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Evaluation of Spirometry Values in Relation to Beta-2-adrenergic Receptor Gene Polymorphism

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Abstract

Introduction: The vagus nerve plays a special role in the control of respiratory system activity which represents the parasympathetic part of the autonomic nervous system. A small bronchial innervation by the sympathetic system also is observed, and there is a significant expression of adrenergic receptors, in particular β_2 receptors, in the airways. The development of genetics and molecular biology allows for a detailed study which can clarify the essential elements in the pathogenesis of many types of lung disease, as well as the physiological phenomena - bronchial smooth muscle tone and their contractile mechanism.

Material and methods: The study involved 148 healthy male volunteers aged 20-26. In all subjects, gene polymorphism at nucleotide position 46 and 79 of β_2-adrenergic receptor (β_2-ADR) was assessed. According to the gene polymorphism data, we divided the whole examined population of males into 6 groups for further studies. Moreover, in all the subjects, we performed spirometry testing to verify their pulmonary functions.

Results: The basic values of spirometry tests in all subjects were in the range of normal values. The frequency of different genotypes in the gene polymorphism of the β_2-adrenergic receptor at nucleotide positions 46 and 79 were typical for the Caucasian population. Analysis of the output values of spirometry, conducted in the particular groups based on their genotype, showed significant inter-group differences in the selected spirometry tests.

Conclusions: Our results may be useful in explaining the differences in the measured values of spirometric indices in healthy subjects in relation to the polymorphism of β_2-ADR, and may also contribute to the verification of standards for spirometric indices for this selected group of young males in the Polish population.

Key words: β_2-adrenergic receptor, gene polymorphism, spirometry

Introduction

The bronchial smooth muscle tonus is determined by the effect of the nervous system and endogenous signal substances (ligands) supplied via the bloodstream, as well as locally secreted substances [22]. Neural regulation is effected with the mediation of parasympathetic fibres in the autonomic nervous system innervating bronchia and one of its parts, defined as the nonadrenergic and noncholinergic system (NANC). Bronchial tonus is maintained by the neurons of the vagus nerve nuclei, whose excitability is modified by impulses received from the respiratory complex in the brain stem. Surprisingly, a very slight bronchial innervation by the sympathetic system is observed with a significant simultaneous expression of adrenergic receptors, in particular β_2 (β_2-ADR) [10, 22, 29]. Changes in bronchial tonus dependent on the activation of β_2-ADR, are determined by the expression and presence of this receptor in isoforms, resulting from the polymorphism of the β_2-ADR encoding gene. In 1986, Dixon et al [8] described a sequence of amino acids for β_2-ADR isolated from hamster tissues, and demonstrated its structural homology with rhodopsin. The β_2-adrenergic receptor is classified to the group of metabotropic membrane receptors interacting with G_s protein [1, 23, 29]. The β_2-ADR gene, cloned in 1987, resides on a long arm of the chromosome 5 (5q31-32), is polymorphic and contains no introns [19, 24, 29, 31]. Up to the present time, 9 gene mutations have been identified in nucleotides (g.): 46, 79, 100, 252, 491, 523, 1053, 1098 and 1239. Five of them concern degenerative changes not affecting the modification of encoded amino acid, while the other four polymorphic positions, located in nucleotides: 46, 79, 100, and 491, are responsible for the modification of amino acids in respective positions 16, 27, 34, and 164 of the receptor polypeptide chain [23, 24]. The structure of the β_2-adrenergic receptor, with the polymorphic regions marked and changes in the receptor chain corresponding to them are presented in Fig. 1.

The most common polymorphism of the β_2-ADR gene concerns codons 16 and 27. The polymorphism at codon 16 is associated with the potential replacement of adenine with guanine (g. 46 A→G) in nucleotide 46 of the gene (g. 46), which results in the replacement of arginine with glycine at position 16 of the receptor polypeptide chain. A more common polymorphism at codon 27 of β_2-ADR, caused by the replacement of cytosine with guanine at position g. 79 (g.79 C→G), results in the replacement of glutamine with glutamate acid at position 27 of the receptor

chain. Polymorphism at codon 16 β_2-ADR is significant for signal transduction by this receptor. The objective of the present study was to evaluate the frequency of β_2-ADR gene polymorphism at codon g. 46 and g. 79 and its effect on the values of spirometric indices in healthy men.

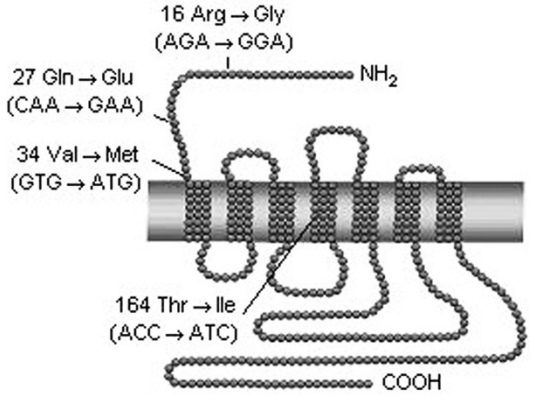

Fig. 1. Structure of the β_2-adrenergic receptor with marked polymorphic regions and corresponding changes in the receptor chain [34].

Material and Methods

The study involved 148 healthy male volunteers aged 20-26. At the first stage, the material for genetic analysis was sampled from the subjects (buccal mucosa smear and oral cavity smear taken after 30 min without intake of food and drink). Genomic DNA was isolated with a Sherlock AX kit from A&A Biotechnology (Gdynia, Poland), according to a method developed by the manufacturer. Afterwards, DNA was amplified in PCR (polymerase chain reaction) to separate obtained DNA fragments electrophoretically; gel photographs were taken under UV (Fig. 2).

Polymorphism of the β_2-ADR encoding gene was evaluated at nucleotide positions 46 and 79. Based on the observed changes subjects were divided into 6 groups: g. 46AA – homozygote, presence of adenine and adenine (at receptor 16Arg/16Arg); g. 46GG – homozygote, presence of guanine and guanine (at receptor 16Gly/16Gly); g. 46AG – heterozygote, presence of adenine and guanine (at receptor 16Arg/16Gly); g. 79CC – homozygote, presence of cytosine and cytosine (at receptor 27Gln/27Gln); g. 79GG – homozygote, presence of guanine and guanine (at receptor 27Glu/27Glu); g. 79GC – heterozygote, presence of guanine and cytosine (at receptor 27Glu/27Gln). Spirometric testing was conducted with Lungtest 1000 apparatus from MES (Cracow, Poland). The obtained values of spirometry indices are presented with relation to the age, sex and body height of examined subjects. The spirometer was calibrated, and current data were entered (temperature, humidity and ambient pressure conditions) before measurements. Testing was carried out according to the recommendations of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) with volumes reported at BTPS parameters (body temperature, pressure saturated) [3, 9, 35]. Vital capacity (VC) and flow/volume loop were measured. The highest measured values of spirometric indices were selected for further analysis.

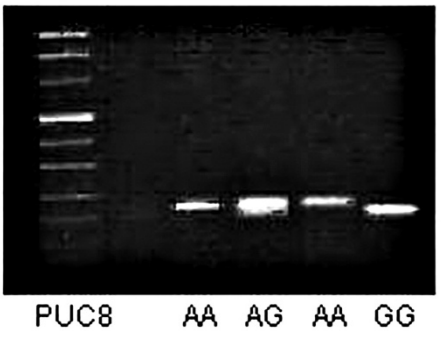

Fig. 2. Polymorphism of g. 46AG (enzyme NcoI-MBI Fermentas). M molecular weight marker; AA-homozygote; AG-heterozygote; GG-homozygote.

The Chi^2 test was used to compare the frequency of genotypes and allele of the gene encoding the β_2-adrenergic receptor. The significance of the differences between the values of measured indices was evaluated in consideration of their correlation and characteristics of sets. Normality of distribution was analyzed with the Shapiro-Wilk test. A non-parametric Mann-Whitney U test was used for distributions other than normal, and a parametric *t*-test for normally distributed data. Significance of correlations between variables was evaluated with Spearman's rank coefficient of correlation.

Results

The distribution of genotypes in the studied group of 148 subjects conformed to the Hardy-Weinberg equilibrium. It was also found that both polymorphisms (SNP) g. 46 and g.v79 are closely correlated: D' = 0.900655; P<0.0001. Three genotypes were found within the nucleotide 46 and three within the nucleotide 79. The distribution of their frequency was as follows: g. 46AA n = 20 (13.5%); g. 46GG n = 65 (43.9%); g. 46AG n = 63 (42.6%); g. 79CC n = 38 (25.7%); g. 79GC n = 31 (21%); and g. 79GG n = 79 (53.3%).

Spirometry testing was performed in 148 subjects. The mean age of the subjects was 22.5 ±1.5SD (range 20-26 years), body height 179.4 ±6.2 (range 165-190 cm), and body mass 77.9 ±10.5 (range 58.0-11.6 kg). Basic values of spirometric indices in all subjects were within the normal range. The mean value of FVCex was 108% and FEV_1 102% predicted. The values of spirometric indices in the individual groups g. 46 and g. 79 are presented in Table 1.

Similarly to the study by Memon et al [31], FEV_1 values in all subjects (n = 148) were significantly correlated with FVC_{ex} (Fig. 3), and the following regression equation was elaborated: $FEV_1 = 0.4862 + 0.7168 \bullet FVC_{ex}$; r = 0.837.

Table 1. FVC_{ex} and FEV_1 values expressed in % of predicted in the individual groups g. 46 and g.79.

g. 46/g. 79	g. 46AA	g. 46GG	g. 46AG	g. 79CC	g. 79GG	g. 79GC
FVC_{ex} (%pred)	108	109	106.9	105.3	111	108
FEV_1 (%pred)	99.6	102.5	101.3	97.8	104	102

Significance of differences between the mean values of spirometric indices recorded in groups g. 46 is presented in Table 2, and between groups g. 79 in Table 3.

Table 2. Significance of differences (P) for the values of spirometric indices in individual groups g. 46.

Spirometric index	Significance of differences between groups within the polymorphism of nucleotide 46		
	AA and GG	AA and AG	GG and AG
VC (L)	0.242	0.268	0.985
FVC_{ex} (L)	0.103	0.270	0.530
$FEV_{0.5}$ (L)	0.007	0.020	0.884
FEV_1 (L)	0.039	0.049	0.987
FEV_2 (L)	0.013	0.040	0.692
FEV_3 (L)	0.058	0.097	0.813
PEF (L/s)	0.026	0.058	0.706
MEF_{75} (L/s)	0.001	0.010	0.236
MEF_{50} (L/s)	0.132	0.084	0.353
MEF_{25} (L/s)	0.075	0.181	0.181
$FEF_{75/85}$ (L/s)	0.150	0.145	0.145
$FEF_{25/75}$ (L/s)	0.049	0.020	0.289
Aex (L^2/s)	0.008	0.021	0.691

Table 3. Significance of differences (P) for the values of spirometric indices in individual groups g.79.

Spirometric index	Significance of differences between groups within the polymorphism of nucleotide 79		
	CC and GG	CC and GC	GC and GG
VC (L)	0.242	0.190	0.879
FVC_{ex} (L)	0.047	0.335	0.418
$FEV_{0.5}$ (L)	0.155	0.045	0.850
FEV_1 (L)	0.183	0.040	0.785
FEV_2 (L)	0.043	0.212	0.595
FEV_3 (L)	0.076	0.222	0.683
PEF (L/s)	0.060	0.250	0.226
MEF_{75} (L/s)	0.007	0.015	0.275
MEF_{50} (L/s)	0.754	0.284	0.498
MEF_{25} (L/s)	0.329	0.090	0.380
$FEF_{75/85}$ (L/s)	0.331	0.052	0.297
$FEF_{25/75}$ (L/s)	0.388	0.077	0.352
Aex (L^2/s)	0.101	0.520	0.666

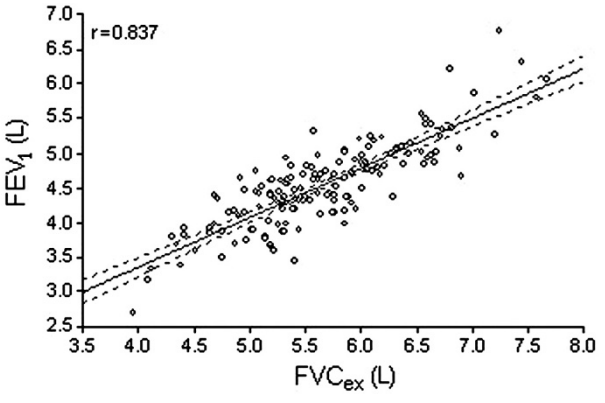

Fig. 3. Ralationship between FEV_1 and FVC_{ex} in the studied group (n = 148).

Discussion

The presence of β_2-ADR genotypes, with attempts to link them with clinical symptoms, has been analyzed in patients with chronic obstructive pulmonary disease, asthma, atopy, hypertension, obesity, dyslipidaemia and diabetes type 2 [5, 12, 19, 21, 31, 36]. Small et al [36] reported that subjects with g. 79GG, particularly women, usually have body mass higher by 20 kg and have by 50% of fatty cells more than subjects with the genotype g. 79CC. Moreover, special importance is attributed to the polymorphism of the β_2-ADR encoding gene at the position of nucleotides 46 and 79 (g. 46 and g. 79) [6, 7, 28, 31, 38]. It has been claimed that these polymorphic positions (g. 46 A→G and g. 79 G→C) may predispose people to the development of pulmonary and circulatory diseases, as well as to diverse responses to treatment [10, 31, 36]. It should be emphasized, however, that the frequency of the genotypes in studied groups of healthy subjects and patients is comparable [13, 34].

In the present investigation, distribution of the studied genotypes conformed to the Hardy-Weinberg equilibrium. Their close correlation was found (D'= 0.9; P<0.0001), which conforms to the results obtained by other authors [13, 14, 16, 31, 39, 40]. Subjects were divided into 6 groups: three based on the polymorphism of the β_2-ADR encoding gene at nucleotide position 46, and three based on the polymorphism of the β_2-ADR encoding gene at nucleotide position 79.

The frequency of genotype g. 46AA was comparable to the results obtained by Taylor et al [39], Hall et al [12], Israel et al [15] Martinez et al [29], Lima et al [26], Liggett [25], and Aynacioglu et al [4]. In studies conducted by Hopes et al [14] and Azis et al [5] this genotype had a lower frequency (6.3-9.3%), while in studies by Xie et al [40] and Kotani et al [19], it was significantly higher – 26.6 and 27.7%, respectively. Differences in the evaluation of the frequency of genotype g. 46AA are significant. However, it is

claimed that this genotype has the lowest frequency within g. 46 [21]. Frequencies of genotype g. 46GG reported by Taylor et al [39], Lima et al [26], Hopes et al [14], Martinez et al [29], and Azis et al [5] are comparable to those observed in our study and ranged from 38.3 to 47.2%. A slightly different range (from 29.1 to 36%) concerned data presented by Hall et al [12], Xie et al [40], Kotani et al [19], Israel et al [15], and Aynacioglu et al [4]. Only Liggett [25] found genotype g. 46GG in 57.3% of studied subjects. In this study, the percentage of subjects heterozygous at codon 16 – g. 46AG, as in the study by Xie et al [40], was comparable with that for homozygotes g. 46GG. Usually, the percentage of individuals with g. 46AG is estimated at 38-49%, which corresponds with the results obtained in our present study [12, 14, 19, 26, 29, 34]. Aynacioglu et al [4], Israel et al [15], and Azis et al [5] recorded a significantly higher frequency of the studied genotype (50%), while Liggett [25], Taylor et al [39] recorded a significantly lower one (29-37%). Individuals with g. 79CC, as in our study, in studies by Hall et al [12], Lima et al [26], Hopes et al [14], and Liggett [25] accounted for 25.7%. Individuals with g. 79GG accounted for 21%. According to Hall et al [12], Lima et al [26], Hopes et al [14], Xie et al [40], and Israel et al [15], their frequency was between 15.4 and 24%. In populations studied by Aynacioglu et al [4] and Martinez et al [29], genotype g. 79GG was significantly less frequent and accounted for, respectively, 10.6% and 13.8%, while in studies by Azis et al [5] and Liggett [25] significantly more frequently (37.5 and 28%). Subjects heterozygous at codon 27 accounted for over a half of the studied population (53.3%), which conforms with results obtained by Hall et al [12], Lima et al [26], Hopes et al [14], Israel et al [15], and Reihsaus et al [34] – range from 49.2 to 59%. In contrast to that, in studies presented by Liggett [25], Martinez et al [29], Xie et al [40], Aynacioglu et al [4], Azis et al [5], and Kotani et al [19], the percentage of individuals with g. 79GC was lower (13.6 - 45%). Data obtained in our present study show, similarly to the cited references, that g. 79GG is the genotype g. 79 of the lowest frequency. Differences in the frequency of the analyzed β_2-ADR genotypes may result from the fact that the studies covered representatives of different ethnic groups, including Caucasian, Hispanic and Latino American, African-American, and Asian [36]. In this study, similar to the available literature data, no individuals were observed with coexisting g. 46AA and g. 79GG [29].

In the present study, spirometric measurements demonstrated the functional efficiency of the respiratory system in all subjects. Values of the analyzed indices did not differ from standard values established with respect to the age and body height of subjects. The mean value of FVC_{ex} was 108% and FEV_1 was 102% of predicted; the mean value of FEV_1 %FVC_{ex} index was 82%. In all six groups, the output values of basic indices were within the normal range; FVC_{ex} 105 - 111% of predicted, FEV_1 98 -105% of predicted value. Currently in Poland, there are no well documented studies available which cover a group of healthy subjects of a wide age range. Therefore, it is recommended to use a set of predicted values according to different authors. However, the most popular predicted values recommended by ERS (unchanged for over 20 years) are not fully representative for the Polish [27], Greek [2], Finish [17] Croatian [37], German [18], or Spain [11] populations.

A comparative analysis of the values of spirometric indices was carried out separately for the genotype-groups. The groups g. 46AA and g. 46GG, as well as g. 46AA and g. 46AG significantly differed for FEV0.5, FEV_1, and FEV_2 values: with the lowest indices recorded in the group g. 46AA and the highest in the group g. 46GG. Peak expiratory flow (PEF) was different only for the groups g. 46AA and g. 46GG, and had the highest values in subjects from the group g. 46GG. From among indices describing the maximum expiratory flow only MEF_{75} was different for the groups g. 46AA and g. 46GG, as well as g. 46AA and g. 46AG: the lowest values were measured in subjects from the group g. 46AA, and the highest from the group g.46GG. Similar to the above results, Aex and $FEF_{25/75}$ values also varied.

The groups g. 79CC and g. 79GG demonstrated significant differences in FVC_{ex} values. $FEV_{0.5}$ and FEV_1 were significantly higher in the group g. 79GC when compared with the group g. 79CC. The FEV_2 index was different for individuals with g. 79CC and g. 79GG, similar to MEF_{75}, which was also varied for the genotypes g. 79CC and g. 79GC. The highest values of the analyzed indices were measured in the group g. 79GG, intermediate - g. 79GC and the lowest for g. 79CC. The present data conform to the results obtained by Joos [16], who found that individuals with g. 46GG achieve the highest FEV_1 and FVC_{ex} expressed as the percent of the predicted value. Israel et al [15] also recorded the highest FEV_1 values in the group g. 46GG. In contrast, Martinez et al [29], in the analysis of polymorphism of g. 79, found the highest FEV_1 values in the group g. 79GG. Taylor et al [39], in the analysis of the polymorphism of g. 46 in asthmatic patients, recorded the highest FEV_1 values in the group g. 46AA, slightly lower in g. 46AG, and the lowest in g. 46GG. Additionally, Martinez et al [29] recorded the highest FEV_1 values in children with g. 46AA, intermediate in g. 46GG, and the lowest in g. 46AG. When analysing the g. 79 polymorphism in the present study we found that, similar to observations by Israel et al [15], heterozygotes achieve intermediate values of spirometric indices, while the highest values are achieved by homozygotes g. 79GG and the lowest by g. 79CC.

In conclusion, we believe the present study may be useful in explaining the differences in normal values of spirometric indices in healthy subjects in relation to the polymorphism of the β_2-ADR gene. Considering the fact that the measured spirometric indices exceeded standard values, further spirometric studies on healthy subjects may contribute to the establishment of new predicted values for relevant age groups in the Polish population.

Conflicts of interest: No conflicts of interests were declared by the authors in relation to this article.

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