The study listed may include approved and non-approved uses, formulations or treatment regimens. The results reported in any single study may not reflect the overall results obtained on studies of a product. Before prescribing any product mentioned in this Register, healthcare professionals should consult prescribing information for the product approved in their country.

GSK Medicine: Fluticasone Propionate / Salmeterol and Fluticasone Furoate / Vilanterol
Study Number: 204661 (also known as PGx7612)
Title: PGx7612: Pharmacogenetic investigation of the association of the ADRB2 rare variant, Thr164Ile, with severe asthma exacerbation
Rationale: Asthma exacerbation remains a major concern for subjects with asthma. The discovery of a genetic marker that predicts those most at risk for asthma exacerbations could potentially be used to prevent, or reduce the incidence of, such exacerbations. Ortega et al, 2014 (Lancet Respiratory Medicine 2014; 2:204-213) described evidence of association between a low frequency genetic variant, Thr164Ile, in the *ADRB2* gene with hospitalization due to asthma exacerbations in non-Hispanic white subjects treated with long-acting beta agonists (LABAs). This project investigated the effect of the *ADRB2* Thr164Ile variant on the less severe, related endpoint of clinically significant asthma exacerbation evaluated in LABA-treated GSK clinical study subjects.
Study Period: 27-February-2015 to 22-February-2016
Objectives: The objective is to determine if there is evidence for the association of *ADRB2* Thr164Ile with clinically significant asthma exacerbation in subjects treated with LABA-containing (salmeterol or vilanterol) regimens.
Indication: Asthma
Study Investigators/Centers: GSK conducted a pharmacogenetic study using DNA samples collected in studies HZA106837, ADA109055, and ADA109057.
Research Methods: This exploratory genetic study used a subset of data from subjects enrolled in three GSK clinical studies HZA106837, ADA109055, and ADA109057 who provided optional written consent and a sample for genetic research and were successfully genotyped. HZA106837 samples were genotyped using the Affymetrix Axiom Biobank Genotyping Array, conducted by BioStorage Technologies'/Bioprocessing Solutions Alliance (Piscataway,NJ, USA). The ADA109055 and ADA109057 samples were genotyped using the Illumina HumanOmni 1-Quad, conducted by Expression Analysis (Durham, NC, USA). The *ADRB2* Thr164Ile variant (C>T), rs1800888, is present on both genotyping arrays.
Data Source: Clinical data from clinical studies HZA106837, ADA109055, and ADA109057
Study Design: Retrospective, non-interventional exploratory genetic investigation of NHW subjects who were treated with inhaled corticosteroid (ICS)+LABA. The effect of the *ADRB2* Thr164Ile (CC / CT) genotype on acute asthma exacerbation rate was assessed. Statistical adjustment was made for relevant clinical and demographic covariates, as well as for genetic prinicipal components.
Study Population: Overall, there were 731 non-Hispanic white subjects with severe asthma in the Genetics Analysis Population (GAP; HZA106837, n= 610; ADA109055/ADA109057, n=121). These subjects provided written informed consent and a blood sample for genetic research, were successfully genotyped, and had clinical outcome data.
Study Exposures, Outcomes: No Investigational product was administered as part of this genetic study. However, all subjects in this study were LABA-treated having received Relvar (n=610) or Advair (n=121) during study conduct while enrolled in HZA106837, ADA109055 or ADA109057. The primary endpoint in this genetic association study was asthma exacerbation rate defined as the number of asthma exacerbation episodes (whether or not the exacerbation resulted in hospitalization). The primary endpoint, clinically significant asthma exacerbation, was explicitly defined within each study as: • "A severe asthma exacerbation is defined as deterioration of asthma requiring the use of systemic corticosteroids (tablets, suspensions, or injection) for at least 3 days or an inpatient hospitalization or emergency department (ED) visit due to asthma that required systemic corticosteroids." (HZA106837) • "Asthma exacerbations are defined as the requirement of treatment with an oral or parenteral corticosteroid or an unscheduled urgent care visit (e.g., unscheduled clinic visit, physician office visit, emergency room visit, hospitalization) for acute asthma symptoms requiring intervention." (ADA109055 and ADA109057). It is acknowledged that the definitions used in HZA106837 and the ADA studies are not identical, however, they are similar enough that they describe a common clinical state.
Data Analysis Methods: The effect of the *ADRB2* Thr164Ile variant on exacerbation rate was modelled as a negative bionomial variable with log time on treatment as an offset variable with 5 genetic principal components and relevant

clinical and demographic variables as covariates. The variables assessed for consideration as covariates were: age, body mass index, diastolic and systolic blood pressure, duration of asthma, baseline eosinophil level (GI/L), baseline FEV_1, baseline FEV_1 percent of predicted, FEV_1 reversibility (%), heart rate, height, weight, country of recruitment, eosinophil category (≥0.15 GI/L or not), and sex. Subject level data were analyzed separately for HZA106837 and for ADA109055/ADA109057. For the HZA106837 analysis, a variable indicating whether subjects were recruited from the Russian Federation, Ukraine, or Romania and the first five genetic prinicipal components were included as covariates. For the analysis of the ADA studies, the first five genetic principal components were included as covariates. Summary results from the two analyses were meta-analyzed. Because the published results of Ortega *et al.* implicated *ADRB2* 164Ile as leading to less beneficial treatment outcomes, conclusions about genetic association were based on one-sided statistical tests in which it was presumed that carriage of the variant T allele (164Ile) would result in higher rates of asthma exacerbation.

Limitations: The sample size of this genetic study meant that statistical power was sufficient to demonstrate a genetic association only if the genetic effect of *ADRB2* Thr164Ile on asthma exacerbation was relatively large (e.g., if the mean exacerbation rate in 164Ile (CT) carriers were more than three times that of non-carriers). Consequently, failure to demonstrate an association between *ADRB2* Thr164Ile and asthma exacerbation rate could be due to lack of statistical power.

Study Results:

Summary statistics for most clinical and demographic variables were similar for Genetic Analysis Population (GAP) and nonGAP subjects within HZA106837 and within ADA109055/ ADA109057, however, there were significant differences ($p<0.05$) for some variables. For HZA106837, Age (years), Baseline FEV_1 Percent of Predicted, Systolic Blood Pressure (mmHg) and Time on treatment (years) had different means among the GAP and non-GAP NHW subjects (Table 1). GAP subjects were older, had lower FEV_1 percent of predicted, had higher systolic blood pressure, and had been on treatment for longer than had non-GAP subjects. There was also a difference in the distribution of countries of recruitment and there was a higher proportion of females in the GAP subjects compared to the non-GAP subjects. (Table 2). For ADA109055 and ADA109057 statistical differences were seen for Baseline FEV_1, Baseline FEV_1 Percent of Predicted, and mean number of exacerbations on treatment (Table 3) and for the distribution of countries of recruitment (Table 4).

In HZA106837, of 610 subjects in the GAP population, there were 18 heterozygous 164Ile (CT) carriers (3.3%). In ADA109055 and ADA109057, of 121 subjects in the GAP population, there were 4 heterozygous 164Ile (CT) carriers (3%). No homozygous 164Ile (TT) carriers were observed in either study.

In HZA106837, though the mean number of exacerbations and the proportion of subjects experiencing one or more exacerbation was greater among the 164Ile (T) carriers than among non-carriers, the effect of the Ile (T) variant on number of exacerbations was non-significant, two-sided p=0.50 (in the pre-specified one-sided test, a two-sided p-value less than 0.10 would have been considered to be significant). The same model was run assuming a Poisson distribution and the effect of the Ile (T) variant was, again, non-significant, two-sided p=0.46. Negative binomial and Poisson regression models were also fit to the rs1800888 genotype without the inclusion of any covariates; the results for these were non-significant, with two-sided p-values of 0.44 and 0.35, respectively. Additional analyses that did not include adjustment for covariates or genetic principal components (PCs) were conducted. Using an alternative analytical approach, the average number of exacerbations per year were compared between carriers and non-carriers of the Ile (T) variant and found to be not significantly different (Wilcoxon rank sum test, two-sided p=0.31). The mean exacerbation rates over 52 weeks were 0.28 for carriers and 0.21 for non-carriers. The proportion of subjects who experienced >1 exacerbations was higher among Ile (T) variant carriers (22%, 4/18) than among non-carriers (14%, 85/592), however these proportions were not significantly different from one another (Fisher's exact one-sided p-value = 0.26).

In ADA109055/ADA109057, there were less exacerbations among 164Ile (T) carriers than among non-carriers, but effect of the Ile (T) variant on number of exacerbations was non-significant, two-sided p=0.76 (in the pre-specified one-sided test, a two-sided p-value < 0.10 with the expected direction of effect would have been considered to be significant). The same model was run assuming a Poisson distribution and the effect of the Ile (T) variant was, again, non-significant, two-sided p=0.76. Negative binomial and Poisson regression models were also fit to the rs1800888 genotype without the inclusion of any covariates; the results for these were non-significant, with two-sided p-values of 0.67 and 0.65, respectively. Additional analyses that did not include adjustment for covariates or genetic PCs were conducted. Using an alternative analytical approach, the average number of exacerbations per year were compared between carriers and non-carriers of the Ile (T) variant and found to be not significantly different (Wilcoxon rank sum test, two-sided p=0.90). The mean exacerbation rates over 52 weeks were 0.25 for carriers and 0.44 for non-carriers.

The proportions of subjects who experienced 1 or more exacerbations were neither nominally nor statistically different in Ile (T) variant carriers (25%, 1/4) and non-carriers (25%, 30/117); Fisher's Exact Test one-sided p-value was 0.70.

Inclusion or exclusion of genetic principal components had minimal impact on the estimate of the effect of Ile (T) variant in models of association with number of exacerbations in either HZA106837 or ADA109055/ADA109057.

Meta-analysis of the results from the two analysis datasets led to an estimate of 1.26 as the ratio between the exacerbation rates in subjects carrying the *ADRB2* 164Ile (T) versus non-carriers. The confidence interval about the ratio was 0.48 – 3.32 and the associated p-value testing for a difference in the rate from 1.0 was 0.64 (two-sided) and 0.32 (one-sided, testing for a value greater than 1.0).

Table 1 Comparison of continuous variables between Genetic Analysis Population (GAP) subjects and non-GAP NHW subjects in HZA106837.

Variable	GAP?	N	Mean	Std Dev	Std Err Mean	P-value (2 sided t-Test)
Age (years)	N	85	38.07	18.57	2.01	0.017
	Y	610	43.21	16.67	0.67	
Body Mass Index (kg/m²)	N	85	26.52	6.30	0.68	0.167
	Y	610	27.53	6.09	0.25	
Diastolic Blood Pressure (mmHg)	N	85	74.52	8.79	0.95	0.313
	Y	610	75.55	8.76	0.35	
Duration of Asthma (years)	N	83	14.46	13.27	1.46	0.893
	Y	606	14.25	12.69	0.52	
Eosinophil Level, Baseline (GI/L)	N	85	0.33	0.30	0.03	0.472
	Y	597	0.31	0.29	0.01	
Baseline FEV_1 (L)	N	85	2.43	0.58	0.06	0.056
	Y	610	2.30	0.66	0.03	
Baseline FEV_1 Percent of Predicted (%)	N	85	74.43	10.46	1.13	0.029
	Y	610	71.74	10.63	0.43	
FEV_1 Reversibility (%)	N	85	22.37	10.23	1.11	0.099
	Y	610	24.41	12.73	0.52	
Heart Rate (beats per minute)	N	85	74.93	8.57	0.93	0.228
	Y	610	73.73	8.12	0.33	
Height (cm)	N	85	166.22	10.05	1.09	0.906
	Y	610	166.09	9.38	0.38	
Systolic Blood Pressure (mmHg)	N	85	116.74	13.81	1.50	0.004
	Y	610	121.47	12.56	0.51	
Weight (kg)	N	85	73.55	19.08	2.07	0.250
	Y	610	76.09	18.40	0.75	
Number of Exacerbations on Treatment	N	85	0.21	0.49	0.05	0.663
	Y	610	0.19	0.50	0.02	
Time on Treatment (years)	N	85	0.80	0.42	0.05	<.0001
	Y	610	1.03	0.18	0.01	

Table 2 Comparison of categorical variables between Genetic Analysis Population (GAP) subjects and non-GAP NHW subjects in HZA106837.

Patient Characteristic		GAP (N=610)	Non GAP (N=85)	Prob>ChiSq (Pearson)
Country	Argentina	10.7%	5.9%	0.01
	Australia	1.3%	2.4%	
	Germany	12.0%	17.6%	
	Poland	12.0%	8.2%	
	Romania	10.2%	16.5%	
	Russian Federation	22.8%	10.6%	
	Ukraine	16.7%	14.1%	
	United States	14.4%	24.7%	
Eosinophils baseline category (≥0.15 GI/L)		70.7%	70.6%	0.99
Percentage of females		64.8%	51.8%	0.02

Table 3 Comparison of continuous variables between Genetic Analysis Population (GAP) subjects and non-GAP NHW subjects in ADA109055 and ADA109057.

Variable	GAP?	N	Mean	Std Dev	Std Err Mean	P-value (2-sided t-test)
Age (years)	N	174	39.78	16.61	1.26	0.241
	Y	121	41.92	14.39	1.31	
Smoking (pack-years)	N	29	3.54	3.01	0.56	0.472
	Y	24	4.16	3.14	0.64	
Baseline FEV_1 (L)	N	174	2.61	0.74	0.06	0.022
	Y	121	2.42	0.65	0.06	
Baseline FEV_1 Percent of Predicted (%)	N	174	76.40	13.72	1.04	0.002
	Y	121	71.55	12.33	1.12	
FEV_1 Reversibility (%)	N	174	23.66	12.79	0.97	0.106
	Y	121	21.52	9.80	0.89	
Number of Exacerbations on Treatment	N	174	0.17	0.46	0.04	0.017
	Y	121	0.34	0.65	0.06	
Time on Treatment (years)	N	174	0.82	0.31	0.02	0.158
	Y	121	0.87	0.27	0.02	
Exacerbation Rate (events/year)	N	174	0.82	0.31	0.02	0.264
	Y	121	0.87	0.27	0.02	

Table 4 Comparison of categorical variables between Genetic Analysis Population (GAP) subjects and non-GAP NHW subjects in ADA109055 and ADA109057.

Patient Characteristic		GAP (N=121)	Non GAP (N=174)	Prob>ChiSq (Pearson)
Country	Argentina	12.4%	6.3%	0.03
	Canada	10.7%	5.2%	
	United States	76.9%	88.5%	
Duration of Disease	>= 6 months to < 1 year	1.7%	0.6%	0.50
	>= 1 year to < 5 years	9.9%	10.3%	
	>= 5 years to < 10 years	10.7%	17.2%	
	>= 10 years to < 15 years	18.2%	18.4%	
	>= 15 years	59.5%	53.4%	
Percentage of Females	Female	53.7%	56.9%	0.59
	Male	46.3%	43.1%	
Smoking History	Former smoker	19.8%	16.7%	0.49
	Never smoked	80.2%	83.3%	

Conclusion: In the LABA-treated, NHW sample, the *ADRB2* Thr164Ile variant was not significantly associated with asthma exacerbation rate in either of two separate analysis datasets (HZA106837: two-sided p=0.50, ADA109055 and ADA109057: two-sided p=0.76) or in the meta-analysis (two-sided p=0.64).
This study did not allow for an assessment of the possible effect of *ADRB2* Thr164Ile on asthma exacerbation rate in subjects not being treated with a LABA-containing regimen.