Original research

Genetic and T2 biomarkers linked to the efficacy of HDM sublingual immunotherapy in asthma

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ABSTRACT

Background Hypersensitivity to house dust mite (HDM) allergens is a common cause of allergic asthma symptoms and can be effectively treated with allergy immunotherapy (AIT).

Objective To investigate whether genetic and type 2 (T2) inflammatory biomarkers correlate with disease severity in subjects with allergic asthma, and whether this can be modified by AIT.

Methods MITRA (NCT01433523) was a phase III, randomised, double-blind, placebo-controlled trial of HDM sublingual immunotherapy (SLIT)-tablets in adults with HDM allergic asthma. Post hoc analyses of the study population (N=742) evaluated associations between T2 inflammatory (blood eosinophils, eosinophil cationic protein (ECP), total IgE and tryptase) and genetic (single-nucleotide polymorphisms, SNP) biomarkers (n=582) for the primary study endpoint (time to first moderate/severe asthma exacerbation). SNP associations were verified in HDM-positive subgroup from an independent 3-year Severe Asthma Research Programme (SARP3) subject cohort.

Results An increased asthma exacerbation risk in subjects homozygous for SNP rs7216389 (chromosomal locus 17q12-21) was reduced (p=0.037) by treatment with HDM SLIT (HR=0.37 (95% CI 0.22 to 0.64), p<0.001). The associations between exacerbation risk and 17q12-21 SNPs were replicated in the SARP3 HDM-positive subgroup. High levels of T2 biomarkers were associated with increased risk of asthma exacerbations in the placebo group. HDM SLIT-tablet treatment reduced this risk (blood eosinophils: HR=0.50 (95% CI 0.30 to 0.85); ECP: HR=0.45 (95% CI 0.29 to 0.87); tryptase: HR=0.45 (95% CI 0.25 to 0.80)). The treatment effect was higher (p=0.006) for subjects with a higher number of elevated T2 biomarkers.

Conclusions HDM SLIT-tablet AIT is efficacious in HDM-sensitised asthma subjects with a genetic asthma predisposition and/or an underlying T2 endotype.

Trial registration number NCT01433523.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Genetic and type 2 (T2) inflammatory biomarkers correlate with disease severity in subjects with allergic asthma. How these biomarkers influence the effect of allergy immunotherapy is unknown.

WHAT THIS STUDY ADDS

⇒ Treatment with sublingual tablet house dust mite (HDM) immunotherapy reduced the risk of asthma exacerbations in subjects homozygous for 17q12-21 single-nucleotide polymorphisms associated with asthma or in subjects with high levels of T2 biomarkers.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Sublingual allergy immunotherapy efficaciously reduced the risk of asthma exacerbation in HDM-allergic asthma patients with a genetic asthma predisposition and/or an underlying T2 endotype in accordance with current international recommendations for asthma management.

INTRODUCTION

Asthma is a global health concern, and its prevalence continues to increase.[1] In the majority of patients with asthma, there is an associated underlying allergic disposition, and sensitisation to house dust mite (HDM) allergens is common.[2 3]

Genome-wide association studies have identified single-nucleotide polymorphisms (SNPs) linked with asthma (online supplemental table I). SNPs at chromosomal locus 17q12-21 have been associated with the risk of developing asthma and exacerbation risk in children,[4] both of which may be modified by environmental factors. For example, exposure to animal sheds or household pets may protect infants carrying the 17q21 risk allele against the development of wheeze and asthma.[5 6]

The availability of biological therapies targeting specific pathways has led to an emphasis in current asthma management guidelines on phenotyping patients with severe asthma, in order to better define their underlying immune status.[1] The most prevalent asthma phenotype in both adults and children, especially in patients with underlying allergy-triggered asthma is type 2 (T2) inflammation, mediated by T-helper cells.[7 8] Biomarkers associated with the T2 response inflammation are serum IgE, fractional exhaled nitric oxide (FeNO), and blood and sputum eosinophil counts.[7 9 10] High levels of these biomarkers predict favourable clinical responses to biologics targeting IgE and interleukin pathways.[11 12] Other allergic response biomarkers (eg, serum tryptase) have also been associated with allergic asthma.[13]

Allergy immunotherapy (AIT) modulates immune mechanisms involved in allergic respiratory disease by immune modulation. Administration of tolerable doses of specific antigens suppresses T2 immune responses[14] and stimulates the production of allergen-neutralising IgG and IgG4 antibodies that inhibit IgE-dependent effector cell activation, promotes the induction of antigen-specific regulatory T cells and drives immune deviation in favour of type 1 immune responses.[14 15]

A sublingual immunotherapy (SLIT)-tablet developed for the treatment of HDM-induced respiratory allergic disease has proven efficacious in reducing symptoms and medication use in both allergic rhinitis and allergic asthma.[16 17] The MITRA trial demonstrated that daily administration of HDM SLIT-tablets significantly reduced the relative risk of moderate or severe asthma exacerbations by 34% compared with placebo, and induced a significant increase in HDM allergen-specific IgG4.[18] The Global Initiative for Asthma (GINA) recommends HDM SLIT-tablets as an add-on treatment option in patients sensitive to HDM and with suboptimally controlled asthma.[1] However, there is relatively little data on the predictive role of biomarkers in relation to AIT. In a recent study of HDM SLIT-tablet treatment in asthma, higher levels of periostin and FeNO predicted improvements in lung function,[19] and a recent study demonstrated the ability of SLIT-tablets to restore the level of regulatory innate lymphoid cells.[20] In the present post hoc analysis, we investigated the association between high-risk subject profiles, defined by the presence of genetic asthma risk loci and biomarkers of the T2 immune response, and the efficacy of HDM SLIT-tablet treatment in subjects enrolled in the MITRA study.

METHODS

MITRA trial

MITRA (NCT01433523) was a phase III, randomised, double-blind, placebo-controlled study conducted in Europe in 834 adult subjects with HDM allergic asthma, comparing two doses of HDM SLIT-tablets (6 SQ-HDM (unit for standardisation on biological potency, major allergen content and complexity of the allergen extract) and 12 SQ-HDM) versus placebo.[18]

HDM SLIT-tablets or placebo were administered once daily to eligible subjects for 7–12 months (dependent on the date of a subject's inclusion relative to the fixed stop date of the initial phase of the trial). This was followed by a further 6-month period on HDM SLIT-tablet or placebo, with predefined stepwise reductions in daily inhaled corticosteroid (ICS) dose by 50% on each occasion for 3 months, followed by complete withdrawal in subjects who did not experience an asthma exacerbation. Efficacy was assessed during the ICS reduction and withdrawal periods in the final 6 months of treatment with the primary outcome of time to first moderate or severe asthma exacerbation. The criteria for moderate asthma exacerbation included ≥2 consecutive nights of awakenings due to asthma requiring short-acting β_2-receptor agonist (SABA) use, two consecutive days of increased SABA use from baseline or a hospital or trial site visit for asthma treatment not requiring systemic corticosteroids. Severe exacerbations were defined as ≥3 days of systemic corticosteroid treatment or hospitalisation for >12 hours for asthma.[18]

MITRA trial subjects

Subjects were eligible for the MITRA trial if they were sensitised to HDMs (confirmed by positive HDM allergen-specific IgE (≥0.7 kU/L) and positive skin prick test (>3 mm) to *Dermatophagoides pteronyssinus* (*Der p*) and/or *Dermatophagoides farinae* allergens), had a clinical history of >1 year of allergic asthma not well controlled on ICS, and had concomitant allergic rhinitis, with HDMs considered the major trigger for both. At randomisation, subjects' forced expiratory volume in the first second (FEV_1) was required to be ≥70% of the predicted value.[18]

The post hoc analyses were performed on subjects with biomarker measurements from the full analysis set. Out of 742 subjects who entered the ICS reduction period, the n per serum biomarker were as follows: eosinophils=731, eosinophil cationic protein (ECP)=702, Tryptase=702 and Total IgE=701. As no significant difference between the efficacy of the two HDM SLIT-tablet treatment doses was reported (online supplemental figure 1),[18] analyses pooled data from these groups to increase statistical power, unless otherwise specified.

Genetic biomarkers

DNA samples for 582 subjects from the MITRA cohort were genotyped with the Infinium Global Screening Array (GSA)-24 V1.0 (Illumina, San Diego, California, USA). Standard quality control was performed using PLINK V.1.9,[21] excluding SNPs if minor allele frequency was <0.01 or Hardy-Weinberg equilibrium test p value was <0.001 for all samples with a SNP call rate ≥95%. After quality control, 22 SNPs known to be associated with asthma were included in the analysis. In order to study the 17q12-21 locus more extensively, we analysed all nine SNPs mentioned by Stein *et al*[4] encoded on the Infinium GSA. The remaining five 17q12-21 SNPs identified by Stein *et al*[4] were imputed using Beagle (V.4.1, 21 January 2017)[22 23] and the 1000 Genomes Project phase 3 reference panel.[24] Altogether, 36 SNPs were analysed (online supplemental table I).

Genetic biomarker verification in the 3-year Severe Asthma Research Programme cohort

The association between the 17q12-21 SNPs encoded on the Infinium GSA and asthma exacerbations was verified in an HDM-positive subgroup (HDM-specific IgE≥0.7 kU/L) of an independently collected prospective cohort of subjects diagnosed with severe asthma from the multisite 3-year Severe Asthma Research Programme (SARP3) study. SARP is a programme funded by the National Heart, Lung and Blood Institute where patients with mild to severe asthma and a subset of controls are being extensively studied.[25 26] Asthma was confirmed based on subjects' responsiveness to β-agonist treatment, and severe asthma was defined as treatment with high-dose ICS for at least six of the previous 12 months.[26] Standard genotyping and quality-control processes of SNPs in SARP3 were performed as described previously.[25]

T2 biomarkers

The relationship between treatment outcomes and levels of T2 inflammatory biomarkers (blood eosinophil counts, serum ECP, tryptase, total IgE, and HDM allergen-specific IgE and IgG4) measured at baseline and prior to the ICS reduction phase was evaluated. Serum levels of HDM (*Der p*) extract-specific IgE and IgG4, and blood eosinophil counts (recorded in multiples of 100 cells/µL, from 0 to 1400 cells/µL) were measured at intervals during the trial as part of the safety monitoring protocol. ECP, tryptase and total IgE in serum samples were quantified post hoc using ImmunoCAP (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's protocols.

For each T2 inflammatory biomarker, subjects were divided into high-level subjects (>80th centile) and normal-level subjects

(≤80th centile). The 80th centile was selected as it approximated a recorded blood eosinophil count ≥400 cells/µL (including all subjects with measured eosinophil count ≥350 cells/µL). Although a threshold of 300 cells/µL has previously been used in a number of studies investigating biological treatment of asthma,[27–30] the low resolution of eosinophil count data recorded meant that a cut-off of ≥400 cells/µL was required to ensure all selected subjects had an eosinophil count over 300 cells/µL. Based on the chosen threshold, 171 (23%) of the subjects were categorised as 'eosinophil high'.

Statistical analysis

The association between individual SNPs and the MITRA primary endpoint, in alignment with the predefined primary endpoint for that study, was tested using a Cox proportional hazards (CoxPH) model stratified for country. Pairwise linkage disequilibrium (LD) calculations for 17q12-21 SNPs were conducted using PLINK v1.9.[21]

Within the confirmatory SARP3 HDM-positive cohort, the association between nine 17q12-21 SNPs and the annual exacerbation rate (mean over three consecutive years) was tested using a negative binomial model adjusted for age, sex and the first five principal components.

The relationship between exacerbation risk and baseline biomarker values was modelled using unadjusted logistic regression and displayed by centile bar plots according to the tail-oriented subpopulation treatment effect pattern plot method.[31] The effect of each biomarker on the primary endpoint was tested by an interaction term in CoxPH model stratified for country as per MITRA trial protocol but modified by adding a term for interaction with the biomarker of interest.[18] The association between the number of 'high' T2 markers per subject and asthma exacerbation risk was tested using unadjusted logistic regression.

All statistical analyses and visualisations were performed using R V.3.6.1.

RESULTS

Subject demographics

At baseline, subjects who went on to enter the ICS reduction period of the MITRA study (n=742) were 34±12 (mean±SD) years old, with a body mass index (BMI, kg/m^2) of 26±5, 98.4% of subjects were Caucasian and 52% were male (online supplemental table II).[18] At randomisation, subjects' FEV_1 was 92%±13% of predicted value, and daily ICS use was 589±249 µg of budesonide.

Among subjects in the confirmatory HDM-sensitised SARP3 cohort (N=160 non-Hispanic white participants), 41% were male, with a mean age of 40±18 years, BMI of 29±7 and FEV_1 of 78%±21% of predicted value at baseline.

Genetic biomarkers

Overall, 582 of the subjects enrolled in the MITRA study were genotyped for a set of 22 SNPs known to be associated with asthma. Three subjects were excluded from the dataset owing to missing data for >5% of the variants encoded on the GSA chip. The SNP rs7216389 at locus 17q12-21 was the only SNP tested that showed a significant interaction with treatment for the primary endpoint (table 1). In individuals with the asthma risk-associated T:T genotype of rs7216389, there was a significant response to treatment (HR 0.37 (95% CI 0.22 to 0.64), p<0.001; $P_{interaction}$=0.037). The risk and number of exacerbations in the placebo group were higher in the subgroup homozygous for the risk allele ('HMZ-risk') compared with subgroups with other allele profiles. The risk of exacerbations associated with rs7216389 in the 'HMZ-risk' subgroup was abrogated by HDM SLIT-tablet treatment (figure 1A,C). The treatment effect in the 'HMZ-risk' subgroup was separately replicated for both doses 6 SQ-HDM (HR 0.34 (95% CI 0.17 to 0.70), p=0.003) and 12 SQ-HDM (HR 0.4 (95% CI 0.21 to 0.76), p=0.005).

LD analysis of nine of the SNPs located in the 17q12-21 locus resulted in the pattern expected for a population of largely European descent: the SNPs upstream of and including rs7216389 showed a high pairwise LD (figure 1B) with a break of linkage downstream of rs7216389. This observed break of linkage was confirmed by the absence of an association between the two SNPs located most downstream, rs3894194 and rs3859192, and a genotype effect on exacerbation risk (figure 1C). Analysis results for imputed genotypes of other functionally implicated 17q12-21 SNPs[4] not encoded on the GSA chip were in agreement with our observations for rs7216389 and genetically linked variants. (online supplemental figure 2).

The association between asthma exacerbation risk and the 17q12-21 SNPs observed in the MITRA study cohort was replicated in the independent SARP3 HDM-positive cohort. Consistent with the MITRA placebo group, five SNPs upstream of the linkage break (and starting with rs8069176) showed an association (nominal p<0.5) with annual asthma exacerbation rate, whereas the two SNPs downstream showed no association (figure 1D).

Serum biomarkers

In the placebo-treated subject group, higher levels of eosinophils, ECP, tryptase and total IgE were associated with a greater risk of asthma exacerbations (figure 2A, online supplemental figure 3). The time to first asthma exacerbation, the primary endpoint of the MITRA study, was shorter in the biomarker 'high' placebo subgroup compared with the biomarker 'normal' active and placebo subgroups, and biomarker 'high' active subgroup (figure 2B). The differences between treatment groups were greatest among subjects in the biomarker 'high' (80th centile) subgroup for each of the biomarkers except for total IgE (online supplemental figure 3). The treatment effect was consistently higher in the biomarker 'high' subgroup than in the biomarker 'normal' subgroup (figure 2C). Compared with those with 'normal' levels of T2 inflammatory biomarkers, the treatment effect was significantly greater in subgroups with high levels of blood eosinophils (n=171), serum ECP (n=141) and serum tryptase 'high' (n=141) subgroups (HR, 95% CI: 0.50 (0.30 to 0.85), 0.45 (0.29 to 0.87) and 0.45 (0.25 to 0.80), respectively) and these differences were statistically significant in the biomarker 'high' groups for blood eosinophil, serum ECP and serum tryptase (figure 2C). No association was observed between time to first asthma exacerbation and biomarker level subgroups for total serum IgE, HDM-specific IgE or IgG4 (figure 2C, online supplemental figure 4).

The total number of 'high' category (values >80th centile) T2 markers for each subject was strongly associated with exacerbation risk in the placebo group (p<0.001), which was abrogated by treatment (figure 3). The interaction value ($P_{interaction}$=0.006) indicates that the treatment effect was significantly higher for subjects with a greater number of elevated T2 markers.

No changes from baseline were observed in biomarker serum levels for ECP and tryptase prior to the ICS reduction phase. A small increase from baseline was observed for total IgE in both HDM SLIT-tablet treatment arms, which was significant compared with placebo (p<0.001) (online supplemental figure

Table 1 Analysed asthma-associated SNPs

SNP	Position	Nearest ENCODE gene	Effect allele*	Minor allele	Minor allele frequency	Interaction with treatment (p value)
rs11071559	chr15:60777789	RORA	C	T	0.142	0.690
rs1233578	chr6:28744470	GPX5, TRIM27	G	G	0.131	0.355
rs1295686	chr5:132660151	IL13	T	C	0.241	0.155
rs1342326	chr9:6190076	IL33	C	C	0.174	0.987
rs1438673	chr5:111131801	WDR36	C	C	0.488	0.112
rs17294280	chr15:67175947	SMAD3	G	G	0.314	0.675
rs17637472	chr17:49384071	ZNF652, PHB	A	A	0.433	0.940
rs1837253	chr5:111066174	TSLP	C	T	0.253	0.468
rs2073643	chr5:132387596	SLC22A5	T	T	0.463	0.175
rs2284033	chr22:37137994	IL2RB	G	A	0.435	0.715
rs2305480	chr17:39909987	GSDMB	G	A	0.399	0.173
rs3771166	chr2:102369762	IL18R1	G	A	0.333	0.284
rs3894194	chr17:39965740	GSDMA	A	A	0.490	0.949
rs4833095	chr4:38798089	TLR1	T	C	0.205	0.137
rs62026376	chr16:11134855	CLEC16A	C	T	0.232	0.095
rs6967330	chr7:106018005	CDHR3	A	A	0.169	0.704
rs7009110	chr8:80379644	ZBTB10	T	T	0.379	0.891
rs7212938	chr17:39966427	GSDMA	G	T	0.492	0.977
rs7216389	**chr17**:39913696	**GSDMB**	**T**	**C**	**0.454**	**0.037**
rs72699186	chr9:6175855	IL33	T	T	0.175	0.976
rs9273349	chr6:32658092	HLA-DQ	C	T	0.371	0.832
rs928413	chr9:6213387	IL33	G	G	0.272	0.738

Asthma-associated allele, minor allele frequency and interaction with treatment (time to first asthma exacerbation) of all tested SNPs in subjects with moderate or severe asthma treated with HDM SLIT-tablets for 7–12 months. P values <0.05 are marked in bold.
*Further details on the association of these SNPs and their alleles with asthma are provided in online supplemental table I.
HDM, house dust mite; SLIT, sublingual immunotherapy; SNP, single-nucleotide polymorphism.

5). Pairwise analysis revealed weak correlations between different biomarker levels at baseline (online supplemental figure 6); as expected, the strongest correlation (r=0.34) was between blood eosinophil counts and serum levels of ECP.

Measurement of HDM-specific IgE and IgG4 levels over time showed no correlation between rs7216389 genotypes and immunological response to treatment (figure 4). The expected steep increase in serum HDM-specific IgE, followed by a gradual decline over time, and an associated gradual increase in HDM-specific IgG4 was seen in response to both doses of HDM SLIT-tablet for all three genotypes of rs7216389. No difference in levels of T2 biomarkers was observed between the three genotypes of rs7216389 (online supplemental figure 7).

DISCUSSION

The MITRA study demonstrated the efficacy of HDM SLIT-tablet treatment in adults with HDM allergic asthma (GINA steps 3–4) not well controlled by ICS.[18] The results of this post hoc analysis provide important novel insights into associations between asthma risk genotypes, T2 biomarkers and clinical response to HDM SLIT-tablet treatment.

Genetic biomarker investigation revealed that among SNPs known to be associated with asthma, only those located in the 17q12-21 locus correlated with an increased exacerbation risk and this risk was modified by HDM SLIT-tablet treatment. Although originally identified as a risk locus for childhood asthma,[32 33] a recent study has confirmed that SNPs in the 17q12-21 locus are also associated with asthma severity and asthma exacerbations in adults with asthma.[25]

Although originally identified as a risk locus for childhood asthma[33 34] a recent study found that SNPs in the 17q12-21 locus are also associated with asthma severity and asthma exacerbations in adults with asthma.[25] The association with asthma exacerbations was confirmed in the current study in the context of HDM allergy and the finding that HDM SLIT therapy can attenuate this genetic risk is interesting and clinically important in light of the large symptom burden associated with uncontrolled asthma. The molecular mechanisms associated with 17q12-21 risk variants are still poorly understood. The locus is characterised by extensive LD and spans several genes with a potential role in asthma pathology, including ORMDL3 and GSDMB, and might involve both respiratory epithelial cells and immune cells.[4] Genetic risk at the 17q12-12 has been associated with increased susceptibility to rhinovirus infections[33] and altered antiviral pathways in the airways.[25] Given that HDM sensitisation also impairs antiviral immunity,[35] it can be speculated that HDM SLIT-tablet treatment interacts with 17q12-21 genotype through mechanisms related to antiviral immunity as recently demonstrated.[36] Other putative mechanisms relate to ORMDL3 and include eosinophilic trafficking and sphingolipid metabolism.[37 38]

It should be noted that the majority of individuals in the current study were of European descent. The 17q12-21 locus

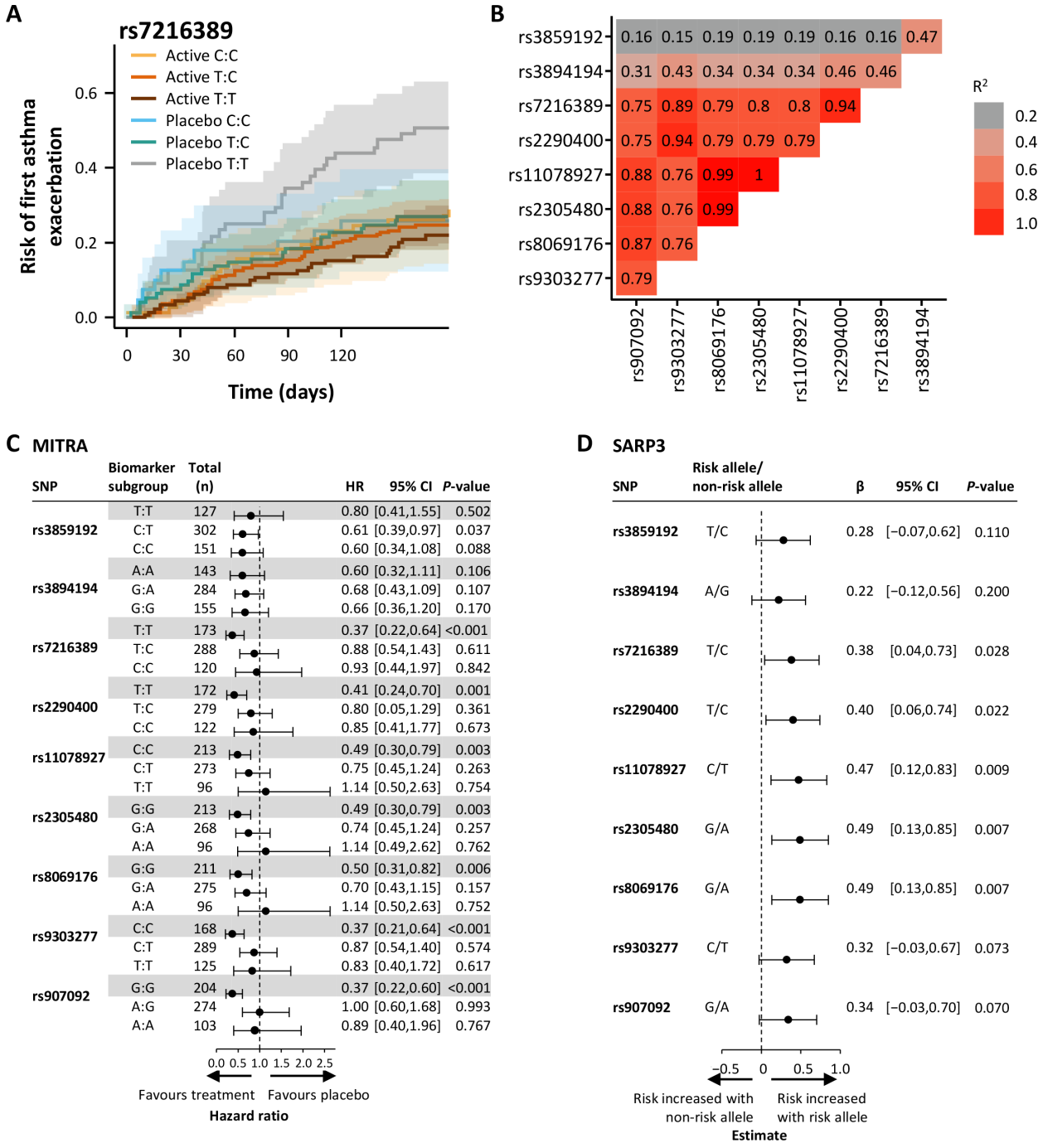

Figure 1 Association between SNPs in chromosomal region 17q12-21 and treatment effect. (A) Cumulative incidence plot for the time to first exacerbation for trial subjects in the active treatment group versus the placebo group in different rs7216389 genotype subsets (T:T, T:C, C:C). (B) Linkage disequilibrium (R^2) of nine SNPs in the chromosomal locus 17q12-21. (C) Impact on time to first exacerbation for nine SNPs in the chromosomal locus 17q12-21. Genotypes associated with asthma risk are highlighted in grey. (D) Association analysis (β) of non-risk allele versus risk allele on annual asthma exacerbation rate in house dust mite-positive SARP3 subjects using a negative binomial model adjusted for age, sex and the first five principal components. SARP3, Severe Asthma Research Programme; SNP, single-nucleotide polymorphism.

has been associated with asthma risk in populations with diverse ancestries but both allele frequencies and LD patterns vary across ancestries[39] and this should be taken into account in future studies involving other populations.

The T2 phenotype is linked to increased severity and exacerbation risk in asthma[9 10] and is also known to predict subject response to treatment with biological therapies that target IgE, IL-4, IL-13 and IL-5 or the IL-5 receptor.[7] The present post hoc analysis of the MITRA study indicates that elevated levels of the T2 biomarkers blood eosinophils, ECP and tryptase correlated with increased exacerbation risk in the placebo group. Additionally, higher values of any of these biomarkers at baseline correlated with a trend towards greater treatment response. The treatment effect was significantly higher for subjects with a greater number of elevated T2 markers ($p<0.01$) suggesting that HDM SLIT-tablet treatment is relevant even for allergic asthma subjects with relatively strong underlying T2 inflammation.

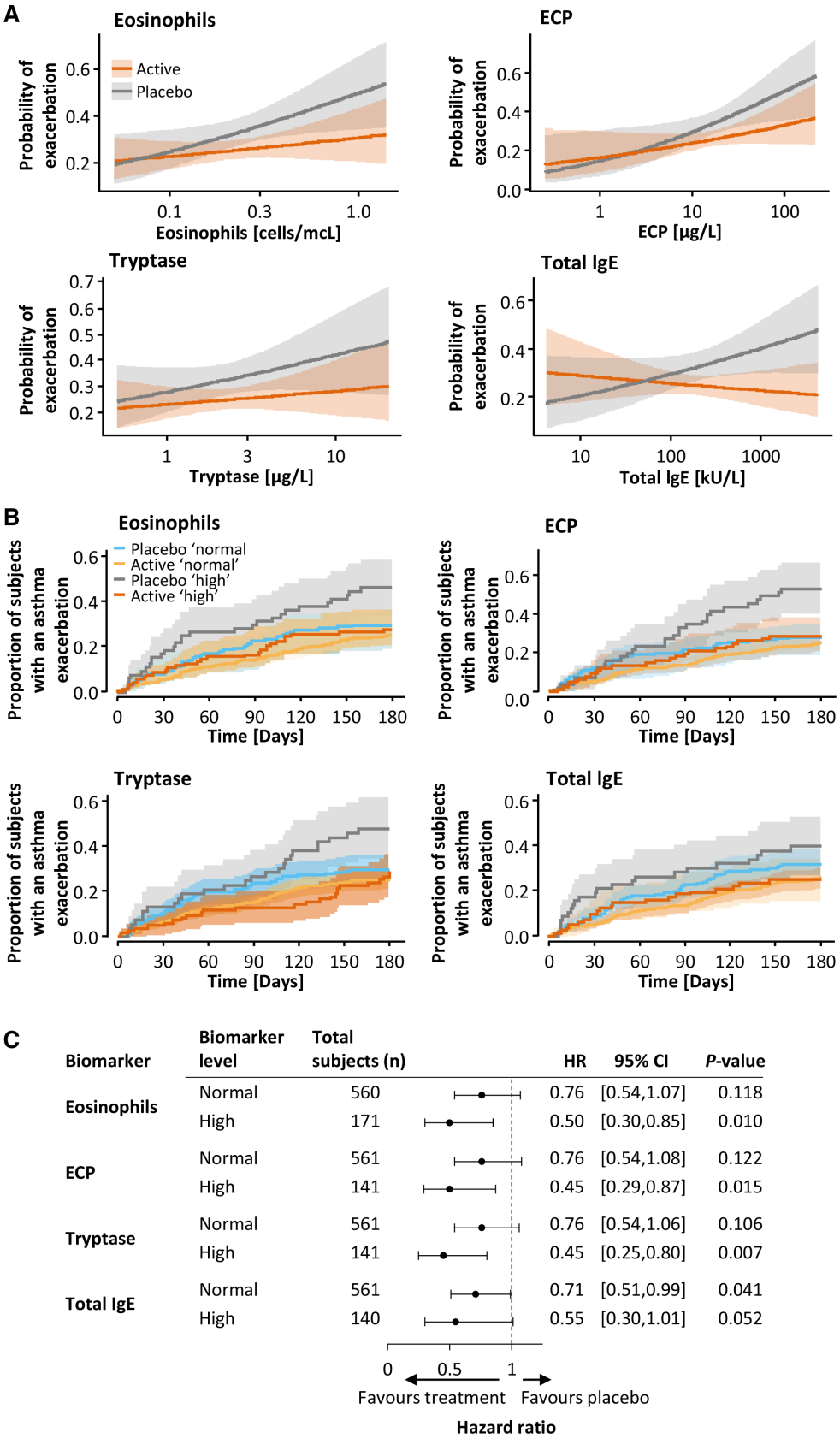

Figure 2 Association between T2 biomarkers and treatment effect. (A) Exacerbation risk by type 2 marker level. Lines show logistic regression per treatment group and shading shows the 95% CI. (B, C) Trial subjects were divided into a biomarker 'high' (>80th centile value) and a biomarker 'normal' (≤80th centile value) subgroup for each of four immunological biomarkers. (B) Time to first asthma exacerbation by risk group. Lines show probability of asthma exacerbation over treatment period and shading shows the 95% CI. (C) P values reflect the statistical significance of reported HRs for treatment groups (active/placebo) in the corresponding subgroup analyses. HR for time to first asthma exacerbation in the active group compared with the placebo group for 'high' and 'normal' biomarker subgroups. ECP, eosinophil cationic protein.

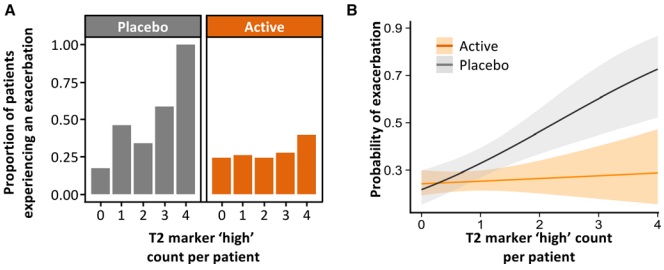

Figure 3 Exacerbation risk based on number of T2 markers (A) Proportion of subjects experiencing an asthma exacerbation where subjects are grouped based on the number of T2 markers for which they fall within the biomarker 'high' subgroup (>80th centile value). (B) Modelling of exacerbation risk by the sum of T2 markers per subject. Lines show logistic regression per treatment group and shading shows the 95% CI. T2, type 2.

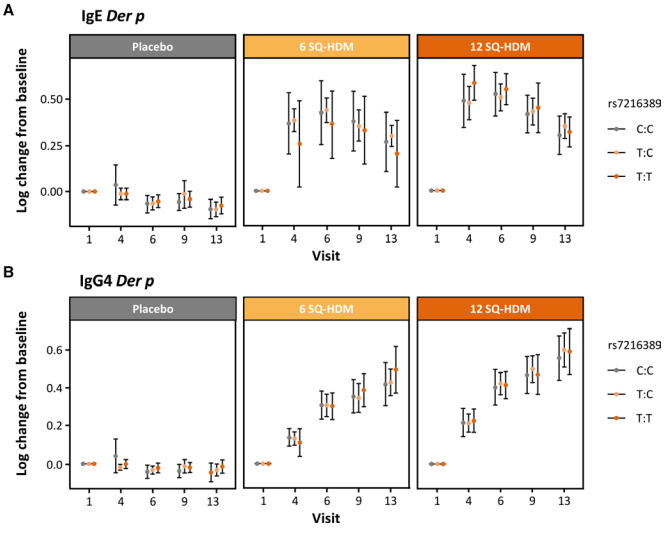

Figure 4 Absence of association between SNP genotype and immunological response to treatment over time in subjects with moderate or severe asthma treated with HDM SLIT-tablets for 7–12 months. (A, B) Change in *Der p*-specific (A) IgE and (B) IgG4 from baseline for different genotypes of rs7216389 and different treatment arms. Plots show the mean log change from baseline and the 95% CI per subgroup defined by genotype, treatment group and trial visit. Visit 1=baseline; visit 4=4 weeks; visit 6=20 weeks; visit 9=start of inhaled corticosteroid reduction; visit 13=end of trial visit. *Der p*, *Dermatophagoides pteronyssinus*; HDM, house dust mite; SLIT, sublingual immunotherapy; SNP, single-nucleotide polymorphism.

No association or interaction between the T2 biomarkers investigated and rs7216389 genotype was detected. Therefore, T2 endotype and 17q12-12 genotype, appear to independently increase the risk of asthma exacerbations in HDM-sensitised subjects. In view of this, the presence of risk alleles of the 17q12-21 locus may serve as an independent and additional biomarker in the analysis of exacerbation risk in asthma. Further, our data are in line with previously observed gene–environment interactions in cohort studies concluding that 17q12-21-associated genetic predisposition to asthma is modifiable by environmental factors such as allergen exposure, independent of inflammatory status.[5 6]

The strengths of this study include the ability to investigate multiple T2 inflammatory and genetic biomarkers, and independent cohort verification of genetic risk associations. Limitations of this study include that measurements of FeNO, a key biomarker of the T2 immune response,[7] were not included in the MITRA trial and hence could not be incorporated in the analysis. Also, the eosinophil measurements formed part of the subject safety assessments in the MITRA protocol, and only involved single-digit resolution (in steps of 100 cells/mL). This may explain the relatively high cut-off value (>300) associated with the asthma outcome. Further, the original study was not powered to detect differences in the efficacy of HDM SLIT-tablet treatment in subpopulations of subjects with different T2 biomarkers levels. A prospective and adequately powered biomarker study including FeNo would be a suitable way to confirm these preliminary findings from this post hoc analysis.

CONCLUSIONS

To the best of our knowledge, this is the first study to demonstrate that AIT with SLIT-tablets may be effective in patients with a genetic predisposition to asthma, independent of inflammatory status. Further, we show that AIT with HDM SLIT-tablets reduces the risk of asthma exacerbations in patients with T2 inflammation.

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Contributors PSA is the guarantor of the study and accepts full responsibility for the finished work. Study conception and design: PSA, KB, IH and TS. Statistical analysis: IH, TS and XL, Sample preparation and biomarker measurements: SB. Cohort data collection: PSA, EDB and DAM. Analysing and interpreting study results: PSA, KB, IH, SB, TS, XL, EDB, DAM, EB and MHS. Contributed to the manuscript from the outset and read and approved the final draft: PSA, KB, IH, SB, TS, XL, EDB, DAM, EB and MHS. Responsibility for the work's integrity as a whole from inception to published article: PSA, KB, IH, SB, TS, XL, EDB, DAM, EB and MHS.

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Supplemental material

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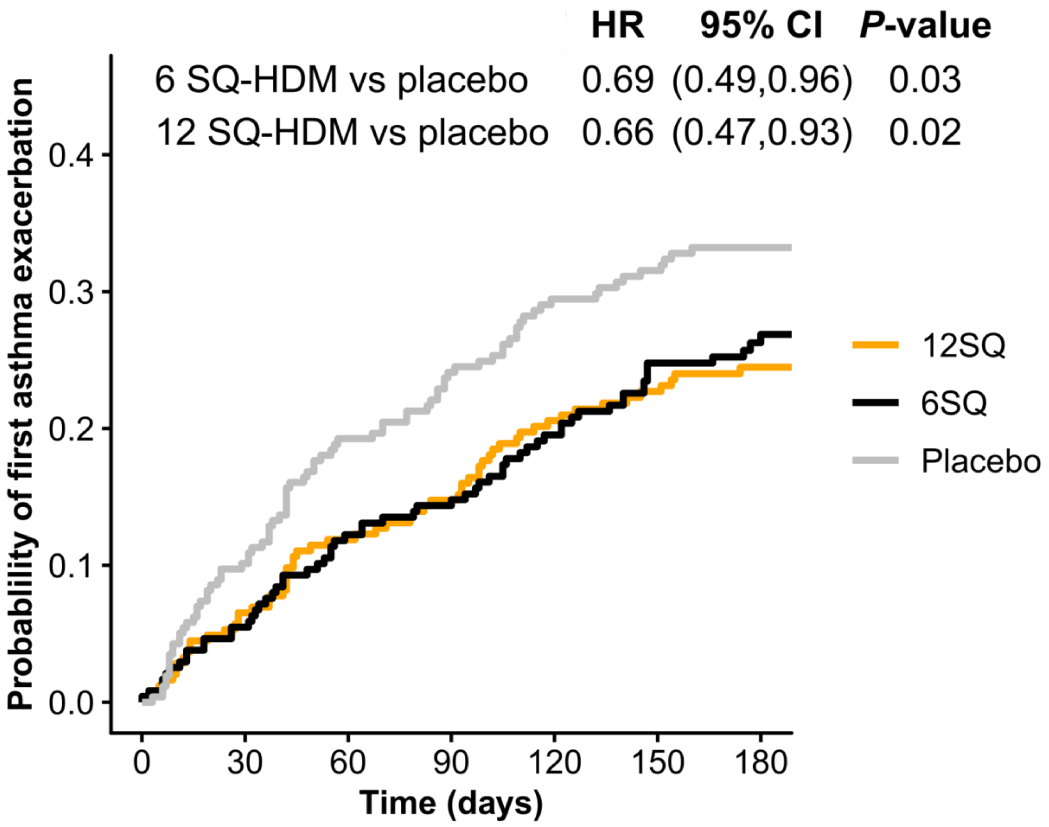
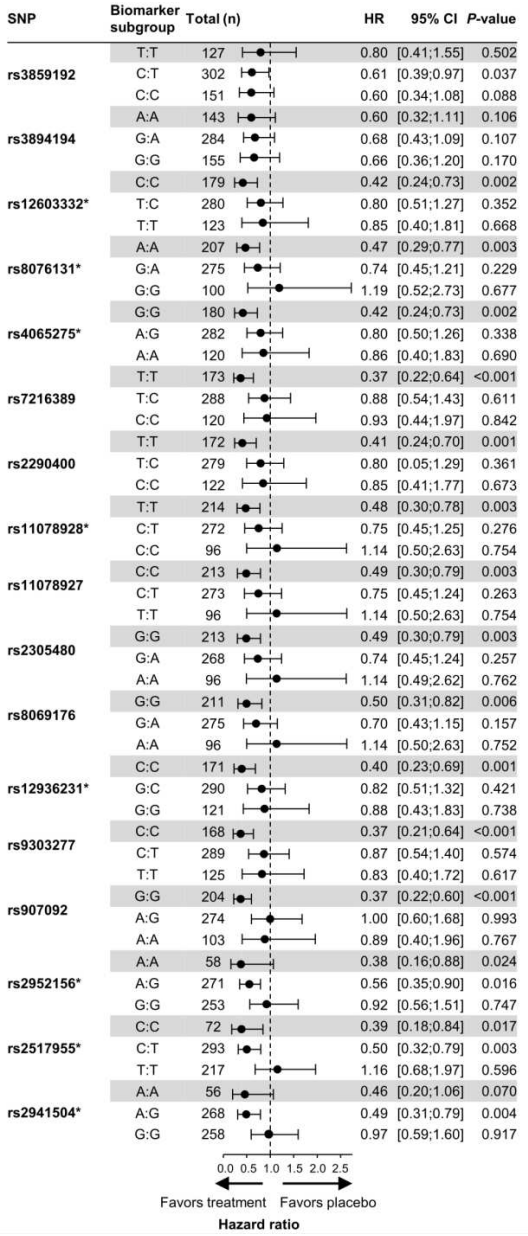
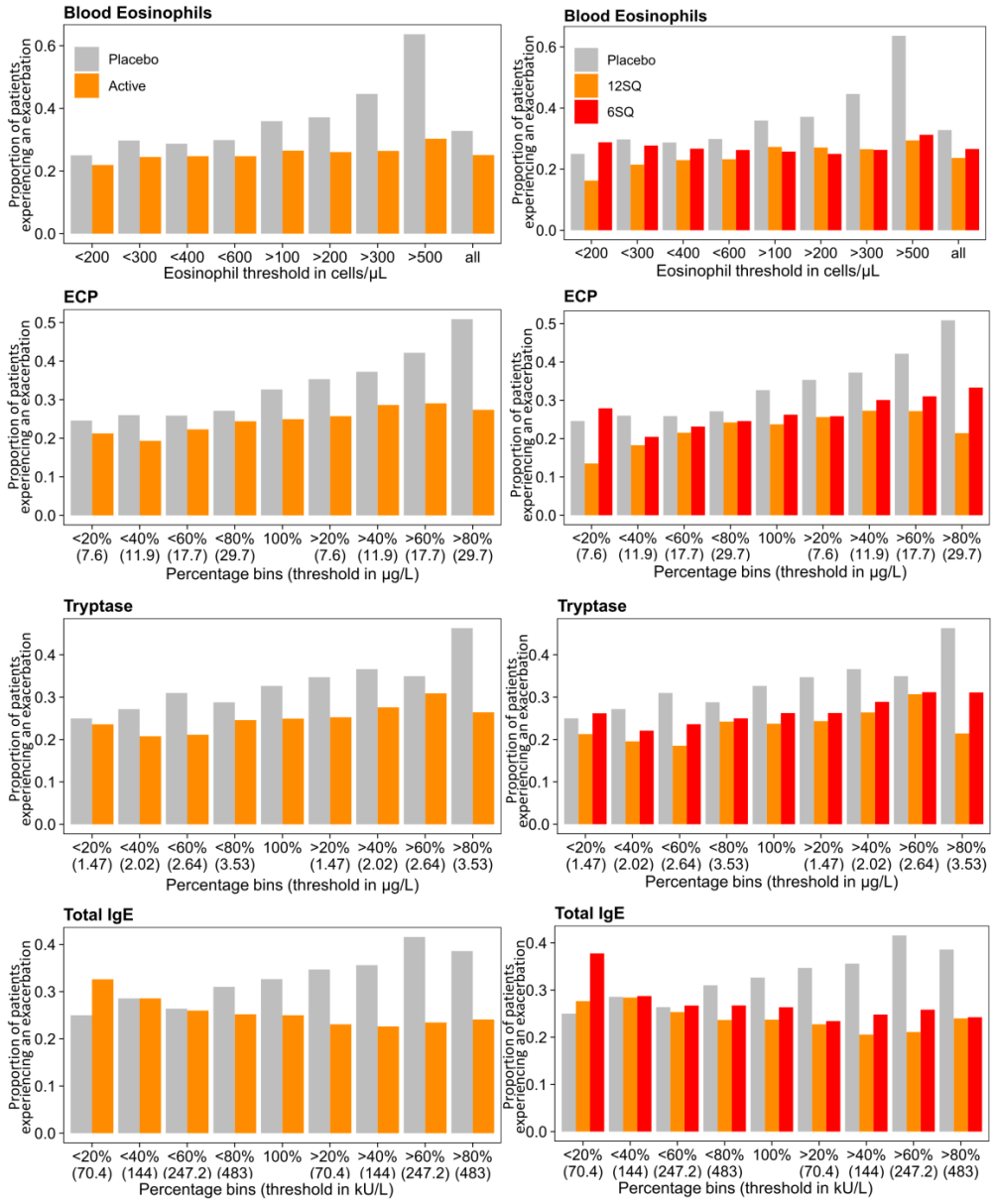


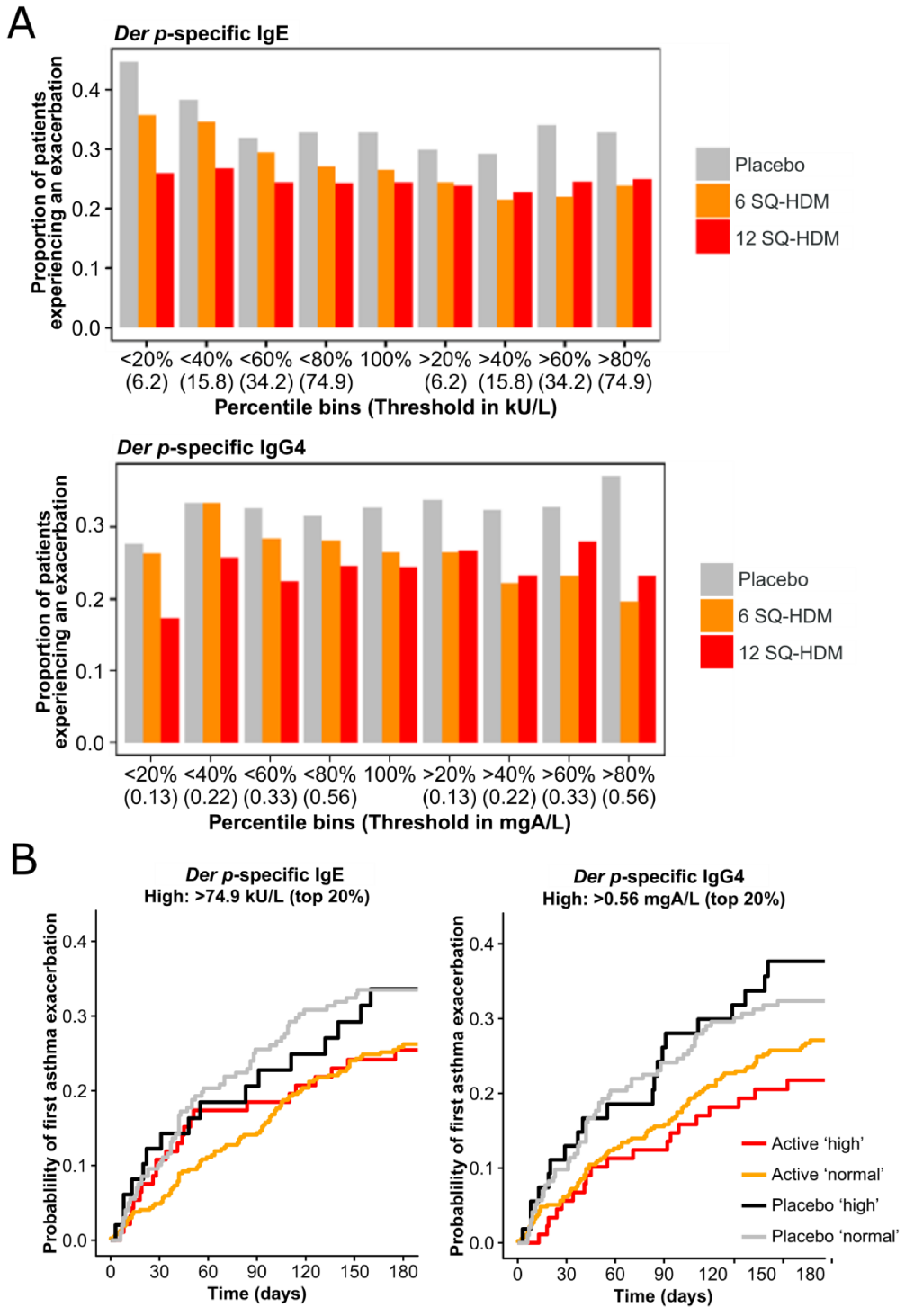
Supplemental Figure 1. Primary endpoint data for full analysis set. Probability of having the first moderate or severe asthma exacerbation in the full analysis set (without imputation, N=742). Primary efficacy analysis of time to first moderate or severe asthma exacerbation was performed using a Cox proportional hazards regression analysis stratified for country. As the two treatment groups produced almost identical HRs with overlapping CIs,[1] results for the two doses were merged in further analyses. CI, confidence interval; HR, hazard ratio; SQ-HDM, sublingual immunotherapy-tablet dose unit.



Supplemental Figure 2. Association between SNPs in chromosomal region 17q12-21 and treatment effect. Impact on time to first exacerbation for 17 SNPs in the chromosomal locus 17q12-21. The association between individual SNPs and the time to first asthma exacerbation (primary endpoint) was tested using a Cox proportional hazard model stratified for country. SNPs for which genotypes were derived by imputation are marked with *. CI, confidence interval; HR, hazard ratio; SNP, single-nucleotide polymorphism.

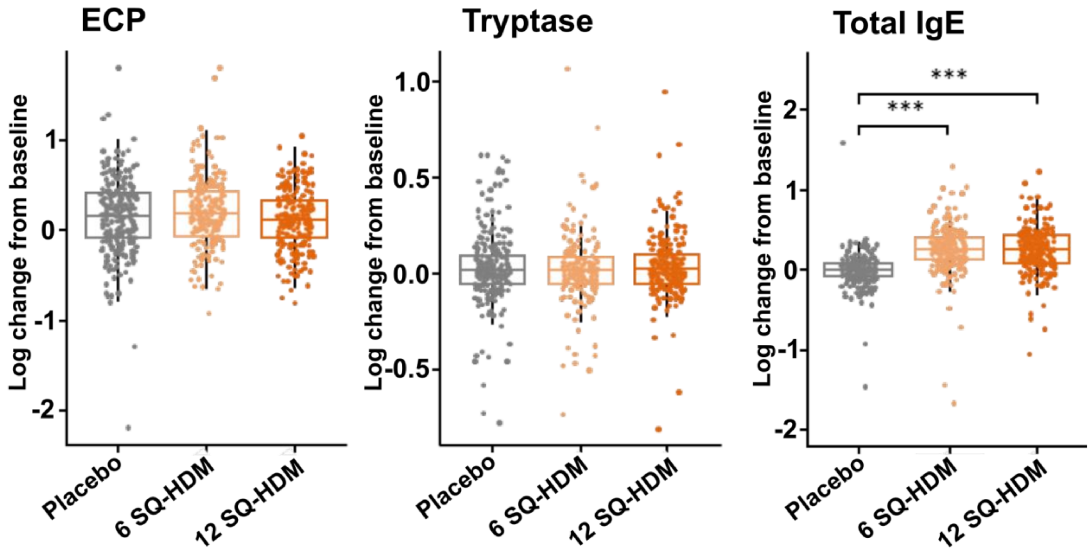


Supplemental Figure 3. Proportion of subjects experiencing an asthma exacerbation for overlapping subgroups of trial subjects with respect to the biomarker of interest. Visualization inspired by the tail-oriented subpopulation treatment effect pattern plot approach.[2] The full analysis set of subjects is shown in the middle of each panel (100%). Starting from the middle, subgroups represent a decreasing centile of subjects: moving to the left along the x axis, subjects with high biomarker values are omitted; moving to the right along the x axis, subjects with low biomarker values are omitted. ECP, eosinophil cationic protein; IgE, immunoglobulin E; SQ-HDM, sublingual immunotherapy-tablet dose unit.

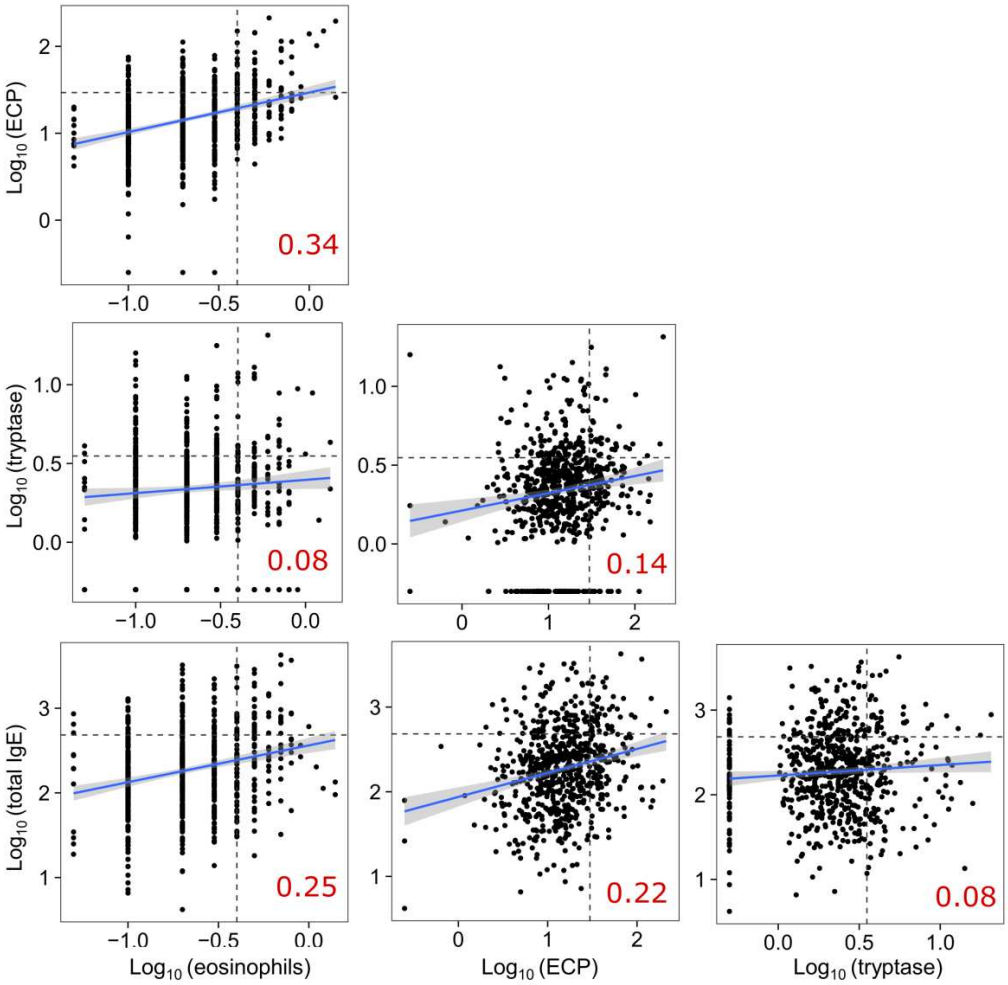


Supplemental Figure 4. Neither proportion of subjects experiencing an asthma exacerbation nor time to first asthma exacerbation are associated with allergen-specific type 2 immune response biomarkers. (A) Proportion of subjects experiencing an asthma exacerbation for overlapping subgroups of trial subjects with respect to the

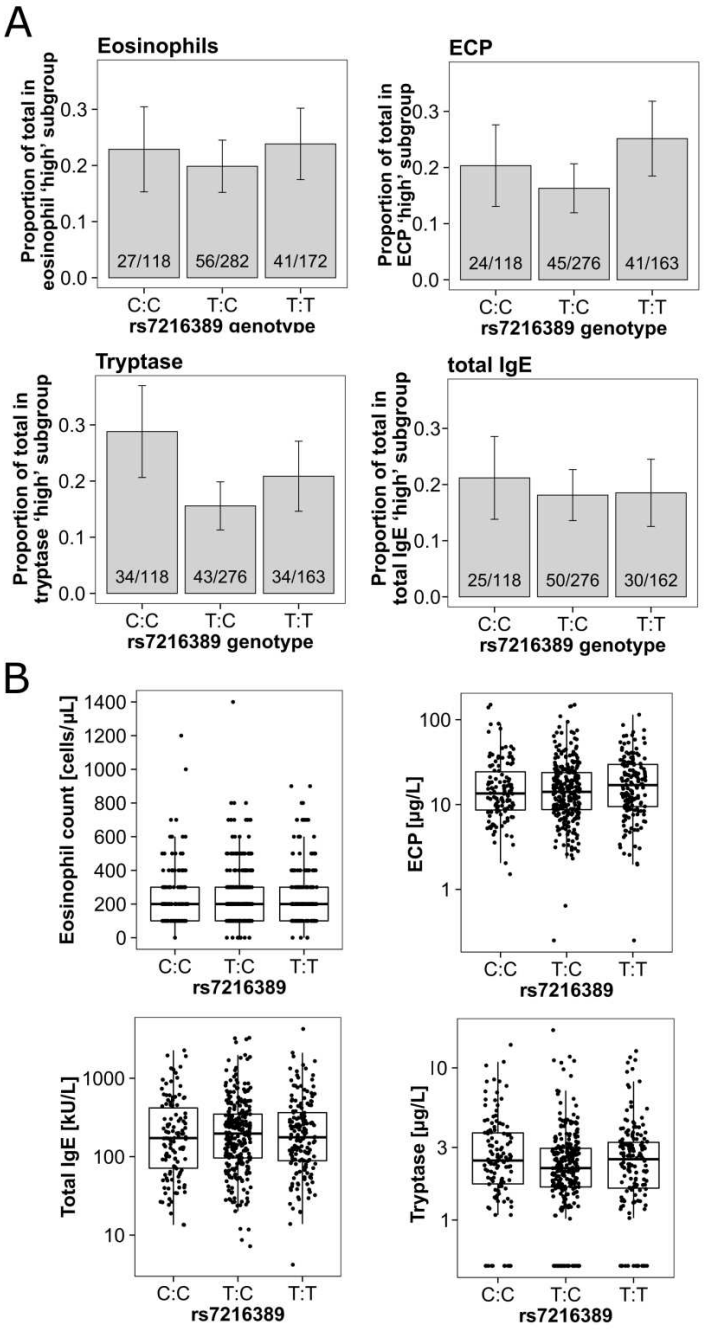
biomarker of interest. Visualization inspired by the tail-oriented subpopulation treatment effect pattern plot approach [2]. The full analysis set of subjects is shown in the middle of each panel (100%). Starting from the middle, subgroups represent a decreasing centile of subjects: moving to the left along the x axis, subjects with high biomarker values are omitted; moving to the right along the x axis, subjects with low biomarker values are omitted. (B) Cumulative incidence plots for HDM-specific IgE and IgG4 showing probability of asthma exacerbation over time in active or placebo group for 'high' and 'normal' biomarker subgroups. *Der p*, *Dermatophagoides pteronyssinus*; SQ-HDM, sublingual immunotherapy-tablet dose unit; IgE, immunoglobulin E; IgG4, immunoglobulin G4.



Supplementary Figure 5. Log change of biomarker levels from baseline to visit 9, the last visit prior to ICS reduction (i.e. after 7–12 months of SLIT-tablets). Boxes show the upper and lower quartiles (25–75%) with a line at the median, whiskers extend from the 10–90th percentiles. ****P*<0.001 for treatment versus placebo. SQ-HDM, SLIT-tablet dose unit; ECP, eosinophil cationic protein; HDM, house dust mite; ICS, inhaled corticosteroid; IgE, immunoglobulin E; SLIT, sublingual immunotherapy.



Supplemental figure 6. Pairwise correlation between baseline biomarkers. Pearson correlation coefficient values (r) are shown at the bottom right of each panel. Blue line shows Pearson correlation line of best fit, grey shows 95% confidence interval. ECP, eosinophil cationic protein; IgE, immunoglobulin E.



Supplemental figure 7. Absence of correlation between endotype and genotype in subjects with moderate or severe asthma treated with house dust mite sublingual immunotherapy-tablets for 7–12 months. (A) Proportion of subgroups carrying a particular rs7216389 genotype that are biomarker 'high'. (B) Box plots showing biomarker levels per genotype-defined subgroup. Boxes show the upper and lower quartiles (25–75%) with a line at the median; whiskers extend from the 10th–90th centiles. ECP, eosinophil cationic protein; IgE, immunoglobulin E.

Supplemental table I. Analyzed asthma-associated single-nucleotide polymorphisms.

				Association with asthma			
Associated trait	**RSID**	**Position (GRCh38.p12)**	**Nearest ENCODE gene**	**Odds ratio**	***P* value**	**Effect allele**	**Source**
Asthma	rs11071559	chr15:60777789	*RORA*	1.18	1×10^{-7}	C	Moffat et al.[3]
Asthma	rs11078927	chr17:39908152	*GSDMB*	n/a	n/a	C	Stein et al.[4]
GSDMB splice variant	rs11078928	17:39908216	GSDMB	n/a	n/a	T	Stein et al.[4]
Asthma	rs1233578	chr6:28744470	*GPX5, TRIM27*	1.11	5×10^{-9}	G	Demenais et al.[5]
Childhood asthma	rs12603332	17:39926554	ORMDL3	n/a	n/a	C	Stein et al.[4]
Asthma	rs12936231	17:39872867	ZPBP2	n/a	n/a	C	Stein et al.[4]
Asthma	rs1295686	chr5:132660151	*IL13*	1.15	1×10^{-7}	T	Moffat et al.[3]
Asthma	rs1342326	chr9:6190076	*IL33*	1.2	9×10^{-10}	C	Moffat et al.[3]
Asthma and rhinitis	rs1438673	chr5:111131801	*WDR36*	1.16	3×10^{-11}	C	Ferreira et al.[6]
Asthma and rhinitis	rs17294280	chr15:67175947	*SMAD3*	1.18	4×10^{-9}	G	Ferreira et al.[6]
Asthma	rs17637472	chr17:49384071	*ZNF652, PHB*	1.08	3×10^{-9}	A	Demenais et al.[5]
Asthma and rhinitis	rs1837253	chr5:111066174	*TSLP*	1.17	1×10^{-9}	C	Ferreira et al.[6]
Asthma	rs2073643	chr5:132387596	*SLC22A5*	1.11	2×10^{-7}	T	Moffat et al.[3]
Asthma	rs2284033	chr22:37137994	*IL2RB*	1.12	1×10^{-8}	G	Moffat et al.[3]
Asthma	rs2290400	chr17:39909987	*GSDMB*	n/a	n/a	T	Stein et al.[4]
Asthma	rs2305480	chr17:39909987	*GSDMB*	1.18	7×10^{-14}	G	Moffat et al.[3]
eQTL for *ORMDL3*	rs2517955	chr17:39687428	*PGAP2*	n/a	n/a	C	Stein et al.[4]
Asthma	rs2941504	chr17:39674647	*PGAP3*	n/a	n/a	A	Stein et al.[4]
Asthma	rs2952156	chr17:39720582	*ERBB2*	n/a	n/a	A	Stein et al.[4]
Asthma	rs3771166	chr2:102369762	*IL18R1*	0.87	3×10^{-9}	G	Moffat et al.[3]

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				Association with asthma			
Associated trait	**RSID**	**Position (GRCh38.p12)**	**Nearest ENCODE gene**	**Odds ratio**	***P* value**	**Effect allele**	**Source**
Asthma	rs3859192	chr17:39972395	*GSDMA*	n/a	n/a	T	Stein et al.[4]
Asthma	rs3894194	chr17:39965740	*GSDMA*	1.17	5×10^{-9}	A	Moffat et al.[3]
Asthma	rs4065275	17:39924612	ORMDL3	n/a	n/a	G	Stein et al.[4]
Asthma and rhinitis	rs4833095	chr4:38798089	*TLR1*	1.2	5×10^{-12}	T	Ferreira et al.[6]
Asthma and rhinitis	rs62026376	chr16:11134855	*CLEC16A*	1.17	1×10^{-8}	C	Ferreira et al.[6]
Childhood asthma	rs6967330	chr7:106018005	*CDHR3*	1.26	3×10^{-14}	A	Bønnelykke et al.[7]
Asthma and rhinitis	rs7009110	chr8:80379644	*ZBTB10*	1.14	4×10^{-9}	T	Ferreira et al.[6]
Asthma and rhinitis	rs7212938	chr17:39966427	*GSDMA*	1.16	4×10^{-10}	G	Ferreira et al.[6]
Asthma	rs7216389	chr17:39913696	*GSDMB*	1.45	9×10^{-11}	T	Moffat et al.[8]
Asthma and rhinitis	rs72699186	chr9:6175855	*IL33*	1.26	2×10^{-9}	T	Ferreira et al.[6]
Childhood asthma	rs8069176	chr17:39900944	*GSDMB*	n/a	n/a	G	Stein et al.[4]
Asthma	rs8076131	17:39924659	*ORMDL3*	n/a	n/a	A	Stein et al.[4]
Asthma	rs907092	chr17:39766006	*IKZF3*	n/a	n/a	G	Stein et al.[4]
Asthma	rs9273349	chr6:32658092	*HLA-DQ*	1.18	7×10^{-14}	C	Moffat et al.[3]
Childhood asthma	rs928413	chr9:6213387	*IL33*	1.24	9×10^{-13}	G	Bønnelykke et al.[7]
Childhood asthma	rs9303277	chr17:39820216	*IKZF3*	n/a	n/a	C	Stein et al.[4]

2 eQTL, expression quantitative trait locus; GSDMB, gasdermin B; n/a, not available; RSID, Reference single-nucleotide polymorphism cluster ID.

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Supplemental table II. Baseline demographics (per biomarker).

			Eosinophils		ECP		Tryptase		Total IgE	
	All (N=742)	SNP analysis (n=582)	High (n=171)	Normal (n=560)	High (n=141)	Normal (n=561)	High (n=141)	Normal (n=561)	High (n=140)	Normal (n=561)
Treatment received										
Placebo, n (%)	257 (35)	196 (34)	65 (38)	188 (34)	57 (40)	188 (34)	54 (38)	191 (34)	57 (41)	188 (34)
6 SQ-HDM, n (%)	237 (32)	187 (32)	57 (33)	176 (31)	42 (30)	179 (32)	45 (32)	176 (31)	33 (24)	187 (33)
12 SQ-HDM, n (%)	248 (33)	199 (34)	49 (29)	196 (35)	42 (30)	194 (35)	42 (30)	194 (35)	50 (36)	186 (33)
Age, years										
Mean (SD)	33.5 (11.9)	33.7 (11.8)	32.9 (11.3)	33.7 (12.1)	34.0 (12.7)	33.6 (11.8)	37.2 (13.6)	32.8 (11.4)	34.0 (13.0)	33.6 (11.7)
Median (IQR)	31 (24, 40)	32 (24,41)	32 (24, 40)	31 (24, 41)	31 (24, 41)	32 (24, 40)	35 (26, 45)	31 (24, 40)	31 (24, 40)	32 (24, 41)
Min–max	17–83	18-83	18–83	17–75	18–83	18–75	18–83	18–75	18–75	18–83
Sex										
Male, n (%)	384 (52)	309 (53)	81 (47)	300 (54)	64 (45)	299 (53)	86 (61)	277 (49)	71 (51)	291 (52)
ICS at randomization, µg of budesonide/d										
Mean (SD)	589 (249)	586 (243)	637 (258)	574 (245)	618 (265)	585 (243)	589 (238)	593 (250)	596 (252)	591 (247)
Median (range)	400 (200–1200)	400 (200-1200)	600 (200–1200)	400 (200–1200)	400 (400–1200)	400 (200–1200)	400 (400–1200)	400 (200–1200)	400 (200–1200)	400 (400–1200)
FEV_1 at randomization, % of predicted value										
Mean (SD)	92.35 (13.11)	92.70 (13.23	92.49 (13.32)	92.22 (13.01)	90.95 (12.44)	92.91 (13.25)	91.85 (13.31)	92.68 (13.06)	92.57 (13.46)	92.50 (13.04)
Median (IQR)	90.9 (82.2, 101.5)	91.0 (82.4, 102.1)	90.0 (81.9, 100.7)	90.8 (82.2, 101.7)	89.2 (82.4, 97.9)	91.6 (82.4, 102.9)	89.3 (82.4, 101.5)	91.4 (82.4, 101.9)	90.6 (83.3, 101.6)	91.2 (82.3, 101.8)
ACQ at randomization										
Mean (SD)	1.23 (0.17)	1.23 (0.17)	1.23 (0.17)	1.23 (0.17)	1.26 (0.17)	1.22 (0.17)	1.22 (0.19)	1.23 (0.17)	1.23 (0.16)	1.23 (0.18)
Median (IQR)	1.29 (1.14, 1.43)	123 (1.14, 1.43)	1.29 (1.14, 1.43)	1.29 (1.14, 1.43)	1.29 (1.14, 1.43)	1.14 (1.14, 1.43)	1.14 (1.14, 1.43)	1.29 (1.14, 1.43)	1.14 (1.14, 1.43)	1.29 (1.14, 1.43)
Total asthma daytime symptom score										
Mean (SD)	2.64 (1.98)	2.65 (1.97)	2.26 (1.62)	2.77 (2.07)	2.45 (1.68)	2.67 (2.03)	2.46 (1.97)	2.66 (1.97)	2.53 (1.79)	2.65 (2.01)

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			Eosinophils		ECP		Tryptase		Total IgE	
	All (N=742)	SNP analysis (n=582)	High (n=171)	Normal (n=560)	High (n=141)	Normal (n=561)	High (n=141)	Normal (n=561)	High (n=140)	Normal (n=561)
Median (IQR)	2.30 (1.00, 4.07)	2.29 (1.00, 4.14)	2.07 (0.93, 3.32)	2.46 (1.00, 4.29)	2.29 (1.10, 3.69)	2.29 (0.93, 4.14)	2.14 (0.85, 3.93)	2.31 (1.00, 4.07)	2.07 (1.15, 3.88)	2.36 (0.93, 4.08)
Asthma nocturnal symptom score										
Mean (SD)	0.61 (0.53)	0.61 (0.53)	0.54 (0.47)	0.63 (0.54)	0.52 (0.45)	0.63 (0.54)	0.62 (0.52)	0.61 (0.53)	0.62 (0.49)	0.61 (0.54)
Median (IQR)	0.52 (0.14, 1.00)	0.54 (0.14, 1.00)	0.43 (0.14, 0.89)	0.56 (0.14, 1.00)	0.46 (0.08, 0.93)	0.54 (0.14, 1.00)	0.54 (0.14, 1.00)	0.50 (0.14, 1.00)	0.50 (0.21, 1.00)	0.54 (0.08, 1.00)
24-hour SABA intake, number of 200 µg puffs										
Mean (SD)	1.31 (1.66)	1.28 (1.56)	1.27 (1.62)	1.32 (1.67)	1.31 (1.37)	1.30 (1.73)	1.54 (1.94)	1.25 (1.58)	1.38 (1.44)	1.29 (1.71)
Median (IQR)	0.71 (0.08, 2.00)	0.77 (0.08, 2.00)	0.71 (0.12, 1.90)	0.72 (0.08, 2.00)	0.86 (0.21, 2.00)	0.64 (0.08, 2.00)	0.85 (0.15, 2.08)	0.71 (0.08, 2.00)	0.92 (0.28, 2.07)	0.64 (0.07, 2.00)

ACQ, Asthma Control Questionnaire; ECP, eosinophil cationic protein; FEV_1, forced expiratory volume in the first second; ICS, inhaled corticosteroids; IgE, immunoglobulin E; IQR, interquartile range; SABA, short-acting β_2-receptor agonist; SD, standard deviation; SQ-HDM, sublingual immunotherapy-tablet dose unit.

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