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ADRB2 Gly16Arg polymorphism, asthma control and lung function decline

C. Rebordosa, M. Kogevinas, S. Guerra, F. Castro-Giner, D. Jarvis, L. Cazzoletti, I. Pin, V. Siroux, M. Wjst, J.M. Antò, R. de Marco, X. Estivill, A.G. Corsico, R. Nielsen and C. Janson

ABSTRACT: Arg/Arg homozygotes for the Gly16Arg polymorphism in the β_2-adrenoreceptor gene (*ADRB2*) have a reduced response to short-acting β_2-agonists but no effect has been associated with long-acting β_2-agonists (LABAs).

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

There was an increased risk of noncontrolled asthma (OR 1.33, 95% CI 1.01–1.75; p=0.046) for each Arg allele. Among nonusers of ICS, the odds ratio of noncontrolled asthma among Arg/Arg *versus* Gly/Gly subjects was 2.73 (95% CI 1.28–5.82; p=0.009). No increased risk of noncontrolled asthma associated with the Arg allele was observed among ICS and/or LABA users. For each Arg allele, a mean±SE decrease in decline in forced expiratory volume in 1 s of 7.7±2.5 mL·yr^{-1} was found (p-value for trend 0.003), irrespective of ICS or LABA use. Arg/Arg subjects had an increased risk of bronchial hyperresponsiveness (BHR) *versus* Gly/Gly subjects, with an odds ratio of 2.51 (95% CI 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 BHR. Absence of genotypic effects on asthma control among ICS users may be due to reversed β_2-adrenoreceptor desensitisation.

KEYWORDS: Asthma control, β_2-adrenoreceptor polymorphisms, bronchial hyperresponsiveness, corticosteroids, lung function

Asthma is a complex disease characterised by reversible airflow obstruction, hyperresponsiveness, airway remodelling and inflammation. In genetically predisposed individuals, environmental factors, such as viral infections or bacterial lipopolysaccharide, may modify the likelihood of developing asthma [1]. Genes such as the β_2-adrenergic receptor gene (*ADRB2*) may modify the response to therapy among asthmatics. *ADRB2* is located on chromosome 5q31–q32 and encodes the β_2-adrenergic receptor (β_2-AR), a G-protein-coupled receptor that is expressed in airway smooth muscle and induces bronchial relaxation [2]. *In vitro* studies have shown that the nonsynonymous single-nucleotide polymorphism at position 46 (rs1042713; herein referred to as Gly16Arg) in the *ADRB2* gene shows an enhanced agonist-promoted downregulation [3]. *In vitro* studies evaluating concomitant use of steroids and β_2-agonists also suggest that inhaled corticosteroids (ICSs) may counteract β_2-AR desensitisation [4]. *In vivo* evidence suggests the presence of a differential response to short-acting β_2-agonist (SABA) treatment according to *ADRB2* Gly16Arg genotype [5]. Other studies have found similar differential responses among Arg/Arg homozygotes regularly treated with long-acting β_2-agonists (LABAs) [6, 7]. However, recent randomised clinical trials show that there is no pharmacogenetic short-term effect associated with LABA use [8, 9]. The long-term consequences of this polymorphism in lung function and whether ICSs may counteract β_2-AR desensitisation have 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 ICSs or LABAs.

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AFFILIATIONS
For a full list of the authors' affiliations, see the Acknowledgements section.

CORRESPONDENCE
C. Rebordosa
Centre for Research in Environmental Epidemiology
Hospital del Mar Research Institute (IMIM)
88 Dr. Aigüader Road
E-08003
Barcelona
Spain
E-mail: crebordo@email.arizona.edu

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METHODS

Study population and design

The European Community Respiratory Health Survey (ECRHS) is a multicentre, longitudinal cohort study that recruited 18,811 subjects in 1991 (ECRHS I) and followed an eligible sample of them (59%) up to 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. Of the 10,933 subjects who participated in ECRHS II, 5,065 subjects who had DNA samples available were genotyped for several polymorphisms, including the *ADRB2* Gly16Arg polymorphism, as part of a more extensive genotyping project. There was no statistically significant differences in the proportion of subjects with asthma or physician-diagnosed asthma between those subjects genotyped and those who were not genotyped. From the 5,065 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 previous 12 months. We excluded all nonasthmatic subjects and those asthmatics who did not fall within our definition of current physician-diagnosed asthma. The mean$\pm$SE follow-up for these subjects was 8.8$\pm$0.7 yrs.

Outcomes and exposures definition

In the assessment of asthma severity, the 2006 Global Initiative for Asthma (GINA) 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 the 2006 GINA guidelines and was previously assessed in this population [12]. Asthma was classified in ECRHS II as controlled if all of the following features were present: diurnal symptoms less than once a week and no asthma attacks in the previous 3 months; no activity (work and other activities) limitations in the previous 12 months; no nocturnal symptoms in the previous 3 months: SABAs twice or less per week in the previous 3 months; no use of oral steroids in the previous 12 months; and forced expiratory volume in 1 s (FEV_1) $\geqslant$80% predicted. Asthma was considered partly controlled if one or two of these features were absent, and uncontrolled if more than two features were absent or if: asthma, shortness of breath or wheezing had caused hospital/emergency department admissions in the previous 12 months; oral steroids were used in short courses or continuously in the last 12 months; or the subject had >12 asthma attacks (one or more per week) in the previous 3 months. In some of the multivariate analyses, partially controlled and uncontrolled asthma were grouped together as ''noncontrolled asthma''. Estimates of risk associated with the Gly16Arg polymorphism for each of these two categories can be found in the online data supplement. Lung function decline was defined as FEV_1 decline in millilitres per year of follow-up between the two surveys. Subjects were defined as having bronchial hyperresponsiveness (BHR) if they had a $\geqslant$20% decrease in FEV_1 with a methacholine dose $\leqslant$1 mg, measured by methacholine challenge test. If the total dose of 1 mg was taken and the FEV_1 fall was <20%, the subject was considered to have no BHR. The methacholine challenge dose–response slope was transformed as 100/log-slope+10 to normalise the data, as previously performed by CHINN *et al.* [13]. Use of inhaled SABAs, LABAs and ICSs were defined as answering ''yes'' to having used the drug in the previous 12 months. Use of ICSs during the whole period within the two surveys was also assessed, as described by DE MARCO *et al.* [14].

Genotyping

Genotyping and quality control for the rs1042713 polymorphism in the *ADRB2* gene were 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

The Chi-squared test (for categorical variables) and ANOVA (for continuous variables) were used to test differences in sociodemographic and clinical characteristics by genotype group. Odds ratios (ORs) of noncontrolled asthma were estimated using logistic regression models. Controlled asthma was used as the reference group to compute relative risks ratios 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 FEV_1. 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-squared exact test. Potential confounders were selected *a priori* based on a 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 <10% change in the genotype effects, except for age and sex, which were forced into the models. Final models were adjusted for age, and sex, body mass index and BHR in the analysis of asthma control, and height, baseline FEV_1, and history of current, former or never tobacco smoking in the analysis of FEV_1 decline. Interactions between ICSs, LABAs and genotypes were tested and defined as significant if $p<0.05$. 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 two previous studies using a genomic control approach [16] and the EIGENSTRAT method [17]. Both methods found no evidence of population stratification with a λ of 1.06 for asthma using the genomic control approach [15] and no subdivisions of populations in the EIGENSRAT analysis [18]. STATA 10 SE (StataCorp, College Station, TX, USA) was used to perform statistical analyses.

RESULTS

Mean$\pm$SD age at the time of the ECRHS II interview was 42$\pm$7.3 yrs and 59% (n=356) of the participants were female. Of the 604 current physician-diagnosed asthmatics, 37% (n=221) were homozygous for the major *ADRB2* allele (Gly/Gly), 46% (n=277) were heterozygous (Gly/Arg) and 18% (n=106) were homozygous for the minor allele (Arg/Arg). Overall, 46.5% (n=281) of asthmatics used ICSs during the previous 12 months, 27.5% (n=166) used ICSs every year during the period between the two surveys, 60.6% (n=358) used SABAs and 16.7% (n=100) used LABAs during the

previous 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 Gly/Gly (45.7%) genotypes (p=0.04). No other statistically significant differences in the distribution of sociodemographic and clinical variables at baseline (1991) and at follow-up (1999) were found between *ADRB2* genotypes (table 1), or between the genotyped and nongenotyped populations (data not shown). At baseline, 65% of the subjects had rare or occasional symptoms, only 9% of the subjects had used oral steroids the previous 12 months and <3% of the subjects had FEV1 ⩽60% pred.

Asthma control

Among the 604 current physician-diagnosed asthmatics, 27.3% (n=156) were considered to have controlled asthma and 72.7% (n=416) noncontrolled asthma at the time of the ECRHS II interview. Among noncontrolled asthmatics, 57.7% (n=240) subjects had partially controlled asthma and 42.3% (n=176) uncontrolled asthma. 32 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).

There was an increased risk of noncontrolled asthma per each Arg allele (OR 1.33, 95% CI 1.01–1.75; p=0.046) (table 3). There was a statistically significant interaction between ICS use and *ADRB2* genotype (p=0.046) on the risk of having noncontrolled asthma. Subjects with the Arg/Arg genotype not using ICSs during the previous 12 months showed a nearly threefold increased risk (OR 2.73, 95% CI 1.28–5.82; p=0.009) of noncontrolled 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 ICSs and LABAs, we observed no differences between Gly16Arg genotypes within users of ICSs, irrespective of LABA use. Among nonusers of ICSs and LABAs, there was an increased OR of 1.61 (95% CI 1.11–2.35; p=0.013) for each Arg allele increase (table 3). Due to small numbers in the users of LABAs alone, no ORs were computed; however, the p-value for the Fisher exact test was 0.1, with 100% (n=12) of the subjects in the Gly/Arg or Arg/Arg groups having noncontrolled asthma *versus* 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 noncontrolled, partially controlled and uncontrolled asthma (see table E1 in the online supplement).

TABLE 1 Distribution of the main sociodemographic and clinical characteristics in subjects with current physician-diagnosed asthma according to *ADRB2* Gly16Arg genotype in the European Community Respiratory Health Survey (ECRHS) II population[#]

	Gly/Gly	Gly/Arg	Arg/Arg	p-value
Subjects	221	277	106	
Age yrs	42.6±7.6	42.2±7.2	41.8±7.4	0.65
Age of onset <16 yrs	84 (38.9)	13 (49.1)	44 (41.5)	0.07
Females	127 (57.2)	168 (60.6)	61 (57.6)	0.73
Body mass index kg·m^{-2}	26.4±5.8	26.0±5.0	25.8±5.2	0.55
Smoking				
Current	65 (29.7)	79 (28.8)	25 (23.8)	0.16
Former	71 (32.4)	66 (24.1)	32 (30.5)	
Never	83 (37.9)	129 (47.1)	48 (45.7)	
Inhaled SABAs	126 (58.3)	160 (59.5)	72 (67.9)	0.23
Inhaled LABAs	38 (17.5)	41 (14.9)	21 (19.8)	0.48
Inhaled steroids	107 (49.5)	120 (43.6)	54 (50.9)	0.29
FEV1 % pred				
At ECRHS I	98.04±18.1	97.6±17.2	94.7±18.0	0.25
At ECRHS II	96.80±16.3	97.6±15.8	95.1±16.5	0.42
BHR	64 (45.7)	95 (55.6)	41 (64.1)	**0.04**
Total IgE >100 kU·L^{-1}	97 (44.9)	148 (54.2)	59 (55.7)	0.07
Chronic cough or phlegm	56 (25.9)	80 (29.3)	30 (29.1)	0.69
Allergic rhinitis	111 (50.7)	168 (61.1)	58 (54.7)	0.06

Data are presented as n, mean±SD or n (%), unless otherwise stated. SABA: short-acting β_2-agonist; LABA: long-acting β_2-agonist; FEV1: forced expiratory volume in 1 s; % pred: % predicted; BHR: bronchial hyperresponsiveness; Ig: immunoglobulin. Bold indicates statistically significant p-values. #: n=604.

Decline in FEV1

The mean±SE decline in FEV1 between ECRHS I and II was 24.6±44.9 mL·yr^{-1} among the 527 subjects with data available in both studies, with a mean duration of follow-up of 8.8±0.7 yrs. *ADRB2* genotype was not associated with FEV1 at the end of follow-up (1999) (p-value for trend (p_{trend})=0.2) or 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·yr^{-1} steeper than that of carriers of the Gly/Gly genotype, respectively (table 4). Similarly, per Arg allele, there was a decrease in FEV1 of 7.7±2.5 mL·yr^{-1} (p=0.003). Reductions in FEV1 among Arg/Arg subjects were observed for nonuse of ICS and nonuse of LABA. Nonusers of LABAs carrying the Arg/Arg genotype had reductions in FEV1 over the 9-yr follow-up that were almost double those of Gly/Gly subjects (22 *versus* 39 mL·yr^{-1}; 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. A similar pattern was seen when evaluating ICS use for each year during the period between the two surveys (data not shown).

Bronchial hyperresponsiveness

The prevalence of BHR was significantly different between genotypes with increased prevalence per Arg allele (table 1). However, only 375 out of 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 OR of 2.11 (95% CI 1.15–3.89; p=0.01) as compared with Gly/Gly subjects; this OR increased to 2.51 (95% CI 1.12–5.63; p=0.025) if they did not use ICS, and the estimates remained the same whether they used LABAs or not, although only nonusers of LABAs remained statistically significant. Similar findings were obtained when evaluating dose–response slope with significant differences between Arg/Arg and Gly/Gly when ICSs or LABAs were not used (see table E2, and figs 1 and 2 in the online supplement).

TABLE 2 Distribution of asthma control and the clinical features used to define asthma control among subjects with current physician-diagnosed asthma according to *ADRB2* Gly16Arg genotype in the European Community Respiratory Health Survey (ECRHS) II population[#]

	Gly/Gly	Gly/Arg	Arg/Arg
Subjects	221	277	106
Asthma control			
Controlled	67 (42.95)	67 (42.95)	22 (14.10)
Noncontrolled	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 previous 3 months ⩾1 per week	53 (24.2)	82 (29.9)	29 (27.4)
Asthma attacks in the previous 3 months			
Yes	64 (29.9)	95 (35.1)	35 (33)
⩾1 per week	27 (12.2)	33 (11.9)	12 (11.3)
Nocturnal symptoms in the previous 3 months	69 (31.5)	99 (36.3)	29 (27.6)
Activity limitation in the previous 3 months	51 (24.3)	70 (27)	23 (23)
SABAs in the previous 3 months			
>2 per week	60 (29.8)	76 (29.6)	33 (32.7)
Use of oral steroids in the previous 3 months			
Yes	13 (6)	20 (7.2)	10 (9.5)
Short courses or continuous	10 (4.6)	11 (4)	5 (4.8)
FEV_1 <80% pred at ECRHS II	32 (15)	37 (14.1)	20 (19.2)
Emergency department visit in the previous 12 months	10 (4.6)	18 (6.5)	7 (6.6)
Hospitalisation in the previous 12 months	2 (0.9)	2 (0.7)	0 (0)

Data are presented as n or n (%). SABA: short-acting β_2-agonist; FEV_1: forced expiratory volume in 1 s; % pred: % predicted. [#]: n=604.

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 noncontrolled asthma and a steeper lung function decline were associated with the *ADRB2* Arg allele, supporting previous findings [5, 19].

TABLE 3 Odds ratios of noncontrolled asthma according to *ADRB2* Gly16Arg genotypes, and stratified by inhaled corticosteroid (ICS) and long-acting β_2-agonist (LABA) use in the previous 12 months as reported in the European Community Respiratory Health Survey II population[#]

			Risk of noncontrolled asthma					
	ICS use	LABA use	Per Arg allele[¶]			Among Arg/Arg[+]		
			Subjects n	OR (95% CI)	p-value	Subjects 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
ICS nonusers	No		287	1.76 (1.21–2.54)	**0.003**	146	2.73 (1.28–5.82)	**0.009**
ICS users	Yes		266	1.02 (0.63–1.68)	0.92	151	1.06 (0.38–2.94)	0.91
LABA nonusers		No	462	1.35 (1.00–1.82)	0.05	245	1.81 (0.96–3.41)	0.07
LABA users		Yes	90	1.27 (0.56–2.91)	0.57	52	1.37 (0.27–6.92)	0.70
ICS or LABA nonusers	No	No	273	1.61 (1.11–2.35)	0.013	139	2.32 (1.07–5.04)	0.033
ICS nonusers + LABA users	No	Yes	16	NA	NA	8	NA	NA
ICS users + LABA nonusers	Yes	No	188	1.06 (0.58–1.93)	0.86	106	1.23 (0.34–4.54)	0.75
ICS and LABA users	Yes	Yes	76	0.97 (0.42–2.28)	0.95	45	0.88 (0.16–4.82)	0.89

Model adjusted for sex, age, body mass index and bronchial hyperresponsiveness. Additive models were estimated by modelling the categorical *ADRB2* genotype variable as continuous. Bold indicates p-values that passed Bonferroni correction for multiple testing (p<0.05/9=0.0056). NA: not available. [#]: n=604; [¶]: additive model; [+]: *versus* Gly/Gly.

TABLE 4 Decline in forced expiratory volume in 1 s (FEV_1) from the European Community Respiratory Health Survey I to II, according to *ADRB2* Gly16Arg genotypes, and stratified by inhaled corticosteroid (ICS) and long-acting β_2-agonist (LABA) use[#]

	ICS use	LABA use	FEV1 decline[¶] mL·yr^{-1}				
			Subjects	Gly/Gly	Gly/Arg	Arg/Arg	p_{trend}
All asthmatics			519	21 (11–30)	29 (201–38)	36 (25–46)	**0.003**
ICS nonusers	No		272	21 (8–34)	31 (20–42)	39 (25–53)	0.007
ICS users	Yes		248	17 (1–32)	28 (13–42)	31 (14–48)	0.062
LABA nonusers		No	438	22 (12–33)	31 (22–41)	39 (27–50)	**0.004**
LABA users		Yes	83	7 (33–20)	18 (44– +9)	18 (48– +12)	0.338
ICS or LABA nonusers	No	No	258	24 (11–38)	32 (21–44)	40 (26–55)	0.022
ICS nonusers + LABA users	No	Yes	14	+13 (55– +81)	47 (122– +28)	25 (115– +66)	0.228
ICS users + LABA nonusers	Yes	No	178	18 (0.7–36)	30 (13–46)	39 (19–59)	0.028
ICS and LABA users	Yes	Yes	68	10 (42– +22)	17 (48– +14)	10 (45– +25)	0.92

Data are presented as n or mean (95% CI), unless otherwise stated. The p-value for the trend (p_{trend}) was calculated by linear regression assuming an additive model and modelling the categorical *ADRB2* genotype variable as continuous. Bold indicates p-values that passed Bonferroni correction for multiple testing ($p<0.05/9=0.0056$). ''+'' indicates an increase (not a decline) in FEV_1. The p-value for the 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. [#]: n=519; [¶]: linear regression models were adjusted for sex, age, height, current, former or never tobacco smoker and FEV_1 at baseline (1991).

The relationship between Gly16Arg genotypes and asthma control was mostly observed among subjects not using ICS, and was not different among subjects taking LABAs 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 Long-Acting β-Agonist Response by Genotype (LARGE) trial [8]. In nonusers of ICS, the Gly/Gly genotype was protective for asthma control. Unlike asthma control, ICS use did not modify the impact of genotype on longitudinal FEV_1 decline. Airway hyperresponsiveness was not different between users and nonusers of LABAs within the 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 with 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 guanosine triphosphate-binding protein (G_s)α- (*i.e.* β_2-AR) and G_sq-coupled receptors producing relaxation and contraction, respectively [20]. Acute desensitisation occurs through phosphorylation of the receptor by G-protein-coupled receptor kinases in the presence of agonists, and by protein kinases A and C in the absence of agonists. As a consequence, the β_2-AR is decoupled from the G-protein. Desensitisation 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 β-agonists [22]. Steroids have shown in experimental *in vitro* and *in vivo* studies to reverse functional desensitisation of β_2-AR [4, 23, 24], increase receptor expression and density, and enhance expression of $G_s\alpha$, producing a dose-dependent increase in cyclic adenosine monophosphate levels [25, 26]. However, in humans, loss of bronchoprotection from regularly administered β_2-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 ICS plus LABA therapy exerts its synergistic beneficial effects is through an increased anti-inflammatory activity and an attenuation of airway remodelling [32]. Additionally, despite 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 suggest that BHR may occur through persistent activation of β_2-AR by LABAs. β_2-AR is a $G_s\alpha$-coupled receptor and persistent activation may lead not only to reduced bronchial relaxation over time but also, as suggested by MCGRAW *et al.* [20], to a cross-talk between $G_s\alpha$ and G_sq pathways that would lead to increased phospholipase Cβ expression, increased inositol 1,4,5-triphosphate production and Ca^{2+} release, inducing increased smooth muscle contraction.

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 FEV_1 of 25 mL, we had 80% power to detect an effect measure of OR 1.47 in asthma control and a 7.5 mL·yr^{-1} difference in FEV_1 decline. Estimates for users of LABAs alone (without ICSs) 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 randomised clinical trials [8, 9].

Similarly, when evaluating partially controlled and uncontrolled asthma separately, the 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 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 FEV_1 of ~97% pred, and preferentially rare and occasional symptoms at both time periods, and this may restrict the generalisability of our results to mild–moderate asthmatics only. Measurement of FEV_1 in two time-points may be inaccurate; however, we do not expect misclassification to be different between genotypic groups. Duration of follow-up was taken into account to evaluate decline in FEV_1 and analyses were also adjusted for initial FEV_1 levels. Sensitivity analysis excluding SABA use from the 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 previous 12 months was defined independently of the dose and duration of use, and based on patients' recall. However, measurement error was probably comparable across genotypes and should not have jeopardised 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 yrs.

In conclusion, in this large, population-based, prospective cohort study, the *ADRB2* gene Gly16Arg substitution 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 β_2-AR desensitisation.

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STATEMENT OF INTEREST

A statement of interest for R. Nielsen can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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The authors' affiliations are: C. Rebordosa, Centre for Research in Environmental Epidemiology and Hospital del Mar Research Institute (IMIM), Barcelona, Spain, and Arizona Respiratory Center, University of Arizona, Tucson, AZ, USA; M. Kogevinas, Centre for Research in Environmental Epidemiology and Hospital del Mar Research Institute (IMIM), Barcelona, and CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain, and National School of Public Health, Athens, Greece; S. Guerra, Centre for Research in Environmental Epidemiology and Hospital del Mar Research Institute (IMIM), Barcelona, and CIBERESP, Madrid, Spain; F. Castro-Giner, Centre for Research in Environmental Epidemiology and Hospital del Mar Research Institute (IMIM), Barcelona, Spain; D. Jarvis, Respiratory Epidemiology and Public Health Group, National Heart and Lung Institute, Imperial College, London, UK; L. Cazzoletti, Dept of Medicine and Public Health, University of Verona, Verona, Italy; I. Pin, Centre Hospitalier Universitaire de Grenoble, Grenoble, France; V. Siroux, INSERM U823, Université Joseph Fourier, Grenoble, France; M. Wjst, Institute of Lung Biology and Disease/Molecular Pneumology, Helmholtz Zentrum München, Munich-Neuherberg, Germany; J.M. Antò, Centre for Research in Environmental Epidemiology and Hospital del Mar Research Institute (IMIM), Barcelona, Spain; R. de Marco, Dept Medicina e Sanità pubblica, Sezione di Epidemiologia e Statistica Medica, Verona, Italy; X. Estivill, Genes and Disease Program, Center for Genomic Regulation, Barcelona, Spain; A.G. Corsico, Division of Respiratory Diseases, IRCCS San Matteo Hospital Foundation, University of Pavia, Pavia, Italy; R. Nielsen, Dept of Thoracic Medicine, Haukeland University Hospital, Bergen, Norway; and C. Janson, Dept of Medical Sciences, Uppsala University, Uppsala, Sweden.

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