ASTHMA

Arginine-16 β_2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol

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Background: The homozygous presence of the arginine-16 variant of the β_2 adrenoceptor gene *ADRB2* reverses the benefits from the regular use of short acting β_2 agonists in asthmatic adults compared with the homozygous glycine-16 genotype. We studied the effect of this polymorphic variation on asthma exacerbations in children and young adults and its relation to long acting β_2 agonists.

Methods: A cross-sectional survey was undertaken using electronic records, direct interviews, and genotype determination of position 16 and 27 of the *ADRB2* gene in DNA from mouthwash samples of 546 children and young asthmatics attending paediatric and young adult asthma clinics in Tayside, Scotland during 2004–5. The primary outcome measure was asthma exacerbations over the previous 6 months.

Results: There was an increased hazard of asthma exacerbations across all treatment steps of the British Thoracic Society (BTS) asthma guidelines when the homozygous genotypes Arg/Arg and Gly/Gly were compared (OR 2.05, 95% CI 1.19 to 3.53, p = 0.010), particularly in patients treated with salmeterol (OR 3.40, 95% CI 1.19 to 9.40, p = 0.022). The Glu27Gln polymorphism had no significant effect on asthma exacerbations in any treatment group.

Conclusions: The arginine-16 genotype of *ADRB2* predisposes to exacerbations in asthmatic children and young adults, particularly in those exposed to regular salmeterol. This may be explained by genotype selective salmeterol induced downregulation and impaired receptor coupling, and associated subsensitivity of the response.

Asthma is one of the most common chronic diseases in the world.[1 2] Scotland tops the ranking list by country for the prevalence of current asthma symptoms in the childhood and teenage years.[3] The guidelines by the Scottish Intercollegiate Guidelines Network/British Thoracic Society[4] and the guidance for best practice in the United States[5] recommends add-on therapy with an inhaled long acting β_2 agonist such as salmeterol in children with asthma when symptoms are not controlled on inhaled short acting β_2 agonists according to need, together with regular inhaled steroids.

A number of polymorphisms in the gene encoding the human β_2 adrenergic receptor have been known to alter its function in the cardiovascular and respiratory systems. In blood vessels, where β_2 adrenergic receptors mediate vasodilatation, the Arg/Arg polymorphism at position 16 of the β_2 receptor is associated with enhanced agonist mediated desensitisation in the vasculature, while the Glu27 polymorphism is associated with increased agonist mediated responsiveness.[6] In the airway the presence of the homozygous Arg/Arg genotype (about 15% of patients with asthma in the US[7] and UK[8]) confers relative protection against downregulation by endogenous catecholamines and reverses the benefits from the regular use of short and long acting β_2 agonists in adults.[7–9] The presence of the Arg16 polymorphism (either Arg/Arg or Arg/Gly) confers bronchoprotective subsensitivity to methacholine and adenosine monophosphate challenge in steroid treated adults with asthma treated with formoterol and salmeterol.[8] However, the consequences of real life prescribing of a long acting β_2 agonist as an add-on medication to inhaled steroids in Arg/Arg and Arg/Gly individuals have not been explored. In addition, although there is significant heterogeneity in bronchoprotection with salmeterol, particularly in children with asthma,[10] it is not known if polymorphisms in the human β_2 adrenergic receptor gene *ADRB2* could contribute to this variation.

In children with asthma, school absences,[11] use of short courses of oral steroids,[12] and asthma related hospital admissions[13] represent well validated measures of asthma exacerbations. We have tested the hypothesis that the Arg16 variant increases the likelihood of asthma exacerbations in children and young people with asthma and have explored the relationship between the Arg16 genotype and exacerbations in the context of ''real life'' prescribing of the short and long acting β_2 agonists salbutamol and salmeterol.

METHODS

Demographic, anthropometric, clinical, and β_2 receptor genotype information were collected from 546 children with physician diagnosed asthma attending primary and secondary clinics in 12 primary care practices and a secondary care asthma clinic in Tayside in 2004–5. A DNA sample was collected by mouthwash after informed consent was given by the patient and the parent/guardian. The study was approved by the Tayside Committee on Medical Research Ethics.

DNA was prepared using the Qiagen Dneasy 96 kit, and genotypes were determined using Taqman based allelic discrimination assays on an ABI 7700 sequence detection system.[14]

For the Gly16Arg variant, the following probes and primers were used:

forward primer: GAACGGCAGCGCCTTCT;
reverse primer: GCACATTGCCAAACACGATG;
Arg16 probe: Cal Orange-CACCCAATAGAAGCCATGCGC CGGACCACGAC-BHQ;

Table 1 Characteristics of study children (n = 546)

Mean (SD) age (years)	10.2 (3.8)
Sex (M/F)	342/204
Mean (SD) body mass index	19.0 (4.3)
Mean (SD) PEFR (% of mean predicted)	89.7 (17.2)
Mean (SD) FEV_1 (% of mean predicted)	97.0 (15.1)
Mean (SD) FVC (% of mean predicted)	93.9 (13.4)
BTS step of asthma treatment*	
Step 1	79 (14.5%)
Step 2	303 (55.5%)
Step 3	92 (16.8%)
Step 4	72 (13.2%)
Number on inhaled salmeterol (steps 3 + 4)	164 (30.0%)
School absences (yes/no) over previous 6 months	191/546 (35%)
Courses of oral steroids (yes/no) over previous 6 months	109/546 (20%)
Hospital admissions (yes/no) over previous 6 months	63/546 (12%)
Overall asthma exacerbations† (yes/no) over previous 6 months	215/546 (39%)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; PEFR, peak expiratory flow rate.
*Step 1 = inhaled β_2 agonists alone; step 2 = step 1 + inhaled steroids; step 3 = step 2 + inhaled long acting β_2 agonists; step 4 = step 3 + montelukast.
†Defined as any one of the following in the previous 6 months: school absences, courses of oral steroids, or hospital admissions.

Gly16 probe: FAM-CACCCAATGGAAGCCATGCGCCGGACCACGAC-BHQ.

For the Glu27Gln variant the forward primer was identical to the forward primer for the Gly16Arg variant and the reverse primer was TGAGAGACATGACGATGCCC.

Gln27 probe: Cal Orange-CCATGCGCCGGACCACGACGTCACGCAGCAAAGGGACGA-BHQ;

Glu27 probe: FAM-CCATGCGCCGGACCACGACGTCACGCAGGAAAGGGACGA-BHQ.

We defined a population taking regular inhaled salmeterol 50 µg twice daily (n = 164) and a cohort not taking salmeterol (n = 382). We also defined the population according to their step of treatment consistent with the British Thoracic Society (BTS) guidelines (step 1 = inhaled β_2 agonists alone; step 2 = step 1 + inhaled steroids; step 3 = step 2 + inhaled LABA; step 4 = step 3 + montelukast)[4] (table 1). The inhaled β_2 agonist use profile for the population was as follows: 351 participants on inhaled salbutamol as required, 31 on regular inhaled salbutamol, 2 on regular salbutamol without any inhaled steroids. and 164 on regular inhaled salmeterol with inhaled steroids.

Any asthma related absence from school, short courses of oral steroids, and asthma related hospital admissions over the previous 6 months were recorded by parental recall for the children aged less than 16 years and by the participants themselves if aged 16 years and above. For simplicity and greater accuracy through recall, only yes/no responses for any of the three options were used for the analysis.

Binary logistic regression was used to calculate odds ratios and p values for asthma exacerbations. In the comparisons of homozygotes previously examined,[7] Gly/Gly16 was coded 0 and Arg16/Arg16 was coded 1. For the co-dominant model, 0 = Gly/Gly16, 1 = Gly/Arg16, 2 = Arg/Arg16. Age, sex, and exposure to tobacco smoke were included in all models as covariates. Other potential covariates such as seasonality, dose of inhaled steroids, and frequency of short acting β_2 agonist use as rescue medication did not contribute significantly to the model and were not associated with genotype in any subgroup tested, so they were excluded from the final analysis. All statistical analyses were performed using SPSS Version 11.

Table 2 Genotype distributions for codons 16 and 27 (total = 546)

Polymorphic variation	No of individuals
Gly16Gly Glu27Glu	109
Gly16Gly Glu27Gln	76
Gly16Gly Gln27Gln	21
Arg16Gly Glu27Glu	0
Arg16Gly Glu27Gln	172
Arg16Gly Gln27Gln	75
Arg16Arg Glu27Glu	0
Arg16Arg Glu27Gln	0
Arg16Arg Gln27Gln	82
Undetermined at position 27	11

Our primary analysis involved testing the association between the genotype of *ADRB2* and exacerbations in all asthma patients on BTS treatment steps 1–4. Our power calculation showed that this analysis had a 99% power to detect an odds ratio (OR) of 1.92 and an 80% power to detect an OR of 1.52. We had also planned a number of secondary analyses. As our sample comprised less than 0.5% (n = 2) of patients who were being treated with regular inhaled salbutamol without inhaled steroids, we were unable to compare genotypes in these patients due to insufficient numbers. We wished, however, to test the associated risk of possessing the Arg16 polymorphism on asthma exacerbations in asthmatics on long acting β agonists and those not on long acting β agonists. In the population taking salmeterol we had a 99% power to detect an OR of 2.73 between the Arg/Arg16 and Gly/Gly16 homozygotes, and an 80% power to detect an OR of 1.90. In the non-salmeterol treated group we had 80% power to detect an OR of 1.75 and 95% power to detect an OR of 2.0.

RESULTS

The characteristics of the study population are shown in table 1. The prevalence of the Arg/Arg16 (15%), Arg/Gly16 (45%), and Gly/Gly16 (38%) genotypes in children and young people with asthma in Tayside was similar to that observed for the US and UK populations.[7 8] Table 2 shows the relative distributions of the Arg/Gly polymorphisms on codon 16 and the Gln/Glu polymorphisms on codon 27 for the population. No individuals were observed with the compound diplotype of both Arg/Arg16 and Glu/Glu27, thus confirming the tight linkage disequilibrium. Arg16Gly or Glu27Gln had no significant effects on baseline pulmonary function (forced expiratory volume in 1 second, forced vital capacity and peak expiratory flow) (data not shown).

We tested the association between the genotype of *ADRB2* and exacerbations over BTS treatment steps 1–4 using a simple genetic contrast comprising homozygous genotypes Arg/Arg16 *v* Gly/Gly16, as has been previously analysed (table 3).[7 8] This showed a significant overall increased

Table 3 Overall effect of genotype on exacerbations in children and young adults with asthma across all four BTS steps of asthma treatment

Exacerbations over the period*	Genotype		
	Gly/Gly16	Arg/Arg16	Total
No	134	43	177
Yes	74	41	115
Total	208	84	292

*Odds ratio for exacerbation risk 2.05 (95% CI 1.19 to 3.53); p = 0.010.

Table 4 Association of asthma exacerbations and polymorphisms according to BTS treatment step

BTS treatment step*	Genotype	Exacerbations over previous 6 months: No	Yes	Total	OR (p value)
Step 1	Gly16Gly	27 (77%)	8 (23%)	35	1.084
	Arg16Gly	24 (75%)	8 (25%)	32	(p=0.852)†
	Arg16Arg	10 (83%)	2 (17%)	12	
	Total	61	18	79	
Step 2	Gly16Gly	70 (64%)	40 (36%)	110	1.281
	Arg16Gly	93 (64%)	53 (36%)	146	(p=0.163)†
	Arg16Arg	24 (52%)	23(49%)	47	
	Total	187	116	303	
Step 3	Gly16Gly	24 (67%)	12 (33%)	36	2.168
	Arg16Gly	24 (56%)	19 (44%)	43	(p=0.023)†
	Arg16Arg	4 (31%)	9 (69%)	13	
	Total	52	40	92	
Step 4	Gly16Gly	13 (48%)	14 (52%)	27	1.617
	Arg16Gly	13 (41%)	20 (61%)	33	(p=0.249)†
	Arg16Arg	5 (42%)	7 (58%)	12	
	Total	31 (44%)	41 (57%)	72	

More asthma exacerbations occurred in association with the Arg16 polymorphism in patients on BTS step 3 treatment, but not in those on BTS treatment steps 1, 2 and 4.
*BTS treatment steps: step 1 = salbutamol according to need; step 2 = step 1 + regular inhaled steroids; step 3 = step 2 + regular inhaled long acting β_2 agonist; step 4 = step 3 + oral montelukast.
†p values were calculated by binary logistic regression analysis corrected for age, sex, and exposure to tobacco smoke using a co-dominant model—that is, a gene/dosage effect for the Arg16 variant. Odds ratios are per genotypic step.

hazard for exacerbations in individuals homozygous for the Arg16 variant (OR 2.05, 95% CI 1.19 to 3.53; p = 0.010). When the entire data including the heterozygotes were examined, the dominant and recessive models did not provide a good fit of the data (data not shown); however, the co-dominant model was significant, confirming the intermediate risk posed by the heterozygote grouping (OR 1.32 per genotypic step, 95% CI 1.03 to 1.71, p = 0.030). As the co-dominant model provided the best fit of the data, this model was used for the remainder of the analysis.

As recent randomised clinical trials have shown that Arg16 is associated with worse lung function in patients on either long acting or short acting β agonists,[15] we explored the genotypic risk associated with the four BTS treatment steps. The associated risk of possessing the Arg16 polymorphism was only significant (p = 0.023) in asthmatics on BTS step 3 (regular inhaled salmeterol + inhaled steroids with inhaled salbutamol on demand, table 4), with a gene/dosage effect of the Arg16 variant in a co-dominant model. This was not significant in the participants on the other BTS steps of treatment including step 4 (regular inhaled salmeterol + inhaled steroids + montelukast with inhaled salbutamol on demand). Although the observed hazard in each BTS step was above 1, the current study was underpowered to detect such small effects in these groups. In contrast, the Glu27 polymorphism and the Glu27Gln variant did not show any effect on asthma exacerbations measured as above, either when considered in isolation or as a covariate with the Gly16Arg variant (data not shown).

The Arg16 variant was associated with a greater frequency of asthma exacerbations in salmeterol treated patients (that is, steps 3 and 4 combined); this was found to hold true for both the co-dominant model (table 5) and for the homozygote only comparison, yielding significant results (Arg/Arg16 *v* Gly/Gly16: OR 3.40, 95% CI 1.19 to 9.40, p = 0.022). The larger group of individuals not on salmeterol also had an increased hazard for exacerbation but this did not reach significance (Arg/Arg16 *v* Gly/Gly16: OR 1.77, 95% CI 0.91 to 3.44, p = 0.093). This may be due to a lack of power as we had only 80% power to detect an OR of 1.75 in this group and 95% power to detect an OR of 2.0.

DISCUSSION

This study shows that there is an increased hazard for exacerbations in young asthmatics homozygous for the Arg16 variant, and regular treatment with inhaled salmeterol has a particular effect on this increased risk.

Table 5 Effect of Arg16Gly polymorphism status on proportion of salmeterol treated patients (BTS step 3 and above) with asthma exacerbations

Treatment	Genotype	Total exacerbations: Yes	No	Total	OR (p values)
Not on salmeterol	GlyGly	48 (33%)	97	145	1.26
	ArgGly	61 (35%)	117	178	(p=0.149)*
	ArgArg	25 (41%)	34	59	
	Total	132	248	382	
On regular salmeterol	GlyGly	26 (41%)	37	63	1.79
	ArgGly	39 (51%)	37	76	(p=0.020)*
	ArgArg	16 (64%)	9	25	
	Total	81	83	164	

*p values were calculated by binary logistic regression analysis corrected for age, sex, and exposure to tobacco smoke, using a co-dominant model. Odds ratios are per genotypic step.

Prospective clinical trials have shown that the homozygous presence of the Arg16 variant of the β_2 adrenoceptor gene *ADRB2* reverses the benefits from the regular use of short acting β_2 agonists in asthmatic adults compared with the homozygous Gly16 genotype.[7] We have confirmed these findings in a population of children and young adults treated across BTS treatment steps 1–4, with poorer overall control in Arg16 than in Gly16 homozygotes.

The previous observations reported in older adults with asthma carrying the homozygous arginine genotype[7 8] suggested that prolonged receptor occupancy by a high affinity agonist such as salmeterol given twice daily may result in downregulation and impaired receptor coupling. This, in turn, would lead to subsensitivity of response and more asthma exacerbations. This is in keeping with the dynamic kinetic receptor regulation hypothesis proposed by Liggett, whereby the arginine genotype would be relatively more resistant than the glycine genotype to downregulation and desensitisation by endogenous catecholamines, so the arginine genotype would be more prone to subsensitivity of response on subsequent exposure to a long acting β agonist such as salmeterol.[16]

While consistent with this hypothesis, our observations extend the genotypic observations as less than 0.5% of our patients were treated with regular short acting β_2 agonists without steroid co-medication, and the primary risk was seen in individuals taking regular long acting β agonists. This is a predictable phenomenon based on the fact that these individuals will have more persistent ligand adrenoreceptors than other treatment groups. A similar adverse effect of salmeterol on lung function and asthma severity scores in Arg16 homozygote adults has recently been reported.[15]

We were particularly interested in testing our hypothesis regarding asthma exacerbations in children and young adults with asthma because salmeterol shows greater heterogeneity of response and seems to be less effective in children than in adults with asthma.[10] We wished to explore whether this ''lack of efficacy'' from genotypic variation was relevant to the practical management of asthma in this population through an effect on asthma control over a period of several months. Our study involved the cross sectional collection of data from parental or patient recall and this process may be open to bias. Although school records are a more accurate source of data on school attendance, they do not specify the cause of the absence. Parental or adolescent recall, the method used for our study, may be more susceptible to bias, but has been shown to be a sensitive measure of school absence caused specifically by asthma.[11] Our data would suggest that the Arg16 genotype may be a clinically important pharmacogenetic paradigm, and that such individuals might gain less benefit from taking regular salmeterol, despite concomitant inhaled steroid administration.

An overall increase in asthma exacerbations resulting in increased school absences (table 1) in children with asthma could have important long term consequences, affecting the social and occupational trajectories of the children in later life.[17] There is evidence, for example, that adults with childhood onset asthma may experience a significant disadvantage in the labour market.[11] Genetic polymorphic variation in children with asthma may thus worsen the social consequences of this disease through adverse drug response.

Our patients, as expected, had well preserved lung function with an overall mean forced expiratory volume in 1 second of 97%. The deleterious effect of salmeterol in genetically susceptible patients therefore seems to be unrelated to airway calibre as such, which suggests that the adverse effects were possibly more defined by bronchoprotective than by bronchodilator subsensitivity during a period of increased airway tone (that is, during asthma exacerbations rather than during the quiescent state). This is also suggested by previous work using bronchial challenge in adults with asthma, where the degree of bronchoprotection conferred by long acting β_2 agonists was less in patients who had the homozygous or heterozygous arginine genotype.[8] However, as we did not perform bronchoprovocation challenges in our cohort, we can only suggest this mechanism as a putative hypothesis.[18]

It would appear that salmeterol is not beneficial, and that other second line controller medication such as montelukast or theophylline may be preferable in susceptible patients with the homozygous arginine genotype when inhaled steroids alone fail to control the symptoms of asthma. Indeed, it was intriguing to note that there was a lower increased risk of excerbations in our homozygous Arg16 patients exposed to regular salmeterol who were also taking montelukast (step 4, table 5), suggesting a possible protective effect of concomitant leukotriene receptor antagonist therapy. However, our study does not have sufficient power to detect the difference between these two groups. Our results suggest the need for further prospective randomised controlled trials to address these key questions.

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CNAP and SM had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: CNAP, SM, BJL. Acquisition of data: IM, TI, DFM. Genotyping: SL. Analysis and interpretation of data: CNAP, SM, BJL. Drafting of manuscript: CNAP, SM, BJL. Critical revision of the manuscript for important intellectual content: CNAP, BJL, SM. Statistical expertise: CNAP, TI. Supervision: CNAP, SM. All authors participated in the writing of the manuscript and have approved the final version for submission.

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LUNG ALERT

Gene profiling to predict recurrence in lung cancer

▲ Potti A, Mukherjee S, Petersen R, *et al*. A genomic strategy to refine prognosis in early-stage non-small-cell lung cancer. *N Engl J Med* 2006;**355**:570–80

Patients with stage I non-small cell lung cancer are traditionally treated with surgical resection alone. However, 30–35% will relapse and these patients may therefore have benefited from adjuvant chemotherapy. The current clinicopathological classification system is an imprecise prognostic indicator, and the authors have evaluated the use of gene expression profiling to predict recurrence.

One hundred and ninety eight tumour samples from three separate groups were used. One group was used to identify gene expression profiles (the lung metagene model), and samples from the other two groups were used to assess the predictive value of the model in comparison with clinical variables known to be of prognostic significance.

The results showed that the lung metagene model was superior to clinical data in predicting recurrence. The model was 93% accurate in the identification group compared with 64% for clinical data alone. The samples in the other two groups were used to validate the predictive model. Using univariate and multivariate analysis, the results once again illustrated the significantly greater accuracy of the model for predicting recurrence ($p<0.001$): 72% (69% positive predictive value, 78% negative predictive value) and 79% (79% positive predictive value, 80% negative predictive value) in the two cohorts, respectively.

The data suggest that patients with stage I disease and likely recurrence may be identified using the lung metagene model. The role of adjuvant chemotherapy in these patients would require a phase 3 clinical trial.

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