LTA4H rs2660845 association with montelukast response in early and late-onset asthma

Maroteau, Cyrielle, Espuela-Ortiz, Antonio, Herrera-Luis, Esther, Srinivasan, Sundararajan, Carr, Fiona, Tavendale, Roger, Wilson, Karen, Hernandez-Pacheco, Natalia, Chalmers, James D, Turner, Steve, Mukhopadhyay, Somnath, Maitland-van der Zee, Anke-Hilse, Burchard, Esteban G, Pino-Yanes, Maria, Young, Simon et al. (2021) LTA4H rs2660845 association with montelukast response in early and late-onset asthma. PLoS One, 16 (9). a0257396 1-17. ISSN 1932-6203

This version is available from Sussex Research Online: http://sro.sussex.ac.uk/id/eprint/101847/

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher’s version. Please see the URL above for details on accessing the published version.

Copyright and reuse:
Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.
RESEARCH ARTICLE

LTA4H rs2660845 association with montelukast response in early and late-onset asthma

Cyrielle Maroteau,1,2,*, Antonio Espuela-Ortiz,3 Esther Herrera-Luis3 Sundararajan Srinivasan5, Fiona Carr2, Roger Tavendale2, Karen Wilson2, Natalia Hernandez-Pacheco3,4, James D. Chalmers5, Steve Turner6, Somnath Mukhopadhyay7, Anke-Hilse Maitland-van der Zee8,9,10, Esteban G. Burchard11,12, Maria Pino-Yanes13,14, Simon Young15,16, Glenda Lassi17, Adam Platt17, Colin N. A. Palmer3, on behalf of the PiCA Consortium

1 Centre for Genomics Research, Discovery Science, BioPharmaceuticals R&D, AstraZeneca, Cambridge, United Kingdom, 2 Division of Population Health and Genomics, School of Medicine, University of Dundee, Dundee, United Kingdom, 3 Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, San Cristobal de La Laguna, Santa Cruz de Tenerife, Spain, 4 Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, San Cristobal de La Laguna, Spain, 5 Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Dundee, United Kingdom, 6 Child Health, University of Aberdeen, Aberdeen, United Kingdom, 7 Academic Department of Pediatrics, Brighton and Sussex Medical School, Royal Alexandra Children’s Hospital, Brighton, United Kingdom, 8 Division of Pharmacopidemiology and Clinical Pharmacology, Utrecht University, Utrecht, The Netherlands, 9 Department of Respiratory Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands, 10 Department of Pediatric Respiratory Medicine and Allergy, Emma’s Children Hospital, Academic Medical Center (AMC), University of Amsterdam, Amsterdam, The Netherlands, 11 Department of Medicine, University of California San Francisco, San Francisco, California, United States of America, 12 Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, United States of America, 13 CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Comunidad de Madrid, Spain, 14 Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, San Cristobal de La Laguna, Santa Cruz de Tenerife, Spain, 15 BioPharmaceutical Precision Medicine, Oncology R&D, AstraZeneca, Cambridge, United Kingdom, 16 Research & Development Centre, Sysmex, Cambridge, United Kingdom, 17 Translational Science and Experimental Medicine, Research and Early Development, Respiratory & Immunology, BioPharmaceuticals R&D, AstraZeneca, Cambridge, United Kingdom

* c.maroteau@dundee.ac.uk

Abstract

Leukotrienes play a central pathophysiological role in both paediatric and adult asthma. However, 35% to 78% of asthmatics do not respond to leukotriene inhibitors. In this study we tested the role of the LTA4H regulatory variant rs2660845 and age of asthma onset in response to montelukast in ethnically diverse populations. We identified and genotyped 3,594 asthma patients treated with montelukast (2,514 late-onset and 1,080 early-onset) from seven cohorts (UKBiobank, GoSHARE, BREATHE, Tayside RCT, PAGES, GALA II and SAGE). Individuals under montelukast treatment experiencing at least one exacerbation in a 12-month period were compared against individuals with no exacerbation, using logistic regression for each cohort and meta-analysis. While no significant association was found with European late-onset subjects, a meta-analysis of 523 early-onset individuals from European ancestry demonstrated the odds of experiencing asthma exacerbations by...
ponents of exacerbation under montelukast treatment, presumably due to the up-regulation of LTA4H activity. These findings support a precision medicine approach for the treatment of asthma with montelukast.

Introduction

Asthma is a common chronic inflammation of the airways manifesting as shortness of breath, wheeze and chest tightness that can lead to death, especially in older adults. With around 339 million affected people worldwide [1] and an increase in incidence over recent decades, asthma is still under-diagnosed and under treated. The wide variation observed within asthma-related traits, including age of onset, respiratory symptoms, risk factors, frequency of exacerbations, lung function, comorbidities, and underlying inflammatory patterns, provide difficulty for diagnosis and are believed to contribute to heterogeneous treatment response.

Although asthma is recognised as a heterogenous disease, conventional stratification of relevant asthma subgroups is mainly limited to Th2-high (eosinophilic) or Th2-low (non-eosinophilic) according to the level of type 2 helper T cell (Th2) cytokine mediated eosinophilic airway inflammation. Other current biomarkers of airway inflammation, such as immunoglobulin E (IgE), or fraction of exhaled nitric oxide (FeNO), are also used clinically to target treatment and predict future risk [2]. In the recent years, new clinical biomarkers of asthma phenotype such as disease severity, exacerbation triggers and comorbidities have been widely used, with the age of onset of asthma and genetic risk factors recently emerging [3–7]. Early-onset asthma is typically Th2-high atopic or allergic and responsive to steroids. Late-onset asthma is more diverse than early-onset asthma and presents with both Th2-high and Th2-low phenotype such as disease severity, exacerbation triggers and comorbidities have been widely used, with the age of onset of asthma and genetic risk factors recently emerging [3–7]. Early-onset asthma is typically Th2-high atopic or allergic and responsive to steroids. Late-onset asthma is more diverse than early-onset asthma and presents with both Th2-high and Th2-low types with the latter being associated to non-atopy or lifestyle (smoking, obesity) and being characterised by a non-response to steroid therapy [8]. Although asthma shares common genetics with allergic diseases [9], none all individuals with allergic asthma respond to steroids [10]. This add to the emerging evidence of asthma genetic heterogeneity defining different underlying biological mechanisms, which should help predict the patients’ likely responses to treatment [11].

Leukotriene modifiers are pharmacological therapies for the treatment of asthma and allergic rhinitis. As established through numerous clinical trials, drugs such as montelukast have been shown to be effective in improving lung function, asthma control and quality of life, by reducing airway hyperresponsiveness and eosinophilia [12]. Recent guidelines for the management of asthma in children and adults from the National Institute for Health and Clinical Excellence (NICE), highlighted leukotriene receptor antagonists (LTRA) as a key part of asthma management and recommended their use as the first line add-on therapy for persistent asthmatic patients who still show symptoms despite low dose of inhaled corticosteroids (ICS) [13]. However, a range of 35 to 78 percent of the patients treated with leukotriene inhibitors such as montelukast were found unresponsive across several independent studies [10, 14–17].
Asthma is a very heterogenous disease resulting from a complex interplay between genetic susceptibility and environment. Despite the ongoing efforts there are many patients with poorly controlled asthma and with treatment based on symptomatology rather than underlying disease pathobiology. A precision medicine approach, based on genetic profiles, biomarkers measures and clinical assessments, is vital to target treatments to the patient population most likely to benefit. Pharmacogenetic studies are therefore essential to the understanding of the genetic interplay in drug response and identification of individuals best targeted treatments.

Pharmacogenetic studies have investigated candidate genes in the leukotriene pathway, showing that interpatient variability might be influenced by genetic variation. Variants in \textit{ALO}X5, \textit{LTA4}H, \textit{LTC4}S, \textit{ABCC}1, \textit{CYSL}TR2, and \textit{SLCO}2B1 genes may contribute to the heterogeneity of response to leukotriene modifiers, including montelukast \cite{14, 18–20}. To date, only the effect of a small number of genetic variants has been validated in independent studies \cite{10, 21}. We focused our analysis on a particular variant in the regulatory region of the leukotriene-\textit{A}4 hydrolase (EC3.3.2.6) gene (\textit{LTA4}H), the single nucleotide polymorphism (SNP) rs2660845. This variant has been shown to influence montelukast response through the prevention of exacerbations in a cohort of young adults with asthma from the United States \cite{14} as well as improvement of pulmonary parameters such as peak expiratory flow (PEF) and forced expiratory volume in one second (FEV1) in a Japanese cohort \cite{16}. However, the sample size of those studies was small, and the association observed between the rs2660845 SNP and responsiveness to montelukast has not been validated.

In this study, we utilised a very large dataset, consisting of previously described, ethnically diverse case-control studies (GALA II and SAGE), asthma cohorts (BREATHE and PAGES), a randomised clinical trial (RCT) (Tayside RCT) and two large real-life cohorts (the UKBiobank and GoSHARE), to explore the association of rs2660845 with lack of montelukast treatment response. We also wished to determine if age of asthma onset status further refined this putative pharmacogenomic association to provide the beginnings of a precision medicine strategy for the treatment of asthma with montelukast.

Methods

Study design

Asthmatic patients treated with montelukast for at least 6 months were included in this study. We defined early-onset as individuals ≤18 years old at asthma diagnosis and late-onset for individuals over 18 years old at asthma diagnosis.

Early-onset were recruited from five cross-sectional studies (PAGES, BREATHE, Tayside RCT, GALA II and SAGE) as well as from one longitudinal study (GoSHARE, the Genetics of Scottish Health Registry \cite{22}). BREATHE, Tayside RCT and PAGES’ patients were recruited from primary and secondary care in Scotland. BREATHE and Tayside RCT details of enrolment have been presented in detail previously \cite{23, 24}. PAGES, the Pediatric Asthma Gene Environment Study, was an exploratory study of the genetic variation and exposure in asthmatic Scottish children from various ethnicities \cite{25}. The Genes-environments and Admixture in Hispanics/Latinos (GALA II) study and the Study of African-Americans, Asthma, Genes, and Environments (SAGE) recruited children from community- and clinic-based centers, with GALA II focused on Hispanics/Latinos and SAGE focused on African Americans \cite{26}. BREATHE, PAGES, GALA II and SAGE studies were all part of the Pharmacogenetics in Childhood Asthma (PiCA) consortium \cite{27}. For all studies from the PiCA consortium and Tayside RCT, asthmatic individuals under montelukast treatment were selected by a physician and information such as age, sex, BMI and OCS use, hospitalization and exacerbation were available from a questionnaire filled at patient enrolment. In GoSHARE, asthma patients were...
selected from a dataset containing complete electronic medical records (EMR), prescription information, hospital, and emergency room records (International Classification of Diseases 10th revision [ICD-10] codes J45) from Tayside, Scotland (S2 Table).

Late-onset individuals were recruited from the UKBiobank [28] and GoSHARE. In the UKBiobank, selected asthma patients were British white individuals from self-reported questionnaires and hospital records (ICD10: J45) released in October 2019 by querying an AstraZeneca in-house protected UKBiobank database server. Other information on UKBiobank individuals such as age of onset of asthma and sex were extracted from self-reported questionnaires whereas montelukast usage was extracted from the recent release of drug prescriptions (October 2019). https://www.sciencedirect.com/science/article/pii/S2213260019300554-bib9

The studies included in this article comply with the Declaration of Helsinki, and locally appointed ethics committee have approved respective research protocols and consent procedures. Study participants for GoSHARE provided written informed consent to their data as well as spare blood, left after routine venepuncture, to be held in NHS databases and used for suitable research projects. For the UKBiobank, written consent form was filled by participants on their enrolment in the different selected centre and witnessed and signed by an NHS staff member. Written informed consent was obtained from the participants and/or parent/guardian as relevant for PAGES, BREATHE, Tayside RCT, SAGE and GALAII studies. All data were fully anonymized before granted access. PAGES was approved by the Cornwall and Plymouth Research Ethics Committee (Plymouth, United Kingdom). GoSHARE, BREATHE and Tayside RCT were approved by the Tayside Committee on Medical Research Ethics (Dundee, United Kingdom). The UKBiobank was approved by the National Research Ethics Committee. 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: 217802 and 210362, respectively).

Asthma treatment was categorized following British Thoracic Society/Scottish Intercollegiate Guideline Network (BTS/SIGN) guidelines [29]. Only individuals on montelukast therapy were selected for further analysis (S2 Table). Both late-onset and early-onset patients were selected from the GoSHARE study. As age of asthma onset was not reported in the EMR for GoSHARE, we use age at first salbutamol, age at first Inhaled corticosteroid and age at first montelukast prescription, all three over 18 years old to characterize the late-onset group GoSHARE(a) and all three under 18 years old to characterize the early-onset group GoSHARE (b).

**DNA collection, extraction and analysis**

For BREATHE and Tayside RCT, saliva was collected in commercially available kits (Oragene, DNA Genotech Ontario, Canada). DNA was prepared using the Qiagen DNeasy 96 kit (Qiagen, Manchester, UK) and genotypes were determined in the University of Dundee laboratory using TaqMan™ based allelic discrimination arrays on an ABI 7700 sequence detection system (ThermoFisher, Waltham, USA). For GoSHARE (a and b) respectively, DNA was extracted from blood samples taken at recruitment or from a prescribed blood test and genotyped using the Infinium Global Screening Array v2 (Illumina, San Diego, USA). Under the UKBiobank project, all recruited individuals were genotyped using a purpose-designed genotypic array: the UKBiobank Axiom Array. As part of the PiCA consortium, PAGES DNA was selected and genotyped in the Spanish National Genotyping Center (CEGEN-PRB3-ISCIII), using the Axiom Precision Medicine Research Array (PMRA) (Affymetrix, Thermo Fisher Scientific Inc, Waltham, MA). GALA II and SAGE were genotyped using the Axiom LAT1 array (World Array 4, Affymetrix, Santa Clara, CA, United States), as described elsewhere [30]. Studies with
genome-wide genotyping data were subjected to standard quality control procedures, ensuring that SNP genotyping call rate was above 95% and the absence of deviations from Hardy-Weinberg equilibrium (p > 10−6) within control subjects. Samples with discrepancy between genetic sex and reported sex and with family relatedness were removed from the analyses.

**Binary risk of having an asthma exacerbation**

The association between the variant of interest and the risk of having an asthma exacerbation was tested. A binary variable related to the absence or presence of at least one exacerbation event in a time frame of 6 to 12 months (0 = no exacerbation, 1 = at least one exacerbation event) was used (Fig 1). Asthma exacerbations were defined based on the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines as episodes of worsening of asthma symptoms which require a short course (3–5 days) of oral systemic corticosteroids (OCS) use, hospitalizations or emergency department (ED) visits [31]. The definition was then adapted to each study design and information available. Cross sectional studies collected data on asthma treatment either from pharmacy records, parent/patient-reported medication use, or completed study questionnaires, 6 months for BREATHE, Tayside RCT and PAGES or 12 months before enrolment for GALAII and SAGE. For PAGES, BREATHE and Tayside RCT, the definition of exacerbation was at least one of the following in the previous six months of recruitment: hospital admission, short course of oral corticosteroids (OCS) or absence from school, all due to asthma symptoms and verified by a general practitioner or a nurse. For GALA II and SAGE, asthma exacerbations were defined by the presence of at least one of the following events in the 12 months preceding the study inclusion as available: need to seek emergency asthma care, hospitalization, or the administration of OCS due to asthma symptoms. Response designed on prescription-based information in GoSHARE and UKBiobank was gathered in a time frame of 12 months after date of first montelukast prescription. For the UKBiobank and GoSHARE (a and b), the definition of exacerbation was at least one of the following within 12 months after first montelukast prescription date: hospital admission for asthma symptoms (ICD10: J45), emergency room visit (ER) for asthma symptoms (ICD10: J45), and two or more prescriptions of OCS. Non responders or cases, under montelukast treatment, have at least one course of oral corticosteroids (OCS), hospitalisation, emergency room visit and/or school absence within 6 months (T = time) before date of enrolment for BREATHE, PAGES and Tayside RCT. For GALA II and SAGE, non-responders or cases have at least one course of OCS, hospitalisation and/or emergency room visit in the 12 months before date of enrolment. For UKB and GoSHARE, non-responders or cases have at least 2 courses of OCS, one hospitalisation and/or one emergency room visit in the 12 months after date of first montelukast prescription.

![Graph of the binary risk of experiencing an exacerbation definition.](https://doi.org/10.1371/journal.pone.0257396.g001)
To verify the effect of rs2660845 on montelukast response and not just on exacerbation, we tested the association between rs2660845 and exacerbation in non-montelukast users. In late-onset, as we have longitudinal data for GoSHARE(a) cohort, individuals previously selected as being under montelukast treatment were used. The binary effect of experiencing an exacerbation was defined 12 months before start of therapy. In early-onset asthma, genetic data from non-montelukast users were only available from PAGES, BREATHE GALA and SAGE cohorts. Individuals were selected as not being under montelukast treatment at enrolment. The binary risk of having an asthma exacerbation was defined as described before for PAGES, BREATHE and GoSHARE. An asthma exacerbation was defined described before as well for PAGES and BREATHE. For GoSHARE (a) and asthma exacerbation was described as at least one of the following within 12 months before first montelukast prescription date: hospital admission, emergency room visit, course of OCS and discontinuation of the drug. Study details are presented in S3 Table.

**Statistical analysis**

Statistical analysis of PAGES, BREATHE, Tayside RCT, the UKBiobank and GoSHARE (a and b) data were performed in SAS 9.3 (SAS Institute, Cary, NC, USA [32]) and PLINK 1.9 [33] for GALA II and SAGE. Binary logistic regression models were used to test the association between rs2660845 and asthma exacerbation status. Information related to age, gender, body mass index (BMI), asthma, age onset and exacerbation status 12 months before the start of therapy as well as the first two principal components of the genotype matrix, to control for population stratification, were included as covariates where appropriate and available for each study (S3 Table). The effect of the LTA4H variant was considered additive for this analysis. Statistically significant associations were considered for p-values (P) lower than 0.05. Regarding the number of individuals selected we were sufficiently powered (>80%) to detect an OR of 1.5 or above based on our power calculation (S1 Table) [34].

**Meta-analysis**

Association results obtained from late-onset and early-onset studies were meta-analyzed separately to investigate the age of onset effect. For early-onset, populations of European origin were meta-analyzed together as well as with Latin/Hispanic and African American populations. Overall pooled odds ratios from all early-onset patients from GoSHARE(b), BREATHE, Tayside RCT, PAGES, SAGE, and GALA II studies, together with 95% confidence interval of the association between rs2660845 genotype and asthma exacerbation status, were obtained using a random effects model, because the phenotype definition as well as study characteristics varied among studies (Table 1). Whereas for late-onset Europeans studies (GoSHARE(b) and the UKBiobank), as the phenotype definition and baseline characteristics (age, gender, exacerbation percentage) were similar, a fixed effects model was used. The analysis was performed using the metafor package in R [35]. We tested the heterogeneity among studies by means of the measure of inconstancy (I²) values as low (0–25%), moderate (25–50%) and high (50–75%). A threshold of P<0.05 was used to assess the statistical significance of the main effect association. All association tests were performed using an additive model.

**Results**

**Study characteristics**

Study type and baseline characteristics of the participants for each study are presented in Table 1. A total of 3,594 individuals (2,514 late-onset and 1,080 early-onset) with asthma from
multiple independent studies were used. More than 50% of the participants were female in SAGE, the UKBiobank and GoSHARE (a and b) (55%, 66.5%, 56% and 79% female respectively) and male in BREATHE, Tayside RCT, PAGES and GALA II (61%, 63%, 60%, 57% male respectively). The proportion of early-onset and late-onset individuals experiencing an exacerbation before being on montelukast was between 12% and 22% for BREATHE, Tayside RCT, GoSHARE and UKBiobank but was 67%, 72% and 73% respectively for PAGES, GALA II and SAGE. The minor allele frequency of rs2660845 in the UKB, GoSHARE, BREATHE, Tayside RCT, PAGES, GALA II and SAGE, study populations was 0.27, 0.27, 0.27, 0.28, 0.26, 0.46 and 0.32 respectively.

As a control analysis, we tested the association between rs2660845 and non-montelukast users in both late-onset and early-onset (S4 Table). No significant association was observed between rs2660845 and exacerbation rate in individuals with early or late onset not under montelukast treatment (Tables 2 and 3).

**Association between rs2660845 and exacerbation status under montelukast treatment in Europeans with early-onset asthma**

We assessed the risk of having an exacerbation whilst on montelukast treatment using an additive model. Table 4 shows the results of logistic regression models assessing the effect of

---

Table 1. Characteristics of the studied cohorts.

| Study          | UKBiobank | GoSHARE (a) | GoSHARE (b) | BREATHE | Tayside RCT | PAGES | GALA II | SAGE |
|----------------|-----------|-------------|-------------|---------|-------------|-------|---------|------|
| N asthmatics   | 72,754    | 10,218      | 546         | 62      | 361         | 3,343 | 3,433   | 977  |
| N montelukast users | 1,561    | 953         | 88          | 210     | 62          | 163   | 486     | 71   |
| Asthma onset   | Late      | Late        | Early       | Early   | Early       | Early | Early   | Early |
| Ethnicity      | Europeans | Europeans   | Europeans   | Europeans | Europeans   | Europeans | Hispanics/Latinos | African Americans |
| % male (n)     | 33.5      | 44          | 21          | 61      | 63          | 60    | 57      | 45   |
| Mean age (SD)  | 43 (12)   | 40 (16)     | 9 (5)       | 10 (4)  | 11 (3)      | 10 (3) | 11.9 (3.0) | 12.2 (3.1) |
| Study type     | Cross-sectional study with Longitudinal prescription data | Longitudinal | Longitudinal | Cross-sectional | Cross-sectional | Cross-sectional | Cross-sectional |
| Exacerbation in 6–12 month | OCS, hospitalization, ER² | OCS, hospitalization, ER² | OCS, hospitalization, ER² | OCS, hospitalization, school absence¹ | OCS, hospitalization, school absence¹ | OCS, hospitalization, school absence¹ | OCS, hospitalization, ER² |
| Exacerbation (%) | 12.6      | 13          | 12          | 22      | 19          | 67    | 72      | 73   |
| Exacerbation 12 months before first montelukast prescription (%) | 29        | 19          | 21          | -       | -           | -     | -       | -    |
| rs2660845 G variant frequency | 0.27      | 0.27        | 0.27        | 0.27    | 0.28        | 0.26  | 0.46    | 0.32 |

¹Exacerbation within 6 months
²Exacerbation within 12 months. OCS: Oral Corticosteroids, ER: Emergency Room visit. Asthma onset is defined as early.

https://doi.org/10.1371/journal.pone.0257396.t001
rs2660845 on exacerbation status. The genotypic effect was noted in three out of four European early-onset asthma studies. In BREATHE and Tayside RCT, individuals carrying at least a copy of rs2660845 G allele had respectively 4.4 (95% CI: 1.77–10.96, P = 0.05) and 9.6 (95% CI:1.00–92.19, P = 0.001) times the odds of having an exacerbation, in an additive model adjusted for age at recruitment and BMI. In GoSHARE (b), and PAGES the associations were not significant in a model adjusted for age at first LTRA prescribed and exacerbation 12 months before date of first montelukast prescription (respectively Odds-ratio (OR) 4.5, 95% confidence interval (CI): 0.77–26.24; P = 0.0913 and OR = 0.959; 95%CI: 0.427–2.15; P = 0.668).

Association of rs2660845 with exacerbation status under montelukast treatment in Latino/Hispanic and African American early-onset asthma ethnic groups

In the Latino/Hispanic population, GALA II, the association was not significant (OR = 1.04; 95%CI: 0.78–1.39; P = 0.788). The African American population (SAGE) presented an opposite nominal association with an OR of 0.27 (95%CI: 0.09–0.80; P = 0.019) in a model adjusted by age of asthma onset, gender and the first two principal components (Table 4).

Meta-analyses of early-onset asthma cohorts

A meta-analysis of European early-onset cohorts PAGES, GoSHARE (b), BREATHE, Tayside RCT revealed that carriers of at least one G allele of rs2660845 SNP have 2.92 (95%CI: 1.04–8.18, P = 0.0412) times the odds of experiencing an exacerbation (Fig 2). When adding GALA II (Latino/Hispanic) and SAGE (African American), where rs2660845 MAF is higher than in European cohorts, no significant association between rs2660845 SNP and exacerbation status was found (OR = 1.60, 95%CI: 0.61–4.19; P = 0.342) (Fig 3).

Table 2. Association between rs2660845 and asthma exacerbation in late-onset GoSHARE(a) individuals 12 months before being on montelukast.

| Study       | GoSHARE(a) (n = 953) | OR (95% CI) | P-value |
|-------------|----------------------|-------------|---------|
|             |                      | 0.85 (0.641–1.127) | 0.6388  |

Odds of exacerbation rate on montelukast using an additive model adjusted for age, gender, age at 1st montelukast prescription or age at asthma onset and BMI when significant and available.

GoSHARE(a): individuals with age at first salbutamol, age at first inhaled corticosteroid and age at first montelukast prescription, all three over 18 years old.

Table 3. Association between rs2660845 and exacerbation in individuals with early-onset asthma in PAGES and BREATHE non-montelukast users and in GoSHARE(b) individuals 12 months before being on montelukast.

| Study       | PAGES (n = 356) | BREATHE (n = 94) | GoSHARE(b) (n = 88) |
|-------------|----------------|-----------------|---------------------|
| OR (95% CI) | 1.97 (0.927–4.187) | 1.14 (0.246–5.36) | 1.49 (0.532–4.18) |
| P-value     | 0.171           | 0.919           | 0.447               |

Odds of exacerbation rate on montelukast using an additive model adjusted for age, gender, age at 1st montelukast prescription or age at asthma onset and BMI when significant and available.

GoSHARE(b): individuals with age at first salbutamol, age at first inhaled corticosteroid and age at first montelukast prescription, all three under or at 18 years old.
Lack of association between rs2660845 genotype and exacerbation status under montelukast treatment in Europeans late-onset asthma and meta-analysis

No association between exacerbation risk and LTA4H rs2660845 was seen in any of the late-onset studies (Table 4).

A meta-analysis of the observed effects across late-onset populations of the UKBiobank and GoSHARE(a) showed no significant association between rs2660845 genotype and exacerbation status under montelukast treatment (OR = 1.02, 95%CI: 0.87–1.19 P = 0.833) (Fig 4).

Discussion

We report the results of the first study evaluating the association of the SNP rs2660845 with asthma exacerbations whilst on montelukast treatment, in late-onset and early-onset asthma patients, from different populations and ethnic backgrounds. We analyzed data from 3,594 individuals with asthma, 2,514 of which were late-onset and 1,080 early-onset.

Although no evidence of rs2660845 association with asthma was found in late-onset cohorts of European ancestry, we observed a significant association (p = 0.0412) and heterogenous

---

Table 4. Association of exacerbation under montelukast treatment with rs2660845 genotype.

| Asthma onset | Late-onset | Early-onset |
|--------------|------------|-------------|
| Ethnicity    | Europeans  | Europeans   | Europeans | Europeans | Europeans | Hispanics/Latinos | African Americans |
| Cohorts      | UKBiobank  | GoSHARE (a) | GoSHARE (b) | BREATHE    | Tayside RCT | PAGES      | GALA II | SAGE |
| Odds ratio (95%CI) for exacerbation using an additive model | 1.04 (0.88–1.23); 0.88 (0.58–1.34); 4.5 (0.77–26.24); 4.40 (1.77–10.96); 9.60 (1.00–92.19); 0.96 (0.43–2.15); 1.04 (0.78–1.39); 0.27 (0.09–0.80); |
| P-value      | 0.965      | 0.33        | 0.0913     | 0.050      | 0.001      | 0.668      | 0.788    | 0.019 |

Odds of exacerbation rate on montelukast using an additive model adjusted for age, gender, age at 1st montelukast prescription or age at asthma onset and BMI when significant and available.

Asthma onset is defined as early for individuals ≤ 18 years old at asthma diagnosis and late for individuals over 18 years old at asthma diagnosis.

https://doi.org/10.1371/journal.pone.0257396.t004

---

Lack of association between rs2660845 genotype and exacerbation status under montelukast treatment in Europeans late-onset asthma and meta-analysis

No association between exacerbation risk and LTA4H rs2660845 was seen in any of the late-onset studies (Table 4).

A meta-analysis of the observed effects across late-onset populations of the UKBiobank and GoSHARE(a) showed no significant association between rs2660845 genotype and exacerbation status under montelukast treatment (OR = 1.02, 95%CI: 0.87–1.19 P = 0.833) (Fig 4).

Discussion

We report the results of the first study evaluating the association of the SNP rs2660845 with asthma exacerbations whilst on montelukast treatment, in late-onset and early-onset asthma patients, from different populations and ethnic backgrounds. We analyzed data from 3,594 individuals with asthma, 2,514 of which were late-onset and 1,080 early-onset.

Although no evidence of rs2660845 association with asthma was found in late-onset cohorts of European ancestry, we observed a significant association (p = 0.0412) and heterogenous

---

https://doi.org/10.1371/journal.pone.0257396.g002
response in early-onset asthma carriers of rs2660845 G allele with the European cohorts PAGES, GoSHARE(b), BREATHE and Tayside RCT studies (heterogeneity P = 0.0364). Higher heterogeneity was found with ethnically different early-onset populations for association between rs2660845 and exacerbation whilst on montelukast treatment (heterogeneity P = 0.0007), with the odds ratio being lower in the African American cohort SAGE and not significant in the Latino/Hispanic cohort GALA II. As our study identifies the rs2660845 genotype as associated with positive, negative and neutral effect on outcome in different populations, mechanistic studies would be required to confirm its relevance to LTRA response in different ethnic groups. However, rs2660845 represents a putative biomarker for prediction of montelukast response in Europeans with early-onset asthma.

Since early-onset and late-onset asthma present fundamental differences both in the establishment of disease, as well as genetic variation and expression [3–7], we chose to analyse the effect of \textit{LTA4H} rs2660845 SNP in each onset group. We found that the age of onset plays a role in the response to montelukast in European early-onset asthma patients, where the SNP

![Fig 3](https://doi.org/10.1371/journal.pone.0257396.g003)

**Fig 3.** Forest plot representing meta-analysis of the association between \textit{LTA4H} rs2660845 and outcomes observed across all early-onset GALA II, SAGE, PAGES, GoSHARE(b), BREATHE and Tayside RCT studies. Study sample size is in parentheses. Squares represent the odds ratio (OR), horizontal lines represent 95% confidence intervals (CIs). The diamond in the bottom represents summary estimate combining the study-specific estimates using a random effects model.

![Fig 4](https://doi.org/10.1371/journal.pone.0257396.g004)

**Fig 4.** Forest plot representing meta-analysis of the association between \textit{LTA4H} rs2660845 and outcomes observed in late-onset UKBiobank and GoSHARE(a) studies. Study sample size is in parentheses. Squares represent the odds ratio (OR), horizontal lines represent 95% confidence intervals (CIs). The diamond in the bottom represents summary estimate combining the study-specific estimates using a random effects model.
rs2660845 was significantly associated with heterogenous montelukast response as measured by exacerbation rate. These results were consistent with previous studies where early-onset asthma is presented as more genetic and allergic driven than late-onset which tends to be more environmental and lung centred [7, 36].

There are significant disparities in asthma prevalence, mortality and drug response between ethnic groups [37]. Here we reported different results from four European early-onset cohorts, one Latino/Hispanic and one African American, with rs2660845 MAF higher in GALA II and SAGE populations (results consistent with gnomAD [38]) compared to the European studies. Previous sequencing of LTA4H in GALA II revealed the prevalence of five polymorphisms [39] differing from those found in a European cohort by Holloway et al [40]. It is possible that ancestry-specific differences in genetic architecture (linkage disequilibrium patterns) may explain why rs2660845 SNP shows no or significant opposite effects in different populations. Furthermore, heterogeneous effects may also be combined with differences in the environmental background between African American, Latino and European populations as well as insufficient sample size [41, 42]. Also as PAGES, GALAII and SAGE, presented a higher exacerbation percentage before montelukast treatment, it is possible that those higher exacerbation percentage explain the high heterogeneity found between early-onset asthma cohorts and so the difference in the outcome. As environmental factors can modulate the effect of genetic variants upon asthma patients [42, 43] or drug response, and we were not able to investigate these cohorts more in depth to determine what environmental factor was driving this difference, it is important to note that we might underestimate the effect of rs2660845 SNP on montelukast response in the European early-onset meta-analysis. Another important driver of the heterogeneity found here is the asthma exacerbation definition. An indirect role of rs2660845 on asthma exacerbation has been suggested by Rao et al., with the inactivation of LTA4H resulting in less exacerbations [44]. Leukotriene-B4 (LTB4), the product of the degradation of leukotriene-A4 by LTA4H, plays a role in recruiting CD4\(^+\), CD8\(^+\) and T-cells and thus triggering lung inflammation and airway responsiveness [45]. Therefore, an inactivation of LTA4H would affect those inflammatory cells recruitment by lowering LTB4 concentration in the blood. As LTB4 levels in the airways have been shown to increase in blood and bronchoalveolar lavage of asthma patients [46–48] and to correlate with asthma severity [49, 50], we examined mRNA expression data from whole blood in the GTEx [51], eQTLGen [52] and BIOSQTL [53] databases. We found that rs2660845 SNP is associated with LTA4H expression, with AA genotype lowering the expression (S2 Fig and S7 Table). rs2660845 is present in the 5'UTR regulatory region of LTA4H, indicative of a putative cis-eQTL effect. However, the gene expression data analyzed was collected from adults of European ancestry (S1A and S1B Fig for GTEx) and no significant association was found between rs2660845 and montelukast response in an adult cohort of early-onset asthma from the UKBiobank (S5 and S6 Tables). As LTA4H expression and effects have been shown to be different in late-onset and early-onset asthma [7], it is possible that the results would be significant in a pediatric study. A possible mechanism in early-onset asthma is presented in Fig 5. By down regulating LTA4H activity, individuals with a rs2660845 AA genotype have a reduced LTB4 blood concentration resulting in less LTB4 driven exacerbations, simultaneously montelukast, acting as a cysteinyl receptor antagonist, will prevent other products of LTA4 degradation by leukotriene C4 synthase from binding to the receptor, thus reducing exacerbations through this arm of the LTA4 pathway. Therefore, LTA4H inhibitors, 5-Lipoxygenase Activating Protein inhibitors and Leukotriene-B4 receptor antagonists might represent a potential therapeutic strategy that could modulate key aspects of early-onset asthma [40, 54].

Our study has limitations that should also be considered. A fine mapping of the LTA4H locus or haplotype analysis as reported by Holloway and colleagues [40] may help our
understand the relationship between genetic variants of \textit{LTA4H}, but was prevented by either GWAS or haplotype data being unavailable for all cohorts. We decided to focus on rs2660845 as literature indicated a putative association with outcomes for asthma treatment \cite{17} and replicated this result in our European early-onset cohorts despite a high heterogeneity between studies. Differences in patient recruitment (GP selection vs prescriptions-base selection, GP selection between asthma studies) and definition of asthma exacerbation despite LTRA treatment such as time frame variation of 6 to 12 months between longitudinal cohorts and asthma study as well as between asthma studies, exacerbation markers used in only one or two cohorts (school absence for BREATHE, PAGES and Tayside RCT, at least two OCS prescriptions for UKB and GoSHARE) explained a large part of this heterogeneity. Within ethnic group differences could also be a driver of the high heterogeneity found here as it can be more significant than heterogeneity across different ethnic groups. Moreover, another potential source of variation came from the use of retrospective information about the occurrence or absence of asthma exacerbations, partly based on self-reporting within asthma studies.

Another important limitation is that we were not able to account for any aetiology of asthma exacerbations as well as exposure to potential environmental triggers such as smoking, one of the most important environmental factors knowing its impact on lungs. Unfortunately, the information was not collected in a similar way for all studies, with only second-hand smoking information available for the pediatric cohorts (PAGES, BREATHE, Tayside RCT, at least two OCS prescriptions for UKB and GoSHARE) and primary smoking status only available for a subset of GoSHARE and the UKBiobank cohorts. Finally, we have also assumed that treatment has been assigned based on similar prescribing criteria and did not take into consideration specific LTRA dose and type or any index of treatment adherence (BTS/SIGN guidelines) since information related to these variables was not available for most of the studies included. We also did not investigate other treatments and it is possible that individuals with increased risk of exacerbation might not have received LTRA treatment which would tend to underestimate the effect of the interaction between montelukast and rs2660845 for exacerbations. As other treatments were not taken into account, the effect found in the early-onset asthma group could also be overestimated and mostly linked to a more serious disease. Altogether, this heterogeneity in data availability could represent a potential interpretation bias in terms of response to asthma treatment.

\textbf{Fig 5.} A possible mechanism of SNPs rs2660845 action on montelukast response in pediatric asthma patients. Reduced risk of exacerbation might be due to the down regulation of \textit{LTA4H} activity, stimulating a montelukast sensitive pathway triggering exacerbation. rs2660845 AA genotype would result in a comparatively reduced \textit{LTA4H} expression, leading to lowered LTB4 concentrations and so reducing exacerbation triggered by BLT receptors. Furthermore, \textit{LTA4} would be more readily converted into LTC4, unable to bind to CysLT1 receptors already blocked by montelukast.

https://doi.org/10.1371/journal.pone.0257396.g005
To our knowledge, this is the only study of leukotriene response to utilize populations of different age of onset and different ethnic backgrounds. These results, together with published studies in other European [40] and Latino/Hispanic cohorts [39], support a strong involvement of age of onset, LTA4H and LTB4 production in the pathophysiology of asthma. Our results add to the growing evidence of age of asthma onset as a robust clinical biomarker and moreover suggest stratifying treatment by age of asthma onset and rs2660845 status can form the basis of a precision medicine strategy to select those patients most likely to benefit from montelukast treatment.

**Supporting information**

**S1 Table. Power of the sample size for combined cohorts to detect increases in OR for asthma exacerbation.** GoSHARE (a) is the late-onset population (>18 years-old); GoSHARE (b) is the early-onset population (≤18 years-old).

(DOCX)

**S2 Table. Selection of asthmatic patients in GoSHARE by treatment steps.** Step 1: inhaled short-acting β2-agonists (SABA) on demand; Step 2: regular inhaled steroids (ICS) plus SABA on demand; Step 3: regular inhaled long-acting β2-agonists (LABA) (salmeterol or formoterol) plus ICS with SABA on demand; Step 4: oral montelukast with SABA on demand (plus ICS plus/or regular LABA).

(DOCX)

**S3 Table. Covariates association with asthma exacerbation binary trait.** For GALA II and SAGE, betas 95%(CI) are reported for quantitative variables; * Traits with a P <0.05 were used as covariates in the logistic regression.

(DOCX)

**S4 Table. Details of late-onset and early-onset in three non-montelukast user populations.**

1Exacerbation within 6 months; 2Exacerbation within 12 months; OCS: Oral Corticosteroids; ER: Emergency Room visit; GoSHARE (a) is the late-onset population (>18 years-old); GoSHARE (b) is the early-onset population (≤18 years-old).

(DOCX)

**S5 Table. Details of early-onset asthma adult montelukast user from the UKBiobank.**

1Exacerbation within 6 months; 2Exacerbation within 12 months; OCS: Oral Corticosteroids; ER: Emergency Room visit; Patients were diagnosed as having early-onset asthma; Montelukast prescription records were only available as adults.

(DOCX)

**S6 Table. Association between rs2660845 and asthma exacerbation in early-onset UKBiobank individuals 12 months after taking montelukast prescription as adults.** Patients were diagnosed as having early-onset asthma; Montelukast prescription records were only available as adults.

(DOCX)

**S7 Table. Cis-eQTL effect of rs2660845 on LTA4H expression in whole blood from adult cohorts.** BIOSQTL: The Biobank-Based Integrative Omics Study Quantitative Trait Locus; eQTLGen: expression Quantitative Trait Loci Genetic; FDR: False Discovery Rate; ID: Gene code Identifier.

(DOCX)
S8 Table. Genotype counts for rs2660845 SNP by exacerbation status. 1 Patients were diagnosed as having early-onset asthma but with montelukast prescription records only available as adults. 2 GoSHARE individuals with exacerbation events in a year before first prescription of montelukast. 3 Individuals from BREATHE and PAGES cohorts not under montelukast treatment. GoSHARE(a): individuals with age at first salbutamol, age at first inhaled corticosteroid and age at first montelukast prescription, all three over 18 years old. GoSHARE(b): individuals with age at first salbutamol, age at first inhaled corticosteroid and age at first montelukast prescription, all three under or at 18 years old.

S1 Fig. Age (a) and ethnicity (b) distributions in the GTEx portal (V8).

S2 Fig. Box plot showing the cis-eQTL effect of rs2660845 on LTA4H expression in whole blood (beta = -0.020, P-value = 0.46). (GTEx v8).

S1 Data.

Acknowledgments
We would like to thank all the participants from the different cohorts, the BREATHE, Tayside RCT, PAGES, UKB, SHARE, GALA II, and SAGE teams as well as nurses, interviewers, technicians, clinicians and researchers’ volunteers and study coordinators, for their help recruiting the participants. CM would like to thank her colleagues from the Centre of Genomic Research (AstraZeneca) and specially Amanda O’Neil, Katherine Smith, Slavé Petrovski, Olympe Chazara, Sophia Cameron-Christie, Eleanor Wigmore, Niedzica Camacho, Dimitrios Vitsios and Sebastian Wasilewski for their help with the UKBiobank data. We acknowledge the support of the Health Informatics Centre, University of Dundee for managing and supplying the anonymized data. The UKBB was accessed through AZ’s MTA (application number 26041). The Pharmacogenomics in Childhood Asthma (PiCA) consortium is a research consortium that has been established in December 2013 by prof. dr. Anke-Hilse Maitland van der Zee and dr. Susanne Vijverberg to bring together asthma studies that have genetic data and treatment outcome measures.

Author Contributions
Conceptualization: Steve Turner, Somnath Mukhopadhyay, Anke-Hilse Maitland-van der Zee, Esteban G. Burchard, Adam Platt, Colin N. A. Palmer.

Data curation: Cyrielle Maroteau, Antonio Espuela-Ortiz, Esther Herrera-Luis, Maria Pino-Yanes.

Formal analysis: Cyrielle Maroteau, Antonio Espuela-Ortiz, Esther Herrera-Luis.

Funding acquisition: Steve Turner, Somnath Mukhopadhyay, Anke-Hilse Maitland-van der Zee, Esteban G. Burchard, Maria Pino-Yanes, Colin N. A. Palmer.

Investigation: Anke-Hilse Maitland-van der Zee, Esteban G. Burchard, Glenda Lassi, Adam Platt.

Methodology: James D. Chalmers, Glenda Lassi.
Project administration: Natalia Hernandez-Pacheco, Somnath Mukhopadhyay, Esteban G. Burchard, Simon Young, Glenda Lassi, Adam Platt, Colin N. A. Palmer.

Resources: Sundararajan Srinivasan, Fiona Carr, Roger Tavendale, Karen Wilson, Maria Pino-Yanes, Simon Young.

Supervision: James D. Chalmers, Anke-Hilse Maitland-van der Zee, Simon Young, Glenda Lassi, Adam Platt, Colin N. A. Palmer.

Validation: Cyrielle Maroteau, Steve Turner, Anke-Hilse Maitland-van der Zee, Simon Young, Glenda Lassi, Adam Platt, Colin N. A. Palmer.

Writing – original draft: Cyrielle Maroteau.

Writing – review & editing: Cyrielle Maroteau, Antonio Espuela-Ortiz, Esther Herrera-Luis, Sundararajan Srinivasan, Fiona Carr, Roger Tavendale, Karen Wilson, Natalia Hernandez-Pacheco, James D. Chalmers, Steve Turner, Somnath Mukhopadhyay, Anke-Hilse Maitland-van der Zee, Esteban G. Burchard, Maria Pino-Yanes, Simon Young, Glenda Lassi, Adam Platt, Colin N. A. Palmer.

References
1. The global asthma report 2018. 2018.
2. Tiotiu A. Biomarkers in asthma: state of the art. Asthma research and practice. 2018; 4(1):10. https://doi.org/10.1186/s40733-018-0047-4 PMID: 30598830
3. Bush A, Menzies-Gow A. Phenotypic differences between pediatric and adult asthma. Proceedings of the American Thoracic Society. 2009; 6(8):712–9. https://doi.org/10.1513/pats.200906-046DP PMID: 2008882
4. Busse W, Banks-Schlegel SP, Larsen GL. Childhood-versus adult-onset asthma. American journal of respiratory and critical care medicine. 1995; 151(5):1635–9. https://doi.org/10.1164/ajrccm.151.5.7735626 PMID: 7735626
5. Dijk FN, de Jongste JC, Postma DS, Koppelman GH. Genetics of onset of asthma. Current opinion in allergy and clinical immunology. 2013; 13(2):193–202. https://doi.org/10.1097/ACI.0b013e32835eb707 PMID: 23407123
6. Holloway JW, Arshad SH, Holgate ST. Using genetics to predict the natural history of asthma? Journal of Allergy and Clinical Immunology. 2010; 126(2):200–9. https://doi.org/10.1016/j.jaci.2010.06.006 PMID: 20688205
7. Pividori M, Schoettler N, Nicolee DL, Ober C, Im HK. Shared and distinct genetic risk factors for childhood-onset and adult-onset asthma: genome-wide and transcriptome-wide studies. The Lancet Respiratory Medicine. 2019; 7(6):509–22. https://doi.org/10.1016/S2213-2600(19)30055-4 PMID: 31036433
8. Kuruvilla ME, Lee FE, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. Clinical reviews in allergy & immunology. 2019; 56(2):219–33.
9. Zhu Z, Lee PH, Chaffin MD, Chung W, Loh PR, Lu Q, et al. A genome-wide cross-trait analysis from UK Biobank highlights the shared genetic architecture of asthma and allergic diseases. Nat Genet. 2018; 50(6):857–64. https://doi.org/10.1038/s41588-018-0121-0 PMID: 2978501
10. Vijverberg SJH, Farzan N, Slob EMA, Neerinck AH, Maitland-van der Zee AH. Treatment response heterogeneity in asthma: the role of genetic variation. Expert review of respiratory medicine. 2018; 12(1):55–65. https://doi.org/10.1080/17476348.2018.1403318 PMID: 29115880
11. Zhu Z, Hasegawa K, Camargo CA Jr., Liang L. Investigating asthma heterogeneity through shared and distinct genetics: Insights from genome-wide cross-trait analysis. The Journal of allergy and clinical immunology. 2021; 147(3):796–807. https://doi.org/10.1016/j.jaci.2020.07.004 PMID: 32693092
12. Wahn U, Dass SB. Review of recent results of montelukast use as a monotherapy in children with mild asthma. Clinical therapeutic. 2008; 30:1026–35. https://doi.org/10.1016/j.clinthera.2008.05.018 PMID: 18640477
13. Health NIf, 80 CEJNG. Asthma: diagnosis, monitoring and chronic asthma management. 2017.
14. Lima JJ, Zhang S, Grant A, Shao L, Tantisira KG, Alayee H, et al. Influence of leukotriene pathway polymorphisms on response to montelukast in asthma. American journal of respiratory and critical care medicine. 2006; 173(4):379–85. https://doi.org/10.1164/rccm.200509-1412OC PMID: 16293801
15. Aimomani B, Hawwa AF, Millership JS, Heaney L, Douglas I, McElney JC, et al. Can certain genotypes predispose to poor asthma control in children? A pharmacogenetic study of 9 candidate genes in children with difficult asthma. PloS one. 2013; 8(4):e60592. https://doi.org/10.1371/journal.pone.0060592 PMID: 23573270

16. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Piñeiro A, Wei LX, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma: a randomized, controlled trial. Annals of internal medicine. 1999; 130(6):487–95. https://doi.org/10.7326/0003-4819-130-6-19990316-00005 PMID: 10075616

17. Lima JJ. Treatment heterogeneity in asthma. Molecular diagnosis & therapy. 2007; 11(2):97–104. https://doi.org/10.1007/BF03262268 PMID: 17398288

18. Sampson AP, Siddiqui S, Buchanan D, Howarth PH, Holgate ST, Holloway JW, et al. Variant LTC4 synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predicts clinical response to zafirlukast. Thorax. 2000; 55(suppl 2):S28–S31. https://doi.org/10.1136/thorax.55.suppl_2.s28 PMID: 10992553

19. Lima JJ, Blake KV, Tantisira KG, Weiss ST. Pharmacogenetics of asthma. Current opinion in pulmonary medicine. 2009; 15(1):57. https://doi.org/10.1097/MCP.0b013e328337a8be PMID: 19077707

20. Kotani H, Kishi R, Mouri A, Sashio T, Shindo J, Shiraki A, et al. Influence of leukotriene pathway polymorphisms on clinical responses to montelukast in Japanese patients with asthma. Journal of clinical pharmacy and therapeutics. 2012; 37(1):112–6. https://doi.org/10.1111/j.1365-2710.2011.01248.x PMID: 21385196

21. Farzan N, Vrijverberg SJ, Kabesch M, Sterk PJ, Maitland-van der Zee AH. The use of pharmacogenomics, epigenomics, and transcriptomics to improve childhood asthma management: Where do we stand? Pediatric pulmonology. 2018; 53(6):836–45. https://doi.org/10.1002/ppul.23976 PMID: 29493882

22. McKinstry B, Sullivan FM, Vasishta S, Armstrong R, Hanley J, Haughney J, et al. Cohort profile: the Scottish Research register SHARE. A register of people interested in research participation linked to NHS data sets. BMJ open. 2017; 7(2):e013351. https://doi.org/10.1136/bmjopen-2016-013351 PMID: 28148582

23. Palmer CNA, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 β2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax. 2006; 61(11):940–4. https://doi.org/10.1136/thx.2006.059335 PMID: 16772309

24. Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA, et al. Tailored second-line therapy in asthmatic children with the Arg16 genotype. Clinical Science. 2013; 124(8):521–8. https://doi.org/10.1042/CS20120528 PMID: 23126384

25. Turner SW, Ayres JG, Macfarlane TV, Mehta A, Mehta G, Palmer CN, et al. A methodology to establish a database to study gene environment interactions for childhood asthma. BMC medical research methodology. 2010; 10(1):107. https://doi.org/10.1186/1471-2288-10-107 PMID: 21134251

26. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. 2013; 188(3):309–18. https://doi.org/10.1164/rccm.201302-0264OC PMID: 23750510

27. Farzan N, Vrijverberg SJ, Andiappan AK, Arianto L, Berce V, Blanca-López N, et al. Rationale and design of the multicentric Pharmacogenomics in Childhood Asthma consortium. 2017; 18(10):931–43.

28. Ollier W, Sprosen T, Peakman T. UK Biobank: from concept to reality. 2005.

29. BTS/SIGN guideline for the management of asthma 2019 [Available from: https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma/.

30. Pino-Yanes M, Gignoux CR, Galanter JM, Levin AM, Campbell CD, Eng C, et al. Genome-wide association study and admixture mapping reveal new loci associated with total IgE levels in Latinos. 2015; 135(6):1502–10.

31. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. Am J Respir Crit Care Med. 2009; 180(1):59–99. https://doi.org/10.1164/rccm.200801-060ST PMID: 19539666

32. Version SAS. 9.3 [Computer software]. SAS Institute, Cary, NC. 2011.

33. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American journal of human genetics. 2007; 81(3):559–75. https://doi.org/10.1086/519795 PMID: 17701901

34. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003; 19(1):149–50. https://doi.org/10.1093/bioinformatics/19.1.149 PMID: 12499305
35. Viechtbauer W. Conducting meta-analyses in R with the metafor package. Journal of statistical software. 2010; 36(3).

36. Sarnowski C, Sugier P-E, Granell R, Jarvis D, Dizier M-H, Ege M, et al. Identification of a new locus at 16q12 associated with time to asthma onset. 2016; 138(4):1071–80.

37. Drake KA, Galanter JM, Burchard EG. Race, ethnicity and social class and the complex etiologies of asthma. 2008.

38. Karczewska KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. 2019;531210.

39. Via M, De Giacom A, Corvol H, Eng C, Seibold MA, Gillett C, et al. The role of LTA4H and ALOX5AP genes in the risk for asthma in Latinos. Clinical & Experimental Allergy. 2010; 40(4):582–9. https://doi.org/10.1111/j.1399-9995.2008.01667.x PMID: 18547289

40. Holloway JW, Barton SJ, Holgate ST, Rose-Zerilli MJ, Sayers I. The role of LTA4H and ALOX5AP polymorphism in asthma and allergy susceptibility. Allergy. 2008; 63(8):1046–53. https://doi.org/10.1111/j.1398-9995.2008.01667.x PMID: 18547289

41. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nature genetics. 2011; 43(9):887. https://doi.org/10.1038/ng.888 PMID: 21804549

42. Bouzigon E, Corda E, Aschard H, Dizier M-H, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. 2008; 359(19):1985–94.

43. Smit L, Bouzigon E, Pin I, Siroux V, Monier F, Aschard H, et al. 17q21 variants modify the association between early respiratory infections and asthma. 2010; 36(1):57–64.

44. Rao NL, Riley JP, Banie H, Xue X, Sun B, Crawford S, et al. Leukotriene A4 hydrolase inhibition attenuates allergic airway inflammation and hyperresponsiveness. American journal of respiratory and critical care medicine. 2010; 181(9):899–907. https://doi.org/10.1164/rccm.200807-1158OC PMID: 20110560

45. Islam SA, Thomas SY, Hess C, Medoff BD, Means TK, Brander C, et al. The leukotriene B4 lipid chemottractant receptor BLT1 defines antigen-primed T cells in humans. Blood. 2006; 107(2):444–53. https://doi.org/10.1182/blood-2005-07-2362 PMID: 16179368

46. Sampson AP, Castling DP, Green CP, Price JF. Persistent increase in plasma and urinary leukotrienes after acute asthma. Archives of disease in childhood. 1995; 73(3):221–5. https://doi.org/10.1136/adc.73.3.221 PMID: 7492159

47. Shindo K, Fukumura M, Miyakawa K. Leukotriene B4 levels in the arterial blood of asthmatic patients and the effects of prednisolone. European Respiratory Journal. 1995; 8(4):605–10. https://doi.org/10.1183/09031936.95.08040610 PMID: 8541568

48. Evans DJ, Barnes PJ, Spaethle SM, van Alstyne EL, Mitchell MI, O’Connor BJ. Effect of a leukotriene B4 receptor antagonist, LY293111, on allergen induced responses in asthma. Thorax. 1996; 51(12):1178–84. https://doi.org/10.1136/thx.51.12.1178 PMID: 8984512

49. Montuschi P, Martello S, Felli M, Mondino C, Barnes PJ, Chiariotti M. Liquid chromatography/mass spectrometry analysis of exhaled leukotriene B4 in asthmatic children. Respiratory research. 2005; 6(1):119. https://doi.org/10.1186/1465-9921-6-119 PMID: 16236169

50. Wenzel SE. Arachidonic acid metabolites: mediators of inflammation in asthma. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 1997; 17(1P2):3S–12S. PMID: 9017783

51. Consortium GT. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science. 2015; 348(6235):648–60. https://doi.org/10.1126/science.1262110 PMID: 25954001

52. Vösa U, Claringbould A, Westra H-J, Border MJ, Deelen P, Zeng B, et al. Unraveling the polygenic architecture of complex traits using blood eQTL meta-analysis. 2018;447367.

53. Zhernakova DV, Deelen P, Vermaat M, Van Iterson M, Van Galen M, Arindarito W, et al. Identification of context-dependent expression quantitative trait loci in whole blood. 2017; 49(1):139.

54. Tulah AS, Beghè B, Barton SJ, Holloway JW, Sayers I. Leukotriene B4 receptor locus gene characterisation and association studies in asthma. BMC medical genetics. 2012; 13(1):110. https://doi.org/10.1186/1471-2350-13-110 PMID: 23167751