI:1.05-1.87, I2 = 0.00%, P = 0.022) and Arg16/Gln27 vs. Gly16/Gln27 (OR:1.43, 95% CI:1.05-1.94, I2 = 0.00%, P = 0.023), were significantly associated with an increased risk of asthma exacerbations (Figure 1). However, Gly16/Gln27 vs. Gly16/Glu27 (OR:0.99, 95% CI:0.71-1.39, I2 = 0.00%, P = 0.946), was not associated with the risk of asthma exacerbations

**Sensitivity analyses**

In patients treated with ICS plus LABA, we repeated the haplotype analysis separately for asthma-related hospitalization/ED visits and for asthma-related OCS use and observed the same trend as the main analysis (see Figures S1 and S2 in the Supporting Information). Furthermore, no association between the *ADRB2* haplotypes and risk of asthma exacerbations was observed in any of the other treatment groups. (see Table S1 in the Supporting Information).

**Functional annotation and eQTL analysis of the *ADRB2* variants**

Functional annotation, using Haploreg v4.1 data,31 showed that rs1042713 and rs1042714 had several proxy variants in strong LD (D` = 1 and r2 > 0.8), but none of them was a non-synonymous proxy (see Table S2 and Table S3 in the Supporting Information). Furthermore, the cis-eQTL data from Genenetwork showed that not only the Arg allele of rs1042713 but also the Gln allele of rs1042714 was associated with reduced levels expression of *ADRB2* in whole blood.32 Therefore, these data indicated that the variants alters the *ADRB2* expression and function.

**DISCUSSION**

In this large multi-ethnic meta-analysis, we observed that the Arg16/Gln27 haplotype vs. the Gly16/Glu27 haplotype and the Arg16/Gln27 haplotype vs. the Gly16/Gln27 haplotype were associated with an increased risk of asthma exacerbations in children and young adults treated with ICS plus LABA. Considering that no statistically significant association between the Gly16/Gln27 haplotype vs. the Gly16/Glu27 haplotype and asthma exacerbations was observed, we might conclude that the combined effect of two polymorphisms at codon 16 and 27 on asthma exacerbation is presumably just driven by the Arg16. Furthermore, we did not find an increased risk for exacerbations in asthmatic children carrying the Arg16 haplotype in any of the other treatment categories. The lack of association in the treatment categories containing ICS, LABA, and LTRA might be due to both the bronchodilation and anti-inflammation effects of LTRA37, as well as to the relatively small sample size.

There was no heterogeneity (I2 = 0.00%) in the main analysis between studies (Figure 1); however, the ORs were slightly different across studies. The proportion of asthma exacerbation largely varied between studies, lowest in PACMAN (recruiting from primary care and community pharmacies) and highest in PASS (recruiting from tertiary care). which might be due to the recruitment of patients from different health care settings (i.e., primary, secondary, tertiary care, or community pharmacies) and thus reflect differences in asthma severity. Also, asthma treatment policy that affects doctors’ underlying tendencies to prescribe OCS varies in different countries, which in turn could influence the proportion of asthma exacerbation.38 In all studies, both SNPs were in complete linkage disequilibrium (D`~1) with each other; as a result, we determined three haplotypes of the four possible haplotypes (Arg16/Glu27 were not reported), which is in line with previous findings.39,40 Furthermore, considering ethnicity variability in our study population, we observed different minor allele frequencies in each SNP that resulted in considerable variations in r2, which indicates the correlation coefficient of the allele frequencies. We also observed the highest risk allele frequencies (the Arg allele at rs1042713 and the Gln allele at rs1042714) in GALA II, SAGE, and SCSGES, the risk allele frequencies were higher, whereas the Gly16/Glu 27 haplotype frequency was substantially the lowest in these three cohorts, consistent with previous work.41-46

A recent systematic review2 reported studies that investigated the association between the *ADRB2* variants and response to LABA in children and adults with asthma. In children, most studies reported an increased risk of asthma exacerbation in carriers of Arg 16, whereas no association was found in adults.4,7,8,10,47 So far, only two studies investigated the effect of rs1042714 on asthma exacerbation in children treated with LABA plus ICS and did not report significant associations.4,9 Similarly, in adults, no association between rs1042714 and response to LABA concerning asthma exacerbation has been shown in a post hoc analysis from a randomized clinical trial.8

A few studies examined the association between these *ADRB2* haplotypes in subjects with asthma. However, they mainly focused on changes in forced expiratory volume in 1 second (FEV1),42 forced vital capacity (FVC), FEV1/FVC ratio,43 and overall mean changes in morning peak flow as primary outcomes.48 To the best of our knowledge, this is the first large meta-analysis investigating the association between the *ADRB2* haplotypes and the risk of asthma exacerbations in patients treated with LABA plus ICS to this date. We know from the literature that Arg16 at rs1042713 is associated with an increased risk for asthma exacerbation; however, this association was not yet investigated in the Arg haplotypes carriers.4,5,10

The exact mechanism by which *ADRB2* polymorphisms confer risk for asthma exacerbations in patients treated with ICS plus LABA is still unknown. The mechanism(s) underlying the association between the Arg16 allele and an increased risk of exacerbation in asthmatic patients treated with LABA plus ICS might involve an enhanced agonist-induced downregulation and uncoupling of airway β2-receptor, resulting in subsensitivity of bronchoprotective response.49 There is some evidence from the literature that *ADRB2* haplotypes regulate receptor transcript and protein expression.42 Previous *in-vitro* findings indicated that the expression of the Arg16/Gln27 haplotype was significantly lower than the Gly16/Glu27 haplotype.42 The latter results42 are in line with eQTL data,32 demonstrating decreased expression levels of *ADRB2* in the carriers of Arg16 and Gln27. Another possible explanation, based on the dynamic baseline receptor model proposed by Liggett,50 could be that the Arg16 genotype would be slightly more resistant than the Gly16 genotype to endogenous downregulation and desensitization. Thus the Arg 16 genotype would remain more susceptible to further subsensitivity to the chronic use of exogenous agonists.50 Hence, the observed weakened response to LABA in carriers of the Arg16/Gln27 haplotype is plausible.

As for all observational research, our study has strengths and limitations. The current study is to be the largest meta‐analysis investigating the combined effect of the *ADRB2* variants in asthmatic patients treated with ICS plus LABA. Also, we used quality-controlled genotyping data, physician diagnosed-asthma, and relevant clinical outcomes (asthma exacerbations). As the first limitation, we did not determine haplotype frequency using gene-counting estimates based on phase-known data. Instead, we obtained haplotype frequency estimates using the expectation-maximization (E-M) algorithm that previous studies have demonstrated the usefulness of this approach (E-M method),51 and the validity of the statistical technique of this method.52 Second, although the *ADRB2* rare variants could affect treatment response to LABA therapy,53 our study was not powered to conduct rare variant analysis. Third, as we lacked information on treatment adherence and dosing in some of the PiCA studies, we could not adjust for these factors in our analyses. Fourth, as gene expression and eQTL are tissue-specific, ideally, they should be examined in the lung tissue of patients with asthma, treated with ICS plus LABA. Finally, in our meta-analysis, we observed a significant OR (1.40), 95% CI (1.05, 1.87) with a P = 0.022, applying a multiple testing correction (P<0.025) to define statistically significant results. We also calculated a prediction interval (PI); the PI in a random-effects model contains a highly probable effect estimate (OR) for a future observation if a new setting is similar to those included in the meta-analysis.54,55 In this case, the 95% PI is (0.96, 2.04), and thus indeed broader than the 95% CI.” In conclusion, we found that the Arg16 haplotype in *ADRB2* , presumably mainly driven by Arg16, increased the risk of asthma exacerbations among users of LABA and ICS. The clinical benefits and risks associated with the use of LABA in patients with the Arg16 haplotype and genotypes need to be evaluated in randomized clinical trials such as the ongoing precision medicine clinical trial (the PUFFIN trial) investigating *ADRB2* genotype-guided (Arg16 genotype) treatment in children with asthma.56

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**CONFLICT OF INTEREST**

Dr. Maitland-van der Zee reports personal fees for participating in advisory boards from Astra Zeneca, Boehringer Ingelheim, unrestricted research grants from Boehringer Ingelheim, and GlaxoSmithKline (GSK). Dr. Vijverberg has received a grant from GSK during the conduct of the study. Dr. Pino-Yanes reports grants and non-financial support from the Spanish Ministry of Economy and Competitiveness, and Instituto de Salud Carlos III (ISCIII); during the conduct of the study. Dr. CHEW reports grants from Singapore Ministry of Education Academic Research Fund, Singapore Immunology Network, National Medical Research Council (NMRC) (Singapore) and the Agency for Science Technology and Research (A\*STAR) (Singapore), during the conduct of the study; consultancy fees from Sime Darby Technology Centre; First Resources Ltd; Genting Plantation, and Olam International, outside the submitted work. Dr. Janssens reports grants from Vectura and personal fees from Vertex, outside the submitted work. Dr. Katia Verhamme reports grants from ZonMw, during the conduct of the study; and KV works for a research group who receives/received unconditional research grants from Yamanouchi, Pfizer/Boehringer Ingelheim, Novartis, and GSK; outside the submitted work. Dr. Engelkes reports grants from ZonMw, during the conduct of the study. Dr. Kabesch reports that his institution received grant from European Union, German Ministry of Education and Research, German Research Foundation, and received personal fees from consultancy and for participating in advisory boards from Bionorica, Sanofi, Novartis, Bencard, European respiratory society (ERS), European Academy of Allergy and Clinical Immunology (EAACI), American Thoracic Society (ATS), Novartis, Glaxo, Nutricia, Hipp; Allergopharma, and Teva; outside the submitted work. Hernandez-Pacheco reports grants from Instituto de Salud Carlos III (ISCIII), during the conduct of the study. The rest of the authors declare that they have no relevant conflicts of interest. The rest authors declare no conflict of interest

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**Table 1:** Characteristics of the study populations

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Characteristics** | **BREATHE** | **ESTATe** | **Follow**  **MAGICS** | **GALA II** | **PACMAN** | **PAGES** | **PASS** | **SAGE** | **SLOVENIA** | **SCSGES** |
| **n** | 998 | 101 | 167 | 1,618 | 791 | 722 | 384 | 740 | 212 | 170 |
| **Male sex, %** | 60.0 | 58.0 | 62.3 | 55.7 | 62.3 | 57.6 | 56.0 | 52.3 | 56.1 | 68.2 |
| **Mean age, y (SD)** | 10.2 (4.0) | 10.6 (4.2) | 17.3 (3.0) | 12.4 (3.2) | 8.7 (2.3) | 9.8 (3.7) | 11 (3.3) | 13.8 (3.5 ) | 10.8 (3.4) | 14.0 (6.4) |
| **Ethnicity, n. (%)** | | | | | | | | | | |
| Caucasian | 998 (100) | 96 (95) | 167 (100) | N/A | 711 (89.9) | 360 (50) | 384 (100) | N/A | 212 (100) | N/A |
| Hispanic | N/A | N/A | N/A | 1,618.(100) | 3 (0.4) | N/A | N/A | 744 (100) | N/A | N/A |
| Asian | N/A | 1 (1) | N/A | N/A | 6 (0.8) | 11 (1.5) | N/A | N/A | N/A | 170 (100) |
| African | N/A | 0 (0) | N/A | N/A | 9 (1.1) | N/A | N/A | N/A | N/A | N/A |
| Mixed | N/A | 2 (2) | N/A | N/A | 53 (6.7) | 15 (2) | N/A | N/A | N/A | N/A |
| Unknown (missing) | N/A | 2 (2) | N/A | N/A | 9 (1.1) | 336 (46.5) | N/A | N/A | N/A | N/A |
| **Treatment group, n. (%)** | | | | | | | | | | |
| SABA alone | 173 (17.3) | 0 (0.0) | 25 (15.0) | 576 (35.6) | 80 (10.1) | 79 (10.9) | 0 (0.0) | 207 (27.9) | N/A | N/A |
| ICS alone | 562 (56.3) | 65 (64.0) | 39 (23.3) | 538 (33.2) | 497 (62.8) | 271 (37.6) | 29 (7.5) | 367 (49.6) | 212 (100) | 170 (100) |
| ICS + LABA | 142 (14.3) | 34 (34.0) | 84 (50.3) | 165 (10.2) | 148 (18.7) | 135 (18.7) | 126 (33.0) | 98 (13.2) | N/A | N/A |
| ICS + LTRA | 37 (3.7) | 0 (0.0) | 4 (2.4) | 208 (12.9) | 21 (2.7) | 65 (9.0) | 0 (0.0) | 35 (4.7) | N/A | N/A |
| ICS + LABA + LTRA | 84 (8.4) | 2 (2.0) | 15 (9.0) | 131 (8.1) | 45 (5.7) | 172 (23.8) | 229 (59.5) | 33 (4.6) | N/A | N/A |
| **Asthma exacerbations in the past year or in the last six months prior to the study visit/enrolment** | | | | | | | | | | |
| Hospitalization/ED\*, n. (%)**#** | 147 (14.7) | 13 (12.9) | 11 (6.6) | 865 (54.8) | 42 (5.5) | 151 (21.7) | 290 (76.0) | 272 (39.0) | 49 (27.7) | 34 (20.0) |
| OCS use\*, n. (%)# | 234 (23.4) | 36 (35.6) | N/A | 587 (37.5) | 46 (5.8) | 316 (45.7) | 198 (52.0) | 162 (22.4) | 23 (12.9) | 36 (21.2) |
| Asthma exacerbation\*, n. (%)**#** | 250 (25.0) | 49 (48.5) | N/A | 1,013(64.3) | 75 (9.7) | 346 (50.0) | 331 (86.0) | 317 (45.8) | 54 (30.3) | 59 (34.7) |

\*ED, emergency department visits; OCS use, oral corticosteroids use; Asthma exacerbations, asthma-related hospitalization/ED visits or oral corticosteroid use. #Data on asthma-related hospitalization/ED visits outcomes were missing in 40 subjects in GALA II, 24 subjects in PACMAN, 27 subjects in PAGES, 43 subjects in SAGE, and 35 subjects in SLOVENIA; data on asthma-related oral OCS use were missing in 49 subjects in GALA II, 30 subjects in PAGES, 16 subjects in SAGE, and 34 subjects in SLOVENIA, data on asthma exacerbations were missing in 44 subjects in GALA II, 21 subjects in PACMAN, 30 subjects in PAGES, 48 subjects in SAGE, and 34 subjects in SLOVENIA.

**Table 2:** *ADRB2* genotype and haplotype data

| **Characteristics** | **BREATHE** | **ESTATe** | **Follow**  **MAGICS** | **GALAII** | **PACMAN** | **PAGES** | **PASS** | **SAGE** | **SLOVENIA** | **SCSGES** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Subjects with rs1042713. n** | 998 | 101 | 167 | 1,618 | 791 | 720 | 384 | 740 | 212 | 170 |
| **Risk allele (Arg) frequency (rs1042713) (A)** | 0.37 | 0.34 | 0.38 | 0.44 | 0.41 | 0.37 | 0.37 | 0.51 | 0.37 | 0.45 |
| **rs1042713 genotype, no. (%)** | | | | | | | | | | |
| Arg/Arg | 154 (15.4) | 14 (13.9) | 25 (15.0) | 306 (18.9) | 124 (15.7) | 101 (14.1) | 59 (15.4) | 198 (26.7) | 35 (16.5) | 28 (16.5) |
| Arg/Gly | 436 (43.7) | 40 (39.6) | 78 (46.7) | 819 (50.6) | 402 (50.8) | 330 (45.8) | 167 (43.5) | 355 (48.0) | 87 (41.0) | 96 (56.5) |
| Gly/Gly | 408 (40.9) | 47 (46.5) | 64 (38.3) | 493 (30.5) | 265 (33.5) | 289 (40.1) | 158 (41.1) | 187 (25.3) | 90 (42.5) | 46 (27.1) |
| **Subjects with rs1042714. n** | 998 | 101 | 167 | 1,622 | 791 | 722 | 384 | 744 | 212 | 169 |
| **Risk allele (Gln) frequency (rs1042714)** | 0.56 | 0.60 | 0.58 | 0.78 | 0.63 | 0.56 | 0.54 | 0.82 | 0.60 | 0.92 |
| **rs1042714 genotype, no. (%)** | | | | | | | | | | |
| Gln/Gln | 307 (30.8) | 36 (35.6) | 57 (34.1) | 971 (59.9) | 313 (39.6) | 232 (32.1) | 115 (30.0) | 497 (66.8) | 81 (38.2) | 144 (84.7) |
| Gln/Glu | 495 (49.6) | 50 (49.5) | 79 (47.3) | 576 (35.5) | 376 (47.5) | 349 (48.4) | 184 (47.9) | 223 (30.0) | 91 (42.9) | 25 (14.7) |
| Glu/Glu | 196 (19.6) | 15 (14.9) | 31 (18.6) | 75 (4.6) | 102 (12.9) | 141 (19.5) | 85 (22.1) | 24 (3.2) | 40 (18.9) | 0 (0.0) |
| **Subjects with both SNPs. n** | 998 | 101 | 167 | 1,618 | 791 | 714 | 384 | 740 | 212 | 169 |
| **Haplotype frequency** | | | | | | | | | | |
| Arg16/Gln27 | 0.37 | 0.34 | 0.38 | 0.44 | 0.41 | 0.37 | 0.37 | 0.51 | 0.37 | 0.56 |
| Gly16/Gln27 | 0.18 | 0.27 | 0.20 | 0.34 | 0.22 | 0.19 | 0.17 | 0.31 | 0.23 | 0.37 |
| Gly16/Glu27 | 0.45 | 0.39 | 0.42 | 0.22 | 0.37 | 0.44 | 0.46 | 0.18 | 0.40 | 0.07 |
| **Linkage disequilibrium between rs1042713 and rs1042714** | | | | | | | | | | |
| **r2 (D`)** | 0.47 (~1) | 0.33 (1) | 0.43 (0.98) | 0.23 (1) | 0.40 (~1) | 0.46 (~1) | 0.50 (~1) | 0.23 (1) | 0.40 (1) | 0.1 (1) |

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**FIGURE LEGENDS**

**Figure1:** Forest plots of *ADRB2* haplotypes and risk of asthma exacerbation (asthma-related hospitalization/emergency department visits or oral corticosteroid use) in patients with asthma treated with ICS plus LABA. These plots describe odds Ratios (OR) and corresponding 95% confidence intervals (95% CI) adjusted for age and sex.

