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ADRB2 Gly16Arg polymorphism, asthma control and lung function decline

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ABSTRACT
Arg/Arg homozygous for the Gly16Arg polymorphism in ADRB2 have a reduced response to short acting β2-agonists but no effect has been associated with long-acting β2-agonists (LABA).

We selected 604 subjects from the European Community Respiratory Health Study with current asthma to evaluate if asthma control and lung function decline were associated with Gly16Arg polymorphism and test if LABA or inhaled corticosteroids (ICS) use modified these effects.

There was an increased risk of non-controlled asthma OR=1.33 (1.01-1.75, p=0.046) for each Arg allele. Among nonusers of ICS, the risk of non-controlled asthma among Arg/Arg vs. Gly/Gly subjects was OR=2.73 (1.28-5.82, p=0.009). No increased risk of non-controlled asthma associated to the Arg allele was observed among ICS and /or LABA users. For each Arg allele a decrease of 7.7 mL/year (SE 2.5) in FEV1 decline was found (p-trend=0.003), irrespective of ICS or LABA use. Arg/Arg subjects vs. Gly/Gly subjects had an increased risk of bronchial hyperresponsiveness with an OR of 2.51 (1.12-5.63, p=0.025) if they did not use ICS.

The Arg allele was associated with poorer asthma control, a steeper lung function decline and bronchial hyperresponsiveness. Absence of genotypic effects on asthma control among ICS users may be due to reversed ADRB2 desensitization.

INTRODUCTION

Asthma is a complex disease characterized by reversible airflow obstruction, hyper-responsiveness, airway remodelling and inflammation. In genetically predisposed individuals, environmental factors such as viral infections or bacterial lipopolysaccharide may modify the likelihood to develop asthma.[1] Genes, such as the β2-adrenergic receptor gene (ADRB2) may modify the response to therapy among asthmatics. ADRB2 is located on chromosome 5q31-q32 and encodes for the β2-adrenergic receptor (β2-AR), a G-protein-coupled receptor that is expressed in airway smooth muscle and produces bronchial relaxation.[2] *In vitro* studies have shown that the non-synonymous single-nucleotide polymorphism (SNP) at position 46 (rs1042713, herein referred to as Gly16Arg) in the ADRB2 gene shows an enhanced agonist-promoted down-regulation.[3] *In vitro* studies evaluating concomitant use of steroids and β2-agonists also suggest that inhaled corticosteroids (ICS) may counteract β2-AR desensitization.[4] *In vivo* evidence suggests the presence of a differential response to short acting β2-agonists (SABA) treatment according to ADRB2 Gly16Arg genotypes. [5] Other studies that have found similar differential response among Arg/Arg homozygous regularly treated with long-acting beta2-agonistst (LABA).[6,7] However, recent randomized clinical trials show that there is no pharmacogenetic short-term effect associated to LABA use.[8, 9] Long term consequences in lung function of this polymorphism and whether ICS may counteract β2-AR desensitization are yet to be determined. We evaluated whether the ADRB2 Gly16Arg polymorphism is associated with short-term asthma control and long-term lung function decline in an asthmatic adult population and whether these effects may be modified by the concomitant use of ICS or LABA.

METHODS

Study population and design

The European Community Respiratory Health Survey (ECRHS) is a multicentre longitudinal cohort study that recruited 18,811 subjects around 1991 (ECRHS I) and followed an eligible sample of them (59%) up to 1999-2001 (ECRHS II). The study included several structured interviews and clinical tests. Details of the study have been described elsewhere.[10] Local ethics committees at each centre approved the study protocols. From the 10,933 subjects who participated in the ECRHS II, 5,065 subjects that had available DNA were genotyped for several polymorphisms, including the ADRB2 Gly16Arg polymorphism, as part of a more extensive genotyping project. There was no statistically significant difference in the proportion of subjects with asthma or physician-diagnosed asthma between those subjects genotyped and those that were not genotyped. From the 5065 genotyped subjects we selected 604 with "current physician diagnosed asthma". Current asthma was defined as self-reported physician diagnosed asthma in combination with having had asthma symptoms or having used asthma medication in the last 12 months. We excluded all non-asthmatic subjects and those asthmatics that did not fall in our definition of "current physician diagnosed asthma". The mean follow-up for these subjects was 8.8 years (SE 0.7).

Outcomes and exposures definition

In the assessment of asthma severity, GINA 2006 guidelines shifted from asthma-severity to asthma control where treatment is no longer part of the classification although most clinical features are still the same.[11] Asthma control was defined according to GINA 2006 guidelines and was previously assessed in this population.[12] Asthma was classified in ECRHS II as controlled if all these features were present: diurnal symptoms less than once a week and no

asthma attacks in the last 3 months, no activity (work and other activities) limitations in the last 12 months, no nocturnal symptoms in the last 3 months, SABA twice or less per week in the last 3 months, no use of oral steroids in the last 12 months, and FEV1 ≥80% of predicted value. Asthma was considered partly controlled if 1 or 2 of the above features were absent and uncontrolled if more than 2 features were absent or if asthma, shortness of breath, or wheezing had caused hospital/emergency department admissions in the last 12 months; or if oral steroids were used in short courses or continuously in the last 12 months; or if the subject had more than 12 asthma attacks (1 per week or more) in the last 3 months. In some of the multivariate analyses partially controlled and uncontrolled asthma were grouped together and indicated as "non-controlled asthma". Estimates of risk associated to Gly16Arg polymorphism for each of these two categories can be found in the online data supplement. Lung function decline was defined as decline in ml per year of follow-up in forced expiratory volume in one second (FEV1) between the two surveys. Subjects were defined to have bronchial hyperresponsiveness (BHR) if they had a 20% or more decrease in FEV1 with a methacholine dose equal or less than 1 mg measured by methacholine challenge test. If the total dose of 1mg was taken and the FEV1 drop was less than 20% the subject was considered to have no BHR. Methacholine challenge dose-response slope was transformed as 100/log-slope+10 to normalize the data as previously done by Chin et al.[13] Use of inhaled SABA, LABA and ICS were defined as answering yes to having used the drug in the last 12 months. Use of ICS during the whole period within the two surveys was also assessed as described by De Marco et al.[14]

Genotyping

Genotyping and quality control of the rs1042713 polymorphism in the ADRB2 gene was performed as a part of more extensive genotyping at the Centre for Genomic Regulation of the Spanish National Genotyping Centre (Barcelona, Spain) as described previously.[15]

Statistical analysis

Chi-square test for categorical variables and ANOVA for continuous variables were used to test differences in socio-demographic and clinical characteristics by genotype group. Odds ratios of non-controlled asthma were estimated using logistic regression models. Controlled asthma was used as the reference group to compute relative risks ratios (RRR) of uncontrolled and partially controlled asthma using multinomial logistic regression (data shown in the online supplement). Linear regression models were used to test genotype effects on decline in FEV1 (mL/year). Both co-dominant and additive genetic models were tested. Hardy-Weinberg equilibrium was confirmed among 'controlled' current physician-diagnosed asthmatics and among the whole population by Chi-square exact test. Potential confounders were selected *a priori* based on the literature review, including those reported to be determinants of asthma control in this population [12] and those potentially related to asthma severity at baseline that may potentially influence later control. Covariates were removed from statistical models if there was less than 10% change in the genotype effects, except for age and sex which were forced into the models. Final models were adjusted for age, sex, and additionally, for body mass index and bronchial hyperresponsiveness in the analysis of asthma control and for height, baseline FEV1 and history of current, former or never tobacco smoking in the analysis of FEV1 decline. Interactions between ICS, LABA and genotypes were tested and defined as significant if p-value<0.05. Subjects included in the analysis were of European-Caucasian origin. The impact of population stratification in our population of self-reported Caucasians was assessed in 2 previous studies

using a genomic control approach [16] and EIGENSTRAT method. [17] Both methods found no evidence of population stratification with an λ of 1.06 for asthma using the genomic control approach [15] and no subdivisions of populations in the EIGENSRAT analysis.[18] Stata 10 S.E package was used to perform statistical analyses.

RESULTS

Mean age at the time of the ECRHS II interview was 42 ±7.3 years and 59% (n=356) of participants were women. Of the 604 current physician diagnosed asthmatics, 37 % (n=221) were homozygous for the major ADRB2 gene allele (Gly/Gly), 46% (n=277) were heterozygous (Gly/Arg) and 18% (106) were homozygous for the minor allele (Arg/Arg). Overall 46.5% (n=281) asthmatics used ICS during the last 12 months, 27.5% (n=166) used ICS every year during the period between the two surveys, 60.6 % (n=358) used SABA and 16.7% (n=100) used LABA during the last 12 months. The proportion of subjects with BHR was higher among those carrying the Arg/Arg genotype (64.1%), than among those carrying the Gly/Arg (55.6%) and the Gly/Gly (45.7%) genotypes (p=0.04). No other statistically significant differences in the distribution of socio-demographic and clinical variables at baseline (1991) and at follow-up (1999) were found between ADRB2 genotypes, (table 1), nor between the genotyped and the non-genotyped population (data not shown). At baseline, 65% of the subjects had rare or occasional symptoms, only 9% of the subjects had used oral steroids the last 12 months and less than 3% of the subjects had a predicted FEV1<=60%.

Table 1. Distribution of the main socio-demographic and clinical characteristics in subjects with current physician diagnosed asthma according to the Gly16Arg genotypes in ECRHS II population (N=604).

	units	Gly/Gly (n=221)		Gly/Arg (n=277)		Arg/Arg (n=106)		p- value
Age in years	mean, sd	42.6	7.6	42.2	7.2	41.8	7.4	0.65
Age of onset before 16 years old	N, %	84	38.9	13	49.1	44	41.5	0.07
Female sex	N, %	127	57.2	168	60.6	61	57.6	0.73
Body mass Index	mean, sd	26.4	5.8	26.0	5.0	25.8	5.2	0.55
Smoking: Current smokers	N, %	65	29.7	79	28.8	25	23.8	0.16
Former smokers	N, %	71	32.4	66	24.1	32	30.5	-
Never smokers	N, %	83	37.9	129	47.1	48	45.7	-
Inhaled short-acting β2-agonists	N, %	126	58.3	160	59.5	72	67.9	0.23
Inhaled long-acting β2-agonists	N, %	38	17.5	41	14.9	21	19.8	0.48
Inhaled steroids	N, %	107	49.5	120	43.6	54	50.9	0.29
FEV1 in ECRHS I (% of predicted)	mean, sd	98.04	18.1	97.6	17.2	94.7	18.0	0.25
FEV1 in ECRHSII (% of predicted)	mean, sd	96.80	16.3	97.6	15.8	95.1	16.5	0.42
Bronchial hyperresponsiveness	N, %	64	45.7	95	55.6	41	64.1	**0.04**
Total IgE >100 kU/L	N, %	97	44.9	148	54.2	59	55.7	0.07
Chronic cough or phlegm	N, %	56	25.9	80	29.3	30	29.1	0.69
Allergic rhinitis	N, %	111	50.7	168	61.1	58	54.7	0.06

Asthma control

Among the 604 current physician-diagnosed asthmatics, 27.3% (n=156) were considered to have controlled asthma, and 72.7% (n=416) non-controlled asthma at the time of the ECRHS II interview. Among non-controlled, 57.7% (n=240) subjects had partially controlled asthma and 42.3% (n=176) uncontrolled asthma. Thirty-two subjects were not classified due to missing data. There were no statistically significant differences in the specific clinical features used to define asthma control between Gly16Arg genotypes (table 2).

Table 2. Distribution of asthma control and of the clinical features used to define asthma control among subjects with current physician diagnosed asthma according to the genotypes in ECRHS II population (N=604).

		Gly/Gly (n=221)		Gly/Arg (n=277)		Arg/Arg (n=106)	
Asthma control:		**N**	**%**	**N**	**%**	**N**	**%**
controlled		67	42.95	67	42.95	22	14.10
non-controlled		138	33.17	196	47.12	82	19.71
partially controlled		80	33.33	109	45.42	51	21.25
uncontrolled		58	32.95	87	49.43	31	17.61
Features used to define asthma control:							
Diurnal symptoms in the last 3 months	>=1 week	53	24.2	82	29.9	29	27.4
Asthma attacks in the last 3 months	yes	64	29.9	95	35.1	35	33
	>=1 week	27	12.2	33	11.9	12	11.3
Nocturnal symptoms in the last 3 months	yes	69	31.5	99	36.3	29	27.6
Activity limitations last 12 months	yes	51	24.3	70	27	23	23
SABA last 3 months	>2 week	60	29.8	76	29.6	33	32.7
Use of oral steroids last 3 months	yes	13	6	20	7.2	10	9.5
	sc or cont*	10	4.6	11	4	5	4.8
FEV1<80%predicted in ECRHS II	% subjects	32	15	37	14.1	20	19.2
Emergency department last 12 months	yes	10	4.6	18	6.5	7	6.6
Hospitalization last 12 months	yes	2	0.9	2	0.7	0	0

*sc or cont, in short courses or continuous.

There was an increased risk of non-controlled asthma per each Arg allele with an odds ratio of 1.33 (95% CI 1.01 to 1.75, p-value=0.046), (table 3). There was a statistically significant interaction between ICS use and ADRB2 genotype (p=0.046) on the risk of having non-controlled asthma. Subjects not using ICS during the last 12 months with the Arg/Arg genotype showed a nearly 3-fold increased risk (odds ratio= 2.73, 95% CI 1.28 to 5.82, p-value=0.009) of non-controlled asthma as compared to Gly/Gly subjects. No interaction between ADRB2 genotype and LABA was observed for asthma control (p=0.879). When stratifying by both ICS and LABA we observed no differences between Gly16Arg genotypes within users of ICS irrespective of LABA use. Among non-users of ICS and LABA there was an increased odds ratio of 1.61 (95% CI 1.11 to 2.35, p-value=0.013) per each Arg allele increase (table 3). Due to small numbers in the users of LABA alone group no odds ratios were computed, however p-value for the Fisher exact test was p=0.1 with 100% (N=12) of the subjects in the Gly/Arg or Arg/Arg groups having non-controlled asthma vs. 50% (N=2) among the Gly/Gly subjects. Similar estimates by genotype and drug exposure were observed when evaluating the risk of different categories of non-controlled asthma; partially controlled and uncontrolled (see table E1 in the online supplement).

Table 3. Odds ratios of non-controlled asthma according to ADRB2 Gly16Arg genotypes, and stratified by ICS and LABA use in the last 12 months as reported in the ECRHS II (N=604).

			Risk of non-controlled asthma								
			per each Arg allele (additive model)					among Arg/Arg (vs. Gly/Gly)			
	Use of ICS	Use of LABA	N	OR	95%CI		p-value	N	OR	95%CI	p-value
all asthmatics	-	-	557	1.33	(1.01 -	1.75)	0.046	300	1.73	(0.97 - 3.09)	0.07
nonusers of ICS	no	-	287	1.76	(1.21 -	2.54)	**0.003**	146	2.73	(1.28 - 5.82)	**0.009**
users of ICS	yes	-	266	1.02	(0.63 -	1.68)	0.92	151	1.06	(0.38 - 2.94)	0.91
nonusers of LABA	-	no	462	1.35	(1.00 -	1.82)	0.05	245	1.81	(0.96 - 3.41)	0.07
users of LABA	-	yes	90	1.27	(0.56 -	2.91)	0.57	52	1.37	(0.27 - 6.92)	0.70
nonusers of ICS or LABA	no	no	273	1.61	(1.11 -	2.35)	0.013	139	2.32	(1.07 - 5.04)	0.033
nonusers of ICS + users of LABA	no	yes	16	n.a	n.a	n.a	n.a	8	n.a	n.a n.a	n.a
users of ICS + nonusers of LABA	yes	no	188	1.06	(0.58 -	1.93)	0.86	106	1.23	(0.34 - 4.54)	0.75
users of ICS and LABA	yes	yes	76	0.97	(0.42 -	2.28)	0.95	45	0.88	(0.16 - 4.82)	0.89

*adjusted for adjusted for sex, age, body mass index and bronchial hyperresponsiveness. Additive models were estimated by modelling the categorical ADRB2 genotype variable as continuous. n.a stands for not available. In bold, p-values that passed Bonferroni correction for multiple testing ($p<0.05/9=0.0056$).

Decline in FEV1

The mean decline in FEV1 between ECRHS I and II was 24.6 ± 44.9 mL/year among the 527 subjects with data available in both studies, with a mean duration of follow-up of 8.8 ± 0 .7 years. ADRB2 genotype was not associated with FEV1 at the end of follow-up (1999) (p-trend=0.2) nor with FEV1 at baseline (1991) (p-trend=0.4). Asthmatics with the Gly/Arg and Arg/Arg genotypes had a decline in FEV1 that was on average 8 and 15 ml per year, respectively, steeper than that of carriers of the Gly/Gly genotype (table 4). Similarly, per each Arg allele there was a decrease in FEV1 of 7.7 mL/year (SE 2.5) (p=0.003). Reductions in FEV1 among Arg/Arg subjects were observed both for non-use of ICS and for non-use of LABA. Nonusers of LABA carrying the Arg/Arg genotype had reductions in FEV1 through the 9 years of follow-up of almost double the magnitude than Gly/Gly subjects (22 mL/y vs. 39 mL/y) (p-trend=0.004). Similarly, nonusers of ICS showed statistically significant trends (p~0.02) by genotype, although estimates of FEV1 decline were very similar to those among users of ICS. Similar pattern was seen when evaluating ICS use every year during the period between the two surveys (data not shown).

Table 4. Mean decline in FEV1 in millilitres per year from ECRHS I to ERCHS II studies, according to ADRB2 Gly16Arg genotypes, and stratified by inhaled corticosteroids and long-acting β2-agonists use (N=519).

	Use of ICS	Use of LABA	N	Decline in FEV1 across ADRB2 Gly16Arg genotypes in mL/year*						p-trend
				Gly/Gly		Gly/Arg		Arg/Arg		
				Mean	95% CI	Mean	95% CI	Mean	95% CI	
all asthmatics	-	-	519	21	(11 to 30)	29	(201 to 38)	36	(25 to 46)	**0.003**
nonusers of ICS	no	-	272	21	(8 to 34)	31	(20 to 42)	39	(25 to 53)	0.007
users of ICS	yes	-	248	17	(1 to 32)	28	(13 to 42)	31	(14 to 48)	0.062
nonusers of LABA	-	no	438	22	(12 to 33)	31	(22 to 41)	39	(27 to 50)	**0.004**
users of LABA	-	yes	83	7	(33 to +20)	18	(44 to +9)	18	(48 to +12)	0.338
nonusers of ICS or LABA	no	no	258	24	(11 to 38)	32	(21 to 44)	40	(26 to 55)	0.022
nonusers of ICS + users of LABA	no	yes	14	+13	(55 to +81)	47	(122 to +28)	25	(115 to +66)	0.228
users of ICS + nonusers of LABA	yes	no	178	18	(0.7 to 36)	30	(13 to 46)	39	(19 to 59)	0.028
users of ICS and LABA	yes	yes	68	10	(42 to +22)	17	(48 to +14)	10	(45 to +25)	0.92

*Linear regression models were adjusted for sex, age, height, current, former or never tobacco smoking and FEV1 at baseline (1991). P-trend was calculated by linear regression assuming an additive model and modelling the categorical ADRB2 genotype variable as continuous. In bold, p-values that passed Bonferroni correction for multiple testing ($p<0.05/9=0.0056$). + is used to indicate where there is an increase (not a decline) in FEV1. P-for interaction between Gly16Arg genotypes and ICS was p=0.9, between Gly16Arg genotypes and LABA was p=0.8, and between LABA and ICS was p=0.5.

Bronchial hyperresponsiveness

The prevalence of BHR was significantly different between genotypes with increased prevalence with each Arg allele (table 1). However, only 375 from the total 604 asthmatics included in our study completed the methacholine test and were included in this analysis. When further investigating this association, taking into account the potential for confounding, we found that Arg/Arg had an increased risk of BHR, with an odds ratio of OR=2.11 (1.15-3.89, p=0.01) as compared to Gly/Gly subjects, and this risk increasing to OR=2.51(1.12-5.63, p=0.025) if they did not use ICS and the estimates remained the same whether they used LABA or not, although only nonusers of LABA remained statistically significant. Similar findings were obtained when evaluating dose-response slope with significant differences between Arg/Arg and Gly/Gly when ICS or LABA were not used (see table E2, Figure 1 and 2 in the online supplement).

DISCUSSION

This study evaluated asthma control, decline in lung function and BHR in relation to the ADRB2 Gly16Arg genotype and the interactions of this gene with asthma medication among subjects with asthma participating in the ECRHS prospective cohort study. An increased risk of having non-controlled asthma and a steeper lung function decline were associated with the ADRB2 Arg allele, supporting previous findings.[5,19] The relationship between Gly16Arg genotypes and asthma control was mostly observed among subjects not using ICS and was not different among subjects taking LABA and those not taking them, in accordance with recent results by Bleecker et al. [9] Our results also support the idea that there is no need to avoid LABA therapy in patients with asthma with the Arg/Arg as suggested by the LARGE trial.[8] In nonusers of ICS, Gly/Gly genotype was protective for asthma control. Unlike asthma control, ICS use did not modify the impact of genotype on longitudinal FEV1 decline. Airway hyperresponsiveness was not different between users and nonusers of LABA within Arg/Arg or Gly/Gly genotypes and these results do not confirm recent findings in the LARGE trial, on the contrary we did find differences between genotypes within nonusers of LABA and ICS, with Arg/Arg subjects having an increased risk of BHR as compared to Gly/Gly subjects. We did not find any differences in BHR among Gly/Gly subjects as reported in the LARGE trial.[8]

Our results also suggest that these genotypic effects on asthma control are not present among users of ICS. One explanation of this could be a reduction of agonist tolerance associated with ICS use, as suggested by experimental data. [4] Airway smooth muscle tone is controlled by Gsα-coupled receptors (i.e. β2-AR) and Gsq-coupled receptors producing relaxation and contraction, respectively.[20] Acute desensitization occurs through phosphorilation of the receptor by G protein-coupled receptor kinases (GRKs) in presence of agonists, and by protein kinase A and protein kinase C in absence of agonists. As a consequence the β2-AR is decoupled from the G-protein. Desensitization over the longer term is associated with a decrease in receptor number as a result of decreased mRNA expression and increased receptor degradation and recycling.[21] It has been suggested that genetically mediated paradoxical bronchial obstruction or hyperresponsiveness may occur with long-term use of beta-agonists. [22] Steroids have shown in experimental *in vitro* and *in vivo* studies to reverse functional desensitization of β2-AR,[4, 23,24] increase receptor expression and density, and enhance expression of GTP-binding protein alpha subunit (Gsα) producing a dose-dependent increase in cAMP levels.[25,26] However, in humans, loss in bronchoprotection to regular administered β2-agonists seems to reverse only with acute high doses of ICS, [27-28] and it is not clear that this happens with chronic use of ICS

at low or medium doses.[29-31] Recent findings suggest that the mechanism by which IC+LABA therapy exerts its synergistic beneficial effects is through an increased anti-inflammatory activity and an attenuation of airway remodelling.[32] Additionally, although a post-hoc finding and high number of missing values in this variable, an increased risk of BHR among carriers of the Arg allele and no interaction with ICS use suggests that BHR may occur through persistent activation of β2-AR by LABA. β2-AR is a Gsα-coupled receptor and persistent activation may lead not only to reduced bronchial relaxation over time but also as suggested by McGraw et al, to a cross-talk between Gsα and Gsq pathways that would lead to phospholipaseCbeta increased expression, increased inositol 1,4,5 triphosphate production (IP3) and Ca^{2+} release, inducing an increased smooth muscle contraction. [20]

Our study has several strengths and limitations. Our sample size may be considered small and the confidence intervals too wide. However, using the additive model, 0.37 cases per control with complete data (N=572), a baseline asthma control disease risk among Gly/Gly subjects of 0.67, and a mean decline in FEV1 of 25 ml we had 80% power to detect an effect measure of OR=1.47 in asthma control and a 7.5 mL/year difference in FEV1 decline. Estimates for users of LABA alone (without ICS) had large standard errors due to small sample sizes and are hard to interpret, although they go in the same direction as results from recent randomized clinical trials. [8, 9] Similarly, when evaluating partially controlled and uncontrolled asthma separately, results do not seem to be additive although this may be due to small numbers in some of the subgroups evaluated. Confounding cannot be ruled out as an alternative explanation for our results. However, analyses were adjusted for known potential confounders and the genotypic groups were comparable for most of the basic demographic characteristics. Similarly, the impact of confounding due to population stratification in this population was assessed in a previous studies and found to be small.[17,18] Thus, our conclusions are limited to this population of Caucasians, and additional studies of the protection of ICS may be warranted in populations from other ethnicities. Additionally, to avoid spurious associations due to multiple comparisons we performed the minimum number of statistical tests that were needed to answer our study questions. Restriction of the analysis to current physician-diagnosed asthmatics and similarity between cases and controls for the outcome variables ensured that we were not evaluating patients other than asthmatics. We acknowledge that this is a very mild population of asthmatics at baseline and at follow-up with a mean FEV1 ~97% predicted and preferentially rare and occasional symptoms at both time periods and this may restrict the generalizability of our results to mild-moderate asthmatics only. Measurement of FEV1 in two time points may be inaccurate, however we do not expect misclassification to be differential between genotypic groups. Duration of follow-up was taken into account to evaluate decline in FEV1 and analyses were also adjusted for initial FEV1 levels. Sensitivity analysis excluding SABA use from asthma control definition was performed and no change in the results was obtained, excluding use of SABA as an explanation for our results. Finally, assessment of drug exposure is likely to have been affected by some measurement error in our study since drug use during the last 12 months was defined independently of the dose and duration of use and based on patients recall. However, measurement error was likely comparable across genotypes and should not have jeopardized our results. Furthermore, the ability to detect a protective effect of ICS even with the limitations of drug exposure assessment may reflect the potency and importance of ICS. The observational nature of this study does not completely exclude potential for confounding when evaluating the effect of drugs use. At the same time, the prospective nature of this study with

adequately long follow-up and minimal losses to follow-up is a major strength and provides a unique setting to evaluate genetic effects on lung function decline over a period of 9 years.

In conclusion, in this large population-based prospective cohort study, the ADRB2 gene Glycine to Arginine substitution at codon 16 was associated with an increased risk of having poorly controlled asthma, an accelerated longitudinal lung function decline and a higher prevalence of airway hyperresponsiveness among physician-diagnosed asthmatics. Genotypic effects on asthma control were not present among ICS users and this may be due to reversed ADRB2 desensitization.

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