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Pharmacogenetics of inhaled corticosteroids and exacerbation risk in adults with asthma

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Abstract

Background: Inhaled corticosteroids (ICS) are a cornerstone of asthma treatment. However, their efficacy is characterized by wide variability in individual responses.

Objective: We investigated the association between genetic variants and risk of exacerbations in adults with asthma and how this association is affected by ICS treatment.

Methods: We investigated the pharmacogenetic effect of 10 single nucleotide polymorphisms (SNPs) selected from the literature, including SNPs previously associated with response to ICS (assessed by change in lung function or exacerbations) and novel asthma risk alleles involved in inflammatory pathways, within all adults with asthma from the Dutch population–based Rotterdam study with replication in the American GERA cohort. The interaction effects of the SNPs with ICS on the incidence of asthma exacerbations were assessed using hurdle models adjusting for age, sex, BMI, smoking and treatment step according to the GINA guidelines. Haplotype analyses were also conducted for the SNPs located on the same chromosome.

Results: rs242941 (*CRHR1*) homozygotes for the minor allele (A) showed a significant, replicated increased risk for frequent exacerbations (RR = 6.11, $P < 0.005$). In contrast, rs1134481 T allele within *TBXT* (chromosome 6, member of a family associated with embryonic lung development) showed better response with ICS. rs37973 G allele (*GLCCI1*) showed a significantly poorer response on ICS within the discovery cohort, which was also significant but in the opposite direction in the replication cohort.

Conclusion: rs242941 in *CRHR1* was associated with poor ICS response. Conversely, *TBXT* variants were associated with improved ICS response. These associations may reveal specific endotypes, potentially allowing prediction of exacerbation risk and ICS response.

KEYWORDS
asthma exacerbations, inhaled corticosteroids, personalized medicine, pharmacogenetics

1 | INTRODUCTION

Asthma is a chronic heterogeneous airway disease, presenting worldwide in different clinical phenotypes and molecular endotypes, affecting up to 300 million people.[1-3] Asthma's hallmark features are airway hyper-responsiveness and reversible airway obstruction. Inflammation not only occurs mainly in the bronchi and conducting trachea, but may also spread up to the alveoli with more severe symptoms. The underlying pathology is not yet fully understood. It is believed that immune-mediated inflammatory processes have a significant role in asthma, especially T helper 2 (Th2) cells and type 2 innate lymphoid cells (ILC2), which mediate inflammation and induce eosinophilia through interleukin mediators.[4] NF-Kb (k is actually greek kappa) is also regarded to be an important pathway of inflammation in asthma, and its upregulation was found to be of special importance in severe uncontrolled asthma.[5]

Asthma is increasingly recognized as a disease with a major genetic component, as heritability is estimated to be 35%-70%.[6,7] According to the GWAS catalog, 646 single nucleotide polymorphisms (SNPs) have been associated with asthma, including variants associated with different phenotypes of the disease (eg childhood-onset asthma, severe asthma).[8,9] Shrine et al.[10] pinpointed genetic loci associated with severe asthma phenotypes, confirming the earlier findings suggesting genetic factors to be involved in disease severity.[11,12] Moreover, environmental factors may also influence the risk of asthma onset and severity, possibly through epigenetic mechanisms.[13,14]

The current asthma guidelines recommend inhaled corticosteroids (ICS) as first-line therapy for persistent asthma, with intensification based on the patient's response.[15] Corticosteroids exert a range of anti-inflammatory effects including by decreasing the expression of pro-inflammatory genes, as well as upregulation of anti-inflammatory genes. Corticosteroids bind to specific glucocorticoid receptors affecting the expression and transcription of a multitude of genes involved in the inflammatory process. Most importantly, ICS anti-inflammatory effects reduce the risk of asthma exacerbations, considered one of the most important components in establishing disease control in patients with asthma.[16-18] ICS may also potentiate beta$_2$-adrenergic agonists.[19]

However, individual response to ICS is widely recognized to be highly variable[20,21] and up to 10% of patients require a maximal dose of ICS.[22] Furthermore, a significant proportion of adults with asthma have exacerbations despite adequate treatment with ICS.[21] This variability may be the result of several factors. Besides adherence and inhaler technique, the mechanism of inflammation underlying the asthma phenotype is important. No standardized clinical test or biomarker exists to predict ICS response. Previous attempts to identify predictors included the age of asthma onset, sex and the fraction of exhaled nitric oxide (FeNO). Additionally, short-term response, defined as six weeks of ICS treatment, and history of exacerbations were also identified as predictors for disease control by ICS, as assessed by reduced risk of further exacerbations in the long term.[23-25]

Identification of individuals with asthma with poor response to ICS therapy may be useful, as these individuals may benefit from earlier interventions with other therapies. This variability of response to ICS treatment in patients with asthma may not only be due to different mechanisms and types of airway inflammation, but may also partly be attributed to pharmacogenetics.[26] Several genome-wide and candidate gene studies have been conducted to evaluate the effects of genetic variants on ICS response.[27-32] McGeachie et al. previously combined two genetic variants in a test to predict ICS response.[33] However, genetic variants are not yet used in clinical practice to predict ICS response, as most discovered loci had a relatively small effect on the drug response, leading to limited potential clinical utility even in the largest GWAS conducted to date.[33-35] Furthermore, few studies have investigated the genetic association with ICS effects on exacerbations in adults with asthma, despite evidence of variability for this important age group as well.[21-37]

Several interesting SNPs may potentially affect patients' response to ICS. For example, two variants (rs28364072 and rs7216389) were previously associated with increased exacerbations risk in children treated with ICS[30-38] and five variants were previously associated with ICS response based on changes in lung function (FEV1).[29-40] In addition, three novel SNPs associated with asthma[41] could affect treatment response through their effects on the expression of three genes affecting important inflammatory pathways: rs17637472, a strong cis-eQTL for G Protein Subunit Gamma Transducin 2 (*GNGT2*); rs7705042 located within an intron of *Nedd4* Family-Interacting Protein 1 (*NDFIP1*); and rs167769, an intron variant of *STAT6* gene.[41]

The main objective of this study was to investigate whether the ten above-described genetic polymorphisms (Table 1) modulate the treatment response to ICS in adult patients with asthma.

TABLE 1 SNPs selected to test for their effects on asthma exacerbations in adults treated with ICS

Gene	Location on chromosome	Variant	Minor allele	Minor allele frequency (European) (%)
Novel variants being investigated				
GNGT2[41]	17q21.33	rs17637472	A	39
NDFIP1[41]	5q31.3	rs7705042	C	36
STAT6[41]	12q13.3	rs167769	T	33
Variants with documented effects on exacerbations (in children)				
FCER2[38]	19p13	rs28364072	G	28
GSDMB[30]	17q12-21	rs7216389	T	13
Variants with documented effects on lung function measured by ΔFEV_1				
GLCCI1[29-34]	7p21.3	rs37973	G	44
CRHR1[34-40]	17q21.31	rs242941	A	28
TBXT (T-box transcription factor) gene locus[39,40]	6q27	rs1134481	T	40
		rs2305089	C	50
		rs3099266	T	42

Abbreviation: FEV1, forced expiratory volume.

2 | METHODS

2.1 | Setting and study population

2.1.1 | Discovery cohort

We investigated all patients with asthma (n = 775) within the Rotterdam study, an ongoing prospective population-based cohort study involving inhabitants of the Ommoord district of Rotterdam, the Netherlands.[42] The rationale and design of the Rotterdam study have been described elsewhere.[43] In short, the Rotterdam study includes three subcohorts RS I, RS II and RS III. Baseline data were collected from 1989 to 1992 in RS I (n = 7983), from 2000 to 2003 in RS II (n = 3011) and from 2006 to 2009 in RS III (n = 3932). Follow-up examinations were conducted periodically based on a home interview and an extensive set of tests at the research facility. In addition, data from the medical records of the general practitioners (GPs), nursing homes and hospitals were collected. The Rotterdam study is approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (registration number MEC 02.1015), and the review board of the Netherlands Ministry of Health, Welfare and Sports (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. Written informed consent was obtained from all participants.

2.2 | Replication cohort

Replication was based on data from the Kaiser Permanente Northern California (KPNC) multi-ethnic Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, which comprises longitudinal electronic health record data on over 100 000 people.[44] Data included longitudinal asthma-related events, such as ambulatory office visits, hospitalizations, emergency department (ED) visits, and fills of ICS and ICS-LABA combination. Follow-up started from the start of their first ICS prescription. The institutional review boards for human subject research of both KPNC and University of California, San Francisco (UCSF), approved the project (AG036607; Schaefer/Risch, PIs). Patients from non-European ancestries were excluded from the analysis, as the Rotterdam cohorts consist mainly of subjects with European Ancestry.

2.3 | Definition of cases and controls

Participants in the Rotterdam study were considered to have asthma if diagnosed by a physician and reported in their medical file.[42] The start date of follow-up was defined as the diagnosis date for incident cases or the date of study enrolment for prevalent asthma subjects. Subjects were followed until death, loss to follow-up or the end of the study period (1 January 2016), whichever came first. In the GERA cohort, subjects were considered to have asthma based on their electronic health records and follow-up started from the start of their first ICS prescription and included up to 15 years of follow-up. Asthmatic subjects experiencing one or more asthma exacerbations during the follow-up period were compared with asthma subjects without an exacerbation during the follow-up period.

2.4 | Asthma exacerbations

In the Rotterdam study, asthma exacerbations were defined as the worsening of asthma requiring the use of short-term systemic corticosteroids for at least three days, according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) statement.[18]

Exposure to systemic corticosteroids was assessed using medication dispensing data (ATC codes: H02). Prescriptions with less than 7 days of difference between end date of the previous prescription and start date of the subsequent one were considered to indicate the same episode. Asthma exacerbations in the GERA replication cohort were defined as an asthma-related ED visit, hospitalization or oral corticosteroid (OCS) burst. OCS bursts were defined as single OCS prescriptions, excluding long-term OCS use as a controller medication.

2.5 | Drug exposure

Medication dispensing data were obtained from all seven fully computerized pharmacies in the study district in the Rotterdam study, and medication filling data were obtained from the electronic records in the GERA cohort. Within the Rotterdam study, records of all filled prescriptions from 1 January 1991 onwards were available and included information on the product name, the ATC codes, the dispensing date, the prescribed dosing regimen and the amount dispensed. The prescribed daily dose of each ICS was expressed in standardized defined daily doses according to the ATC/DDD system of the World Health Organization (Anatomical Therapeutic Chemical Classification System/ defined daily doses).[45] The studied inhaled corticosteroids included ICS monotherapy (ATC codes: R03BA01-9) and ICS combination therapy (ATC codes: R03AK06-13). ICS exposure was assessed based on pharmacy dispensing data on start of and during the follow-up period. Subjects were considered ICS users if their prescribed ICS covered 80% or more of their total follow-up time.

2.6 | Genetic variants

We extracted the dosage of 10 SNPs in the Rotterdam study with minor allele frequency ≥5% (Table 1). The SNPs were selected based on a systematic review of the available literature on SNPs associated with asthma. The search strategy is described in the File S1. Genotyping was performed using Illumina 500 (+duo) and Illumina Human 610-Quad BeadChips. Imputation quality for the investigated SNPs was high (at least ≥0.85). For the functional annotation of the variants, we checked their predicted functions, including effects on gene regulation, protein structure and splicing by using the HaploRegv4.1 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php). Haploview 4.2 and LDLINK were both used to estimate haplotype population frequency and linkage disequilibrium between SNPs.[46] DNA extraction, genotyping, array design and population structure analyses for the GERA cohort have been previously described.[44-49]

2.7 | Statistical analysis

Descriptive statistics were calculated using means and standard deviations for continuous variables and using percentages for categorical variables. Given the primary outcome zero-inflated distribution in the discovery cohort, hurdle regression models were used to calculate the odds ratios for the first exacerbation and rate ratios for further exacerbations during the follow-up period. Interaction terms were used to check whether genetic variants modify the ICS treatment response on exacerbation risk. The hurdle model consists of two stages, the first step models the risk of having an exacerbation count above zero, and the second step models the risk of having more frequent exacerbations (ie risk of additional exacerbations for subjects with at least one exacerbation).[50] As follow-up periods varied between subjects, logarithm of the follow-up times was used as an offset variable in the model. The study design and follow-up of both discovery and replication cohorts have been illustrated in Figure S1. Full models were adjusted for covariates that were significantly (or clinically) related to the exposure and changed the point estimate. Significant difference was calculated using the t test for continuous variables (age, BMI, pack-years of cigarette smoking) and the chi-square test for categorical variables (sex, medication use and smoking status). To account for potential confounding by disease severity, treatment steps—according to GINA guidelines—were categorized using level of ICS use and other asthma comedications.[51] The final models were therefore adjusted for BMI, sex, age at baseline, smoking and the highest GINA treatment step. Finally, interaction terms were included in the full model, to investigate the effects of genotype on drug response according to the following formula:

$$E\left[\frac{\text{Total number of exacerbations}}{\text{Years of follow up as offset term}}\right] = \beta 0 + \beta 1 SNP \times ICSuse + \beta 2 SNP + \beta 3 ICSuse + \beta 4 Age + \beta 5 Sex + \beta 6 BMI + \beta 7 Smoking + \beta 8 GINA\ Treatment\ step$$

All statistical analyses were performed using SPSS version 25 (SPSS Inc., Chicago, IL) and R Statistical Software version 1.1.463 (R Foundation for Statistical Computing, Vienna, Austria).[52] The package pscl was used to fit the hurdle models.[53] Haplo.stats, an R package, was used for haplotype analysis.[54] Because we tested 10 SNPs, a Bonferroni-corrected *P* value below 0.005 (0.05/10) was considered statistically significant. Subjects with missing genotype or covariable data were excluded.

3 | RESULTS

Within the Rotterdam study population, 12,453 subjects were genotyped, 11,496 of them passed the quality control, and 11,385 participants gave informed consent for retrieving follow-up data. There were 325 asthmatic cases with at least one exacerbation during follow-up and 272 adults with asthma without an exacerbation during follow-up with available genotype and covariable data (Figure 1). From the 13761 asthmatic subjects within the GERA replication cohort, 2787 were excluded due to their different ancestries, 775 were excluded for being followed for less than a year, and 357 were not included in the analysis due to missing data. From the remaining, 7978

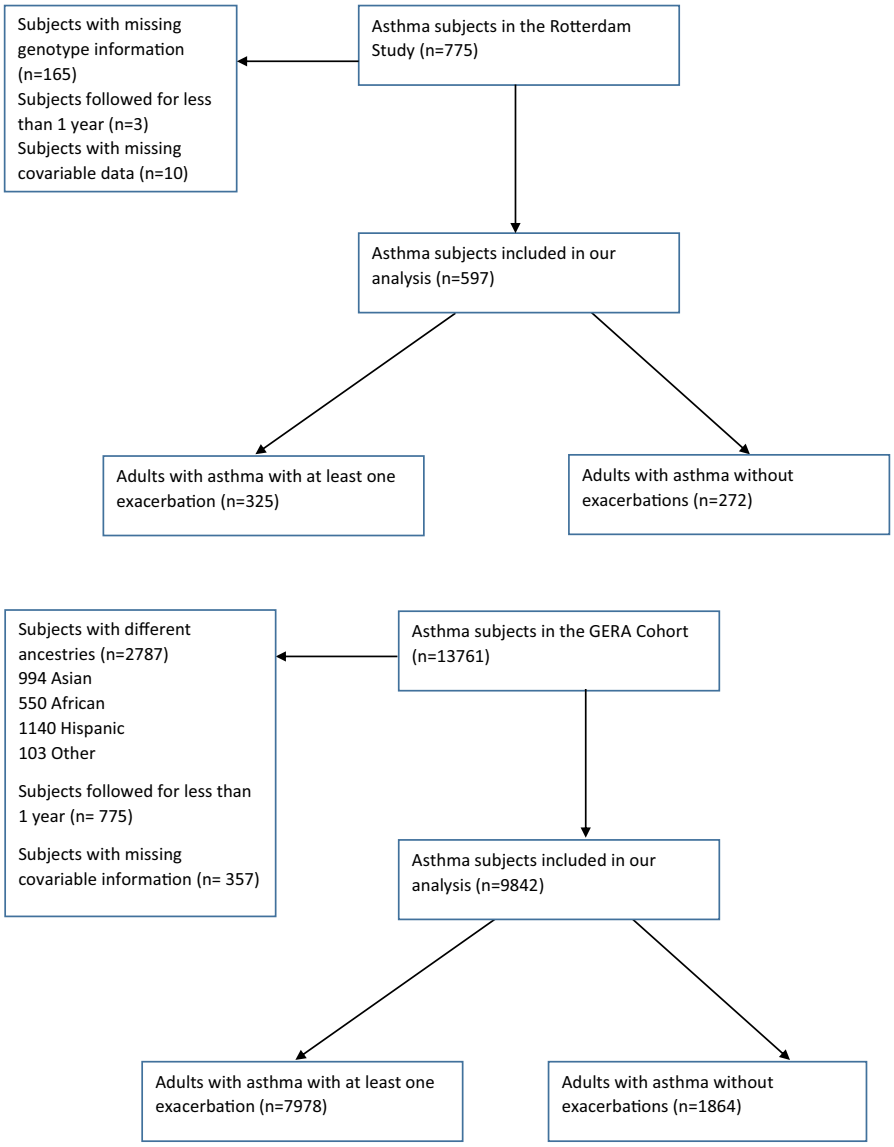

FIGURE 1 Study flow, including both the discovery and the replication phases

adults with asthma had exacerbations and 1864 adults with asthma had no exacerbations during the follow-up starting from their first ICS prescription. Adults with asthma with exacerbations were older, used more (frequently) ICS and were therefore more likely to be in higher GINA treatment steps in both the discovery and replication cohorts (Table 2).

3.1 | Pharmacogenetic effects on exacerbation risk (zero model)

Only rs17637472 heterozygotes showed a nominally significantly improved response with ICS in both crude and full models (Table 3, Table S1) compared with wild-type individuals (OR: 0.24, $P = 0.012$).(Table 3) Despite a similar direction of effect for this SNP in the zero model of the replication (Table 4), no SNPs were significantly associated with ICS response to prevent the first exacerbation, adjusted for age, sex, smoking, BMI and GINA treatment step (Tables 3 and 4).

3.2 | Pharmacogenetic effects on recurrent exacerbation risk (positive count model)

Three variant alleles were associated with an increased risk of recurrent exacerbations by ICS use in the positive count model (Table 3). rs242941 homozygotes for the minor allele (A) of *CRHR1* were at more than sixfold increased risk for exacerbations when using ICS (RR:6.11; $P < 0.005$; Figure 2), and rs7705042 homozygotes for the minor allele (C) had an incidence rate ratio of 2.44 ($P < 0.005$) when using ICS. The minor allele (G) of rs37973 showed increased risk by ICS use for both homozygotes and heterozygotes carriers, but only heterozygotes passed the Bonferroni threshold. Three SNPs (rs1134481, rs2305089 and rs3099266)—associated with *TBXT* gene—showed better response with ICS within the population-based Rotterdam study. A significantly decreased risk of subsequent exacerbations was also observed for heterozygous rs167769 ICS users, but this effect was not significantly extended to the homozygous variant carriers, nor was it replicated (Tables 3 and 4, Table S2).

TABLE 2 Baseline characteristics of the discovery and replication cohort study subjects

Parameter	Rotterdam adults with asthma with exacerbations (n = 325)	Rotterdam adults with asthma without exacerbations (n = 272)	*P* value	GERA adults with asthma with exacerbations (n = 7978)	GERA adults with asthma without exacerbations (n = 1864)	*P* value
Age (y)	64.0 (9.1)	62.0 (8.3)	0.004	65.1 (12.0)	62.1 (13.1)	<0.005
Females	235 (72.3%)	185 (68.0%)	0.292	5329 (66.8%)	1240 (66.5%)	0.843
Adherent ICS use	67 (20.6%)	33 (12.1%)	0.007	606 (7.6%)	85 (4.6%)	<0.005
Smoking (ever)	203 (62.4%)	186 (68.3%)	0.154	3852 (48.3%)	709 (38.0%)	<0.005
BMI	28.2 (4.6)	28.1 (4.7)	0.873	28.5 (6.2)	27.5 (5.9)	<0.005
Smoking (pack-years)	14.8 (20.3)	14.7 (19.9)	0.932	—	—	-
Follow-up time (years)	13(6)	11(6)	<0.005	7(3)	6(3)	<0.005
GINA treatment step						
GINA step 1: No ICS maintenance treatment	14 (4.3%)	70 (25.7%)	<0.005	—	—	-
GINA step 2:ICS monotherapy/ LTRA	29 (8.9%)	51 (18.8%)	0.014	3926 (49.2%)	1364 (73.2%)	<0.005
GINA step 3:low-dose ICS-LABA/ high-dose ICS/low-dose ICS-LTRA	93(28.6%)	95 (34.9%)	0.884	2066 (25.9%)	241 (12.9%)	<0.005
GINA step 4 or 5: medium- to high-dose ICS-LABA	189 (58.2%)	56 (20.6%)	<0.005	1986 (24.9%)	259 (13.9%)	<0.005

[a]ICS = Inhaled Corticosteroids, BMI = Body Mass Index, GINA = Global Initiative for Asthma, LTRA = Leukotriene Receptor Antagonist. Categorical variables are represented as percentages, while means and standard deviations are used to represent continuous variables.

TABLE 3 Results of the adjusted hurdle models for the 10 selected SNPs in the discovery cohort (n = 597)

SNP	Gene	Genotype frequency (%)	SNP by ICS interaction effect on exacerbation risk (Zero part of the hurdle model)		SNP by ICS interaction effect on frequent exacerbations risk (Count part of the hurdle model)	
			OR	*P* value	RR	*P* value
rs17637472	*GNGT2*					
		47.7	0.24	0.011	0.96	0.635
GAAA		17.4	0.71	0.666	1.09	0.777
rs7705042	*NDFIP1*					
AC		45.0	1.09	0.864	0.93	0.609
CC		13.4	1.49	0.687	**2.44**	<0.005
rs167769	*STAT6*					
CT		47.6	0.65	0.413	**0.52**	<0.005
TT		13.2	2.03	0.462	0.86	0.498
rs28364072	*FCER2*					
		43.0	1.09	0.868	0.84	0.188
CG AG		7.7	1.27	0.787	1.12	0.744
rs7216389	*17q21*					
CT		52.8	1.13	0.836	1.36	0.069
TT		21.1	1.17	0.829	1.19	0.374
rs37973	*GLCCI1*					
GA		52.2	1.86	0.277	**1.88**	<0.005
GG		15.6	0.64	0.532	1.54	0.043
rs242941	*CRHR1*					
CA		51.2	0.59	0.330	1.27	0.079
AA		5.5	0.46	0.506	**6.11**	<0.005
rs1134481	*TBXT*					
GT		44.2	1.17	0.768	0.68	0.006
TT		17.9	3.87	0.135	**0.36**	<0.005
rs2305089	*TBXT*					
TC		47.7	1.00	0.993	**0.42**	<0.005
CC		25.9	3.25	0.149	**0.37**	<0.005
rs3099266	*TBXT*					
GT		47.9	1.20	0.732	**0.58**	<0.005
TT		19.1	4.40	0.103	**0.29**	<0.005

Odds ratios, rate ratios and their corresponding *P* values of the association between the SNPs and increased or decreased risks in both zero and count models, adjusted for age, sex, smoking, BMI and GINA treatment step. Underlined associations are nominally significant, bolded associations have passed the Bonferroni threshold.

Abbreviations: ICS, inhaled corticosteroid; OR, odds ratio; RR, rate ratio; SNP, single nucleotide polymorphism.

Results for the replication analysis for the 8 SNPs with significant associations in the discovery cohort are outlined in Table 4. Effects of SNPs rs242941 and rs1134481 by ICS use showed the same direction of association as the discovery cohort in their count model and passed the Bonferroni threshold. rs37973 showed a very significant association but in the opposite direction compared with the discovery cohort.

Three of our selected SNPs were located on the same chromosome (chromosome 6) in the T-box transcription factor (*TBXT)* gene locus (Figure S2). The three SNPs were in high linkage disequilibrium (LD) (R^2>0.67, D'> 0.96). Haplotype frequencies for a European population were estimated as follows: GTC: 49.4%; TCT: 39.0%; GCC = 8.7%; GCT = 2.2%. In the discovery cohort, 17.6% of subjects

TABLE 4 Results of the adjusted hurdle models for 8 SNPs in the replication cohort (n = 9842)

SNP	Gene	Genotype frequency (%)	SNP by ICS interaction effect on exacerbation risk (Zero part of the hurdle model)		SNP by ICS interaction effect on frequent exacerbations risk (Count part of the hurdle model)	
			OR	*P* value	RR	*P* value
rs17637472	*GNGT2*					
GA		47.6	0.68	0.163	1.04	0.174
AA		16.1	0.74	0.415	1.03	0.428
rs7705042	*NDFIP1*					
AC		46.1	1.18	0.508	0.98	0.628
CC		13.2	1.43	0.354	0.96	0.429
rs167769	*STAT6*					
CT		47.6	1.25	0.396	1.05	0.071
TT		15.1	1.06	0.851	1.10	0.023
rs37973	*GLCCI1*					
GA		49.2	1.44	0.176	**0.92**	<0.005
GG		18.9	1.25	0.528	**0.82**	<0.005
rs242941	*CRHR1*					
CA		42.6	1.27	0.327	0.99	0.924
AA		9.9	3.07	0.074	**1.16**	0.004
rs1134481	*TBXT*					
GT		48.3	0.81	0.416	**0.92**	<0.005
TT		14.3	1.13	0.764	1.02	0.563
rs2305089	*TBXT*					
TC		50.8	0.71	0.241	1.01	0.782
CC		26.3	0.80	0.533	0.95	0.171
rs3099266	*TBXT*					
GT		48.8	0.92	0.764	0.97	0.383
TT		16.0	0.95	0.899	0.99	0.975

Legend: Odds ratios, rate ratios and their corresponding *P* values of the association between the SNPs and increased or decreased risks in both zero and count models, adjusted for age, sex, smoking, BMI and GINA treatment step in the replication cohort. Underlined associations are nominally significant, bolded associations have passed the Bonferroni threshold.

Abbreviations: RR, rate ratio, OR, odds ratio ICS, inhaled corticosteroid, SNP, single nucleotide polymorphism.

had the TCT haplotype. Subjects homozygous for the haplotype (TCT) using ICS were at a decreased risk of recurrent exacerbations compared with the wild haplotype (RR: 0.41; $P < 0.005$) (Table S3). However, this association was not replicated in the replication cohort. To better reflect the same exacerbation definition in the replication as the discovery cohort, we have performed a sensitivity analysis excluding exacerbators based on ED visits and hospitalization only (n = 1600) and observed similar effect estimates for the three replicated results.

Regarding the SNP main effects (Tables S4 and S5), homozygotes of the minor allele of rs7216389 and rs37973 had a significantly increased frequent exacerbation risk in both discovery and replication cohort. rs242941 heterozygotes had a significantly decreased risk of frequent exacerbations in the discovery cohort (Table S4), yet a significantly increased risk in the replication cohort (Table S5). Homozygotes of the minor allele of rs1134481, rs2305089 and rs3099266 within the *TBXT* gene demonstrated a significantly increased frequent exacerbation risk in the discovery cohort (Table S4).

4 | DISCUSSION

In this largest pharmacogenetic candidate gene study on exacerbations in adults with asthma to date, eight of 10 investigated polymorphisms showed significant interactions with ICS on exacerbation risk

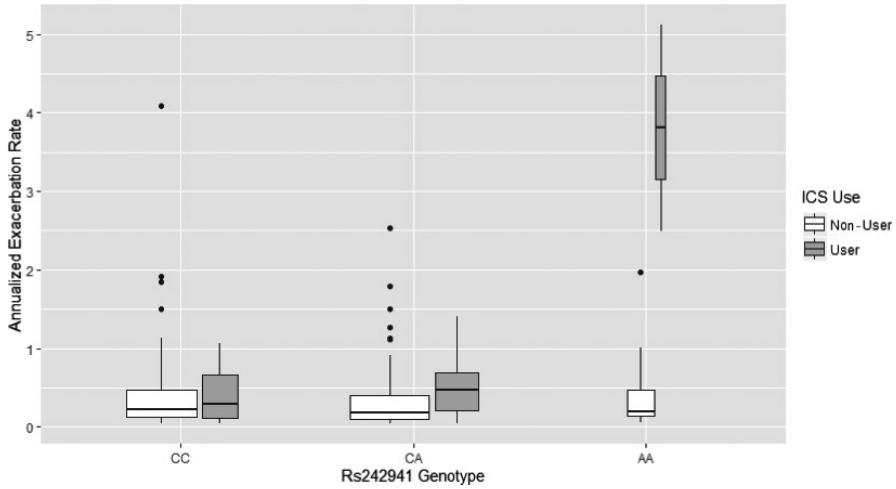

FIGURE 2 Exacerbation rate per rs242941 genotype (Discovery cohort). Legend: Boxplot illustrating exacerbation rates per rs242941 genotype (CC: 135 patients, CA: 177 patients, AA:17 patients) in subjects with at least one exacerbation, stratified by ICS use. The boxplots width represents the proportion of the genotype among the subjects in their respective strata. The minor allele (A) homozygotes (17 patients, 2 ICS users) show a significantly increased exacerbation rate, despite their small proportion

in adults with asthma. In an independent replication cohort, three of these eight SNP by ICS interactions passed the Bonferroni threshold with the same direction of effect for two polymorphisms: rs242941 (*CRHR1*) homozygotes for the minor allele were at increased risk for frequent exacerbations upon ICS treatment, while rs1134481 (*TBXT* gene) minor allele carriers showed better response with ICS in both discovery and replication cohort. Interestingly, rs37973 *(GLCCI1)* significantly increased exacerbation risk by ICS use in the Dutch discovery cohort, but had an opposite direction of effect in the American replication cohort.

Homozygotes of the rs1134481—located within the T-box transcription factor T (*TBXT*) gene—minor allele showed better response with ICS in both cohorts. rs1134481 homozygous mutants have previously been shown to have a favourable response to ICS.[39] The *T gene* (also called *T Brachyury*) belongs to a family of genes called the T-box family, a highly conserved family of transcription factors with widespread roles in embryonic and stem cell development.[55] Mutations may result in developmental syndromes in humans and in other vertebrates, such as mice.[56] *T Brachyury* encodes for a protein that binds to a palindromic site (called T-site), upregulating genes required for mesoderm formation and differentiation.[55-57] *T Brachyury* is expressed in lung tissue[58] as well as other members of the T-box family, including *TBX4*, *TBX5* and *TBX21*, a family member linked to childhood asthma,[59] aspirin-induced asthma [60] and improvement in FEV1 during an asthma exacerbation in children receiving high-dose ICS.[40] The effects of this gene on exacerbation risk in adult ICS users may represent a highly corticosteroid responsive asthma phenotype, as its SNPs' main effects showed increased frequent exacerbation risk, which seems to be effectively reduced with ICS use. This phenotype might already benefit from interactions with endogenous glucocorticoids during fetal and neonatal lung development. Endogenous glucocorticoids were found to be crucial to early lung development through their effects on lung cell maturation, differentiation and the production of surfactant-related proteins.[61] It has also been suggested that decreased *T gene* expression may inhibit chondrogenesis, through mediator proteins involved in corticosteroid resistance.[31-63] In the Dutch population, ICS was more effective in the count model for minor allele homozygotes of the haplotype consisting of rs1134481, rs2305089 and rs3099266, further implicating a potential role for this genetic locus in ICS response on exacerbations in adults with asthma.

Corticotrophin-releasing hormone receptor 1 *(CRHR1)* rs242941 mutant carriers demonstrated the strongest association with a more than sixfold increased exacerbation risk upon ICS use (RR: 6.11). Although the effect was less pronounced but still Bonferroni significant in the replication analysis, it is important to note that its effect was already threefold increased in the replication zero model (OR: 3.07). Therefore, though the portions of the model may be different, the risk allele may be globally associated with a substantial increase in exacerbation risk while on ICS. rs242941 is an intronic variant previously linked to improved lung function response to ICS over 8 weeks in asthma,[64] although it was not statistically significant in a large GWAS studying ICS response in patients with asthma.[35] Moreover, rs242941 was also associated with good initial (sustained for 60 minutes) response to inhaled corticosteroids in asthmatic Indian children experiencing an acute exacerbation.[65] Independent of treatment, rs242941 heterozygotes demonstrated a significantly decreased risk of frequent exacerbations in the Dutch discovery cohort but a significantly increased risk in the American replication cohort. Since the SNP is linked to corticotrophin-releasing hormone, the long-term effects by interfering these inflammatory pathways within asthma's pathophysiology by anti-inflammatory treatment warrant further investigation.[66] *CRHR1* is highly expressed in brain tissues (hippocampus, cortex and cerebellum).[67,68] It encodes a G protein–coupled receptor, essential for activating signal transduction pathways affecting the hypothalamic-pituitary-adrenal pathway and the normal hormonal responses to stress and anxiogenic stimuli.[69] Besides regulation of stress and immune responses, involvement into obesity and cAMP-dependent protein kinase and glucocorticoid pathways has also been described.[70-72] Interestingly, variation in *CRHR1* has also been linked to the incidence of depression,[68-74] an important asthma comorbidity [75] associated with increased risk of hospitalization in patients with asthma.[76] Additionally, *CRHR1* variants have been associated with variability in antidepressant response.[77,78]

Though the direction of our long-term effects for this SNP in adults and elderly with asthma differs from previously published studies

on acute effects in mainly asthmatic children,[64,65] it is worth noting that our results are in line with effects observed in COPD patients.[79] Therefore, this contrast may be either due to difference in outcome defined as long-term recurrent exacerbations versus short-term lung function response or due to different age groups. It may be suggested that subjects carrying the variant show a good initial pulmonary response to ICS, but fail to improve in long-term outcomes. It may also be suggested that the subset of frequent exacerbators carrying these genotypes represent a subgroup of patients with severe irresponsive asthma, who may benefit from early treatment with additional agents or only respond to higher corticosteroid doses. Alternatively, since the previous studies were mainly conducted on children cohorts, environmental exposures resulting in severe asthma and frequent exacerbations may be affecting the genotype-phenotype associations over the course of life.[80] Finally, the number of patients with minor allele homozygotes (AA) who were classified as ICS users in the discovery cohort was small. Nevertheless, the variant may still be relevant for 5%-10% of patients with asthma, or for populations with an even higher frequency of the risk allele. Interestingly, the A allele has an even higher frequency in African-ancestry populations (64%),[81] and further studies may shed light on the variant role in African-ancestry patients with asthma.

Some associations initially detected in the Dutch cohort were not replicated in the independent American cohort. rs37973 within the Glucocorticoid-Induced Transcript 1 (*GLCCI1*) showed a significantly poor ICS response in line with previous observations of decreased response to ICS in *GLCCI1* polymorphic asthmatic patients in the Dutch cohort, but had a significantly yet opposite direction of effect in the American cohort.[29] Interestingly, the SNP main effect showed a significantly increased frequent exacerbation risk in both the discovery and replication cohort. The gene expression of *GLCC1* is known to be induced by glucocorticoids, and it has been described to be an essential mediator of glucocorticoid-induced T-cell apoptosis.[82] Non-replicated results could still be in part due to different patient recruitment and follow-up strategies. Since the follow-up in the replication cohort started from their first registered ICS prescription (instead of study start or asthma incident date in the discovery cohort), it is likely that for some, the zero model already describes the SNP by adherent ICS effect on subsequent exacerbations if an exacerbation already occurred before the first registered ICS prescription. Moreover, exacerbations in the GERA cohort included both hospitalizations and moderate-to-severe exacerbations, while exacerbations in the Dutch cohort only included moderate-to-severe (OCS bursts) exacerbations. In addition, our analysis only included patients of non-Hispanic European White ancestry, and therefore, the results may differ among other ethnicities.[83-85] Moreover, our models did not include other potential factors that could have affected response to inhaled corticosteroids, including atopy status and environmental effects, and adding these could have increased our explained proportion of the variance in treatment response further, although differences in environment during the study period are minimized in our discovery cohort since all participants were recruited from the same district in the city of Rotterdam. Additionally, while our calculations assumed an additive model, it is possible that some of our loci follow different models (eg recessive or dominant).[86] Finally, it should be noted that while the use of pharmacy prescription records provides accurate information on the drugs prescribed by physicians and dispensed by pharmacies, this may not fully reflect patients' adherence to their therapeutic regimen.

Our study uniquely focuses on the long-term risk of exacerbations in real-life adults and elderly with asthma, and takes for the first time the important impact of ICS adherence into account when investigating the attributable role of pharmacogenetics into variable treatment response.[87] Although this reduced number of true ICS users may have limited our power to detect or replicate some interaction effects, we are convinced that the gained exposure specificity is a strength and important in estimating true effect sizes of variants directly affecting medication response. We used special types of count models—hurdle models—to be able to assess the real-life variability in asthma severity over a longer time period, adding to potential clinical implications, as patients who experience one exacerbation are at a high risk of a second exacerbation.[88] Our analysis divides risk into two different questions: first, factors affecting initial risk of having an exacerbation (primary prevention in the general population); and second, factors affecting subsequent exacerbation risk, meaning how often will a participant have exacerbations once they already have had one (secondary prevention in a clinical asthma population). We believe this to be an important strength of our study, as exacerbations are a major burden for patients and health systems, but their primary prevention may differ from secondary prevention.[89] Previous studies have investigated the associations between rs37973, rs242941 or *TBXT* gene locus variants and ICS response using FEV1 as marker of short-term response to therapy.[90] However, a large GWAS could not confirm any of the previously reported associations.[35] Although long-term outcomes may be modelled through lung function response as well,[91] establishment of genetic predictors for exacerbations, an important long-term clinical outcome of asthma, has a significant potential for classification and prediction of asthmatic response to maintenance therapy. Medication effects have also been shown to vary between long- and short-term outcomes. For example, long-acting beta-agonists improve lung function, but their single use leads to increased risk of exacerbations and mortality.[92,93] It may also be suggested that the SNPs previously associated with asthma exacerbations in children (rs7216389 and rs28364072) were differently associated with the outcome in our study due to age differences, highlighting the heterogeneity in the disease mechanism between adult and childhood asthma, possible gene-environment interactions and therefore the limitations of extrapolating genetic results between children and adults in asthma.

5 | CONCLUSION

Genetic variants may affect the efficacy of ICS in reducing the risk of exacerbations in adult asthma. rs242941 (a *CRHR1* variant) was associated with reduced ICS effects on exacerbations, while variants in *TBXT* were associated with increased response. *GLCCI1* minor

allele, previously linked to FEV_1 change in adults with asthma using ICS, leads to an increased risk of recurrent exacerbations only in the discovery cohort. Further research on these genetic loci, and their potential effects in adults with asthma, may help clarify their predictive role in ICS response and the development of asthma phenotypes and endotypes.

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CONFLICT OF INTEREST

The authors have no conflict of interests to disclose. Outside of the scope of this study, KGT has received funding for work in asthma pharmacogenomics from the US National Institutes of Health, but the NIH does not stand to gain or lose financially from the results or conclusions of this article. Katia Verhamme works for a research department that receives/received unconditional research grants from Yamanouchi, Pfizer/Boehringer Ingelheim, Novartis, GSK, UCB, Amgen and Chiesi, none of which are related to the content of this work. G. Brusselle has, within the last 5 years, received honoraria for lectures from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Novartis and Teva; he is a member of advisory boards for Amgen, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Novartis, Sanofi/Regeneron and Teva. LL reports Society awards sponsored by AstraZeneca and Chiesi and expert consultation for Boehringer Ingelheim GmbH and Novartis, outside the submitted work.

AUTHOR CONTRIBUTIONS

AE and LL conceived of the presented idea, designed the study and performed the analyses. EDR, MGM, BHC, AWC and KGT contributed to data collection and validation. AE and LL took the lead in writing the manuscript. All authors critically revised the final manuscript.

DATA AVAILABILITY STATEMENT

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data are not available. The analysis code is available upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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