ARG16GLY POLYMORPHISM OF BETA 2 ADRENERGIC RECEPTOR GENE, ADRB2, IN ALBANIAN POPULATION

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Abstract

ß-2 adrenergic receptors mediate bronchial and vascular smooth muscle relaxation and play important roles in regulating cardiac, vascular, pulmonary and metabolic functions. Polymorphism at codon 16 has a clinical importance for treatment of various diseases. In this study we used allele specific PCR, in order to identify Arg16Gly polymorphism in 47 healthy Albanian individuals. The frequency of Arg and Gly allele was found to be 0.33 and 0.67 respectively. The genotype frequencies of Arg16Arg, Arg16Gly and Gly16Gly of ADRB2 gene in our control group were 0.17, 0.32 and 0.51 respectively. The genotype frequencies at locus 16 were in Hardy-Weinberg equilibrium. We found out that there is a 95% probability that the real frequency of Arg allele in our population vary at range (0.23-0.43), whereas the frequency of Gly allele vary at range (0.57-0.77). This study is the first analysis of Arg16Gly polymorphism in healthy Albanian population.

Përmbledhje

Receptorët β-2 adrenergjik mundësojnë relaksimin e muskulaturës së lëmuar të bronkeve dhe enëve të gjakut si dhe luajnë një rol të rëndësishëm në rregullimin e funksioneve kardiake, vaskulare, pulmonare dhe metabolike. Polimorfizmi në kodonin 16 ka një rëndësi klinike për trajtimin e sëmundjeve të ndryshme. Në këtë studim, ne kemi përdorur teknikën PCR alel specifike, me qëllim identifikimin e polimorfizmit Arg16Gly në 47 individë të shëndetshëm shqiptarë. Frekuencat e aleleve Arg dhe Gly janë përkatësisht 0.33 dhe 0.67. Frekuencat e gjenotipeve Arg16Arg, Arg16Gly dhe Gly16Gly janë përkatësisht 0.17, 0.32 dhe 0.51. Frekuencat gjenotipike të lokusit 16 janë në ekuilibër Hardy-Weinberg. Ne konstatuam se me nivel besueshmërie 95%, frekuenca reale e alelit Arg luhatet në popullatën tonë nga 0.23 në 0.43, ndërsa frekuenca për alelin Gly luhatet nga 0.57 në 0.77. Ky studim analizon për herë të parë polimorfizmin e Arg16Gly në popullatën e shëndetshme shqiptare.

Keywords: ADRB2 gene, Arg16Gly polymorphism, AS-PCR, Healthy Albanian population.

Introduction

Beta-2 Adrenergic Receptors (ADRB2), are members of G Protein-Coupled Receptor (GPCR) superfamily, which mediate the action of catecholamines in multiple tissues. ADRB2 are widely distributed over several cell types, including bronchial smooth muscle cells in the lung, mast cells and various immune cells, epithelial cells and nerve terminals (Drysdale *et al*. 2000).

They are activated by β-adrenergic agonists that stimulate the production of cAMP through the stimulation of adenylyl cyclase. Activation results in vasodilatation, bronchodilation, relaxation of the gastrointestinal tract (GIT), glycogenolysis in the liver, tremor in skeletal muscle and inhibition of histamine release from mast cells Maxwell *et al*. (2005). The ADRB2 is encoded by an intronless gene located on the long arm of chromosome 5q31–32 (Brodde *et al*. 2005).

Several single nucleotide polymorphisms (SNPs) have been described to date in ADRB2 gene, four of which result in amino acid substitutions in the receptor and appear to be of functional importance (Gao *et al*. 2004). The four polymorphisms occur in nucleic acid residues 46 (A to G), 79 (C to G), 100 (G to A) and 491 (C to T). Some of these SNPs, as Arg16Gly and Gln27Glu are the more common polymorphisms of the ADRB2 gene in populations or patients studied to date and have been implicated in the pathogenesis of cardiovascular diseases, asthma, cystic fibrosis, obesity, diabetes mellitus type II etc (Brodde *et al*. 2005).

Many researchers have found interethnic differences in the frequency of genotypes of these two *ß2AR* polymorphisms. Frequency of functionally important beta-2 adrenergic receptor polymorphisms varies markedly among African-American, Caucasian and Chinese individuals (Maxwell, *et al*. 2005) and (Xie *et al*. 1999).

Considering the data from other authors, we propose this study with the aim to evaluate Arg16Gly polymorphism of ADRB2 gene in healthy Albanian population. These results will serve as point of reference for the next study related to ADRB2 gene polymorphisms in Albanian patients with Cystic fibrosis disease.

Materials and methods

Study Participants

Forty seven individuals with a median age of 30 years without clinical evidence of disease were studied; medical history and physical examination were performed in order to exclude under-lying disease in all studied individuals. This

study was done in the Center of Molecular Diagnosis and Genetic Research, at University Hospital of Obstetrics and Gynecology "Queen Geraldine", in Tirana.

DNA Extraction

Genomic DNA was isolated from peripheral blood using Qiagen extraction kit and following manufacturer's instructions Maniatis *et al.(*1989). The purified DNA was safely stored at -20°C for later use.

Detection of Arg16Gly polymorphism

Arg16Gly polymorphism of ADRB2 gene was performed by using allele-specific polymerase chain reaction (PCR) that depends on the usage of two different primer pairs that precisely matches with arginine but mismatches with glycine. By performing two PCR reactions on every DNA sample, using two different primer pairs, we could determine the genotypes of each individuals.

In order to detect two polymorphisms of ADRB2 gene, Arg16 and Gly16, we used two forward primers: 5'-CTT CTT GCT GGC ACC CAA AA-3' and 5'-CTT CTT GCT GGC ACC CAA AG-3'. The reverse primer was the same 5'-TGA TGA AGT AGT TGG TGA CC-3' for both reactions. By using these primers we get PCR products approximately 158 bp long. In addition we used this primer pair: 5'-GAA CTG CCA CTT CAG CTG TCT-3' and 5'-CAG CTG CAT TTG GAA GTG CTC-3', in order to amplify a 320-bp DNA fragment of CYP1A1 gene, which served as the internal positive control.

PCR reactions were carried out in a volume of 50μL, which included 5 μL of genomic DNA, 1,5mM of $MgCl_2$, 200 μmol/L of each dNTP, 1μmol of each primer, 0,2 μL of Taq Gold Polymerase and reaction buffer. A modification of PCR program was done as below: an initial denaturation step at 94 ºC for 10 minutes, followed by 40 cycles of 94 ºC for 60s, 57 ºC for 60s and 72 ºC for 60s, with a final extension step of 7 min at 72 ºC. Ten μL of the PCR mixture was then electrophoresed on 2% agarose gels and visualized with ethidium bromide staining and ultraviolet illumination.

Identification of Arg16/Gly16 genotypes in ADRB2 gene

For every DNA sample were carried out 3 PCR reactions, one using primers that amplify only Arg16 allele, the second using primers that amplify only Gly16 allele and the third amplification was carried out by using the primers that amplify CYPA1 gene, which served as internal positive control of PCR reactions.

In case that DNA was amplified only by the first PCR reaction, we concluded that the genotype was homozygote for Arg allele, Arg16/Arg16. If DNA was not amplified by the first PCR reaction but it was amplified by the second reaction, we concluded that the genotype was homozygote for Gly allele, Gly16/Gly16. In

case that DNA was amplified by the first and second reaction, we concluded that the genotype was heterozygote for both alleles, Arg16/Gly16. The third PCR reaction should produce an amplification fragment of 320 bp long, showing that the PCR procedure was done correctly.

Statistics

Calculation of allele frequencies of Arg and Gly was done according to the standard formula used in population genetics. The Hardy-Weinberg equilibrium by using Chi- Square test was calculated according to the online Encyclopedia for Genetic Epidemiology (OEGE) software :

(*http://www.oege.org/software/hwe-mr-calc.shtml*). We tested also the reliability of allele frequencies by calculating the standard deviation with this formula:

$$\sigma = \sqrt{pxq/2N}$$

where p-was the frequency of Arg16 allele, q-the frequency of Gly16 allele and N was the total number of the analyzed individuals.

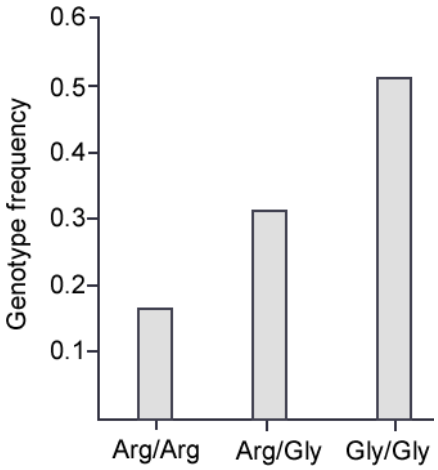


Figure 1. Genotype frequency of Arg16Gly polymorphism in healthy Albanian individuals.

Results and discussion

Determination of Arg16Gly polymorphism in ADRB2 gene

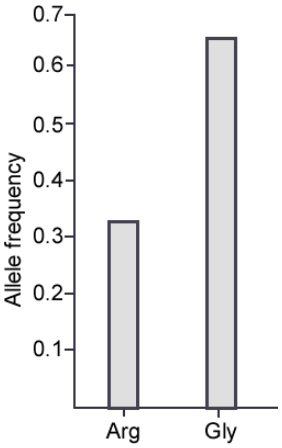
We analyzed 47 healthy individuals by using allele specific PCR method. According to the results of electrophoresis, we found 8 genotypes of Arg/Arg, 15 genotypes of Arg/Gly and 24 genotypes of Gly/Gly.

Genotype frequencies of Arg/Arg, Arg/Gly and Gly/Gly were 0.17, 0.32 and 0.51 respectively (Figure 1).

In our study the Arg/Arg genotype had the lowest frequency, whereas the Gly/Gly genotype had the highest one. The ADRB2 genotype frequencies in 47 healthy Albanian individuals were in proportion p^2:2pq:q^2, which means that our sample was in Hardy-Weinberg equilibrium, by using Chi-Square test. According to this test we concluded that the observed and expected genotype frequencies were not significantly different from one another:

$$\chi^2 = \frac{\sum(o-e)2}{e} = 3.63 << \chi^2_{(0.05)}[2] = 5.99$$

Determination of allele frequencies



In our study the frequencies of Arg16 and Gly16 allele were 0.33 and 0.67 respectively. Fig.2.

Standard deviation related to our allele frequencies was 0.048. By using this value, we found out that there is a 95% probability that the real frequency of Arg allele in our population vary at range 0.23-0.43.

We concluded that there is also a 95% probability that the real frequency of Gly allele in our population vary at range 0.57-0.77.

Figure 2. Frequencies of Arginine 16 and Glycine 16 alleles in 47 healthy Albanian individuals

This is the first study related to Arg16Gly polymorphism of ADRB2 gene in healthy Albanian population. There was also another study related to Gln27Glu polymorphism of ADRB2 gene in healthy Albanian population, made by other authors (Zaçe & Zoraqi 2011).

Polymorphisms of ADRB2 gene, as a modifier gene, has attracted the attention of many researchers with the aim to elucidate the conection between different genotypic variants of ADRB2 gene, clinical features of patients with Cystic Fibrosis disease (CF), hipertension, diabetes, obesity, asthma etc Daghestani *et al*. (2012), Taylor *et.al*. (2001). The codon 16 and 27 polymorphisms are the more common polymorphisms of the *ß2AR* gene in populations or patients studied to date. These studies aim to adapt the best medical treatment with the genetic profile of patients suffering from diseases where ADBR2 receptors are implicated.

Genotype frequencies of Arg16Arg and Arg16Gly in healthy Albanian population are similar with values reported in Greek, Turkish, Polish, Italian, Swedish population etc (Efstratios *et al.* 2013; Aynacioglu *et al.* 1999; Pączkowska *et al.* 2009; Covoloa *et al.* 2004; Maxwell, *et al.* 2005). On the other hand the frequency of Gly16Gly genotype tent to be higher, but quite similar with those reported in Swedish, Polish, Greek population etc. (Bengtsson *et al.* 2001; Pączkowska *et al.* 2009; Efstratios *et al.* 2013).

In this study we found that the frequency of Arg allele was 0.33 (0.23-0.43), whereas the frequency of Gly allele was 0.67 (0.57-0.77). These values are quite similar with those reported by Bengtsson *et al.* (2001) in Swedish population.

In the next study we are going to present the allele and genotype frequencies at locus 16 in the patients suffering from Cystic fibrosis disease.

Our data of Arg16Gly polymorphism and data of Gln27Glu polymorphism reported by other authors in healthy Albanian population will serve as a reference for future studies, which will focus in the patients suffering from different diseases where ADBR2 receptors play an important role.

Conclusion

- The genotype frequencies of Arg16Arg, Arg16Gly and Gly16Gly of ADRB2 gene in 47 healthy Albanian population were 0.17, 0.32 and 0.51 respectively.
- The allele frequencies of arginine and glycine in ADRB2 gene were 0.33 and 0.67 respectively. The real frequencies of these alleles (95% probability) varies at range 0.23-0.43 for Arg16 and 0.57-0.77 for Gly16.
- The genotypic distribution at 16 locus was consistent with Hardy-Weinberg equilibrium.
- Our results related to genotype and allele frequencies in healthy Albanian individuals provide a basis for clinical studies in ADRB2 gene.

References

Aynacioglu AS, Cascorbi I, Gu¨ngor K, Ozkur M, Bekir N, Roots I. (1999): Population frequency, mutation linkage and analytical methodology for the Arg16Gly, Gln27Glu and Thr164Ile polymorphisms in the b2-adrenergic receptor among Turks. Blackwell Science Ltd Br J Clin Pharmacol, 48: 761–764

Bengtsson K, Orho-Melander M, Melander O, Lindblad U, Ranstam J, Råstam L, Groop L. (2001): b2-Adrenergic Receptor Gene Variation and Hypertension in Subjects With Type 2 Diabetes. American Heart Association, Hypertension, 37: 1303-1308

Brodde OE, Leineweber K. (2005): Beta2-adrenoceptor gene polymorphisms. Pharmacogenet Genomics 15: 267–27

Buscher R, Eilmes KJ, Grasemann H, Torres B, Knauer N, Sroka K, Insel PA, Ratjen F. (2002): Beta 2-adrenoreceptor gene polymorphisms in cystic fibrosis lung disease. Pharmacogenetics. 12(5): 347–353

Covolo L, Gelattia U, Metrab M, Nodari S, Picciche A, Pezzali N, Zania C, Alberti A, Donato F, Nardi G, Dei Cas L. (2004): Role of b1- and b2-adrenoceptor polymorphisms in heart failure: a case-control study. European Heart Journal 25, 1534–1541

Daghestani MH, Warsy A, Al-Odaib AN, Eldali A, Al-Eisa NA, Omer SA and Hassan ZK. (2012): Arginine 16 glycine polymorphism in β2-adrenergic receptor gene is associated with obesity, hyperlipidemia, hyperleptinemia, and insulin resistance in Saudis. International Journal of Endocrinology, vol. 2012, Article ID 945608; 8

Deichmann KA, Schmidt A, Heinzmann A, Kruse S, Forster J, Kuehr J. (1999): Association studies on beta2-adrenoceptor polymorphisms and enhanced IgE responsiveness in an atopic population. Clin Exp Allergy 29: 794-799

Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G and Liggett SB. (2000): Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci USA 97: 10483-10488

Efstratios KTh, Konstantinos DB, Brian EG, Taimour YL, Timotheos GK , Antonios A P, Julie AJ, Harisios B. (2013): Beta-adrenergic receptor polymorphisms: A basis for pharmacogenetics. World Journal of Cardiovascular Diseases, 3, 406-411

Gao JM, Lin YG, Qiu CC, Liu YW, Ma Y, Liu Y. (2004): Beta2-adrenergic receptor gene polymorphism in Chinese Northern asthmatics. Chin Med Sci J 19: 164–169

Gök İ, Çeleb İ, Hüseyinoğlu N, and Özic C. (2014): Roles of beta2-adrenergic receptor gene polymorphisms in a Turkish population with obstructive sleep apnea syndrome or obesity. Genet. Mol. Res. 13 (4): 8511-8518

Maniatis T, Fritsch EF, Sambrook J. (1989): Molecular cloning: A laboratory manual. (2nd ed.). Cold spring harbor laboratory, New York, U.S.A

Maxwell TJ, Ameyaw M-M, Pritchard S, Thornton N, Folayan G, Githang'a J, Indalo A, Tariq M, Mobarek A, Evans DP, Ofori-Adjei D, Templeton AR and McLeod HL. (2005): Beta-2 adrenergic receptor genotypes and haplotypes in different ethnic groups. Intern J. Mol. Med. 16: 573-580

Pączkowska A, Szperl M, Małek Ł, Mazurkiewicz Ł, Skóra E, Grzybowski J, Roszczynko M, Bilińska Z, Tesson F, Rużyłło W.(2009): Polymorphisms of the beta-1 and beta-2 adrenergic receptorsin Polish patients with idiopathic dilated cardiomyopathy. Kardiol Pol: 67: 235-241

Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Pérusse L, Bouchard C.(2006): The human obesity gene map: The 2005 update. Obesity (Silver Spring) 14:529-644

Steagall WK, Barrow BJ, Glasgow CG, Mendoza JW, Ehrmantraut M,Lin JP, Insel PA and Moss J.(2007): Beta-2-adrenergic receptor polymorphisms in cystic fibrosis. Pharmacogenet Genomics.17(6): 425–430

Taylor DR, Kennedy MA. (2001): Genetic variation of the beta(2)- adrenoceptor: its functional and clinical importance in bronchial asthma. Am J Pharmacogenomics; 1 (3): 165-174

Tsvetkova-Vicheva V, Velkova A, Ivanov P, Komsa-Penkova R. (2013): The Arg16Gly Polymorphism in the B2-Adrenergic Receptor Gene is Associated with Bronchial Hyperresponsiveness and Allergic Rhinitis in the Bulgarian Population. Biotechnology & Biotechnological Equipment. 27:2, 3700-3702.

Xie HG, Stein CM, Kim RB, Xiao ZS; He N; Zhou HH; Gainer JV; Brown NJ; Haines JL; Wood AJJ;(1999): Frequency of functionally important beta-2 adrenereceptor polymorphisms varies markedly among African-American, Caucasian and Chinese individuals. Pharmacogenetics 9: 511-516, 1999

Zaçe O, Zoraqi G. (2011): Genetic polymorphism Gln27/Glu27 of the β2-adrenergic receptor gene, ADRB2, in Albanian population. BSHN (UT) 151-157