

Association between Arg16Gly Polymorphism in the B_2 -Adrenergic Receptor Gene and Obesity Risk in Patients with Bronchial Asthma

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Abstract

The objective of our study was to investigate the association between the Arg16Gly polymorphism in the β_2 -AR gene and body mass index (BMI) in patients with bronchial asthma (BA) with regard to the age of onset. **Materials and methods:** 553 patients were examined. The control group consisted of 95 apparently healthy individuals. The patients were divided into 2 groups depending on the age of BA onset: 282 patients with late onset (Group I) and 271 patients with early onset (Group II). Arg16Gly polymorphism in the β_2 -AR gene (rs1042713) was determined using polymerase chain reaction-restriction fragment length polymorphism analysis. Statistical analysis of obtained results was performed using SPSS-17 program. **Results:** There was no significant difference in the distribution of genotypes for Arg16Gly polymorphism in the β_2 -AR gene depending on BMI ($\chi^2 = 5.74$; $p = 0.22$), and no association was found with obesity risks disregarding the age of BA onset. The study on the frequency of genotypes for this polymorphism with regard to the age of asthma onset and BMI found statistically significant differences between early-onset ($\chi^2 = 11.27$; $p = 0.02$) and late-onset ($\chi^2 = 10.66$; $p = 0.03$) asthma. It was found that the risk of obesity in Group I patients was 1.57 times higher in the additive model and 2.67 times higher—in the recessive model of inheritance. In patients with late-onset asthma, Gly16 allele in the recessive model of inheritance was found to have a protective role against obesity. **Conclusions:** There was no significant difference in the distribution of genotypes for Arg16Gly polymorphism in the β_2 -AR gene with regard to BMI, and no association was found with obesity risks in BA patients disregarding the age of BA onset. The risk of obesity in patients with early-onset asthma was 1.6 times higher in the additive model and 2.7 times higher—in the recessive model of inheritance.

Keywords

Bronchial Asthma, Onset, Obesity,
Arg16Gly Polymorphism in the β_2 -Adrenoceptor Gene

1. Introduction

Genetic factors are known to be important for the development of bronchial asthma (BA) and obesity. The results of genetic studies on the comorbidity of these diseases show the presence of common genetic factors that are associated with pleiotropic effects of the genes of the β_2 -adrenoceptor (AR), glucocorticoid receptors and leptin receptors, TNF- α , and others. Specific regions of the human genome have been identified, which are associated with both asthma and obesity [1] [2]. In particular, polymorphic variants in the β_2 -AR gene were associated with asthma [3] [4] and obesity [5] [6] [7] in some studies, because the β_2 -AR gene is associated with lipid mobilization. Therefore, single nucleotide polymorphisms in the β_2 -AR gene require further study in the context of BA-obesity phenotype, because bronchial hyperreactivity and poor response to bronchodilator treatment are related to the same defect that stimulates lipid mobilization through adipocyte lipolysis. The study by Hallstrand *et al.* on the common genetic origin of asthma and obesity showed a strong association between asthma and obesity ($p < 0.001$) and a significant hereditary impact for asthma (53%) and obesity (77%), indicating an additive genetic effect with these diseases. In addition, about 8% of genetic factors for obesity were also characteristic of asthma, which confirms genetic pleiotropy [1]. According to Liu Z. Q., 40% of body weight variations may be related to genetic changes in patients with asthma and obesity [8].

Asthma is a heterogeneous respiratory disease involving different pathobiological mechanisms that are based, at least in part, on different genetic factors. These factors may be common for many pathological conditions, including asthma and obesity. The study of common genetic factors allows a better understanding of the mechanisms that represent phenotypic features of the disease. The results of large-scale genome-wide association studies demonstrated a genetic contribution to the association between asthma subtypes and comorbidities. The studies revealed both common and distinguishing genetic components of asthma subtypes, which suggests an association between heterogeneity and genetic features [2] [9]. Distinguishing genetic features in early-onset vs. late-onset asthma were demonstrated in UK Biobank study ($n = 447,628$), which identified 96 variants of genetic risk specific for early-onset asthma and three variants strongly characteristic of late-onset asthma [10]. This explains the differences in the pathogenesis between early-onset and late-onset asthma.

A few *in vivo* studies on the association of polymorphic gene variants with obesity and asthma showed conflicting results [11] [12]. Genetic-level associations with BMI and asthma were found, but none of them were significant after

being adjusted for multiple testing [11]. The analysis of known candidate genes demonstrated some evidence of common genetic grounds for asthma and obesity, but common genetic determinants are also likely to be identified at new loci.

The studies conducted on the association between the Arg16Gly polymorphism in the β_2 -AR gene and BA-obesity phenotype could not reveal the corresponding association. It may be explained by the failure to take account of the phenotypic features of the BA-obesity association, in particular the age of onset. Further study on genomic associations with the age of asthma onset as a key factor in identifying risk options for a particular disease phenotype [10] can help understand the differences in the pathogenesis, clinical course, and treatment approaches between early-onset and late-onset asthma. Thus, the heterogeneity of the obtained data related to the role of β_2 -AR gene polymorphisms was due to different study objectives and design, and different populations. These contradictory results substantiate the advisability of further study of molecular and genetic mechanisms of the pathogenesis of BA-obesity association and pleiotropic effects of β_2 -AR genes in order to develop options for predicting these diseases.

The objective of our study was to investigate the association between the Arg16Gly polymorphism in the β_2 -AR gene and body mass index (BMI) in patients with BA with regard to the age of onset.

2. Materials and Methods

553 patients with bronchial asthma were examined. All of them had previously signed an informed consent form. The control group consisted of 95 apparently healthy individuals with no individual and family history of asthma symptoms, symptoms of allergies and atopy, hypersensitivity to aspirin and nonsteroidal anti-inflammatory drugs; the subjects were non-smokers and had no history of smoking, no acute or chronic somatic diseases in the acute stage within 3 months prior to the enrollment, or chronic infectious diseases of the upper airways, or autoimmune or oncological diseases. The patients were divided into 2 clinical groups depending on the age of BA onset. Group I included 282 patients with late-onset asthma (late-onset asthma phenotype) and group II included 271 patients with early-onset asthma (early-onset asthma phenotype). BA diagnosis and BA severity were determined according to the GINA recommendations-2016 and its later versions. Obesity was diagnosed in accordance with the Order of the Ministry of Health of Ukraine № 574 dated 05.08.2009 “On approval of the protocols for medical care for patients with endocrine diseases” and the WHO recommendations (1999), the European Association for the Study of Obesity (EASO, 2016). BMI of 18 kg/m² to 24.9 kg/m² was regarded as normal body weight (healthy weight), 25 kg/m² to 29.9 kg/m²—as overweight, BMI of higher than 30 kg/m²—as obesity. Anthropometric studies included waist-to-hip ratio (WHR) determination. WHR ratio of greater than 0.85 in women and 0.9 in men, as well as WC of greater than 94 cm in men and 80 cm in women were regarded as visceral adiposity.

The study was approved by the Bioethics Committee of Medical Institute of Sumy State University. Polymorphism of β_2 -adrenergic was determined by polymerase chain reaction followed by restriction analysis of amplified fragments. Reaction was performed containing an allele-specific primer (F:5'...C|C A T G G...3', R:3'...G G T A C|C...5'). PCR was performed in a volume of 20 μ l in a thermal cycler GeneAmp 61 PCR System 2700 ("Applied Biosystems", USA). PCR products were incubated in the presence of appropriate restriction enzymes (restriction endonucleases) under the conditions recommended by the manufacturer ("Fermentas", Lithuania). Post-restriction amplifications were separated in a 2.5% agarose gel containing 10 μ g/ml ethidium bromide. Horizontal electrophoresis (0.13 A; 200 V) was performed for 20 minutes. Visualization of DNA after electrophoresis was performed using a transilluminator ("Biocom", Russia). Statistical analysis of obtained results was performed using SPSS-17 program. Comparison of the distribution of genotypes in the studied groups was performed using Pearson's chi-squared test. In order to determine the risk of BA and obesity, the odds ratio (OR) and 95% confidence interval (CI) for dominant, recessive, superdominant and additive inheritance models were calculated. The relevance of the obtained results was assessed with Akaike's information criterion. All tests were two-sided, the p-value < 0.05 was considered statistically significant.

3. Results

The analysis of anthropometric parameters showed that among the examined patients with asthma, there were 152 (27.5%) patients with healthy weight, 206 (37.3%)—with overweight and 195 (35.2%)—with obesity. In all cases, visceral adiposity was verified. Due to the fact that the studies proved the association of certain polymorphic variants in the β_2 -AR gene, including Arg16Gly polymorphism in the β_2 -AR gene, with the development of obesity [1] [7] [10], we analyzed the distribution of genotypes and alleles for this polymorphism in the examined BA patients with regard to BMI (Table 1).

Table 1. Genotype and allele distribution for the Arg16Gly polymorphism in the β_2 -adrenoceptor gene in patients with bronchial asthma with regard to the body mass index.

rs 1042713	Body mass index, n = 553					
	Normal body mass, n = 152		Overweight, n = 206		Obesity, n = 195	
Genotype	n	%	n	%	n	%
Arg/Arg	39	25.7	63	30.6	71	36.4
Arg/Gly	71	46.7	97	47.1	85	43.6
Gly/Gly	42	27.6	46	22.3	39	20.0
$\chi^2 = 5.74; p = 0.22$						
Arg allele	49.0		54.1		58.2	
Gly allele	51.0		45.9		41.8	

Based on the data obtained no significant difference was found in the distribution of genotypes for the Arg16Gly polymorphism in the β_2 -AR gene in BA patients with regard to BMI ($p = 0.22$). The regression analysis of the correlation between the Arg16Gly polymorphism in the β_2 -AR gene and the risk of obesity also showed the lack of statistically significant association in all models of inheritance: dominant model (OR 1.38; 95% CI: 0.84 - 2.28; $p = 0.2$), additive model (OR 1.26; 95% CI: 0.9 - 1.77; $p = 0.19$), and recessive model (OR 1.33; 95% CI: 0.71 - 2.63; $p = 0.39$).

The study on the frequency of genotypes for this polymorphism given the age of asthma onset and BMI found statistically significant differences between early-onset ($\chi^2 = 11.27$; $p = 0.02$) and late-onset ($\chi^2 = 10.66$; $p = 0.03$) asthma (**Table 2**). In the patients with early onset of the disease, the major allele homozygotes were 1.6 and 1.7 times more frequent in obese patients, while the Gly/Gly genotype was more frequent in patients with healthy weight vs. patients with overweight and obesity. In those having late-onset asthma, the frequency of the minor allele homozygotes was twice as high in healthy weight patients vs. obese patients. However, the frequency of Gly/Gly homozygotes among patients with early onset was significantly higher (34%) vs. patients with late onset of asthma (5.3%), while Arg/Gly heterozygotes were more frequent among patients with late-onset BA (56.8%) vs. patients with early-onset BA (31%).

Table 2. Genotype and allele distribution for the Arg16Gly polymorphism in the β_2 -adrenoceptor gene in patients with bronchial asthma with regard to the body mass index and age of onset.

Early onset, n = 271						
Genotype	Normal body mass, n = 84		Overweight, n = 87		Obesity, n = 100	
	n	%	n	%	n	%
Arg/Arg	19	22.6	18	20.7	35	35.0
Arg/Gly	30	35.7	44	50.6	31	31.0
Gly/Gly	35	41.7	25	28.7	34	34.0
$\chi^2 = 11.27$; $p = 0.02$						
Arg allele	40.5		46		50.5	
Gly allele	59.5		54		49.5	
Late onset, n = 282						
Genotype	Normal body mass, n = 68		Overweight, n = 119		Obesity, n = 95	
	n	%	n	%	n	%
Arg/Arg	20	29.4	45	37.8	36	37.9
Arg/Gly	41	60.3	53	44.5	54	56.8
Gly/Gly	7	10.3	21	17.6	5	5.3
$\chi^2 = 10.66$; $p = 0.03$						
Arg allele	59.6		60.1		66.3	
Gly allele	40.4		39.9		33.7	

The relative risk estimation for the additive model showed that the Gly16 allele carriers (heterozygotes and the minor allele homozygotes) have 1.6 times higher risk of developing obesity in the patients with early-onset asthma (95% CI: 1.09 - 2.28; $p = 0.02$) vs. the major allele homozygotes. In case of the recessive model, the Gly16 allele carriers (the minor allele homozygotes) have 2.7 times higher risk of developing obesity in the patients with early-onset asthma (95% CI: 1.36 - 5.43; $p = 0.01$) vs. the major allele homozygotes. In patients with late-onset asthma, Gly16 allele in the recessive model of inheritance was found to have a protective role against obesity ($p = 0.02$) (**Table 3**).

4. Discussion

The established role of the Arg16Gly polymorphism in the β_2 -AR gene in the development of asthma and obesity separately [3] [4] [5] [6] [7] made us think about its possible pleiotropic properties. Gjesting A.P. *et al.* found no convincing evidence of association between the Arg16Gly polymorphism in the β_2 -AR gene and obesity in the study which included 7808 unrelated Caucasians, but the Gly16-allele showed a nominal association with systolic blood pressure, type 2 diabetes mellitus, metabolic syndrome ($p = 0.003$) [13]. The association of the Gly16-allele with the development of metabolic syndrome was confirmed in the study of Masuo K. [7]. The study by Kawaguchi H. involving men with overweight and obesity showed that Gly16-allele was associated with further weight gain [6]. However, Large V. reported that Arg16Gly polymorphism in the β_2 -AR gene is largely associated only with the lipolytic function of β_2 -AR in adipose tissue, but no clear link with obesity was found [14]. Subsequent meta-analyses of the findings on the role of Arg16Gly polymorphism in the β_2 -AR gene in obesity development showed the lack of statistical significance in all genetic models (as well as associations with obesity by sex) [12] [15]. In our study, we also found

Table 3. Analysis of the association between Arg16Gly polymorphism in the β_2 -adrenergic receptor gene and obesity risk in patients with early-onset and late-onset asthma.

Model	P_{obs}	OR _{obs} (95% CI)	AIC
Early onset			
Dominant	0.16	1.5 (0.85 - 2.71)	23.98
Recessive	0.01	2.7 (1.36 - 5.43)	17.72
Super-dominant	0.26	0.7 (0.4 - 1.28)	24.76
Additive	0.02	1.6 (1.09 - 2.28)	19.97
Late onset			
Dominant	0.38	1.3 (0.73 - 2.33)	24.66
Recessive	0.02	0.3 (0.09 - 0.8)	19.62
Super-dominant	0.02	1.9 (1.11 - 3.54)	20.02
Additive	0.66	0.9 (0.58 - 1.4)	25.24

neither significant difference in the distribution of genotypes for the Arg16Gly polymorphism in the β_2 -AR gene in BA patients depending on BMI, nor significant association with the risk of obesity in all models of inheritance. Previously, we found differences in the distribution of genotypes for the Arg16Gly polymorphism in the β_2 -AR gene depending on the age of disease onset, and increased risk of early-onset asthma in the recessive model, which was 2.83 times higher ($p = 0.001$), and additive model, 1.87 times higher ($p = 0.001$). These findings showed that Gly16 allele carriers (heterozygotes and homozygotes for the minor allele) had higher risk of developing early-onset asthma as compared with the major allele homozygotes. This became the basis for the analysis of obesity risks in patients with asthma with regard to the age of onset, which showed increased relative risk of obesity in patients with early-onset asthma in the additive (by 1.6 times) and recessive (by 2.7 times) inheritance models. On the contrary, Gly16 allele in the recessive model of inheritance was found to have a protective role against obesity in patients with late-onset asthma. The results we obtained in the previous study indicate the increased risk of early-onset asthma in Gly16 allele carriers (heterozygotes and the minor allele homozygotes) and the results of this study indicating the increased risk of obesity in Gly16 allele carriers suggest a common genetic origin of this phenotype. The studied polymorphism presents pleiotropic features with early-onset asthma and BMI in contrast to late-onset asthma and may become a target for the prevention and treatment of asthma and obesity in the future. The observed association in BA-obesity comorbidity in the case of early-onset BA with Arg16Gly polymorphism in the β_2 -AR gene presents limited evidence of pleiotropy and requires further detailed study.

5. Conclusions

1) There was no significant difference in the distribution of genotypes for Arg16Gly polymorphism in the β_2 -AR gene with regard to BMI, and no association was found with obesity risks in BA patients disregarding the age of BA onset.

2) The relative risk of obesity in patients with early-onset asthma was 1.6 times higher in the additive model and 2.7 times higher—in the recessive model of inheritance.

3) In patients with late-onset asthma, Gly16 allele in the recessive model of inheritance was found to have a protective role against obesity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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