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Polymorphism of the β_2-adrenoceptor and the response to long-term β_2-agonist therapy in asthma

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Polymorphism of the β_2-adrenoceptor and the response to long-term β_2-agonist therapy in asthma. R.J. Hancox, M.R. Sears, D.R. Taylor. ©ERS Journals Ltd 1998.

ABSTRACT: Polymorphisms affecting amino acids 16 and 27 of the β_2-adrenoceptor alter receptor regulation *in vitro*. Whether these polymorphisms alter the response to β_2-agonist therapy in asthma is unknown. In a previous study of 64 asthmatics, most experienced a deterioration in asthma control during regular inhaled β_2-agonist (fenoterol) treatment, while a minority improved. We have determined the β_2-adrenoceptor genotypes in these subjects, to establish whether changes in asthma control during the earlier study were influenced by β_2-adrenoceptor polymorphism.

The genotypes coding for amino acids 16 and 27 were identified in 60 subjects using allele-specific polymerase chain reaction. The effects of regular β_2-agonist treatment on asthma control were compared between genotypes.

There was no association between genotype and change in overall asthma control during regular β_2-agonist treatment. Only two of 10 markers of asthma control showed changes that were significantly associated with genotype: subjects homozygous for glycine at position 16 had no increase in bronchial responsiveness to methacholine during regular treatment; subjects homozygous for glutamic acid at position 27 had no increase in evening peak expiratory flow rates during regular treatment. These differences are the opposite of those that would have been predicted by the results of *in vitro* studies.

In these subjects, the deleterious response to regular inhaled β_2-agonist treatment was not related to β_2-receptor polymorphism.

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In 1990 we reported a randomized controlled trial in which the administration of regular inhaled β_2-agonist therapy was associated with a deterioration in asthma control in a majority of subjects when compared to as-required β-agonist treatment [1]. There were more asthma exacerbations, reductions in the forced expiratory volume in one second (FEV_1) and morning peak expiratory flow rates (PEFRs) and increased bronchial responsiveness to methacholine during regular fenoterol therapy [2].

The mechanism responsible for the deterioration in asthma control during regular β-agonist therapy is unknown, but agonist-induced down-regulation of β_2-adrenoceptor function is one possible explanation. In our study there was no evidence of loss of the acute bronchodilator response to β-agonist after prolonged therapy, suggesting that down-regulation of airway smooth muscle β_2-receptors was not a significant problem [2]. However, tolerance to the nonbronchodilating properties of inhaled β_2-agonists during regular treatment has been demonstrated in other studies [3–5]. It may be that down-regulation of β_2-receptors in other cells contributed to the overall deterioration in asthma control in our original study.

Down regulation of the β_2-adrenoceptor *in vitro* has been shown to be influenced by two common polymorphisms of the receptor gene which change the amino-acid sequence of the N-terminal domain of the receptor [6]. One results in the substitution of glycine (Gly) for arginine (Arg) at amino acid position 16 (Gly 16) of the receptor protein while the other results in the substitution of glutamic acid (Glu) for glutamine (Gln) at position 27 (Glu 27). In cell culture studies the Gly 16 variant of the receptor undergoes enhanced down-regulation during incubation with isoprenaline, whereas the Glu 27 variant is relatively resistant to down-regulation [7, 8]. Receptors with both Gly 16 and Glu 27 polymorphisms down-regulate to a similar extent to the Gly 16 variant alone [7].

Although these polymorphisms occur with similar frequency in asthmatics and nonasthmatics, among asthmatic patients they have been linked to certain clinical features. Gly 16 has been associated with an increased requirement for oral corticosteroids [6] and nocturnal asthma symptoms [9]. In contrast, Glu 27 has been associated with reduced bronchial responsiveness to methacholine [10]. Recently asthmatic patients homozygous for the Gly 16 polymorphism have been shown to undergo a greater loss of the bronchodilator response to β-agonist during treatment with formoterol. Subjects heterozygous for Arg 16/Gly 16 showed intermediate desensitization [11].

There have been no reports on the influence of β_2-adrenoceptor polymorphism on changes in asthma control during prolonged β_2-agonist therapy. We now report an analysis of the β_2-adrenoceptor genotype in the subjects

from our previous study [1], in which we have sought to establish whether the change in asthma control during regular β_2-agonist treatment was related to β_2-adrenoceptor polymorphism at amino acids 16 and 27. Our hypothesis was that the polymorphism which gives rise to enhanced down-regulation of the β_2-adrenoceptor (Gly 16) would be associated with a deterioration in asthma control during prolonged β_2-agonist therapy, whereas the polymorphism which is relatively resistant to down-regulation (Glu 27) would be associated with improved or unchanged asthma control.

Methods

Study design

The randomized controlled study of regular *versus* as-needed β-agonist has been reported previously [1]. Briefly, 89 patients with a history of mild to moderate asthma were recruited to a double-blind, randomized, placebo-controlled, cross-over study of treatment with inhaled dry-powder fenoterol (400 μg *q.i.d.*) or matching placebo for 24 weeks each. Throughout the study, subjects were allowed to use additional known β-agonist by metered dose inhaler as required for symptom relief. All subjects had demonstrated bronchial hyperresponsiveness to methacholine (provocative concentration causing a 20% fall in FEV_1 (PC_{20}) <8 $mg \cdot mL^{-1}$) and a significant response to inhaled bronchodilator (>20% rise in FEV_1).

Inhaled corticosteroids or cromoglycate were continued providing they had been used at a constant dose for three months before the study. Exacerbations of asthma were treated with increased β_2-agonist and short courses of oral prednisone if needed. All other asthma treatment was withdrawn prior to entry into the study.

Asthma control was monitored from a diary of asthma symptom scores recorded twice daily, morning and evening PEFRs, and additional bronchodilator use. Every 4 weeks, each subject performed spirometry followed by either a methacholine challenge [12] or a bronchodilator response test (repeat spirometry 10 min after nebulized β_2-agonist) at alternate visits. Subjects withheld all bronchodilator medication (including the blinded trial medication) for 6 h before attending the laboratory.

Sixty eight patients completed the study. Of these, four were excluded from analysis because of protocol violations [1]. Data from 64 subjects were analysed.

The period of better asthma control was judged by within-subject comparisons of data from weeks 9–24 of each treatment period using predefined criteria: the need for short courses of prednisone, morning PEFR, additional nocturnal bronchodilator use, nocturnal symptoms, daytime symptoms, evening PEFR and additional daytime bronchodilator use.

Further analyses compared the effects of regular β-agonist with placebo on lung function, methacholine responsiveness, bronchodilator responsiveness, morning and evening PEFR, diurnal PEFR variation and asthma exacerbations [2].

Identification of polymorphisms

All 64 subjects who had satisfactorily completed the trial were invited to provide venous blood samples for the identification of their β_2-receptor genotype. Two were living overseas and unable to participate and one refused. Samples were obtained from the remaining 61 subjects. Informed consent was obtained from all participants. The study was approved by the Southern Regional Health Authority Ethics Committee (Otago).

Deoxyribonucleic acid (DNA) was extracted from the citrated blood sample using a commercial kit (QIAamp; Qiagen, Hilden, Germany) within 48 h of obtaining the sample. DNA specimens were kept frozen until transfer to the University of Cincinnati for genotyping. Identification of the polymorphisms at nucleic acids 46 and 79 of the β_2-receptor gene (coding for the amino acids at position 16 and 27 of the β_2-receptor protein) was performed using allele-specific polymerase chain reaction as previously described [9].

Statistical analysis

The frequencies of the different polymorphisms of the group judged to have had an overall improvement in asthma control during regular fenoterol treatment in the earlier study were compared with the frequencies for the group whose asthma deteriorated and the group whose asthma showed no change during regular treatment (Chi-squared). In addition, the changes in morning PEFR, evening PEFR, diurnal variation of PEFR, daytime and night-time asthma symptoms, additional bronchodilator use, FEV_1, methacholine responsiveness and bronchodilator response that occurred between the regular compared to the as-required treatment periods were calculated for each of the genotypes at position 16 and 27. Differences in the between-treatment changes were compared using a repeated-measures analysis of variance (MANOVA) (Statistical Products and Service Solutions; SPSS Inc., Chicago, IL, USA).

Results

The genotype for position 16 was determined in all 61 subjects. The allelic frequency (percentage of the total 122 alleles) of Gly 16 was 66% (49% of subjects were homozygous for Gly 16). The genotype for position 27 could

Table 1. – Number of subjects with the polymorphisms at position 16 and position 27

Position 27	Position 16			Total
	Gly/Gly	Gly/Arg	Arg/Arg	
Glu/Glu	17	1	0	18
Glu/Gln	10	14	1	25
Gln/Gln	2	4	11	17
Total	29	19	12	60

The genotype for position 27 could not be identified in one subject (not included in table). For position 16 the "wild-type" is arginine (Arg). For position 27 the "wild-type" is glutamine (Gln). Chi-squared is 45.7 with 4 degrees of freedom ($p < 0.0001$). Gly: glycine; Glu: glutamic acid.

not be determined in one subject. In the remainder the allelic frequency of the Glu 27 polymorphism was 51% (30% homozygous). There was a strong linkage between the polymorphisms (table 1) (Chi-squared, p<0.0001).

The genotype of those whose asthma was better controlled during the 24 weeks in which they used regular β-agonist therapy (n=16) did not differ significantly at either position 16 or 27 from those whose asthma showed no change (n=7) or deteriorated (n=38) during the regular treatment period (table 2).

When the responses to treatment were compared between genotypes, significant differences were found for two outcome variables (table 3):

1) The change in bronchial responsiveness to methacholine during regular compared to as-required β-agonist treatment was significantly different between genotypes (p=0.029). Subjects homozygous for Gly 16 did not show a deterioration in bronchial responsiveness during regular treatment (+0.08 doubling dose shift in PC_{20}), whereas heterozygotes and homozygotes for Arg 16 did (-0.71 and -0.88 doubling dose shifts, respectively). Change in methacholine responsiveness during treatment was not associated with polymorphism at position 27.

2) Subjects homozygous or heterozygous for Gln 27 had higher evening PEFRs during regular fenoterol treatment (attributable to the measurements being made 1–2 h after the evening dose of fenoterol). Subjects homozygous for Glu 27 did not show this increase in evening PEFR during regular treatment (p=0.037 for difference in evening PEFR change between groups). Change in evening PEFR was not affected by the polymorphisms at position 16.

No relationships were found between the genotypes at either position 16 or 27 and between-treatment changes in any of the following measures: morning PEFR; diurnal variation in PEFR; FEV_1; additional bronchodilator use; bronchodilator response; or asthma symptom scores (table 3).

Table 2. – Treatment period associated with best overall asthma control by genotype

	Position 16 (n=61)			Position 27 (n=60)		
Best treatment period	Arg/Arg	Arg/Gly	Gly/Gly	Gln/Gln	Gln/Glu	Glu/Glu
Regular	2 (17)	4 (21)	10 (33)	4 (24)	7 (28)	5 (28)
No difference	3 (25)	3 (16)	1 (3)	3 (18)	3 (12)	1 (6)
As required	7 (58)	12 (63)	19 (63)	10 (59)	15 (60)	12 (67)
Total	12	19	30	17	25	18

Values are presented as the number of subjects, and percentage in parenthesis, with each genotype whose asthma control was better during regular or as required β-agonist treatment or in whom there was no difference between treatment periods. For definitions see legend to table 1.

Table 3. – Mean values during as-required and regular beta-agonist treatment for each of the genotypes controlling for amino acid residues 16 and 27, and for all subjects

			PEFR L·min^{-1}						Symptom score		
		FEV_1 L	am	pm	Diurnal	PC_{20}† mg·mL^{-1}	BDR %$^{+}$	Extra BD puffs·day^{-1}	Day	Nocturnal	Total
Position 16											
Arg/Arg	As req.	2.44	415	442	7.13	1.25	29.1	2.15	1.48	0.36	1.84
(n=12)	Reg.	2.22	405	466	17.86	0.68	30.9	2.32	1.82	0.48	2.29
	Δ	-0.22	-10	24	10.73	-0.88	1.8	0.17	0.34	0.12	0.45
Arg/Gly	As req.	2.55	396	422	8.52	2.37	21.4	2.04	1.78	0.30	2.08
(n=19)	Reg.	2.48	391	444	17.54	1.45	23.6	1.77	1.85	0.32	2.17
	Δ	-0.07	-5	22	9.02	-0.71	2.2	-0.27	0.07	0.02	0.09
Gly/Gly	As req.	2.44	396	426	11.01	1.33	29.9	3.00	1.54	0.56	2.09
(n=30)	Reg.	2.26	386	436	17.15	1.41	33.5	2.72	1.61	0.57	2.18
	Δ	-0.18	-10	10	6.14	0.08§	3.6	-0.28	0.07	0.01	0.09
Position 27											
Gln/Gln	As req.	2.35	386	415	9.06	1.21	32.6	2.30	1.54	0.32	1.86
(n=17)	Reg.	2.17	380	444	20.19	0.84	34.8	2.38	1.79	0.42	2.20
	Δ	-0.18	-6	29	11.13	-0.53	2.2	0.08	0.25	0.10	0.34
Gln/Glu	As req.	2.53	401	427	8.17	2.06	21.6	2.17	1.88	0.40	2.28
(n=25)	Reg.	2.44	394	444	16.25	1.59	23.8	1.73	1.80	0.39	2.19
	Δ	-0.09	-7	17	8.08	-0.37	2.2	-0.44	-0.08	-0.01	-0.09
Glu/Glu	As req.	2.52	409	443	12.29	1.45	30.1	3.39	1.36	0.62	1.98
(n=18)	Reg.	2.31	396	446	17.12	1.28	34.1	3.29	1.64	0.67	2.31
	Δ	-0.21	-13	3$^{\#}$	4.83	-0.18	4.0	-0.10	0.28	0.05	0.33
All subjects											
(n=60)	As req.	2.48	399	428	9.66	0.47	27.2	2.57	1.63	0.44	2.07
	Reg.	2.32	391	445	17.62	0.22	30.0	2.38	1.75	0.48	2.23
	Δ	-0.15	-8	17	7.96	-0.28	2.8	-0.19	0.12	0.04	0.16

The values are presented as overall means during each treatment period. †: geometric mean values, difference expressed as doubling dose shifts; $^{+}$: percentage improvement in forced expiratory volume in one second (FEV_1) following bronchodilator; §: p=0.029, comparing Gly/Gly with Arg/Arg and Arg/Gly; $^{\#}$: p=0.037, comparing Glu/Glu with Gln/Gln and Gln/Glu. PEFR: peak expiratory flow rate; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1; BDR: bronchodilator response; Extra BD: mean bronchodilator use during treatment period; Arg: arginine; Gly: glycine; Glu: glutamic acid; Gln: glutamine; As req.: as required; Reg.: regular; Δ: difference.

Because of the strong linkage between the genotypes (see table 1) it was not possible to assess the effects of different combinations of the 16 and 27 polymorphisms or to separate the independent effects of each polymorphism. However, it was possible to compare subjects who were heterozygous for Gln 27 but homozygous for Gly 16 (n= 10) with those who were heterozygous for both Gln 27 and Gly 16 (n=14) (*i.e.* differing only at position 16). In this comparison, those who were homozygous for Gly 16 did not experience an increase in bronchial responsiveness to methacholine during regular treatment, whereas those who were heterozygous did (0.35 and -0.91 doubling dose shifts, respectively, p=0.008). No other significant differences were found. Comparison was also made between subjects who were homozygous for both Gly 16 and Glu 27 (n=17) and those who were homozygous for Gly 16 but heterozygous for Glu 27 (n=10) (*i.e.* differing only at position 27). No significant differences occurred between these groups for any outcome variable.

To establish whether the simultaneous inheritance of both polymorphisms altered to response in β-agonist therapy, a comparison was made between the 17 subjects homozygous for both Gly 16 and Glu 27 and the 11 subjects homozygous for Arg 16 and Gln 27 (*i.e.* differing at both the 16 and 27 position). Subjects with the Gly 16/ Glu 27 combination had a smaller increase in diurnal PEFR variation during active treatment than subjects with Arg 16/ Gln 27 (3.9 and 10.9%, respectively, p=0.045). No other significant differences were identified.

Discussion

This study did not find an association between β_2-adrenoceptor genotype and change in asthma control during regular high dose inhaled β_2-agonist therapy. We had hypothesized that the β_2-receptor polymorphism associated with enhanced receptor down-regulation *in vitro* (Gly 16) would be associated with a greater deterioration of asthma control during regular β-agonist treatment. However, we found that the majority of subjects showed a deterioration in overall asthma control regardless of their genotype.

When individual outcome variables were considered separately, significant differences in response between the genotypes were found for only two of the 10 measured variables. Subjects homozygous for Gly 16 did not have the increase in bronchial responsiveness during fenoterol treatment seen in other genotypes. Since the Gly 16 polymorphism leads to increased down regulation *in vitro*, this finding is the opposite of that which we had predicted. We also found that, unlike other genotypes, subjects homozygous for Glu 27 had no increase in evening PEFR during regular fenoterol. *In vitro* this polymorphism confers resistance to down regulation and we would, therefore, have expected individuals with this polymorphism to have a greater increase in evening PEFR than other genotypes. Thus, neither of the two statistically significant associations that we found are consistent with our original hypothesis. Although these findings are interesting, the importance of these two results in the context of a large number of analyses is uncertain

Throughout the study, all subjects were permitted to use extra inhaled β-agonist as required. During the "as-required" phase of the study subjects used a mean of 2.6 puffs·day^{-1} of known β-agonist, although the total β-agonist use was much higher in the regular treatment group (mean 10.0 puffs·day^{-1}). This raises the possibility that there may have been significant down-regulation of pulmonary β_2-adrenoceptors during both the placebo and active treatment periods. Thus, although we cannot exclude the possibility that the response to low-dose β-agonist treatment is determined by β_2-receptor genotype, the change in asthma control associated with the switch from low to high dose (placebo phase *versus* active phase) β-agonist therapy does not appear to be explained by a further increase in genetically determined down-regulation.

The power of this study to detect an association between genotype and treatment outcome was considered. Based on a 50% occurrence of homozygotes for the Gly 16 polymorphism (actual occurrence = 49%) this study had a greater than 80% chance of detecting a difference of 0.7 standard deviations in any of the continuous variables. Smaller differences may have been missed by this study, but the data do not even show a consistent trend for difference between the genotypes. The existence of a small association between β_2-receptor genotype and response to regular β-agonist treatment cannot be excluded by this study, but the genotype was clearly not an important factor in determining the overall response.

An association was identified between the Gly 16 and Glu 27 polymorphisms. *In vitro* these polymorphisms have opposite effects on down-regulation and the simultaneous occurrence of both polymorphisms *in vivo* may confound their individual effects. We were only able to examine the effects of each individual polymorphism while "controlling" for the other in a very limited way. In the subgroup who were heterozygous at position 27 it appeared that the Gly 16 polymorphism protected against deterioration in bronchial responsiveness during regular β-agonist treatment, thus confirming one of the findings from the whole group. No other significant association was identified. *In vitro* the simultaneous expression of both polymorphisms results in enhanced down-regulation to a similar extent to the Gly 16 polymorphism alone [7]. Whether the interaction between these polymorphisms is different *in vivo* is unknown. In this study, the group who were homozygous for both polymorphisms (Gly 16 and Glu 27) had a smaller increase in diurnal peak flow variation during regular β-agonist treatment compared to those with neither polymorphism, but there were no other differences.

The role of β_2-receptor regulation in asthma is unclear. While tachyphylaxis to the bronchodilator effects of β-agonists occurs in nonasthmatic individuals, it has been difficult to demonstrate in asthmatics [13–17]. However, tolerance to the bronchoprotective effects of β-agonists in asthma has been demonstrated [3–5]. Thus, the mechanisms of β_2-receptor regulation in asthma appear to differ between cell types and may be altered by disease states [18]. It has recently been reported that asthmatic subjects homozygous for Gly 16 demonstrate greater tachyphylaxis to the long-acting β-agonist formoterol [11]. In the present study we found no difference in bronchodilator tachyphylaxis between genotypes. Against this background, the clinical importance of *in vitro* differences in regulation of β_2-receptors conferred by polymorphisms of the receptor protein is difficult to interpret.

In conclusion, polymorphism of the β_2-receptor in 61 New Zealand asthmatics did not appear to determine the response to long-term inhaled β_2-agonist treatment. Subjects of all genotypes demonstrated poorer control of asthma when treated with regular fenoterol compared with as-required therapy. The apparent protective effect of the Gly 16 polymorphism against deterioration in bronchial responsiveness with regular β-agonist treatment warrants further investigation. The mechanism underlying the overall deterioration in asthma control during regular β_2-agonist treatment is still to be explained.

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