# Fluctuation analysis of lung function as a predictor of long-term response to β2-agonists

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**ABSTRACT**

Response to $\beta_2$-agonists differs between asthmatics and has been linked to subsequent adverse events, even death. Possible determinants include $\beta_2$-adrenoceptor genotype at position 16, lung function, and airway hyperresponsiveness. Fluctuation analysis provides a simple parameter $\alpha$ measuring the complex correlation properties of day-to-day peak expiratory flow. We investigated whether $\alpha$ predicts clinical response to $\beta_2$-agonist treatment, taking into account other conventional predictors.

Analysis was performed on previously-published twice-daily peak expiratory flow measurements in 66 adult asthmatics over three six-month randomised order treatment periods – placebo, salbutamol and salmeterol. Multiple linear regression was used to determine the association between $\alpha$ during placebo period and response to treatment (change in number of days with symptoms), taking into account other predictors namely $\beta_2$-adrenoceptor genotype, lung function, its variability, and airway hyperresponsiveness.

We found that $\alpha$ during placebo period considerably improved the prediction of response to salmeterol treatment, taking into account genotype, lung function or its variability, or airway hyperresponsiveness.

We provide further evidence that response to $\beta_2$-agonists is related to the time correlation properties of lung function in asthma. We conclude that fluctuation analysis of lung function offers a novel predictor to identify patients who may respond well or poorly to treatment.

## INTRODUCTION

The $\beta_2$-agonist controversy is based on substantial evidence relating $\beta_2$-agonist use to adverse asthma outcomes, including mortality[1-5]. Responses to $\beta_2$-agonists differ among individuals[2, 6]. This heterogeneity may partly be related to $\beta_2$-adrenoceptor (ADRB2) genotypes[2, 6-12]. The homozygous Arg-16 polymorphism has been associated with poorer outcomes[7, 8], although findings are not entirely consistent[13, 14]. Additionally, airway hyperresponsiveness, lung function and its variability have been proposed to predict treatment response[15]. However, often only a weak relationship exists between these factors and treatment response, suggesting that conventional pathophysiological measurements are inadequate to predict the behaviour of complex diseases such as asthma[16, 17]. Therefore, a crucial target for investigation remains: to find new predictors which allow clinicians to identify patients who would or would not benefit from treatment**.**

The complexities in asthma could be characterised in a novel way, by considering it as a dynamical disease[16, 18-23]. In this context, the likelihood of loss of asthma control may be better characterised by fluctuation analysis[24] applied to time series of daily variations in peak expiratory flow (PEF)[18]. The method yields a single parameter $\alpha$, which quantifies the strength of long-range correlations present in the time series. The presence of long-range correlations means that PEF on any given day is dependent on its values on previous days even over longer time intervals[18], consistent with fractal behavior. Hence $\alpha$ can be thought of as a measure of complexity arising from the intrinsic control of the system producing the fluctuations, and likely influenced by external stimuli. Fluctuation analyses have been applied to heart rate variability[24], end-tidal oxygen and carbon dioxide concentrations[25], and tidal volume[25, 26]. Analysis of heart rate fluctuations have even been used to predict tachycardia following a myocardial infarction[27]. Using twice-daily PEF, we have reported that in a group of mild to moderate asthmatic adults in a randomised placebo-controlled three-way

crossover study[28], $\alpha$ tended to increase, and thus asthma control improved, during long-term salmeterol treatment. In contrast, $\alpha$ significantly decreased during long-term salbutamol treatment[18]. Thus, $\alpha$ has value in characterising asthma control during treatment.

In this study, we aimed to determine whether $\alpha$ measured in the absence of regular treatment, using data obtained during the placebo period ($\alpha$(PL)), has value in predicting clinical response to long-term $\beta_2$-agonist treatment. To answer this, we determined the association between $\alpha$(PL) and changes in number of days with clinical symptoms during treatment, taking into account other important predictors of treatment response, namely ADRB2 genotype, lung function and its variability, and airway hyperresponsiveness.

## MATERIAL AND METHODS

*Study population and design*

This study was a retrospective analysis of a randomised, double-blind, double-dummy, crossover study, approved by the Otago and Canterbury ethics committees[28, 29]. Briefly, 157 mild to moderate asthmatic adult subjects underwent a four-week run-in period involving assessment of spirometric lung function ($FEV_1$) and airway responsiveness to methacholine (provocative concentration causing 20% fall in $FEV_1$, $PC_{20}FEV_1$). This was followed by three six-month randomised treatment periods during which they received salmeterol 50 μg twice daily, salbutamol 400 μg four times daily, or lactose placebo, with intervening four-week placebo washout periods. Subjects maintained the same dosage of their inhaled corticosteroids, if applicable, for at least three months prior to and during the study, but were allowed on-demand rescue bronchodilators and emergency oral corticosteroids for exacerbations as appropriate.

Patients were instructed to record their PEF and respiratory symptoms twice daily in a diary card. Symptoms recorded included day- and night-time chest tightness/wheeze/dyspnoea, cough, sputum production, exercise, and nocturnal wakening, rated on a 0 to 3 scale or a yes/no response where applicable. A composite asthma score taking into account symptoms, morning PEF, and rescue bronchodilator use was computed for each study day, ranging from 0 for stable asthma to 4 for major exacerbation/medical emergency. Details of the asthma score calculation have been previously published[28].

Of the 157 subjects in the database (80 of whom have had their fluctuation analysis data reported previously[18]), 66 were identified who had undergone genotyping for ADRB2 position 16[29], lung function and airway hyperresponsivess testing, and for whom fluctuation analysis[18] data were available in all three treatment periods.

*Fluctuation analysis*

Fluctuation analysis was undertaken using custom-written software (Matlab, The Mathworks Inc, MA) as described previously[18]. The analysis was limited to PEF time series with 300 data points for each treatment period, corresponding to the first 150 days of each treatment period, in order to standardise the data length across subjects. The time series was first integrated and then divided into non-overlapping windows of size n. The local trend in each window was removed by fitting and subtracting a regression line from the integrated data. The root-mean-square values of the detrended signal was calculated for a given window length n to yield the detrended fluctuation function, F(n). This calculation was then repeated for increasing n, and log F(n) was plotted against log n. Typically, F(n), a measure of the fluctuations, increases with n. A linear relation between log F(n) versus log n indicates the presence of scaling, which can be characterised by the slope $\alpha$ of the regression line fit.

A PEF time series with $\alpha$ of 0.5 indicates a system that is not deterministic and prone to instabilities and exacerbations, whereas higher $\alpha$ values imply more deterministic behavior with stronger correlations present and hence are more likely to be the expression of stable and more predictable asthma control[18].

*Clinical outcome*

From the asthma score we derived the total number of symptom days, i.e. days when the asthma score was greater than zero within the same 150-day period which was used for fluctuation analysis. This outcome was chosen because, unlike total asthma score, it was not directly dependent on PEF. Our primary outcome was the difference in the number of symptom days between placebo and either the salbutamol or salmeterol treatment period (i.e., treatment – placebo). Thus, an increase (positive difference) in the number of symptom days

indicates deterioration in clinical condition, while a decrease (negative difference) indicates improvement. This outcome was less dependent on PEF than the asthma score (which included morning PEF as one dimension).

*Statistical analysis*

All statistical analyses were performed using Intercooled Stata 8.1 for Windows (Stata Corporation, College Station, Texas, USA). Multiple linear regression models were used to examine associations between potential predictors of interest and clinical outcome. To test the robustness of our findings, we performed two sensitivity analyses using slightly different statistical approaches. First, we looked at number of symptom days during treatment *per se* (i.e. not the difference from placebo) using a negative binomial regression model instead of linear regression, because the number of symptom days was not normally distributed but conformed to that expected from count or rate data. The more conventional Poisson regression (a special case of negative binomial regression) was not appropriate due to overdispersion, i.e. high variance compared to the mean. Second, we repeated the linear regression using differences in the mean asthma score between placebo and treatment as outcome, instead of differences in number of symptom days.

A standard model was defined as the model in which adjustments were made for age, sex, treatment order, and number of symptom days during the placebo period. The possible effect of treatment order was adjusted for using the position of the relevant treatment period within the sequence of treatments. All continuous, i.e., non-categorical variables such as age and number of symptom days during the placebo period were centered to the mean.

In a first, simple model, potential predictors of outcome were examined separately by adding each respective predictor to the standard model. Predictors of interest were: α(PL), ADRB2

genotype, lung function expressed as percentage of the predicted value for the subject[30] (%predPEF, %predFEV$_1$), coefficient of variation of PEF (CVPEF), and airway hyperresponsiveness (PC$_{20}$FEV$_1$). To facilitate interpretation, these were stratified into low, medium and high tertiles, and comparison made with the lowest tertile as baseline, except for ADRB2 genotype, for which Gly/Gly genotype was used as the reference group. Thus, the coefficients reported are relative to the baseline group of subjects with low $\alpha$(PL), low %predPEF, low %predFEV$_1$, etc, with the baseline group effect represented by the constant term of the regression.

Predictors which were found to be significantly associated with the outcome (p < 0.05) were then included into a fully-adjusted, multivariable model. Potential interaction between $\alpha$(PL) and genotype within the multivariable model was tested for statistical significance using the F-test.

To evaluate how well $\alpha$(PL) predicted response to treatment relative to other parameters, we compared the adjusted R$^2$ value of the standard model with models which included one or more of the predictors of interest ($\alpha$(PL), ADRB2 genotype, %predPEF and CVPEF during the placebo period, and %predFEV$_1$ and PC$_{20}$FEV$_1$ during run-in). Predictors were tested for significance using the F-test.

*Sensitivity to PEF quality control criteria*

For fluctuation analysis we used 300 PEF data points (150 days) with less than 3% missing data. We investigated the effect of relaxing this strict quality control criteria for inclusion of PEF time series into the study (by using shorter PEF series or allowing greater percentage of missing data). Details and results are found in the Appendix.

**RESULTS**

*Subject characteristics*

[18][29][18]Characteristics, spirometric lung function and airway hyperresponsiveness of the 66 subjects obtained at run-in ($FEV_1$, $PC_{20}FEV_1$), as well as potential predictors obtained during the placebo periods ($\alpha$(PL), PEF, CVPEF), are summarised in Table 1. The mean (SD) values of the low, medium and high tertiles for the predictors are also shown. The three genotype groups were: homozygous Gly-16 (Gly/Gly), heterozygous (Gly/Arg) and homozygous Arg-16 (Arg/Arg). The genotype frequencies in the original population have been shown to be consistent with the Hardy-Weinberg equilibrium[29]. Clinical assessment parameters, i.e. the mean asthma score and number of symptom days during the placebo and treatment periods are given in Table 2.

*Association of $\alpha$(PL) with clinical response to treatment*

For salmeterol, $\alpha$(PL), %predPEF during placebo period and Arg/Arg genotype were significantly associated with response to treatment, both in the simple and the multivariable regression models (Table 3). The association between $\alpha$(PL) and change in days with symptoms during salmeterol treatment became stronger in the multivariable model, with a mean improvement of -17.9 and -16.1 days with symptoms in individuals with medium and high $\alpha$(PL), respectively, compared to those with low values. The association with genotype was not significant for Gly/Arg (p = 0.278) and was weak for Arg/Arg (p = 0.044). There was no evidence for an interaction between $\alpha$(PL) and genotype in our data (p for interaction = 0.739).

For salbutamol, the only predictor which was significantly associated with treatment outcome was %predPEF during placebo period, in both the simple and multivariable regression models (Table E1 in the online supplement).

The changes in the number of symptom days in response to both salbutamol and salmeterol are illustrated in Figure 1. With increasing $\alpha$(PL) from the low, medium to high tertiles, relatively small change in symptom days is observed with salbutamol while progressively fewer symptom days are observed with salmeterol.

*Comparison of $\alpha$(PL) with other predictors*

The $R^2$ values of the standard regression model as well as models including one or more of the predictors of interest are shown in Table 4. Adding $\alpha$(PL) to the model improved the goodness-of-fit from 0.563 in the standard model to 0.604. This was a similar improvement to adding %predPEF into the model, and better than for genotype or $PC_{20}FEV_1$. The goodness of fit increased to 0.662 when both $\alpha$(PL) and %predPEF were included in the model, suggesting independent effects.

Similar results were obtained with the two alternative statistical approaches (predicting number of symptom days *per se* using a negative binomial model, and predicting the difference in mean asthma score with a linear regression model). Results for genotype became weaker with asthma score as the outcome, but the associations with $\alpha$(PL) and %predPEF remained highly significant. These are presented in Tables E2 and E3 in the online supplement, respectively.

**DISCUSSION**

In this study, we set out to determine the clinical utility of fluctuation analysis of PEF, specifically whether it helps to predict treatment outcome over and above conventionally-used predictors. We found that $\alpha$(PL) was strongly and independently related to the change in clinical symptoms in response to salmeterol treatment. When coupled with %predPEF, $\alpha$(PL) adds considerable predictive power to using CVPEF, %predFEV$_1$, PC$_{20}$FEV$_1$ and ADRB2 position 16 genotype.

*Interpretation of findings*

The strength of $\alpha$ as a predictor of treatment response lends further support to the idea that asthma is a complex dynamic disease[16, 18-21], well-characterised by a parameter which quantifies long-range correlations. In practical terms, if a patient has either a medium (or high) $\alpha$ during the placebo period, then that patient would likely have 18 (or 16) fewer days with symptoms during the 150 days of salmeterol treatment, i.e. 11% of treatment days. This compares with only 1% (from the constant term in the regression) of treatment days in a patient with a low $\alpha$ and %predPEF (based on data from Table 3). It is notable that a medium $\alpha$ appears to be as good as a high $\alpha$, as the high tertile does not appear to result in further improvement compared to the medium tertile [18].

Interestingly, if a patient had a high %predPEF during the placebo period, he would have 23 (15%) more symptom days with treatment compared to low %predPEF, corrected for the number of symptom days during placebo. At first glance this seems counter-intuitive but could be due to a "ceiling" effect, whereby a person with low %predPEF has greater room for improvement with treatment and thus a very large negative change in symptom days, while a person with high %predPEF has only a modest negative change in symptom days.

There was a lack of predictive power of $\alpha$(PL) for outcomes during salbutamol treatment. A possible explanation, consistent with our previous findings[18], is that the frequent short-term stimuli provided by salbutamol results in deterioration in asthma control independently of baseline $\alpha$. As a consequence, the PEF pattern becomes irregular in time, which in turn results in a significant loss of predictive power.

Combining $\alpha$(PL) and %predPEF explained almost 10% of the total goodness-of-fit in modelling individual responses to treatment. This is especially beneficial as it does not require an additional measurement procedure for the patient, since the two are calculated from the same peak flow measurements. It is worth making the distinction between $\alpha$ and PEF here. A high $\alpha$ is not the only criterion for stability, since it is an indicator of time correlation properties of the PEF, as opposed to the properties of the PEF magnitude distribution. A patient with a high $\alpha$, but low mean PEF or high CV in PEF, may still have poorly-controlled asthma, since these are independent properties of the daily PEF behaviour, both of which contribute independently to predicting whether a patient reamains stable or not[20].

*Significance of findings*

In our previous study, we found presence of long-range correlations in PEF, and that $\alpha$ was useful in characterising long-term treatment response[18]. Here, we show that it is also useful in *predicting* long-term treatment response, even when measured in the absence of such treatment (in this case during the placebo period). Also of note in our previous findings was the suggestion that there might be some "optimum" value of $\alpha$, since any positive or negative deviation in $\alpha$ from a mean of 0.78 during the placebo period corresponded to a tendency for $\alpha$ to return to 0.78 with treatment[18]. In the present study, we see more evidence for this,

although in this case a plateau is more apparent: improvement in symptoms was optimally predicted when $\alpha$ was in the medium or high tertiles. Additionally, using multiple regression we were able to complement the value of $\alpha$ by taking into account other factors, notably %predPEF, and show that it compares favourably with other conventional predictors.

Our results add value to the use of daily peak flow measurements in assessing and monitoring patients with asthma [31] by looking at its variability in a new way. Previously there has been doubt that regular measurements of peak flow add value in monitoring asthma given that they do not appear to be consistently related to current symptoms [32-34]. Zhang and coworkers have pointed out that multiple measurements of lung function over a length of time are a minimum criterion for being able to establish end points for asthma control[17]. With the advent of electronic peak flow monitoring and appropriate software for analyses, their predictive role in assessing asthma control may deserve renewed attention. Furthermore, a multi-dimensional approach to monitoring asthma, taking into account various factors and their relative contributions to asthma, is increasingly being advocated[16, 20, 32, 36]. The characterisation of time correlation properties of lung function complements this approach.

*Limitations and open questions*

Our study has a number of limitations. First, the numbers are small. One drawback of fluctuation analysis is that a large number of data points, e.g. 150 days, is necessary for the determination of long-range correlations. We have used very strict quality control criteria (see Appendix) to determine acceptability of individual PEF time series data into our study. This restricted the total number of eligible patients from the original study. We investigated relaxing these criteria, but found that as the length of the PEF series decreased, the association of $\alpha(PL)$ with outcome became less significant compared to %predPEF. This is not surprising given that the interpretation of long-range correlations becomes less important when the time

range of the data is decreased. Similarly as the percentage of missing data increased, a similar but less pronounced pattern emerged. Thus fluctuation analysis is to some extent dependent on the completeness of the data, and its use is limited to long-term monitoring of asthma. However, in such cases we provide an extra dimension of usefulness to data which would already be available[31]. Note that the actual time over which the data is collected is not the principal limitation of the method, rather it is the number of data points required to characterise the fluctuation dynamics. Analysis of variability from respiratory data collected with greater frequency and over shorter time-scales has been done in relation to asthma[19, 23, 37], but the time scale over which interpretation of the results is made would likely have to be adjusted accordingly. It may be that fluctuations over short times scales can predict behaviour at longer time scales, which would be a great advantage, but this has yet to be shown.

Second, corticosteroids and on-demand $\beta_2$-agonist bronchodilators were permitted throughout the study. There is evidence that concurrent use of corticosteroids mitigates the adverse effect of long-acting $\beta_2$-agonists[38]. However, in our study the contribution of $\alpha(PL)$ and PEF to predicting better or worse response to salmeterol was apparent even in the presence of corticosteroid use. Third, we did not adjust for a past history of smoking, which could be a potential confounder given recent evidence for interaction between passive smoking and ADRB2 genotype[39]. However current and ex-smokers (>5 pack years) were excluded from the study.

Finally, we calculated $\alpha$ using data obtained from the placebo period. Arguably, the run-in period would have been more suitable as it was prior to any treatment, unfortunately it was too short to allow adequate fluctuation analysis to be performed. However both periods would be comparable, given that during the run-in the subjects were taking placebo, there was a

washout of four weeks between treatment periods, and we have corrected for the possible effect of treatment order on the outcome in our regression analysis. Despite these limitations, what is clear from our study is that the relationship between $\alpha(PL)$ and symptom response to treatment remained strongly significant using different adjustments, outcomes and regression models.

*Conclusion*

Having previously identified the utility of long-range correlations in daily lung function for characterising $\beta_2$-agonist treatment responses, here we report their utility in *predicting* response to treatment. We found that fluctuation analysis of baseline lung function measurements was strongly associated with changes in symptoms with long-term treatment, in this instance using salmeterol. Baseline $\alpha$, when coupled with mean daily peak flows, added considerably to the prediction of outcome compared to other conventional single measures of spirometric lung function, variability of lung function, airway hyperresponsiveness, as well as ADRB2 genotype, and contained information which seemed to be distinct and independent of these factors. This novel approach of looking at the time history of PEF recordings, distinct from studying its mean or variability, not only provides additional insight into asthma control, but also offers a potential new parameter to predict whether a patient will respond favourably or adversely to treatment in the future. This is particularly important in light of the potential detrimental response to $\beta_2$-agonists. It also constitutes a step towards the multi-dimensional approach to asthma monitoring, which is increasingly valid in such a complex disease.

**APPENDIX. Sensitivity to quality control criteria of PEF time series**

For our study and in previous work, we have used strict acceptability criteria to determine if a time series was to be included into the analysis. The criteria were as follows: at least 300 PEF data points (150 days) available, less than 3% missing data, and furthermore these two criteria had to be met in all treatment and placebo periods for a subject to be included into the study. Where there were missing data, they were replaced by the PEF value of the previous corresponding day/night, as detailed previously[18].

We repeated the regression analyses of Tables 3 and 4, while comparing $\alpha(PL)$ calculated using the following criteria: using 300, 200 and 100 data points, and allowing less than 3%, 5% and 10% missing data in the PEF time series. To allow comparison across different data lengths, the outcome (change in number of symptom days from placebo to treatment period) was calculated over the entire 150 day observation period regardless of data length used to calculate $\alpha(PL)$, but normalised to one year.

We found that the association of $\alpha(PL)$ with outcome became less significant as the length of the PEF series decreased from 300 to 100 data points (from $-14.2$ days per observation period, $p = 0.009$ to $-8.9$ days per observation period, $p = 0.096$, both for the highest $\alpha(PL)$ tertile), as %predPEF during placebo period became more important. The association of $\alpha(PL)$ with outcome also became less significant as the percentage of missing data increased from 3% (from $-14.2$ days per observation period, $p = 0.009$ to $-9.8$ days per observation period, $p = 0.058$, both for the highest $\alpha(PL)$ tertile) to 10%, although the effect was marginally less pronounced than with the data length. However, $\alpha(PL)$ and %predPEF still yielded the greatest increase in model fit from the standard model, regardless of data length or amount of missing data.

Relaxing the criterion from 300 to 100 data points resulted in the percentage of acceptable subjects changing from 71% to only 72% out of the total subjects with otherwise complete data (n = 108). When relaxing the criterion from 3% to 10% missing data allowable, the percentage of acceptable subjects changed from 71% to 87%.

**ACKNOWLEDGEMENTS**

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**TABLES**

**Table 1.** Characteristics of the study population including $\beta_2$-adrenoceptor (ADRB2) genotype, lung function and airway hyperresponsiveness parameters obtained during the run-in period prior to treatment randomisation ($FEV_1$ and $PC_{20}FEV_1$), and parameters calculated from peak expiratory flow during the placebo period ($\alpha$, PEF, CVPEF). See text for full descriptions of parameters.

| Characteristics | Summary (n = 66) | | |
|---|---|---|---|
| Age (years)* | 45 (19 to 64) | | |
| Sex (M:F) | 33:33 | | |
| Height (cm)* | 170 (152 to 194) | | |
| ADRB2 genotype | Gly/Gly: Gly/Arg: Arg/Arg 30: 26: 10 | | |
| | **Tertiles** | | |
| **Run-in** | **Low** | **Medium** | **High** |
| %predFEV$_1$ (%) † | 57.4 (10.4) | 77.7 (4.7) | 95.0 (9.4) |
| PC$_{20}$FEV$_1$ (mg mL$^{-1}$)‡ | 0.26 (0.19 to 0.35) | 1.21 (1.04 to 1.40) | 3.67 (3.11 to 4.33) |
| **Placebo period** | | | |
| $\alpha$ † | 0.59 (0.06) | 0.74 (0.03) | 0.96 (0.15) |
| PEF (L min$^{-1}$) † | 304 (34) | 405 (22) | 520 (49) |
| %predPEF (%) † | 58.1 (5.2) | 75.4 (4.5) | 91.9 (7.0) |
| CVPEF † | 0.042 (0.010) | 0.068 (0.007) | 0.098 (0.021) |

* Median with ranges shown

† Mean with standard deviation shown

‡ Geometric mean with 95% confidence intervals shown.

**Table 2.** Asthma score and number of days with symptoms obtained during the placebo, salbutamol and salmeterol treatment periods (medians and interquartile ranges).

| n = 66 | Placebo | Salbutamol | Salmeterol |
|---|---|---|---|
| Mean asthma score * | 0.15 (0.05 to 0.33) | 0.15 (0.09 to 0.39) | **0.05 (0.01 to 0.13)** |
| Median number of days with symptoms † | 22 (7 to 46) | 22 (12 to 56) | **8 (2 to 19)** |

Outcomes were calculated over an observation period of 150 days. Numbers in bold indicate statistical significance (Wilcoxon signed rank test, $p < 0.05$), compared to placebo.

* Mean over the observation period of a composite asthma score comprising symptoms, morning PEF, and rescue bronchodilator use computed for each study day, ranging from 0 for stable asthma to 4 for major exacerbation/medical emergency. See online supplement for more details.

† Median over the observation period for the number of days on which a non-zero asthma score was recorded.

**Table 3.** Association between patient characteristics at baseline and response to salmeterol treatment (defined as change in number of symptom days from the placebo period).

| Effect | Simple model | | | Multivariable* | | |
|---|---|---|---|---|---|---|
| | Coeff. | 95% CI | P value | Coeff. | 95% CI | P value |
| α(PL) † | | | | | | |
|   Medium | -15.3 | -27.4 to -3.1 | 0.015 | -17.9 | -29.4 to -6.4 | 0.003 |
|   High | -15.4 | -28.2 to -2.7 | 0.018 | -16.1 | -28.5 to -3.8 | 0.012 |
| ADRB2 genotype | | | | | | |
|   Gly/Arg | -2.8 | -14.3 to 8.8 | 0.633 | -5.6 | -16.0 to 4.7 | 0.278 |
|   Arg/Arg | -17.2 | -32.9 to -1.4 | 0.034 | -14.6 | -28.9 to -0.4 | 0.044 |
| %predPEF during PL | | | | | | |
|   Medium | 11.6 | -1.0 to 24.3 | 0.071 | 8.3 | -3.3 to 19.9 | 0.158 |
|   High | 17.6 | 4.1 to 31.1 | 0.012 | 22.9 | 10.4 to 35.4 | 0.001 |
| CVPEF during PL | | | | | | |
|   Medium | -1.5 | -14.8 to 11.7 | 0.815 | - | - | - |
|   High | -11.2 | -26. 9 to 4.8 | 0.158 | - | - | - |
| %predFEV$_1$ | | | | | | |
|   Medium | 7.7 | -5.1 to 20.6 | 0.234 | - | - | - |
|   High | 16.1 | 2.9 to 29.3 | 0.018 | - | - | - |
| PC$_{20}$FEV$_1$ | | | | | | |
|   Medium | 2.6 | -10.9 to 16.1 | 0.702 | - | - | - |
|   High | 6.4 | -7.0 to 19.9 | 0.343 | - | - | - |
| Constant ‡ | - | - | - | -1.5 | -15.5 to 12.4 | 0.825 |

Results derived from multiple linear regression. All models were adjusted for age, sex, sequence of treatment, and number of symptom days during placebo period (PL) by default.

All non-categorical variables such as age and number of symptom days during the placebo period were centred to the corresponding mean. Comparisons were made against having low $\alpha$(PL), Gly/Gly genotype and low %predPEF, CVPEF during the placebo period, %predFEV$_1$ and PC$_{20}$FEV$_1$ during run-in period.

* $R^2$ for the fully-adjusted multivariable model was 0.677. Due to collinearity, only one lung function parameter was included in the multivariable model.

† If a patient has either a medium (or high) $\alpha$ during the placebo period, then that patient would likely have 18 (or 16) fewer days with symptoms during the 150 days of salmeterol treatment, i.e. 11% of treatment days, compared to the placebo period.

‡ A patient with Gly/Gly genotype, a low $\alpha$ and %predPEF during the placebo period will have a mean improvement in number of symptom days of 1.5 days (1%) during the 150 days of salmeterol treatment compared to the placebo period.

**Table 4.** Comparison of goodness-of-fit between linear regression models with different potential predictors of the change in number of symptom days from the placebo period with salmeterol treatment.

| Potential predictor | Adjusted $R^{2*}$ | P value † |
|---|---|---|
| Standard model | 0.563 | - |
| $\alpha$(PL) | 0.604 | 0.022 |
| ADRB2 genotype | 0.582 | 0.102 |
| %predPEF during PL | 0.599 | 0.030 |
| CVPEF during PL | 0.565 | 0.328 |
| %predFEV$_1$ | 0.590 | 0.059 |
| PC$_{20}$FEV$_1$ | 0.555 | 0.632 |
| $\alpha$(PL) and %predPEF during PL | 0.662 | 0.001 |
| Multivariable model (fully-adjusted) | 0.677 | 0.001 |

All models were adjusted for age, sex, sequence of treatment, and number of symptom days during placebo period (PL) by default. The predictors $\alpha$(PL), %predPEF, CVPEF, %predFEV$_1$ and PC$_{20}$FEV$_1$ were categorised into tertiles of low, medium and high values. For ADRB2 position 16 genotype, Gly/Gly was the reference group.

* Note that each parameter was added to the model separately, i.e. the adjusted $R^2$ values are not cumulative.

† P values are shown for the F test, where the null hypothesis was that the relevant parameter was equal to zero (i.e., not associated with the outcome).

**FIGURE LEGENDS**

**Figure 1.** Boxplots showing change in number of symptom days from placebo during treatment with salbutamol and salmeterol, in subjects with values of $\alpha$ during placebo ($\alpha$(PL)) within the low, medium and high tertiles. The median is shown for each boxplot, with the box boundaries denoting the 25th and 75th percentiles, and the error bars denoting the 10th and 90th percentiles. Dots indicate outliers. A negative change indicates improvement of symptoms due to decreased number of symptom days with treatment compared to placebo. With increasing $\alpha$(PL) from the low, medium to high tertiles, relatively little changes in symptom days are seen with salbutamol and progressively less symptom days for salmeterol.