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Original Research

Beta2-adrenergic receptor polymorphisms among healthy Jordanian population

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Abstract

Background: Several polymorphisms of the β2-adrenergic receptor gene have been identified. These polymorphisms affect receptor function and significantly reduce expressed receptors in the cells. The main objective of this study is to determine the frequency of β2 adrenergic receptor polymorphism among a healthy Jordanian population and compare it with those of different ethnic groups. Blood samples were collected from 96 healthy Jordanians. β2-adrenergic receptor gene polymorphism at codon 16 and codon 27 was assessed by Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism RFLP. The minor allele frequency of β2-adrenergic receptor polymorphism at position 46 (46 G/A) was 31%, while the polymorphism at position 79 (79 C/G) was 14% in the Jordanian population. The genotype frequencies for 46 (46 G/A) were 51% for (GG), 13% for (AA) and 36% for (GA). In case 79 C/G, the genotype frequencies were 87.5% 7.3 % 5.2% CC, GG and CG genotypes, respectively. This study's results indicate marked interethnic differences in the genotype and allele frequencies of the two β2-adrenergic receptor polymorphisms among healthy Jordanians, Caucasians, African Americans, and Asians.

Keywords: β2-Adrenergic Receptors; healthy; jordan; pcr; polymorphism

INTRODUCTION

β1, β2 and β3-adrenergic receptors are G protein-coupled receptors expressed throughout the body and serve as receptors for the catecholamines; epinephrine and norepinephrine. They are targets for therapeutic agonists and/or antagonists in treatment of heart failure and asthma,[1] Each type of b-ARs is mainly expressed in certain tissues. B1-adrenergic receptors are predominant in the heart and β2-adrenergic receptors are predominant in the respiratory system. β3-adrenergic receptor are expressed primarily in adipose tissues and may be important in regulation of body weight. B3-adrenergic receptors increase energy expenditure and increases lipolysis.[2]

The β2-adrenergic receptor is coded by an intronless gene called ADBB2. This gene is a small gene located on chromosome 5q31-q32, a region genetically linked to asthma.[1] There are 13 validated SNPs in the ADRB2 gene localized to coding regions of the gene.[1] Four of these polymorphisms predict amino acid sequence changes: Gly16Arg, Gln27Glu, Val34Met and Thr164Ile. The two most common deleterious polymorphisms in the ADRB2 gene are Arg16Gly (+46A>G; rs1042713) and Gln27Glu (+79C>G; rs1042714). The rs 1042714 and rs1042713 are associated with susceptibility to obesity and asthma.[1] The Arg16Gly and Gln27Glu polymorphisms are near the receptor's ligand binding site. The frequency of Gly16 is greater than that of Arg16, which is considered the normal allele. The allelic frequency described for the Arg16 variant ranges from 67% to 72% in different populations.[1] Variation in ADRB2 has been associated with varying risks for obesity, diabetes mellitus, essential hypertension, and asthma.[3,4]

The β2-adrenergic receptors are widely expressed in the respiratory tract, particularly in the airway smooth muscles.[5] The most clinically relevant effect of the β2-adrenergic receptors in the pulmonary smooth muscle is relaxation, which may be caused by β2-adrenergic receptor agonists. Chronic exposure to these agonists leads to a significant reduction in the number of β2-adrenergic receptors on the cell surface.[5]This down regulation is reflected as a tolerance to the effect of the β2-adrenergic receptor agonists. β-2adrenergic receptors also present in number of regions of the lung, so they may contribute to the pathophysiology of asthma.[6] Most studies found an association between the presence of nocturnal asthma and polymorphism (Gly16) of the β2- adrenergic receptor.[7-9]

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However not all studies support the previous findings.[5-10] β2-adrenergic receptor polymorphisms frequencies differ in ethnic groups such frequencies are listed in Table (1).

In this study, we sought to determine the frequency of Gly16Arg and Gln27Glu β2-adrenergic receptors polymorphism among a healthy Jordanian population and compare it with those of different ethnic groups.

MATERIALS AND METHODS

Study population

The study was conducted in the faculty of Medicine at the University of Jordan and was approved by the University of Jordan Ethics Committee (Reference Number 67/2016/2226); all participants were of Jordanian origin, and written informed consent was filled and submitted by all participants. 96 individuals (32 males, 64 females) with a median age of 45 years (range from 18 to 80) without clinical evidence of disease were enrolled in this study. The study participants were volunteers that were non-blood relatives. Whole blood was withdrawn from each subject using standard venous puncture in which 2 mL of blood was collected into EDTA anti-coagulant tubes in order to determine β2 adrenergic receptor polymorphisms.

Table 1. Summary of minor allele frequency in different population and functional consequences of the β 2- receptor Gly16Arg and Gln27Glu polymorphisms according t[o1]1

Gene Symbol	Polymorphism	Minor Allele	Frequency of minor allele by race	Functional consequences
ADRB2	Gly16Arg	Arg	Caucasians (39%), African-American (49%) , Asians (51%)	Gly16 allele has greater receptor down-regulation with agonist stimulation
	Gln27Glu	Glu	Caucasians (25%), African-Americans (19%), Asians (9%)	Glu allele is resistant to receptor down-regulation

DNA extractionGenomic DNA was isolated and purified from whole blood using a commercially available kit (Wizard Genomic DNA Purification Kit, Promega Corporation, Madison, WI,USA) and protocol provided by the manufacturer , The purified DNA was safely stored at -20ºC for later use.

Genotyping

The Arg16Gly and Gln27Glu polymorphisms were detected by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). The primers were selected according to a previous study by[12] were purchased from Gene Link, USA.[13]

Arg16Gly polymorphism

The PCR reaction was carried out in 20 µL volume reaction containing 1µL of genomic DNA and 19 µL of ready mix solution (Solis Bio Dye, 5x FIREPol® Master Mix) with 10µM primers , the forward primer was 5'-ATGGGGCAACCCGGGAAC-3' and the reverse primer was 5'- CAGGCCAGTGAAGTGATGAA -3' . After the initial denaturation step at 94ºC for 5 minutes, amplification was carried out for 35 cycles with denaturation at 94ºC for 40 second, annealing at 64ºC for 40 second and extension at 72ºC for 50 second, with a final extension step at 72ºC for 5minutes. 5 µL of the PCR mixure was then electrophoresed on 2% agarose stained with red safe dye and visualized under gel documentation system. The size of generated PCR product was 168 base pairs (bps).

Arg16Gly polymorphism was carried out using 5 µL of PCR and 1 U of NcoI digestion enzyme (New England Biolabs), at 65ºC for 1 hour. The restriction digests fragments were electrophoresed on 4% agarose gels stained with red safe dye and visualized under gel documentation system.

Gln27Glu polymorphism

The PCR reaction was carried out in 20 µL volume reaction containing 1µL of genomic DNA and 19 µL of ready mix solution (Solis Bio Dye, 5x FIREPol® Master Mix) with 10µM primers , the forward primer was 5'-GGCCCATGACCAGATCAGCA-3' and the reverse primer was 5'- GAATGAGGCTTCCAGGCGTC -3' . After the initial denaturation step at 94ºC for 5 minutes, amplification was carried out for 35 cycles with denaturation at 94ºC for 1 minute, annealing at 63ºC for 1 minute and extension at 72ºC for 1 minute, with a final extension step at 72ºC for 5 minutes. 5 µL of the PCR mixure was then electrophoresed on 2% agarose gels stained with red safe dye and visualized under gel documentation system . The size of generated PCR product was 353 base pairs (bps). Gln27Glu polymorphism was carried out using 5 µL of PCR and 1 U of Fnu4HI digestion enzyme (New England Biolabs), at 37ºC for 1 hour. The restriction digests were electrophoresed on 4% agarose gels stained with red safe dye and visualized under gel documentation system.

RESULTS

This study emphasis the importance of determining ADBR2 polymorphism and the allele frequency for the different alleles in different populations. Polymorphisms of b2-adrenergic receptor in healthy populations has been studied and discussed by several studies.[1-3] The current study is the first to determine the polymorphism of β2-adrenergic receptor in a healthy Jordanian population. The results of this investigation indicate that there are marked interethnic differences in the frequencies of alleles for the two common β2 adrenergic receptor polymorphism among healthy Jordanian, Caucasians, African Americans, Asians.[12]

The results showed that Jordanians has 51% genotype frequency of the wild ADRB2 gene Arg16Gly (GG), and 13% of the mutant genotype frequency of ADRB2 Arg16Gly (AA) and 36% genotype frequency of the heterozygous ADRB2 gene Arg16Gly (GA). The allele frequency for minor allele of this polymorphism is 31 %, which differs from the frequency in other populations: (Caucasians 39%, African Americans 49% and Asians 51%). Comparing the population minor allele frequency for this polymorphism shows that Jordanians MAF

is closer to the Caucasians, while it is lower than African Americans and Asians.

Regarding the ADRB2 Gln27Glu polymorphism, the study showed that the genotype frequency of ADRB2 Gln27Glu CC is 87%, 8% for the GG and 5% for CG genotype. The minor allele frequency for this polymorphism is 11% in the Jordanian population while it is 25% in Caucasians, 19% in African Americans, 9% in Asians. The study shows that Jordanians has a close frequency to the Asians and lower frequency than Caucasians, African Americans.[11]

To have clear image, we compared the genotype frequencies in Jordanian and Greek. The frequency of the mutant ADRB2 gene Arg16Gly (AA) allele in Jordanian and Greek populations are close (13% in Jordanian and 12% in Greeks). The frequency of the ADRB2 gene Arg16Gly (GG) is higher in Jordanians than in Greek (51% in Jordanians and 39% in Greeks), however the frequency of the heterozygous ADRB2 gene Arg16Gly (GA) allele is higher in Greeks than in Jordanians (36% in Jordanians and 48% in Greeks).[12] Regarding the ADRB2 Gln27Glu polymorphism, the allele.

the mutant ADRB2 Gln27Glu (GG) allele is close to the Greeks (5.2% in Jordanians and 8% in Greeks), the frequency of the wild ADRB2 Gln27Glu (CC) is higher in Jordanian than in Greeks (87% in Jordanians and 44 % in Greeks), however the frequency of the heterozygous ADRB2 Gln27Glu (CG) is higher in Greek than in Jordanians (5.2 % in Jordanians and 47% in Greeks).[12]

Studies have shown that some of these polymorphisms significantly alter function expression, or regulation of the receptor.[1] Most clinical studies have shown that β2-adrenergic receptor polymorphisms act as disease modifiers or are a determinant of responsiveness to β-agonist therapy; determination of β2-adrenergic receptor polymorphism in clinical practice can potentially provide important information due to variability in receptor response.[12-18] The allelic frequencies of β2-adrenergic receptor polymorphisms can be taken in consideration when using β2-receptor agonist and antagonist therapies. As well as comparing frequency of these important polymorphisms of β2-adrenergic receptors in various diseases to better-tailored therapies. Recently,[19] have studied the Association of multiple genetic variants with level of asthma control in the Arab population. The variants that were studied included the ADRB2 46 (G/A) and 79 (C/G) and most of the include subjects were Jordanians. The authors found both studied polymorphisms to be significantly associated with asthma control. Importantly, the genotype frequency for ADRB2 46 (G/A) in asthmatic patient were 10.8 % for the GG, 45.3 % for the GA, 44 % for the AA, and for ADRB2 79 (C/G) it was 8.7 % for the GG, 29.3 % for the CG and 51.4 % for the CC. In term of minor allelic frequency, they were 33.4 % for ADRB2 46 (G/A) and 29.7% for ADRB2 79 (C/G). Interestingly, comparing their finding with our findings reveal a clear differences in the both genotype and allele frequency especially in case ADRB2 79 (C/G) polymorphism. For example, the minor allele frequency for ADRB2 79 (C/G) is 14 % in the healthy subject while it is 29.7% in the asthmatic subject. Such differences perhaps is a results of the high frequency of the GC frequency in the asthmatic subject than the healthy one (38.9 % verses 5.2 %).[19]

DISCUSSION

This study emphasis the importance of determining ADBR2 polymorphism and the allele frequency for the different alleles in different populations. Polymorphisms of β2-adrenergic receptor in healthy populations has been studied and discussed by several studies.[1-3] The current study is the first to determine the polymorphism of β2-adrenergic receptor in a healthy Jordanian population. The results of this investigation indicate that there are marked interethnic differences in the frequencies of alleles for the two common β2 adrenergic receptor polymorphism among healthy Jordanian, Caucasians, African Americans, Asians.[12] The results showed that Jordanians has 51% genotype frequency of the wild ADRB2 gene Arg16Gly (GG), and 13% of the mutant genotype frequency of ADRB2 Arg16Gly (AA) and 36% genotype frequency of the heterozygous ADRB2 gene Arg16Gly (GA). The allele frequency for minor allele of this polymorphism is 31 %, which differs from the frequency in other populations: (Caucasians 39%, African Americans 49% and Asians 51%). Comparing the population minor allele frequency for this polymorphism shows that Jordanians MAF is closer to the Caucasians, while it is lower than African Americans and Asians.

Regarding the ADRB2 Gln27Glu polymorphism, the study showed that the genotype frequency of ADRB2 Gln27Glu CC is 87%, 8% for the GG and 5% for CG genotype. The minor allele frequency for this polymorphism is 11% in the Jordanian population while it is 25% in Caucasians, 19% in African Americans, 9% in Asians. The study shows that Jordanians has a close frequency to the Asians and lower frequency than Caucasians, African Americans.[11] To have clear image, we compared the genotype frequencies in Jordanian and Greek. The frequency of the mutant ADRB2 gene Arg16Gly (AA) allele in Jordanian and Greek populations are close (13% in Jordanian and 12% in Greeks). The frequency of the ADRB2 gene Arg16Gly (GG) is higher in Jordanians than in Greek (51% in Jordanians and 39% in Greeks), however the frequency of the heterozygous ADRB2 gene Arg16Gly (GA) allele is higher in Greeks than in Jordanians (36% in Jordanians and 48% in Greeks).[12] Regarding the ADRB2 Gln27Glu polymorphism, the allele.

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Many factors may have contributed to a source of errors and produced some conflicting date in this investigation. Some can be statistical factors that caused these discrepancies such as small sample size as well as uncorrected multiple comparisons. Non-statistical factors includes differences in study design and population and inaccurate self-medical evaluation by the participate individuals. These factors should be taken in consideration in future pharmacogenetic studies.

CONCLUSIONS

In conclusion, The results of this investigation indicate that there are marked interethnic differences in the frequencies of alleles for the two common β2 adrenergic receptor polymorphism among healthy Jordanian, Caucasians, African Americans, and Asians. Moreover, the obtained allele frequencies significantly differ from its counterpart in asthmatic patients in Arab population.

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INFORMED CONSENT STATEMENT: all participants were of Jordanian origin, and written informed consent was filled and submitted by all participants.

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