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 he 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 2009;124:1188-94.)

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 β2-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 admissions[16] 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.

METHODS

We have continued the recruitment of children with physician-diagnosed asthma for the BREATHE study beyond the publication of our initial results.[7]

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

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 admissions[16] 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 previously[7] 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 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