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RESEARCH ARTICLE

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

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Data Availability Statement: Restrictions apply to datasets. GoSHARE, SAGE, BREATHE, PAGES, Tayside RCT and GALAII datasets presented in this article are not readily available as they contain individual-level identifiable information. All analysis of anonymised data is performed in an International Organization for Standardisation 27001 – and Scottish Government accredited, secure safe haven. For the Pharmacogenetics in Childhood Asthma consortium studies (PAGES, GALAII, SAGE, BREATHE and Tayside RCT) data requests can be initiated by contacting Dr. Susanne

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

carriers of at least one G allele, despite montelukast treatment, were increased (odds-ratio = 2.92, 95%confidence interval (CI): 1.04–8.18, I2 = 62%, p = 0.0412) compared to those in the AA group. When meta-analysing with other ethnic groups, no significant increased risk of asthma exacerbations was found (OR = 1.60, 95% CI: 0.61–4.19, I2 = 85%, p = 0.342). Our study demonstrates that genetic variation in *LTA4H*, together with timing of asthma onset, may contribute to variability in montelukast response. European individuals with early-onset (≤18y) carrying at least one copy of rs2660845 have increased odd 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 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 *ALOX5*, *LTA4H*, *LTC4S*, *ABCC1*, *CYSLTR2*, and *SLCO2B1* genes may contribute to the heterogeneity of response to leukotriene modifiers, including montelukast [14, 18–20]. To date, only the effect of a small number of genetic variants has been validated in independent studies [10, 21]. We focused our analysis on a particular variant in the regulatory region of the leukotriene-A$_4$ hydrolase (EC3.3.2.6) gene (*LTA4H*), 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 [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 [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 [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 [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 [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 [26]. BREATHE, PAGES, GALA II and SAGE studies were all part of the Pharmacogenetics in Childhood Asthma (PiCA) consortium [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 within 12 months before date of enrolment. For UKB and GoSHARE, non-responders or cases have at least 2 course of OCS, one hospitalisation and/or one emergency room visit within 12 months after date of first montelukast prescription.
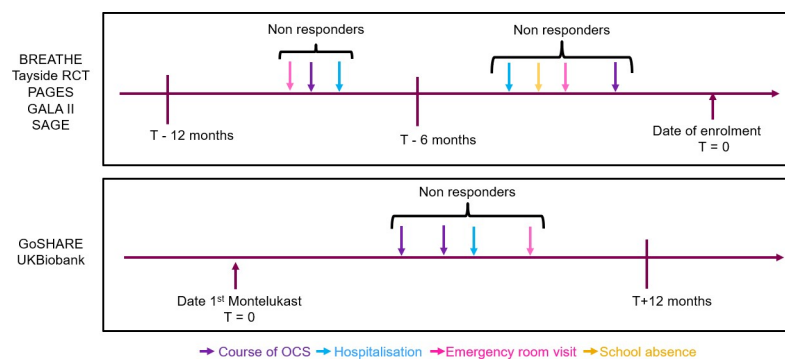

Fig 1. Graph of the binary risk of experiencing an exacerbation definition.

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 where 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(a) 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 (I^2) 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

Table 1. Characteristics of the studied cohorts.

	UKBiobank	GoSHARE (a)	GoSHARE (b)	BREATHE	Tayside RCT	PAGES	GALA II	SAGE
N asthmatics	72,754	10,218		546	62	361	3,343	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) years	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	Cross-sectional
Exacerbation in 6–12 month	OCS, hospitalization, ER[2]	OCS, hospitalization, ER[2]	OCS, hospitalization, ER[2]	OCS, hospitalization, school absence[1]	OCS, hospitalization, school absence[1]	OCS, hospitalization, school absence[1]	OCS, hospitalization, ER[2]	OCS, hospitalization, ER[2]
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

[1]Exacerbation within 6 months

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

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 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)	0.85 (0.641–1.127)
P-value	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.

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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 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.

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

Asthma onset	Late-onset		Early-onset					
Ethnicity	Europeans	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 $\leq$ 18 years old at asthma diagnosis and late for individuals over 18 years old at asthma diagnosis.

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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

Fig 2. Forest plot representing meta-analysis of the association between *LTA4H* rs2660845 and outcomes observed across early-onset 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.
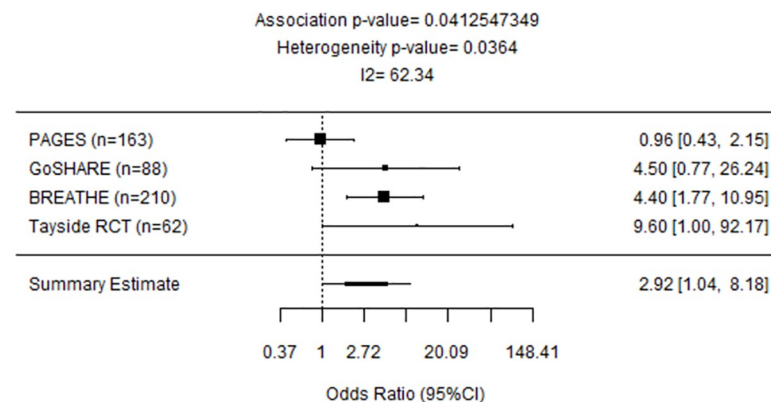
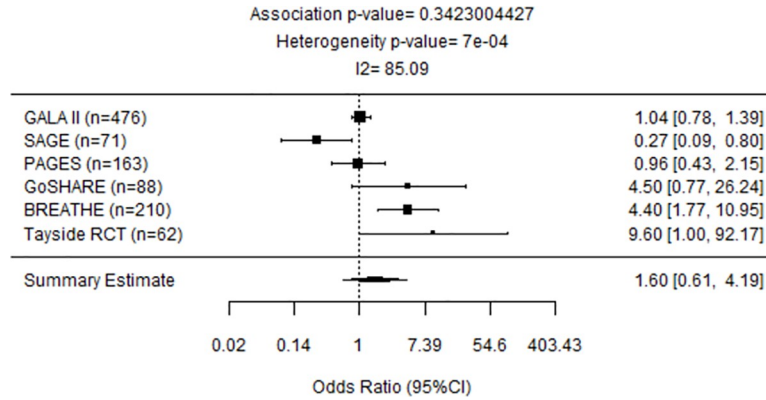https://doi.org/10.1371/journal.pone.0257396.g002

Fig 3. Forest plot representing meta-analysis of the association between *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.

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response in early-onset asthma carriers of rs2660845 G allele with the European cohorts PAGES, GoSHARE(b), BREATHE and Tayside RCT (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 *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
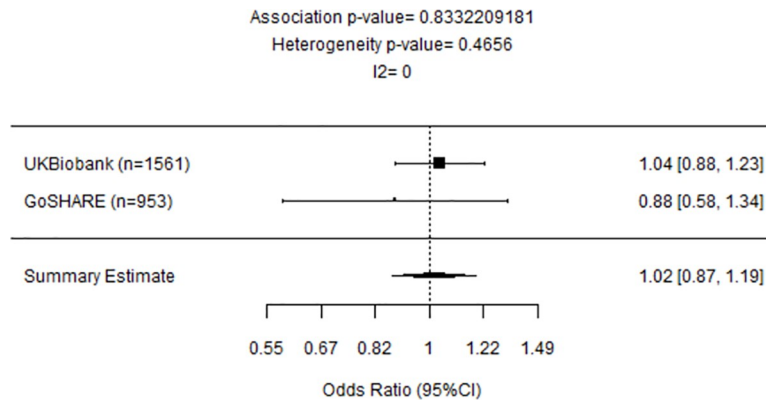
Fig 4. Forest plot representing meta-analysis of the association between *LTA4H* rs2660845 and outcomes observed in late-onset UKBiobank and GoSHARE(a) studies. Study sample size is in parentheses. Squares represent the odds ratio (OR), horizontal lines represent 95% confidence intervals (CIs). The diamond in the bottom represents summary estimate combining the study-specific estimates using a random effects model.

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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-B_4 (LTB_4), the product of the degradation of leukotriene-A_4 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 LTB_4 concentration in the blood. As LTB_4 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 LTB_4 blood concentration resulting in less LTB_4 driven exacerbations, simultaneously montelukast, acting as a cysteinyl receptor antagonist, will prevent other products of LTA_4 degradation by leukotriene C4 synthase from binding to the receptor, thus reducing exacerbations through this arm of the LTA_4 pathway. Therefore, LTA4H inhibitors, 5-Lipoxygenase Activating Protein inhibitors and Leukotriene-B_4 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
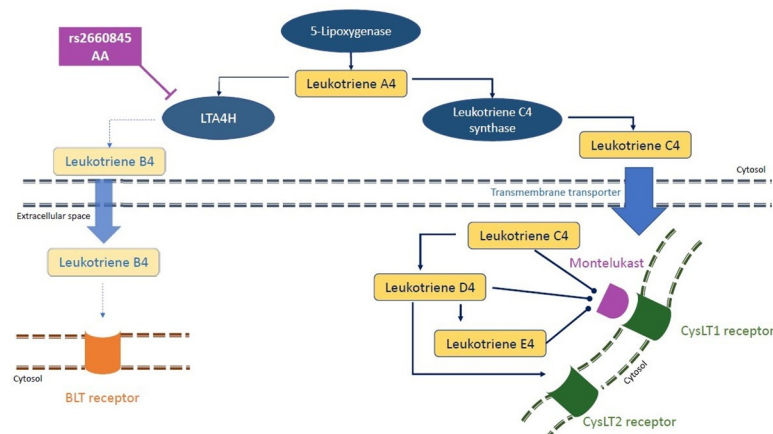
Fig 5. A possible mechanism of SNPs rs2660845 action on montelukast response in pediatric asthma patients.
Reduced risk of exacerbation might be due to the down regulation of LTA4H activity, stimulating a montelukast
sensitive pathway triggering exacerbation. rs2660845 AA genotype would result in a comparatively reduced LTA4H
expression, leading to lowered LTB4 concentrations and so reducing exacerbation triggered by BLT receptors.
Furthermore, LTA4 would be more readily converted into LTC4, unable to bind to CysLT1 receptors already blocked
by montelukast.

understand the relationship between genetic variants of *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 [17] and rep-
licated 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 treat-
ment 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 differ-
ences 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 varia-
tion 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 smok-
ing information available for the pediatric cohorts (PAGES, BREATHE, Tayside RCT, GALAII
and SAGE) 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.

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. [1]Exacerbation within 6 months; [2]Exacerbation 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. [1]Exacerbation within 6 months; [2]Exacerbation 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: Gencode 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.
(DOCX)

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

S2 Fig. Box plot showing the *cis*-eQTL effect of rs2660845 on *LTA$_4$H* expression in whole blood (beta = -0.020, P-value = 0.46). (GTEx v8).
(TIF)

S1 Data.
(DOCX)

Acknowledgments

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