



DOI: 10.1111/all.13499



17q21 variant increases the risk of exacerbations in asthmatic children despite inhaled corticosteroids use

To the Editor,

Approximately 25% of the asthmatic children suffer from uncontrolled asthma despite regular use of inhaled corticosteroids (ICS).¹ Variation within the 17q21 locus is the strongest genetic determinant for childhood-onset asthma.² Recently, the influence of this locus on treatment outcomes has been shown in several studies.³⁻⁴ The Pharmacogenomics in Childhood Asthma (PiCA) consortium is a multiethnic consortium that brings together data from ≥14 000 asthmatic children/young adults from 12 different countries to study the pharmacogenomics of uncontrolled asthma despite treatment.⁵ In 14 PiCA populations (with over 4000 asthmatic patients), we studied the association between variation in the 17q21 locus, and asthma exacerbations despite ICS use. We specifically focused on rs7216389, a single nucleotide polymorphism (SNP) in the 17q21 locus strongly associated with childhood asthma and initially identified by Moffatt et al.²

Ten PiCA studies included patients with non-Hispanic European origins, two included Hispanic patients, one African American, and one included East Asian patients. Additional details of the study populations can be found in the Data S1. Two outcomes were assessed: (i) asthma-related hospitalizations/emergency department visit (ED) visits and (ii) short courses of oral corticosteroid (OCS) use reported by the parent/child at the study visit or based on completed study questionnaires. Age, gender, genotype data, and exacerbation data were available for 4529 steroid-treated children and young adults (Table 1). Logistic regression analysis was used to assess the risk of exacerbations when carrying rs7216389. Due to potential heterogeneity between cohorts, the odds ratios (ORs) were meta-analyzed with the inverse variance weighting method assuming random effects. See Data S1 for more detail.

The risk allele (T) frequency was highest in East Asians ($n = 182$, $T = 0.81$), followed with African Americans ($T = 0.79$, $n = 468$) and Hispanics ($T = 0.66$, total $n = 916$), and it was less frequent in patients with European ancestry (ranged between 0.54 and 0.62, total $n = 2963$). The genotype distribution of the SNP was in Hardy-Weinberg equilibrium in all cohorts. There was a low to moderate heterogeneity between studies (Figure 1). Exacerbation rates ranged between 6.5% (PACMAN) and 77.2% (HPR) for OCS use and 6% (PACMAN) and 58% (GALA II and HPR) for hospitalizations/ED visits.

Thirty percent (1378 out of 4454) of the patients reported hospitalizations/ED visits. In the meta-analysis of 13 studies, rs7216389 was statistically significantly associated with asthma-related ED visits/hospitalizations, (summary OR per increase in risk allele: 1.32, 95% CI: 1.17- 1.49, $P < .0001$, $I^2 = 3.9\%$) (Figure 1A). In a subgroup analysis, the effect estimates for hospitalizations/ED visits were approximately the same for both non-Hispanic whites ($n = 2888$, OR: 1.33, 95% CI: 1.10-1.61, $P = .004$, $I^2 = 30.2\%$) and Hispanics ($n = 916$, OR: 1.31, 95% CI: 1.06-1.63, $P = .01$, $I^2 = 0.00\%$). Thirty-one percent (1269 out of 4050) of the patients reported OCS use/high-dose ICS. In the meta-analysis of the nine studies, the rs7216389-T was statistically significantly associated with an increased risk of OCS use/high-dose ICS (summary OR per increase in variant allele: 1.19, 95% CI: 1.04-1.36, $P = .01$, $I^2 = 22.8\%$) (Figure 1B). Rs7216389 was associated with OCS use in the meta-analysis of seven European studies ($n = 2492$, OR: 1.26, 95% CI: 1.09-1.45, $P = .002$, $I^2 = 6.2\%$) but not in Hispanics ($n = 916$, OR: 0.96, 95% CI: 0.76-1.22, $P = .7$, $I^2 = 0.00\%$). Differences in the minor allele frequencies and LD structures among different ethnicities can influence results of the association studies.⁶ This could be one of the potential explanations why we did not find a significant association in African Americans and patients from Singapore.

A sensitivity analysis was performed to investigate this association in children ≥5 years of age. When excluding children <5 years of age in the meta-analysis, the results remained significant. In the meta-analysis of 13 studies, the SNP was associated with asthma-related hospitalization/ED visits ($n = 4254$, OR: 1.32, 95% CI: 1.18-1.49, $P < .0001$, $I^2 = 0.0\%$) (Figure S1). Regarding OCS use, 10 studies collected data on patients ≥5 years of age ($n = 3771$). In the meta-analysis of 10 studies, rs7216389 was associated with the OCS use (summary OR: 1.20, 95% CI: 1.05-1.38, $P = .01$, $I^2 = 21.7\%$) (Figure S2).

We also performed a meta-analysis of the studies that had sufficient data available on preschool children (2-4 years of age). Although the effect estimates in younger children were in the same direction for both outcomes, the results were not statistically significant (Figures S3 & S4). All preschool studies solely included European children.

Altered expression of *ORMDL3* and *GSDMB* by 17q21 locus variants may play a key role in childhood asthma onset.^{2,7} Two 17q21 asthma-risk variants (rs4065275 and rs12936231) in high

Farzan and Vijverberg equally contributed to this study.

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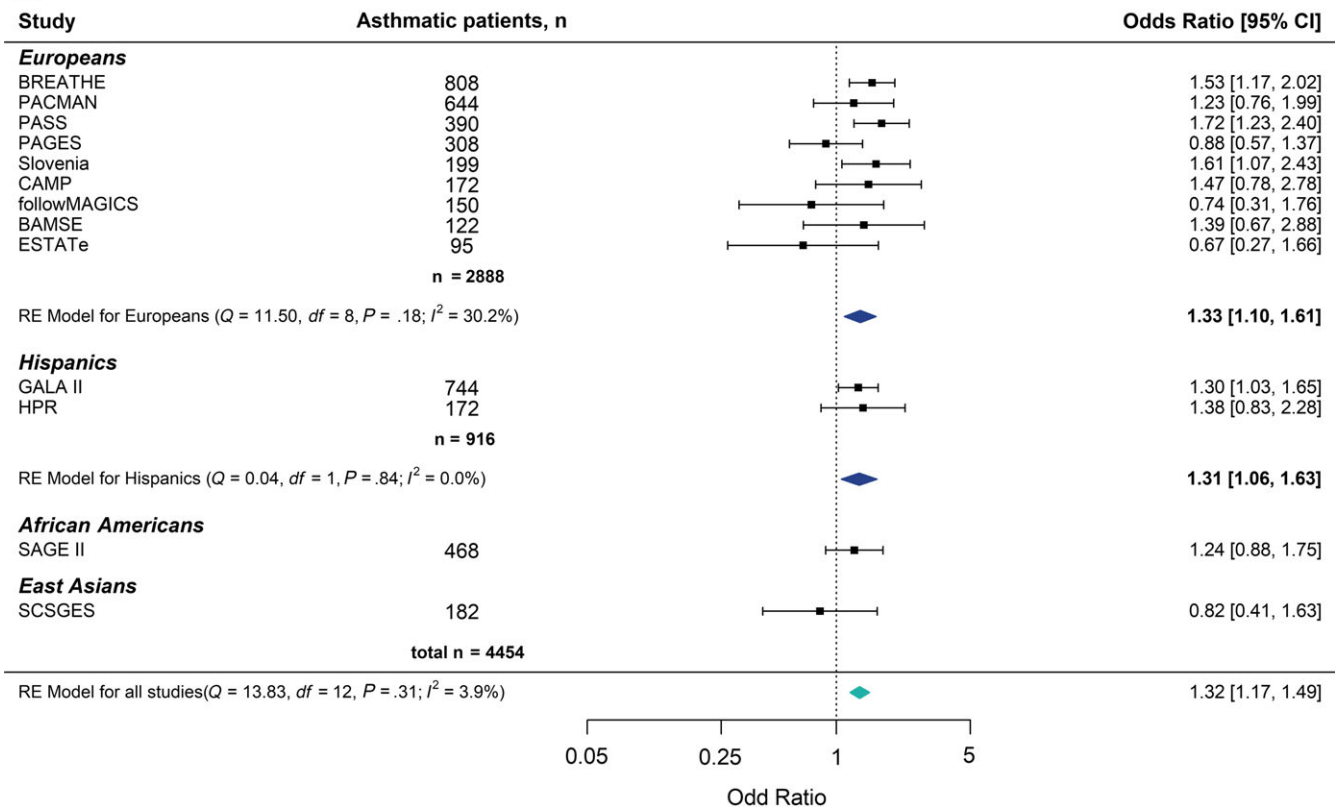
TABLE 1 Characteristics of the study populations

Patient characteristic	BAMSE ^a (n = 122)	BREATHE (n = 808)	CAMP ^a (n = 172)	COPSAC2000 ^{a,b} (n = 54)	ESTaTe (n = 95)	followMAGICS ^a (n = 150)	GALA II ^a (n = 744)	HPR ^a (n = 172)	PACMAN (n = 665)	PAGES (n = 308)	PASS ^a (n = 390)	SAGE II ^a (n = 468)	SCSGES ^a (182)	Slovenia ^a (n = 199)
Age (y), mean (SD)	8.37 (0.41)	9.8 (4.0)	8.8 (2.1)	3.3 (1.0)	10.8 (4.3)	17.13 (3.03)	12.1 (3.2)	9.8 (2.7)	8.7 (2.3)	9.2 (3.8)	11.1 (4.0)	13.5 (3.4)	13.35 (5.09)	10.9 (3.41)
Male gender, % (n)	79 (96)	60.8 (491)	55.2 (95)	54 (29)	57.9 (55)	59.3 (89)	56.8 (423)	49.4 (85)	61.1 (406)	56.8 (175)	55.9 (218)	54.1 (253)	67.6 (123)	54.8 (109)
Asthma exacerbations in past year														
Asthma- related ED visit/hospital admission, % (n) ^a	14.7 (18)	19.0 (154)	13.4 (23)	–	10.5 (10)	8.6 (13)	58.3 (434)	58.1 (100)	6.0 (39/644)	15.6 (48)	76 (296)	44.7 (209)	20.3 (37)	35.6 (71)
Oral corticosteroid use, % (n)	–	31.7 (256)	47.1 (81)	11.1 ^b (6)	36.8 (35)	–	42.3 (315)	77.3 (133)	6.5 (43)	43.2 (133)	52 (203)	29.3 (137)	20.3 (37)	–
BTS treatment step														
2, %	–	65.6	^c	–	60	28.7	41.1	60.7	72	25.6	–	68.6	–	–
3, %	–	18.4	–	–	37.9	60.7	43.6	36.4	22.3	61	–	25	–	–
4, %	–	16.0	–	–	2.1	10.6	15.3	2.9	5.7	13.3	–	6.4	–	–
Rs7216389														
T-allele frequency	0.57	0.56	0.58	0.62	0.60	0.58	0.66	0.66	0.59	0.58	0.60	0.79	0.81	0.54

–, data not available; BTS, British Thoracic Society; ED, emergency department; SD, standard deviation; y, years.

^aDNA was extracted from peripheral blood samples in these studies, and in the remaining, DNA was extracted from saliva samples.^bPatients with exacerbations were treated with oral corticosteroid or high-dose inhaled corticosteroids.^cCAMP is randomized clinical trial of mild to moderate asthmatics, and all children were on 200 µg of budesonide (ICS) plus SABA as needed.

(A)



(B)

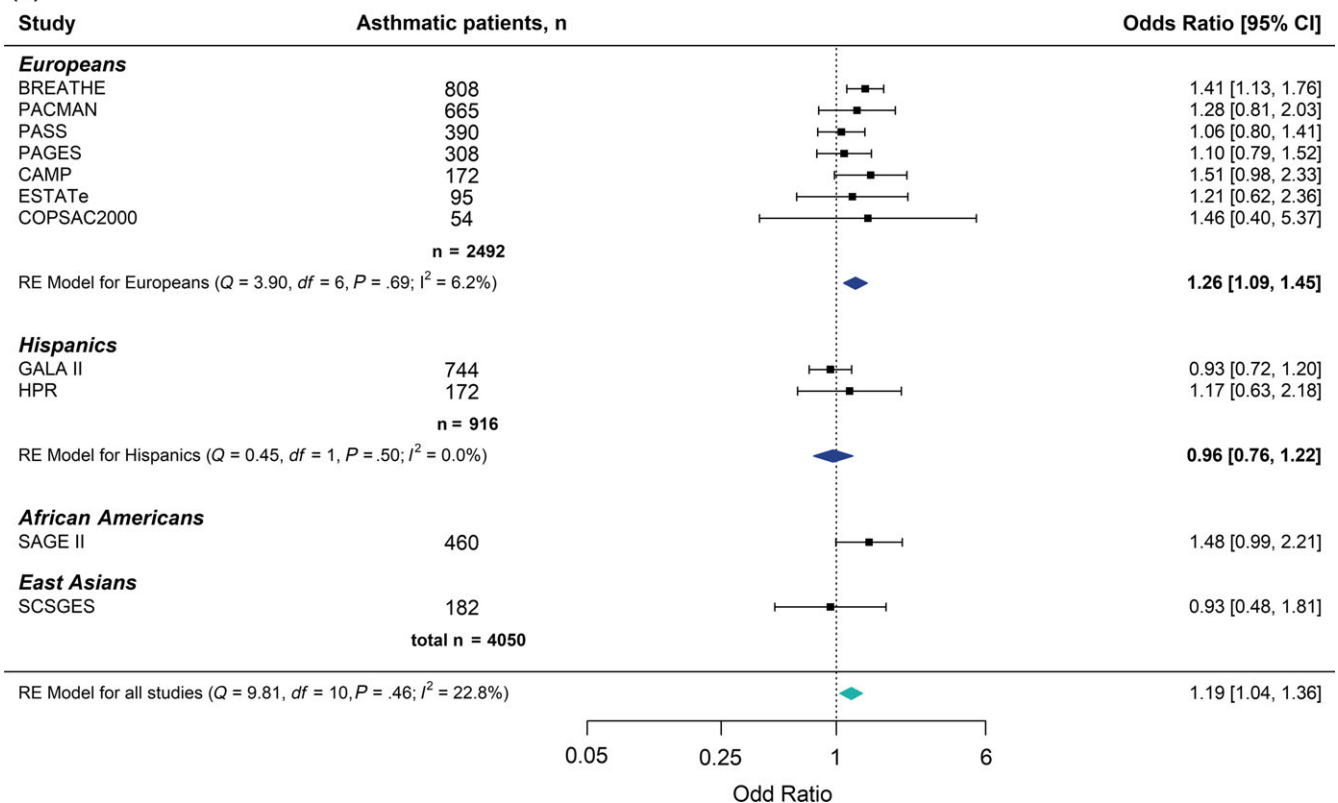


Figure 1. Forest Plots of rs7216389 for (A) asthma-related hospitalizations/ED visits in thirteen studies and (B) OCS/high-dose ICS use in eleven studies. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals with rs7216389, controlling for age, gender, and BTS treatment step [Colour figure can be viewed at wileyonlinelibrary.com]

linkage disequilibrium (LD) with rs7216389 were reported to switch CTCF-binding sites that resulted in increased expression of ORMDL3 in CD4⁺ T cells which subsequently reduced interleukin-2 production.⁸

Rs7216389 has previously shown to be associated with exacerbations³ and poor lung function in Caucasian children using ICS.⁴ Even though in our study Caucasians were the largest group, this study is the largest multiethnic population evaluating the association between 17q21 variant and asthma exacerbations in ICS users. Rs7216389 seems to increase bronchial responsiveness and therefore exacerbation rates in children,⁹ suggesting that carriers of rs7216389 might have a more severe form of asthma. However, by adding British Thoracic Society (BTS) treatment steps as a marker of disease severity to the model, we argue that the association reflects, at least partly, poor response to ICS.

Limitations of the study include the use of retrospective reporting of exacerbations in the observational cohort studies. However, the effect was also observed in a clinical trial population (CAMP), where exacerbations were reported prospectively. Hence, we do not believe that using retrospective data has significantly influenced the results. As not all studies had data available on both hospitalizations/ED visits and OCS use, we did not combine the two outcomes in our analysis. Furthermore, as information on treatment adherence was not available in all included studies, it was not considered in the analysis.

We show that 17q21, a widely replicated asthma susceptibility locus, is also associated with an increased risk of exacerbations in children/young adults treated with ICS. A better understanding of the molecular mechanisms underlying exacerbation-prone phenotype of pediatric asthma could lead to a better classification of different pediatric asthma phenotypes and the identification of novel treatment targets.

CONFLICTS OF INTEREST

AHM reports an unrestricted research grant from GSK, during the conduct of the study; she was a member of an advisory board for AstraZeneca, outside the submitted work. MP-Y reports grants from Spanish Ministry of Economy and Competitiveness (RYC-2015-17205), from Instituto de Salud Carlos III (ISCIII, AC15/00015), and from ERA-CoSysMed 1st Joint Transnational Call (SysPharmPedia), during the conduct of the study. NHP reports grants from Instituto de Salud Carlos III (ISCIII) and cofunded by the European Social Funds from the European Union (ESF) "ESF invests in your future", during the conduct of the study. KGT reports grants from U.S. National Institutes of Health, during the conduct of the study. SJV reports grants from Stichting Astma bestrijding, during the conduct of the study; and PACMAN cohort was funded by a strategic alliance between Utrecht Institute for Pharmaceutical Sciences and GSK. The other authors have no other conflict of interests that are directly relevant to the content of this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13530

Association of mast cells with clinical, endoscopic, and histologic findings in adults with eosinophilic esophagitis

To the Editor,

Eosinophilic esophagitis (EoE) presents with diverse features which can differ between children and adults, as well as among adults, but eosinophils alone do not account for this heterogeneity.^{1,2} Mast cells are involved in EoE pathogenesis, are highly increased in EoE compared to non-EoE controls, and mast cell-associated genes are upregulated in EoE.²⁻⁵ Mast cells also produce the profibrotic factor TGF- β 1 and promote smooth muscle contraction.⁶ However, the extent to which mast cell presence correlates with

clinical features of EoE is unknown. We aimed to determine whether esophageal mast cell levels correlated with clinical symptoms, endoscopic findings, and histologic features in newly diagnosed adults with EoE.

We performed a secondary analysis of data and biospecimens collected during a prospective cohort study of EoE cases, details of which have been previously described.⁷ The parent study included adults (>18) undergoing outpatient endoscopy for symptoms of esophageal dysfunction. EoE was diagnosed by consensus guidelines.⁸

TABLE 1 Patient characteristic, and characteristics stratified by the highest and lowest mast cell quartile, and by mast cell to eosinophil ratios ≤ 1 and >1

Characteristic	Overall EoE population (n = 96)	Lowest mast cell quartile (n = 24)	Highest mast cell quartile (n = 24)	P	Mast cell to eos ratio ≤ 1 (n = 72)	Mast cell to eos ratio > 1 (n = 24)	P
Mast cell density (mean cells/mm ² $\pm$ SD)	266.8 $\pm$ 180.0	88.7 $\pm$ 32.6	515.3 $\pm$ 157.4	-	235.1 $\pm$ 142.2	361.6 $\pm$ 242.6	0.002
Eosinophil density (eos/mm ² ; mean $\pm$ SD)	555.9 $\pm$ 478.1	304.2 $\pm$ 212.0	717.5 $\pm$ 501.1	<0.001	664.6 $\pm$ 498.8	229.8 $\pm$ 168.8	<0.001
Age at diagnosis (mean $\pm$ SD)	37.2 $\pm$ 12.7	38.0 $\pm$ 14.2	38.4 $\pm$ 13.6	0.91	37.1 $\pm$ 12.6	37.6 $\pm$ 13.2	0.88
Male (n, %)	54 (56)	10 (42)	15 (63)	0.15	38 (53)	16 (67)	0.24
White (n, %)	91 (95)	23 (96)	24 (100)	0.31	68 (94)	23 (96)	0.79
Symptoms							
Dysphagia (n, %)	94 (98)	24 (100)	23 (96)	0.31	71 (99)	23 (96)	0.41
>5 y of dysphagia (n, %)	53 (60)	13 (56)	12 (55)	0.51	37 (57)	16 (70)	0.67
VAS ^a score (mean $\pm$ SD)	3.5 $\pm$ 2.7	2.4 $\pm$ 1.9	3.6 $\pm$ 2.8	0.12	3.5 $\pm$ 2.6	3.5 $\pm$ 3.0	0.97
Heartburn (n, %)	8 (8)	1 (4)	2 (8)	0.55	7 (10)	1 (4)	0.39

(Continues)

Supplementary material

17q21 variant increases the risk of exacerbations in asthmatic children using inhaled corticosteroids

Methods

Studies were eligible to participate in the current study if:

- Included patients were on British Thoracic Society (BTS) treatment step 2 or higher [1].
- Data were available on exacerbations in the last 6 or 12 months of the study.
- Genotype data of rs7216389 were available in their study population.

From 22 studies currently participating in PiCA, 14 met the inclusion criteria and were included in the meta-analyses.

Study populations

European cohorts

BAMSE (Stockholm, Sweden) is a prospective birth cohort including children born between 1994 and 1996 in Stockholm. In the current study, Non-Hispanic white children at the age of 8 years with physician-diagnosed and parental-reported asthma were included in the analysis [2]. The ethnicity was defined based on the principal component analysis from genome-wide genotyping data. BREATHE is an observational study and included physician-diagnosed asthmatic children/young adults (age: 3-22 years) [3], through primary and secondary health centers in either Tayside or Dumfries (Scotland, United Kingdom). The majority of the patients were considered to be non-Hispanic whites. COPSAC2000 is a

prospective birth cohort including children (born between 1998 and 2001) of asthmatic mothers[4]. For this study, self-reported non-Hispanic whites were included in the analyses. ESTATE (Effectiveness and Safety of Treatment with Asthma Therapy in children) is a case-control study including children and young adults with physician-diagnosed asthma recruited from primary care units in the Netherlands (age: 4-19 years). Self-reported non-Hispanic whites were included in the analyses. FollowMAGICS (Germany, age: 7-25 years) is the follow-up study of the MAGICS cohort approximately 5 years after recruitment (Multicentre Asthma Genetics in Childhood Study), which is an observational study and includes physician diagnosed asthmatic children and young adults recruited from secondary and tertiary centers in Germany and Austria. The patients were self-reported non-Hispanic whites and their ethnicity was further validated through principal component analysis using genome-wide genotyping data. The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) study (the Netherlands, age: 4-12 years) [5], is an observational cohort study including children with a reported regular use of asthma medication through community pharmacies. In PACMAN, self-reported ethnicity was categorized as African, Asian, white, Hispanic, or mixed. In this study, non-Hispanic white patients were included in the analyses. Given that genome-wide genotyping data was available for PACMAN, principal component analyses was performed (Figure 1) to check whether the results could be affected by differences in the ancestral composition among individuals. The effect sizes of the association analyses in PACMAN with and without including three principal components as covariates, varied slightly (data not shown). Furthermore, since genome-wide analysis of ICS response did not result in genomic inflation due to population stratification (λ value = 0.98) (Figure 2), and the model was not adjusted by ancestry. The Paediatric Asthma Gene Environment Study (PAGES) (Scotland, United Kingdom, age: 2-16 years) [6], is an observational cohort study including children/young adults with physician-diagnosed asthma through primary, secondary and tertiary centers. For this study, patients with self-reported ancestry other than non-Hispanic white were excluded. Pharmacogenetics of adrenal suppression (PASS) (United Kingdom, age: 5-18 years) is a multicenter cohort of asthmatic children [7]. Children were included in the study based on asthma diagnosis by a

secondary care paediatrician experienced in the treatment of asthma, severe asthma that requires inhaled steroids and clinical concern about adrenal suppression sufficient to warrant a Low Dose Short Synacthen Test (LDSST). The ethnicity of two patients was self-reported mixture of non-Hispanic white and another ancestry, the remaining were self-reported non-Hispanic whites, all validated through principal component analysis from genome-wide genotyping data. The SLOVENIA cohort (Slovenia, age: 5-19) is an observational study and includes asthmatic children/young adults recruited from tertiary health centers. Asthma was defined by physician diagnosis and hospital records. Patients were self-identified Central European Caucasians of Slavic origin.

North American clinical trial

CAMP is a multi-center trial including 1,041 children (age: 5-12 years at the start of the trial) with mild-to-moderate asthma randomized to ICS (budesonide), nedocromil, or placebo twice daily [8]. In this study, patients with the self-identified non-Hispanic white of European origin were analyzed.

Hispanic/Latino and African American cohorts

The Genes-Environment and Admixture in Latino Americans (GALA II) study (the United States, age: 8-21 years) [9] is an ongoing multi-center study of Hispanic children/ young adults with and without asthma. Asthma was defined by physician diagnosis and report of symptoms in the two years preceding enrollment. Patients were self-identified Hispanics with four Hispanic grandparents and their ethnicity was validated with principal component analysis from genome-wide genotyping data. The Study of asthma in Puerto Rican children (HPR) (the United States, age: 6-17 years) [10, 11], is a case-control study including patients from tertiary care. Asthma was defined based on physician diagnosis and symptoms or medication use in the past 12 months. All participants had to have four Puerto Rican grandparents. The study of African Americans, Asthma, Genes Environments (SAGE II) study (the United States, age: 8-21 years) [9], is an ongoing multi-center study of children and young adults with African American ancestry with and without asthma. Asthma was defined by physician diagnosis and report of symptoms in the two years preceding enrollment. Patients had four African-American

grandparents and their ethnicity was validated with principal component analysis from genome-wide genotyping data.

East Asian cohort:

The Singapore Cross-Sectional Genetic Epidemiology Study (SCSGES) (Singapore, age: 6-31 years) is an on-going cross-sectional genetic epidemiology study on allergic diseases among Singapore Chinese individuals [12]. Self-identified Chinese ethnicity was further validated through principal component analysis from genome-wide genotyping data. Volunteers, including asthmatic and non-asthmatic individuals, were recruited from two different recruitment centers within Singapore. Asthma was defined by physician diagnosis and report of symptoms in the year preceding enrollment. In this study, we included patients up to 25 years of age.

All studies participating in this study received ethical approval from their local medical ethics committees.

Medication data

Data collection on ICS use in the last 6/12 months of the study or first year of the trial:

BAMSE/ COPSAC2000/followMAGICS/PACMAN: Pharmacy records and parent/patient-reported medication use at the study visit or based on completed study questionnaires.

BREATHE/GALA II/HPR/PAGES/SAGE II/ SCSGES: Parental/patient-reported medication use at the study visit or based on completed study questionnaires.

ESTATe/PASS/SLOVENIA: Physician prescriptions and pharmacy records.

In CAMP patients received budesonide 200 µg twice daily.

Outcomes

The following outcomes were studied:

- 1) Any asthma-related hospitalizations/ED visits reported by the parent/child at the study visit or based on completed study questionnaires:
 - BREATHE/PAGES/PASS: hospitalizations in the past 6 months preceding the study visit.
 - PACMAN: ED visits in the past 12 months preceding the study visit.
 - BAMSE/ ESTATE/followMAGICS/GALA II/ HPR/ SAGE II/ SCSGES /SLOVENIA: ED visits/hospitalizations in the past 12 months preceding the study visit or filling in the questionnaire.
 - CAMP: hospitalizations/ED visits in first 12 months of the trial.
- 2) Any course of OCS reported by the parent or child at the study visit or based on completed study questionnaires:
 - BREATHE/PAGES/ PASS: in the past 6 months preceding the study visit.
 - ESTATE/GALA II/ HPR/ PACMAN/ SAGE II/ SCSGES: in the past 12 months preceding the study visit.
 - CAMP: in the first 12 months of the trial
 - COPSAC2000: in the past 6 months preceding the study visit. In COPSAC2000, OCS and acute treatment with high-dose inhaled steroids (4 times normal dose) were used interchangeably for treatment of severe asthma exacerbations.

Genotyping

Genotyping in BAMSE, BREATHE and PAGES was performed using the Sequenom Mass Array platform (Sequenom, San Diego, California, USA). Genotyping was performed using Axiom LAT1 array

World Array 4 (Affymetrix, Santa Clara, CA, USA) in GALA II and SAGE II. In COPSAC2000 genotyping was performed using Illumina Infinium HumanOmniExpressExome chip. Genotyping in CAMP was performed using Illumina Infinium II 550 K SNP Chips and 610 Quad Chip (Illumina, Inc, San Diego, California) and rs7216389 was imputed based on 1000 Genomes Project. Genotyping in ESTATE was performed using Human Core-24 BeadChip Marker information and the SNP was imputed from the Haplotype Reference Consortium. For followMAGICS, the Illumina Sentrix HumanHap300 BeadChip used for genotyping. In HPR genotyping was performed using Illumina HumanOmni2.5 BeadChip. Genotyping in PACMAN was performed using Human Core-24 BeadChip Marker information and the Sequenom Mass Array platform (Sequenom, San Diego, California, USA). Furthermore, a subset of the PACMAN samples was previously genotyped using the Sequenom Mass Array platform (Sequenom, San Diego, California, USA). For this study, the genotype data from these two methods were pooled together for the analyses. Genotyping in PASS was performed using Illumina Omni Express 8v1, 1000 Genomes was used as reference panel and the SNP was imputed with ShapeIT and Impute2. The imputation quality was estimated by “INFO” score (≥ 0.5 indicating high imputation quality). Genotyping in SCSGES was performed using the 2-staged method, with each of discovery and validation stages focuses on separate sets of human participants. In discovery stage of genotyping, Illumina Bead Xpress Assay was performed, while validation stage used Sequenom platform with MassARRAY and iPLEX technology. For SLOVENIA, real-time PCR instrument LightCycler 480 (Roche) used for genotyping.

Statistical analysis

Logistic regression analysis was used to assess the risk of exacerbations when carrying rs7216389. Odds ratios (OR) were calculated per study. The model was adjusted for age (in years), gender and for an adjusted form of the BTS guideline treatment steps (step 2: ICS plus short-acting beta agonist as needed, step 3: step 2 plus long-acting beta agonist, step 4: step 3 plus leukotriene antagonist). The ORs were meta-analyzed with the inverse variance weighting method assuming random-effects, this was due to

consideration of potential heterogeneity between cohorts and their sampling. Two statistical tests of heterogeneity, I^2 and Cochran's Q-test, are reported along with other results of the meta-analysis [13]. Forest plots were made with R (version 3.3.3) and the 'metafor' package [14]. An additive genetic model was assumed for the analysis. In addition, separate meta-analyses were performed for European studies and Hispanics. Furthermore, since younger children might have a different phenotype of the disease and a different response to ICS, a sensitivity analysis was performed to investigate whether the association would be different by categorizing the patients based on their age at the time of the outcome measurement (2-4 years of age and ≥ 5 years). Deviation from Hardy-Weinberg equilibrium (HWE) in the studies with genome-wide genotyping data was assessed with standard quality control for GWAS. For the remaining studies (BAMSE, BREATHE, PAGES, SLOVENIA and SCSGES), we used a web program (<http://www.oege.org/software/hwe-mr-calc.shtml>) which uses Pearson chi-squared test for HWE testing [15]. The results are reported according to the STREGA guidelines.

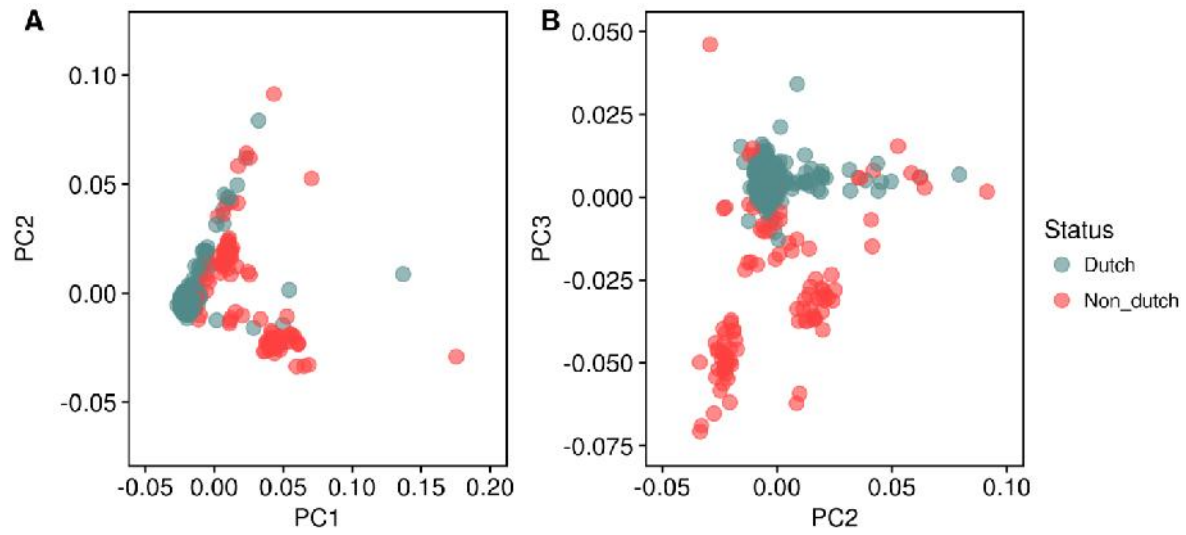


Figure 1. Principal components for PACMAN individuals classified by being dutch/non-dutch. A) PC1 versus PC2; B) PC2 versus PC3.

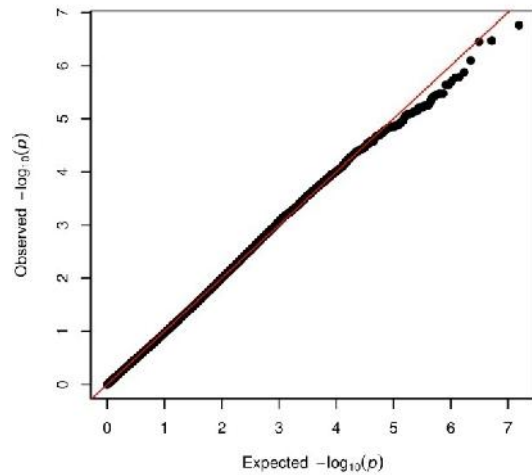


Figure 2. Quantile-quantile plot of GWAS of ICS response not adjusted by principal components.

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Figure legends:

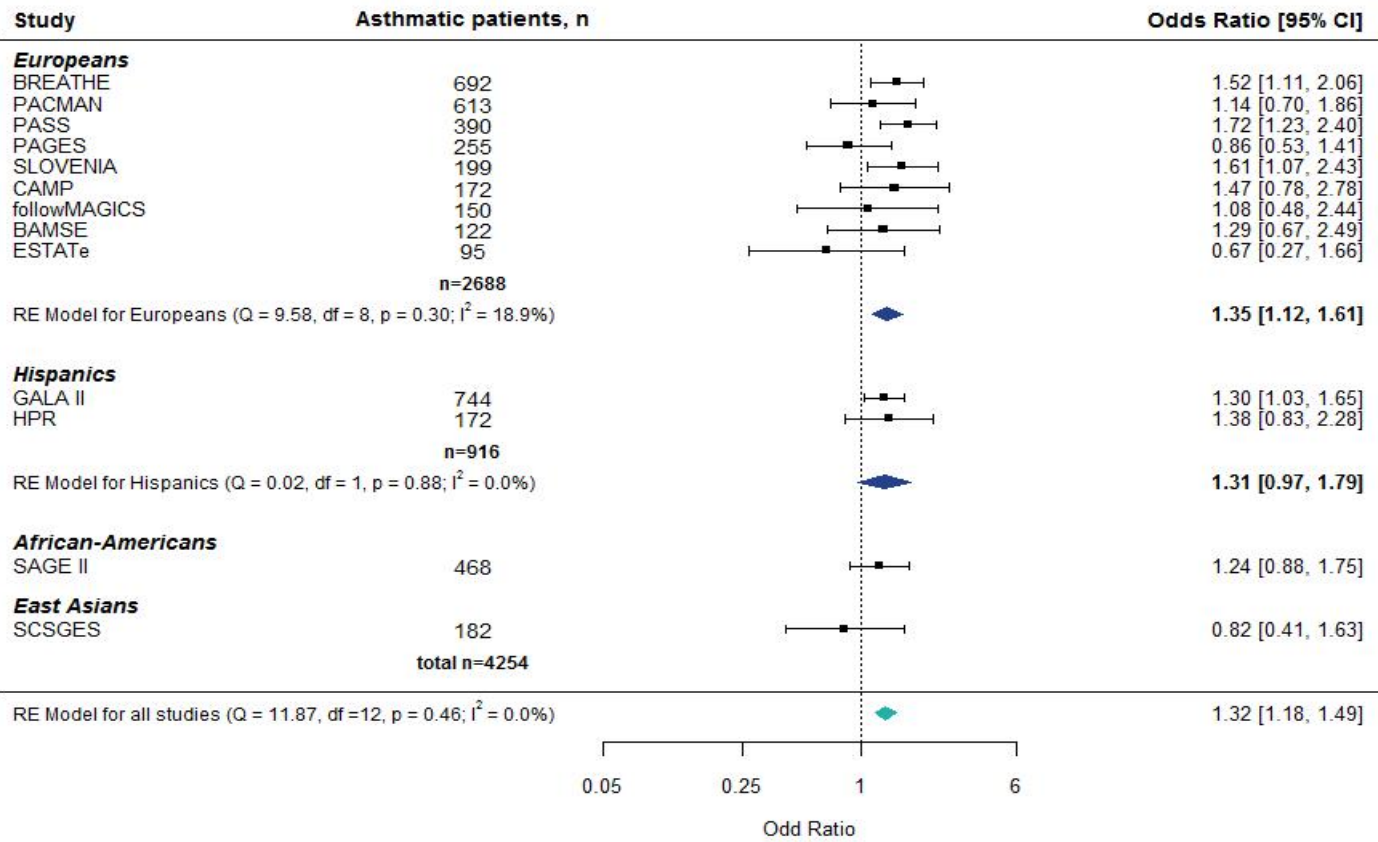
Supplementary figure 1: Forest Plots of rs7216389 for asthma-related hospitalizations/ED visits in patients older than 5. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals carrying the risk allele (T), controlling for age, gender, and BTS treatment steps.

Supplementary figure 2: Forest Plots of rs7216389 for OCS/high dose ICS for patients older than 5. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals carrying the risk allele (T), controlling for age, gender, and BTS treatment steps.

Supplementary figure 3: Forest Plots of rs7216389 for OCS/high dose ICS in patients younger than 5. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals carrying the risk allele (T), controlling for age, gender, and BTS treatment steps.

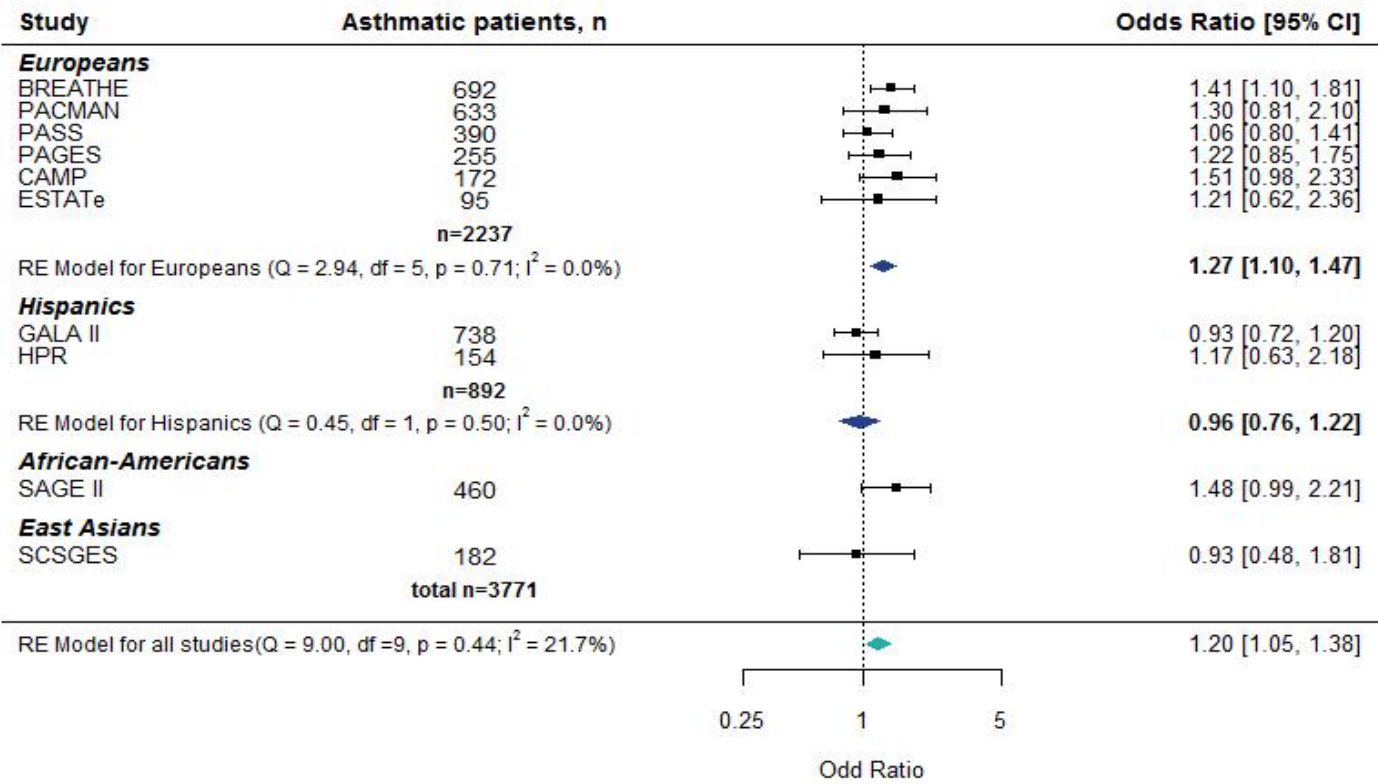
Supplementary figure 4: Forest Plots of rs7216389 for asthma-related hospitalizations/ED visits in patients younger than 5. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals carrying the risk allele (T), controlling for age, gender, and BTS treatment steps.

Supplementary figure 1.



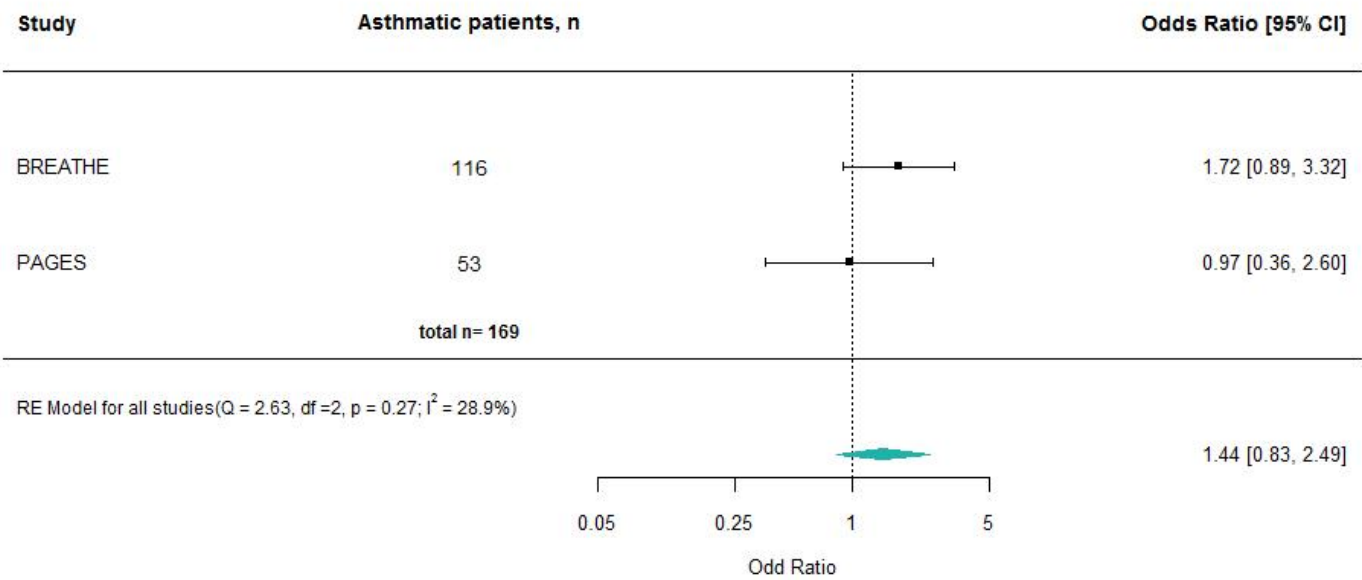
Forest Plots of rs7216389 for asthma-related hospitalizations/ED visits in patients 5 years and older. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals with rs7216389, controlling for age, gender, and BTS treatment step.

Supplementary figure 2.



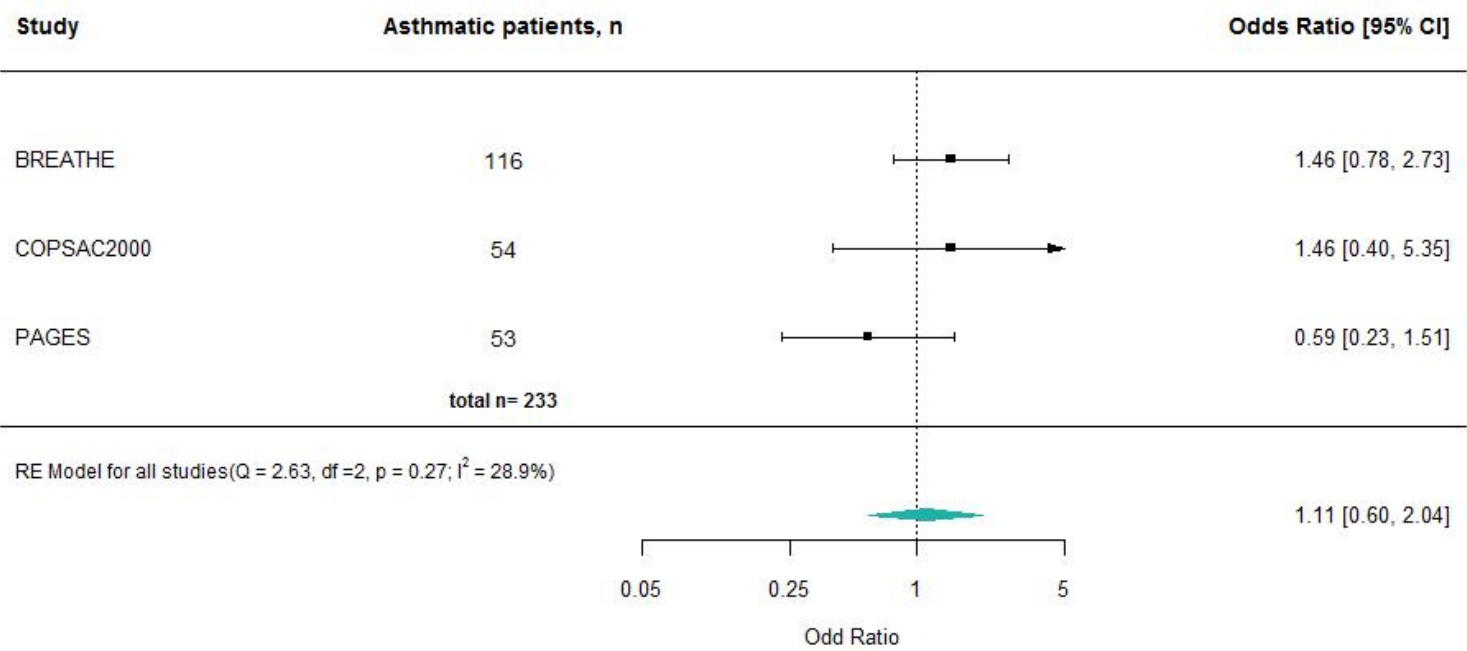
Forest Plots of rs7216389 for OCS use in patients 5 years and older. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals with rs7216389, controlling for age, gender, and BTS treatment step

Supplementary figure 3.



Forest Plots of rs7216389 for asthma-related hospitalizations/ED visits in patients younger than 5. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals carrying the risk allele (T), controlling for age, gender, and BTS treatment steps.

Supplementary figure 4.



Forest Plots of rs7216389 for OCS/high dose ICS in patients younger than 5. Odds Ratios (OR) and corresponding 95% Confidence Intervals (95% CI) for individuals carrying the risk allele (T), controlling for age, gender, and BTS treatment steps.