

Adrenergic β_2 -receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol

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Background: On-demand inhaled albuterol is commonly prescribed worldwide. We have shown that the Arg16 allele of the adrenergic β_2 -receptor agonist gene (*ADRB2*) predisposes to exacerbations in young asthmatic patients taking regular salmeterol.

Objective: We have now extended our previous population by 636 patients and explored the role of the Arg16 allele on asthma exacerbations in the context of the use of on-demand albuterol and regular salmeterol.

Methods: Arg/Gly status at position 16 of *ADRB2* was assessed in 1182 young asthmatic patients (age, 3-22 years) from Scotland. Asthma exacerbations, use of β -agonists and other medications over the previous 6 months, and lung function were also studied.

Results: An increased risk of exacerbations per copy of the Arg16 allele was observed in asthmatic patients, regardless of treatment regimen (odds ratio [OR], 1.30; 95% CI, 1.09-1.55; $P = .003$). This appears to be largely due to exposure to β_2 -agonists because the risk of exacerbations observed in patients with the Arg16 allele was only observed in those receiving daily inhaled long- or short-acting β_2 -agonist treatment (OR, 1.64; 95% CI, 1.22-2.20; $P = .001$). In contrast, there was no genotypic risk for exacerbations in patients using inhaled β_2 -agonists less than once a day (OR, 1.08; 95% CI, 0.85-1.36; $P = .525$). The Arg16 genotype-associated risk for exacerbations

was significantly different in those exposed to β_2 -agonists daily versus those that were not (test for interaction, $P = .022$).

Conclusion: The Arg16 genotype of *ADRB2* is associated with exacerbations in asthmatic children and young adults exposed daily to β_2 -agonists, regardless of whether the exposure is to albuterol or long-acting agonists, such as salmeterol. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, child, polymorphism, asthma exacerbations, albuterol, salmeterol, β_2 -adrenoceptor, adrenergic β_2 -receptor agonist gene

Asthma is one of the most common chronic diseases in the world.^{1,2} The on-demand use of inhaled short-acting β_2 -agonists during asthma exacerbations is recommended by national guidelines and represents the cornerstone of asthma management worldwide.³⁻⁶ We have previously shown that there is an increased risk for exacerbations in asthmatic children and young adults homozygous for the Arg16 variant of the Arg16Gly *ADRB2* polymorphism.⁷ In addition, the risk of possessing the Arg16 polymorphism was significant in asthmatic patients taking regular inhaled long-acting β_2 -agonists with a gene/dosage effect of the Arg16 variant.⁷

Irrespective of regular salmeterol use, asthmatic patients are routinely advised to take inhaled short-acting β_2 -agonists on demand, such as before exercise, after allergen or cold air exposure, or when they are experiencing exacerbations.^{3,5,6} This is a treatment strategy that is globally adopted in current guidelines; regularly scheduled use of albuterol is no longer recommended because it might aggravate underlying airway hyperreactivity.^{3,8} Several studies on older adults have suggested that homozygous Arg16 status is associated with reduced peak flows and increased exacerbations in asthmatic patients treated with regularly scheduled but not on-demand short-acting β_2 -agonists.⁹⁻¹² However, the role of the Arg16 allele on exacerbations in steroid-treated asthmatic children using on-demand short-acting β_2 -agonists *per se* has not been assessed.

Asthma exacerbations, which can be severe, account for the largest proportion of health costs of asthma.¹³ In children with asthma, school absences caused by asthma,¹⁴ use of short courses of oral steroids,^{3,15} and asthma-related hospital admissions¹⁶ represent well-validated measures of asthma exacerbations. We have combined these measures to develop a tool for defining asthma exacerbations that we have now validated through several studies.^{7,17-22} In respect to our previous study,⁷ we have now doubled the size of our cohort, aiming to address a number of additional questions, and have examined the association of the Arg16 variant with exacerbations on exposure to both long- and short-acting β_2 -agonists.

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Abbreviations used

ADRB2: Adrenergic β_2 -receptor agonist gene
 BTS: British Thoracic Society
 OR: Odds ratio

METHODS

We have continued the recruitment of children with physician-diagnosed asthma for the BREATHE study beyond the publication of our initial results.⁷ The current dataset includes information about demographic, anthropometric, and clinical details from 1182 patients attending 29 primary care practices and 2 secondary care asthma clinics in Tayside, Scotland, from 2004–2008 (age, 3–22 years).

The study was approved by the Tayside Committee on Medical Research and Ethics. Informed consent was provided by the patients and parents/guardians as relevant. The methods have been described in detail.^{21,22} The patients were seen in the asthma clinic setting, in which a detailed history was obtained, including information on school absences, use of oral steroids, and hospital admissions over the previous 6 months. For simplicity and greater accuracy through recall, only yes/no responses for any of the 3 options were used for analysis.

The asthma prescribing level was determined in accordance with the British Thoracic Society (BTS)³ guidelines for physician-led management of asthma, as follows: step 0, no use of inhaled albuterol on demand within the past month; step 1, inhaled albuterol on demand; step 2, regular inhaled steroids plus inhaled albuterol on demand; step 3, regular inhaled salmeterol plus inhaled steroids with inhaled albuterol on demand; and step 4, regular inhaled salmeterol plus inhaled steroids plus oral montelukast with inhaled albuterol on demand. From these data, a global index of asthma severity was derived through construction of a composite variable, as reported in our previous publications.¹⁸ Pulmonary function was measured by means of spirometry as per the standard procedure described previously.¹⁸

A DNA sample was collected by using mouthwash after informed consent was provided by the patient and the parent/guardian. DNA was prepared with the Qiagen Dneasy 96 kit (Qiagen GmbH, Hilden, Germany), and genotypes were determined by using Taqman-based allelic discrimination assays on an ABI 7700 sequence detection system.²³ For the Gly16Arg variant, the primers and Gly16 probe were used as before,⁷ and the fluorophore on the Arg16 probe was either Cal Orange or VIC.

All statistical analyses were performed with SPSS for Windows version 15 (SPSS, Inc, Chicago, Ill).

In children with asthma, school absences caused by asthma,¹⁴ use of short courses of oral steroids,^{3,15} and asthma-related hospital admissions¹⁶ represent well-validated measures of asthma exacerbations. We have combined these measures to develop a tool for defining asthma exacerbations that we have now validated through several studies.^{7,17–22} Binary logistic regression was used to calculate odds ratios (ORs) and *P* values for asthma exacerbations. Measures for asthma exacerbations were grouped according to severity to calculate the ORs for comparison of risk. Thus school absences, intake of oral steroids, and admission to the hospital because of severity of asthma symptoms were grouped as present (minimum of once over the previous 6 months) or absent. The total asthma exacerbation response was calculated as any of these measures during the same period. This was again grouped as present or absent.

To explore the associations, we used a codominant model (0 = Gly/Gly16, 1 = Gly/Arg16, and 2 = Arg/Arg16), as has been previously examined.⁷ Age, sex, and exposure to tobacco smoke were included in all models as covariates after stepwise removal procedures (covariates at *P* < .2 were retained). Seasonality, another potential covariate, did not contribute significantly to the model (*P* > .4) and was not associated with genotype in any subgroup tested and hence was excluded from the final analysis.

We tested the effect of the Arg16 allele on asthma exacerbations in relation to inhaled β_2 -agonist use from a number of different perspectives. First, we tested the overall effect of the Arg16 allele on the individual measures of asthma exacerbations and on the overall risk of exacerbations (Table I).

Second, we also tested the association of the genotype of the adrenergic β_2 -receptor agonist gene (*ADRB2*) and exacerbations over asthma treatment steps 0 to 4 and in participants using regular salmeterol versus those not using regular salmeterol to identify any treatment steps in which the effect could be more prominent than others (Table II and see Table E1 in this article's Online Repository at www.jacionline.org). Finally, we tested the hypothesis that there is an interaction between genotype and the daily use of any inhaled β_2 -agonist (as-required albuterol, regular salmeterol, or both) on the risk of asthma exacerbations (Table III). We also tested the association of the Glu27Gln genotype with exacerbations conditioned on Arg16Gly polymorphic variation (see Table E2 in this article's Online Repository at www.jacionline.org). Significance was assessed at a *P* value of less than .05.

RESULTS

The population characteristics are typical of children and young adults with well-controlled asthma derived from both primary and secondary care (Table IV). We had collected data on 546 children previously⁷ and have now increased our study population to 1190 participants, of whom 1182 were white. Further analysis was performed on data from white participants. The data from other ethnic groups were not sufficient for further analysis. The prevalence of the Arg/Arg16 genotype was 15.3%, the prevalence of the Arg/Gly16 genotype was 43.8%, and the prevalence of the Gly/Gly16 genotype was 40.8% in children and young persons with asthma within Tayside (minor allele frequency, 0.37) and was similar to that observed for the US and United Kingdom populations^{7,10,24} (minor allele frequency of 0.35 and 0.36, respectively^{25,26}; Table V).

The data in Table I demonstrate an increased prevalence of exacerbations in patients per copy of the Arg16 allele in the total population, regardless of medication (OR, 1.30; 95% CI, 1.09–1.55; *P* = .003). An increased risk was also observed per copy of the Arg16 allele for the individual measures of exacerbation, oral steroid intake, and school absences caused by asthma exacerbations over the previous 6 months. However, there was no increase in the risk of hospital admission caused by asthma exacerbations in the children with the Arg/Arg16 genotype. The Glu/Gln27 genotype did not show any increase in the risk of exacerbations in this population (see Table E2). Because the codominant model provided the best fit of the data based on the highest likelihood ratio obtained for the model (data not shown), this model was used for the remainder of the analysis.

In our present study, which has been extended to 1182 participants, we observed a significant increase in exacerbation risk per copy of the Arg16 allele in participants on treatment step 2 (regular inhaled corticosteroids and inhaled albuterol on demand: Gly/Gly, 97/271 [36%]; Arg/Gly, 112/293 [38%]; Arg/Arg, 47/95 [49%]; OR, 1.32; 95% CI, 1.04–1.67; *P* = .02; Table II). We also observed an increase in exacerbation risk per copy of the Arg16 allele on step 3 (regular inhaled steroids, the long-acting β_2 -agonist salmeterol, and inhaled albuterol on demand: Gly/Gly, 25/63 [40%]; Arg/Gly, 34/65 [52%]; Arg/Arg, 19/29 [65%]; OR, 1.74; 95% CI, 1.09–2.80; *P* = .02; Table II). This effect was not observed in participants with asthma at steps 0, 1, and 4.

These data suggested that exposure to β_2 -agonist *per se* rather than the nature of the β_2 -agonist was most relevant, and therefore we studied the association of the Arg/Gly16 genotype for asthma exacerbations in patients with infrequent versus daily exposure to albuterol, salmeterol, or both (Table III). In asthmatic participants taking any inhaled β_2 -agonist less than once a day, there was no effect of Arg16 allele copy on the risk of exacerbations. In

TABLE I. Overall effect of the Arg/Gly16 genotype on oral steroid intake, school absences, and hospital admissions caused by asthma exacerbations and overall asthma exacerbations in children and young adults with asthma regardless of treatment

		Genotype				OR (95% CI)	P value
		Gly/Gly16	Arg/Gly16	Arg/Arg16	Total		
Oral steroid intake	No	383	404	131	918	1.27 (1.04-1.56)	.02
	Yes	99	113	50	262		
	Total	482	517	181	1180		
School absence caused by asthma exacerbations	No	338	355	107	800	1.29 (1.07-1.54)	.007
	Yes	144	162	74	380		
	Total	482	517	181	1180		
Hospital admission caused by asthma exacerbations	No	417	452	154	1023	1.04 (0.82-1.34)	.73
	Yes	65	65	27	157		
	Total	482	517	181	1180		
Overall asthma exacerbations	No	310	321	95	726	1.30 (1.09-1.55)	.003
	Yes	172	196	86	454		
	Total	482	517	181	1180		

P values were calculated by means of binary logistic regression corrected for age, sex, perennial rhinitis, smoking, and exposure to tobacco smoke. ORs are per copy of the Arg16 allele (additive model).

TABLE II. Association of asthma exacerbations and polymorphisms according to BTS steps of management of asthma

BTS treatment step	Genotype	Exacerbations over previous 6 months			OR (95% CI), P value
		No	Yes	Total	
Step 0	Gly/Gly16	11	2	13	0.62 (0.09-4.22), .63
	Gly/Arg16	19	1	20	
	Arg/Arg16	5	1	6	
	Total	35	4	39	
Step 1	Gly/Gly16	70	19	89	1.02 (0.57-1.83), .93
	Gly/Arg16	71	17	88	
	Arg/Arg16	23	4	27	
	Total	164	40	204	
Step 2	Gly/Gly16	174	97	271	1.32 (1.04-1.67), .02
	Gly/Arg16	181	112	293	
	Arg/Arg16	48	47	95	
	Total	403	256	659	
Step 3	Gly/Gly16	38	25	63	1.74 (1.09-2.80), .02
	Gly/Arg16	31	34	65	
	Arg/Arg16	10	19	29	
	Total	79	78	157	
Step 4	Gly/Gly16	16	26	42	1.41 (0.77-2.55), .26
	Gly/Arg16	19	32	51	
	Arg/Arg16	8	15	23	
	Total	43	73	116	

P values were calculated by means of binary logistic regression corrected for age, sex, perennial rhinitis, smoking, and exposure to tobacco smoke by using a codominant model (ie, a gene/dosage effect for the Arg/Arg16 variant). ORs are per copy of the Arg16 allele. There was no difference in the distribution of the Arg16 variant between the treatment steps (χ^2 test for 8 *df* = 5.8, *P* = .666).

participants using any inhaled β_2 -agonist at least once daily (as-required albuterol taken at least once daily, regular inhaled salmeterol, or both), there was an increased risk of asthma-related exacerbations (OR, 1.64; 95% CI, 1.22-2.20; *P* = .001) per copy of the Arg16 allele. The interaction term between genotype and daily use of any inhaled β -agonists (as-required albuterol, salmeterol, or both) was significant (*P* = .022, Table III). In the group exposed to β_2 -agonists daily, the codominant model of risk was clearly observed, with the Arg/Gly16 heterozygotes having an OR for exacerbations of 1.63 (95% CI, 1.02-2.60; *P* = .04) and the Arg/Arg16 homozygotes having an OR of 2.70 (95% CI,

1.46-4.99; *P* = .002) when compared with the individuals with the common Gly/Gly16 genotype.

The Arg16 variant did not appear to be associated with general asthma severity because there was no difference in the Arg16 allele frequency across the different treatment steps (Table II; χ^2 test for 8 *df* = 5.8, *P* = .666).

The Arg16 variant had no significant effects on measures of pulmonary function (FEV₁, forced vital capacity, and peak expiratory flow; data not shown).

DISCUSSION

The study shows for the first time that there is an increase in risk of exacerbations per copy of the Arg16 allele in children and young adults with asthma taking frequent (once daily or more) as-required doses of inhaled albuterol. This effect is not observed in participants with asthma who are not exposed to β_2 -agonist on a daily basis. The study also extends our previous findings of an increase in the risk of exacerbations per copy of the Arg16 allele in children and young adults with asthma taking the regular long-acting β_2 -agonist salmeterol because the number of patients taking salmeterol in the previous article was 164, and this group now comprises 273 patients taking salmeterol.

We have only investigated 2 of the single nucleotide polymorphisms in the *ADRB2* gene. Other haplotypes of the *ADRB2* gene have been described.^{26,27} However, a closer study of the haplotype data shows that only 3 of the haplotypes are found in relevant numbers in the white population, and these are completely tagged by codons 16 and 27.^{26,27} It is of particular note that the promoter polymorphisms are in complete linkage disequilibrium with the Arg16 variant. This linkage disequilibrium is exclusively relevant in the white population. Thus in practical terms, in a study such as ours that exclusively involves white patients, it is only possible to study the association of the 16 of 27 diplotypic variation with clinical outcomes. However, the possibility remains that associations with the Arg16 variant might only be due to an uncharacterized and tightly linked causal variant.

Both retrospective and prospective analyses of data from a number of randomized controlled trials have demonstrated the adverse effects of the Arg16 variant on pulmonary function (peak flow rates¹⁰⁻¹² and FEV₁^{11,12}), asthma symptoms,^{10,11} methacholine

TABLE III. Association and drug-genotype interaction of the Arg/Gly16 genotype with asthma exacerbations in children with and without daily exposure to β -agonists

Exacerbations		No	Yes	Total	OR (95% CI), <i>P</i> value
Albuterol/salmeterol use less than once per day	Gly/Gly16	228	100	328	1.08 (0.85-1.36), .525*
	Arg/Gly16	241	98	339	
	Arg/Arg16	69	37	106	1.27 (0.77-2.08), .353†
	Total	538	235	773	
Daily use of any β -agonist (as-required albuterol, salmeterol, or both)	Gly/Gly16	81	69	150	1.64 (1.22-2.20), .001*
	Arg/Gly16	80	97	177	2.68 (1.46-4.94), .002†
	Arg/Arg16	25	49	74	
	Total	186	215	401	
	Test for interaction between genotype and daily use of any β -agonist on exacerbations				.022*
					.049†

P values were calculated by means of binary logistic regression corrected for age, sex, perennial rhinitis, smoking, and exposure to tobacco smoke by using a codominant model.

*ORs (95% CIs) and *P* values are shown for the codominant model (OR per copy of the Arg16 allele).

†ORs (95% CIs) and *P* values are shown for the comparison of Arg16 homozygotes versus Gly16 homozygotes.

TABLE IV. Characteristics of the study children (n = 1182)

Age (y), mean (SD)	10.3 (4.0)
Sex (male/female)	703/479
Body mass index, mean (SD)	19.1 (4.5)
PEFR (% of mean predicted value), mean (SD; n = 915)	87.6 (17.8)
FEV ₁ (% of mean predicted value), mean (SD; n = 915)	95.8 (15.5)
FVC (% of mean predicted value), mean (SD; n = 915)	92.2 (14.4)
FEV ₁ /FVC ratio (% of mean predicted value), mean (SD; n = 915)	1.02 (0.97)
Modified BTS step of asthma treatment*	0 = 39 (3.3%); 1 = 204 (17.3%); 2 = 660 (56%); 3 = 157 (13.3%); 4 = 117 (10%)
Mean dose of inhaled corticosteroids (beclomethasone dipropionate equivalent)	432.3 μ g (SEM, 12.6)
No. of children using more than once weekly on-demand inhaled short-acting β -agonists	1044 (79%)
Atopic eczema (yes/no; n = 1182)	607/575 (51.4%)
Exposure to smoke (yes/no; n = 1182)	401/744 (35%)
Inhaled bronchodilator use†	0 = 137 (11.6%); 1 = 827 (70%); 2 = 186 (15.7%); 3 = 31 (2.6%)
School absences (yes/no) over previous 6 months (n = 1182)	382/800 (32.3%)
Courses of oral steroids (yes/no) over previous 6 months (n = 1182)	263/919 (22.3%)
Hospital admissions (yes/no) over previous 6 months (n = 1182)	157/1025 (13.3%)
Overall asthma exacerbations‡ (yes/no) over previous 6 months (n = 1182)	456/726 (38.6%)

PEFR, Peak expiratory flow rate; FVC, forced vital capacity.

*Step 0 = No use of inhaled albuterol within the past month; step 1 = inhaled β_2 -agonists alone; step 2 = step 1 + inhaled steroids; step 3 = step 2 + inhaled long-acting β_2 -agonists; and step 4 = step 3 + montelukast.

†Inhaled bronchodilator use: 0 = none; 1 = occasional (more than once a week and less than daily use); 2 = daily (200 μ g/d required for symptom control); and 3 = excessive use (use of >1 dose of 200 μ g/d for symptom control).

‡Defined as any one of the following in the previous 6 months: school absences, courses of oral steroids, or hospital admissions.

responsiveness and exacerbations,^{9,12,28} and reliever bronchodilator use¹¹ in adults with asthma taking regular short-acting β_2 -agonists. However, inhaled short-acting β_2 -agonists used on an on-demand basis constitute one of the most commonly prescribed asthma medications in the world.³ This method of use results in a very different pattern of administration of short-acting β_2 -agonists compared with the regular use of this medication, with short-acting β_2 -agonists typically being used before activity (eg, football), during exposure to cold (eg, going to school in the early morning), or as frequent doses to afford relief during developing asthma exacerbations.

Importantly, the interaction of Arg16 status with inhaled albuterol on exacerbation risk is observed in a population who are not taking regular doses of inhaled albuterol that might be comparable with regular 4 times daily administration. Seventy percent of the population on BTS steps 2, 3, and 4 were using less than 1 daily dose (200 μ g/d) of inhaled albuterol. Only 3% of the

population was using inhaled albuterol several times daily (classified as “3” or “excessive”: this represents the use of >1 dose of 200 μ g/d for the control of symptoms). Against this background of relatively sparse on-demand inhaled short-acting β_2 -agonist administration, we have identified a significant deleterious effect on asthma exacerbations per copy of the Arg16 allele (Table III). The interaction between genotype and daily use of any inhaled β_2 -agonists (as-required albuterol, salmeterol, or both) had a significant effect on the risk of asthma exacerbations (*P* = .022, Table III).

We have also identified a significant increase in the risk of exacerbations in children and young adults with mild persistent asthma (ie, receiving inhaled steroids with inhaled short-acting β_2 -agonists according to need) per copy of the Arg16 allele (Table II). No similar associations were observed in patients with mild intermittent asthma (in whom inhaled albuterol only controls intermittent symptoms or in whom there has been no

TABLE V. Genotype distributions for codons 16 and 27 (n = 1182)

Polymorphic variations	No. of patients
Gly16Gly Glu27Glu	232
Gly16Gly Glu27Gln	197
Gly16Gly Gln27Gln	45
Arg16Gly Glu27Glu	3
Arg16Gly Glu27Gln	372
Arg16Gly Gln27Gln	132
Arg16Arg Glu27Glu	0
Arg16Arg Glu27Gln	0
Arg16Arg Gln27Gln	175
Undetermined at position 27	26

requirement for inhaled albuterol within the past month, [Table II](#)). Indeed, this increased risk per Arg16 allele copy was also present in step 3 patients who were also taking regular long-acting β_2 -agonists (13.3% of the overall population, [Table II](#)). However, in step 4 patients (receiving montelukast in addition to regular long-acting β_2 -agonists, inhaled steroids, and albuterol according to need; 9.9% of the overall population), there was no increase in the risk of exacerbations per Arg16 allele copy, despite a higher exposure to short-acting β_2 -agonists ([Table II](#)). However, there was a significant increased risk of exacerbations in the asthmatic patients when steps 3 and 4 were combined (OR, 1.51; 95% CI, 1.06–2.17; $P = .02$). One possible explanation for this apparent disconnect ([Table II](#)) between the genotype associations for steps 3 and 4 could be the concomitant use of montelukast or the use of oral steroids (BTS step 3, 34%; BTS step 4, 42%) or higher dose of inhaled steroids (BTS step 3, 533 μ g of beclomethasone dipropionate equivalent; BTS step 4, 561 μ g of beclomethasone dipropionate equivalent), with the latter protecting against β -agonist-induced downregulation and desensitization through the glucocorticoid response element on the *ADRB2* gene and the former conferring nonsteroidal anti-inflammatory protection.²⁹

Our previous study had shown an increased hazard for exacerbations per copy of the Arg16 allele in young asthmatic patients, with a particular effect of regular inhaled salmeterol on this increase in risk. Now that we have doubled our study size, previous effects remain consistent. However, the effect has not become more significant with greater numbers. We believe, however, that the increased hazard for exacerbations per copy of the Arg16 allele in asthmatic patients taking salmeterol observed in the previous study and subsequently in this extended study is a true effect. This is because, in our previous study, the children were mostly recruited from the secondary care setting and were on higher steps of asthma management (40% of the study population was on BTS step 3 or greater). When we extended our study, we recruited mainly from the primary care setting, and a smaller proportion of the new recruits are taking inhaled salmeterol (in the combined analysis, 23% of the study population is on BTS step 3 or greater). Hence a smaller proportion of the overall participants are taking salmeterol in the extended analysis in comparison with our previous article. This accounts for the observation that although the effect is consistent, it has not become any stronger with the increase of numbers. Our strategy for recruiting participants from primary care to recruit more patients taking inhaled short-acting β_2 -agonists on demand and not regular inhaled long-acting β_2 -agonists

was guided by the hypothesis that we primarily wished to test for this study, which is that inhaled short-acting β_2 -agonists interact with the Arg16 genotype to increase exacerbation risk. We wished to specifically recruit a large population of asthmatic patients taking inhaled short-acting β_2 -agonists on demand who were not exposed to regular inhaled long-acting β_2 -agonists to fulfill this goal.

We have further demonstrated that there is an increase in risk of the individual constituents of the asthma exacerbation score (school absences and requirement for courses of oral steroids) per copy of the Arg16 allele over a 6-month period of reporting in addition to the overall effect on asthma exacerbations, as has been reported in our previous analysis ([Table I](#)). These individual measures represent different aspects of asthma exacerbations that have different effects on children and young adults in the community. School absences have an effect on children's education, and oral steroid courses represent the use of primary care, whereas hospital admissions represent the effect on secondary care services. Therefore our findings appear to have a greater relevance for the burden of asthma in the community and primary care than hospital settings.

The observation of an adverse effect per copy of the Arg16 allele of on-demand short-acting β_2 -agonists on asthma exacerbation risk raises a further question. Because patients taking inhaled long-acting β_2 -agonists are also taking short-acting β_2 -agonists, what proportion of the adverse effect of increased risk of exacerbations in the real-life scenario results through use of long-acting β_2 -agonists in comparison with short-acting β_2 -agonists? This question might be resolved through a study that involves the randomization of asthmatic children carrying the Arg16 allele who are taking regular salmeterol to comparator arms of reliever medication (ie, short-acting β_2 -agonist versus ipratropium bromide), with risk of asthma exacerbations as the primary outcome measure. Another strategy might be to randomize patients carrying the Arg16 allele to receive either salmeterol or montelukast as an add-on to inhaled corticosteroids with albuterol reliever in both arms on the basis that any potential adverse effect of albuterol could be seen in both randomized arms.

Our participants used inhaled short-acting β_2 -agonists as reliever medications and did not use non- β_2 -agonists as reliever medication for their asthma. Thus we cannot provide the data in this study to address the hypothesis that the use of non- β_2 -agonist relievers is not associated with the deleterious effects that have been reported with inhaled β_2 -agonist relievers. This is a major limitation of the study. Thus because these necessary controls are missing, it is too early to draw conclusions in this area of research. Therefore although our data are of interest, a proper study involving a non- β_2 -agonist reliever is now required. This could involve either of 2 strategies.

First, if we could identify a population of asthmatic patients with significant use of non- β_2 -agonist relievers, we could compare the interactions of the Arg16 genotype with inhaled β_2 -agonist reliever use versus non- β_2 -agonist reliever use. Alternatively, it could involve a strategy for the prospective randomization of a population of asthmatic patients carrying the Arg16 genotype to inhaled β_2 -agonist versus non- β_2 -agonist reliever therapy with an exploration of the hypothesis that there is an increased risk of asthma exacerbations in participants taking inhaled β_2 -agonists in comparison with participants taking non- β_2 -agonists.

A recent analysis of 2 separate studies (mean age of participants around 40 years for both studies) has failed to demonstrate a

role for the Arg16 genotype on clinical outcomes, including exacerbations, pulmonary function, and asthma control measures, in patients receiving inhaled long-acting β_2 -agonists.³⁰ However, the selection criteria for the first study raise questions about its generalizability. In that study all patients had to demonstrate 12% or greater reversibility to inhaled terbutaline, and bronchodilator reversibility for the patients had a very high mean value of 24.5%. Selecting such a population has the potential to bias the population toward a group that is disproportionately responsive to all β_2 -agonists (ie, both short and long acting). Such a population might be less likely to demonstrate the pharmacogenetic effect under study for the analysis. In addition, we³¹ and others³² have found that mean FEV₁ values are much higher (between 90% and 97% of mean predicted value) in asthmatic children attending clinics in northern Europe; we have also observed that these children have characteristically much smaller responses to inhaled short-acting β_2 -agonists (data not shown). A *post hoc* analysis of a randomized controlled trial on adults with asthma shows that, relative to patients with the Gly/Gly genotype with asthma, patients with the Arg/Arg16 genotype might have an impaired therapeutic response to salmeterol measured as improvement in lung function either in the absence or presence of inhaled corticosteroids.^{33,34}

In addition, results from studies on adult patients with asthma³⁰ cannot often be extrapolated to children and teenagers with the disease. Long-term cohort studies have shown that asthma in childhood differs markedly from asthma during adult life.³⁵ For example, early sensitization to inhaled allergens constitutes a significant risk factor for asthma until the teenage years but not for adult asthma.^{33,36,37} Other aspects of asthma, such as eosinophil response³⁶⁻³⁸ and effects of maternal smoking,³⁹ show major differences between children and adults with asthma. This suggests that the effects of polymorphic variations that are important from the pharmacogenetic perspective could be different between children and adults with asthma. The development of tolerance to the nonbronchodilator (eg, mast cell-mediated) actions of inhaled β_2 -agonists in patients with relatively mild asthma might also interact with these effects.⁴⁰ With the vast majority of our participants being aged less than 15 years (86%) and with the maximum age of participants being 22 years, our study firmly focuses on children with this disease. We believe that our exploration of the role of the *ADRB2* genotype primarily in childhood thus complements the observations of other studies on adults, providing a more complete picture of the effects of this genotypic variation across asthmatic patients of different age groups.

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Clinical implications: The diminished efficacy of frequent, inhaled on-demand albuterol and regular salmeterol in young asthmatic patients with the Arg16 allele of *ADRB2* might contribute to an increased risk of asthma exacerbations.

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TABLE E1. Effect of Arg16Gly polymorphism status on the proportion of patients with asthma exacerbations according to salmeterol treatment status

Treatment	Genotype	Total exacerbations			OR (95% CI), <i>P</i> value
		No	Yes	Total	
No salmeterol	Gly/Gly16	255	118	373	1.23 (0.99-1.52), .05
	Gly/Arg 16	271	130	401	
	Arg/Arg 16	76	52	128	
	Total	602	300	902	
Salmeterol treated	Gly/Gly16	54	51	105	1.51 (1.06-2.17), .02
	Gly/Arg 16	50	66	116	
	Arg/Arg 16	18	34	52	
	Total	122	151	273	

P values were calculated by means of binary logistic regression corrected for age, sex, perennial rhinitis, smoking, and exposure to tobacco smoke by using a codominant model. ORs are per copy of the Arg16 allele.

TABLE E2. Overall effect of the Glu/Gln27 genotype on oral steroid intake, school absences, and hospital admissions caused by asthma exacerbations and overall asthma exacerbations in children and young adults with asthma across all steps of asthma management conditioned on Arg/Gly16 genotype

		Genotype				OR (95% CI), <i>P</i> value, for Arg16Gly	OR (95% CI), <i>P</i> value, for Glu27Gln
		Glu/Glu27	Glu/Gln27	Gln/Gln27	Total		
Oral steroid intake	No	192	264	442	898	1.21 (0.91-1.61), .19	1.11 (0.82-1.48), .50
	Yes	43	88	127	258		
	Total	235	352	569	1156		
School absence caused by asthma exacerbations	No	167	224	393	784	1.24 (0.96-1.59), .10	1.08 (0.83-1.39), .57
	Yes	68	128	176	372		
	Total	235	352	569	1156		
Hospital admission caused by asthma exacerbations	No	207	303	492	1002	0.95 (0.68-1.34), .79	1.18 (0.83-1.66), .35
	Yes	28	49	77	154		
	Total	235	352	569	1156		
Overall asthma exacerbations	No	153	200	357	710	1.27 (0.99-1.63), .06	1.06 (0.82-1.36), .65
	Yes	82	152	212	446		
	Total	235	352	569	1156		

P values were calculated by means of binary logistic regression corrected for age, sex, perennial rhinitis, smoking, and exposure to tobacco smoke by using a codominant model. ORs are per genotypic step.

TABLE E3. Association of asthma exacerbations and Glu27Gln polymorphism according to BTS steps of management of asthma conditioned on the Arg/Gly16 genotype

BTS treatment steps	Genotype	Exacerbations over previous 6 months			OR (<i>P</i> value)
		No	Yes	Total	
Step 0	Glu/Glu27	5	1	6	0.17 (.11)
	Glu/Gln27	16	2	18	
	Gln/Gln27	12	1	13	
	Total	33	4	37	
Step 1	Glu/Glu27	32	11	43	1.50 (.33)
	Glu/Gln27	90	17	107	
	Gln/Gln27	38	11	49	
	Total	160	39	199	
Step 2	Glu/Glu27	90	48	138	0.99 (.95)
	Glu/Gln27	199	125	324	
	Gln/Gln27	107	80	187	
	Total	396	253	649	
Step 3	Glu/Glu27	20	8	28	1.30 (.41)
	Glu/Gln27	32	32	64	
	Gln/Gln27	25	34	59	
	Total	77	74	151	
Step 4	Glu/Glu27	6	11	17	0.74 (.48)
	Glu/Gln27	19	36	55	
	Gln/Gln27	17	26	43	
	Total	42	76	115	

P values were calculated by means of binary logistic regression corrected for age, sex, perennial rhinitis, smoking, and exposure to tobacco smoke by using a codominant model. ORs are per genotypic step.