Population frequency, mutation linkage and analytical methodology for the Arg16Gly, Gln27Glu and Thr164Ile polymorphisms in the β_2 -adrenergic receptor among Turks

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Aims Inherited polymorphisms of codons 16, 27, and 164 of the β_2 -adrenergic receptor (B2AR) gene may result in significantly changed functions of this receptor. The aim of the present study was to investigate the frequencies of the main mutations of the B2AR gene in Turks.

Methods A group of 104 unrelated Turkish subjects were analysed for the Arg16Gly, Gln27Glu, and Thr164Leu polymorphisms of B2AR by a newly designed polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. A 242-bp amplicon including the Arg16Gly and Gln27Glu polymorphisms was generated including an arbitrary restriction site, allowing the cleavage with *Eco130I* or *Fnu4HI* for analysis of the two polymorphic sites, respectively. For the amino acid polymorphism at codon 164 a 280-bp fragment was generated and digested by *MnlI*.

Results The allelic frequencies of mutated Gly16, Glu27, and Ile164 alleles were found as 59.6%, 31.7%, and 0.5%, respectively. Although, the frequencies of the polymorphisms at codon 16 and 164 were found similar as in various Caucasian populations, the frequency of the Glu27 variant was found significantly lower than these populations.

Conclusions Obviously there are interethnic differences in functionally relevant B2AR variants which may have implications for drug treatment and disease susceptibility.

Keywords: β_2 -adrenergic receptor, molecular epidemiology, polymorphism, Turkish population

Introduction

The human β_2 -adrenergic receptor (B2AR) is a primary target of bronchodilatory drug treatment and may be relevant in pathogenesis and drug treatment of cardiovascular disease and obesity. The coding region of the B2AR is located on chromosome 5q31 and was firstly sequenced by Kobilka *et al.* [1]. More recently, nine single nucleotide polymorphisms have been identified [2]. Four of these variants were found to cause changes of amino acids at residues 16, 27, 34, and 164. Most frequent were the arginine to glycine (Arg16Gly) and glutamine to glutamic acid (Gln27Glu) polymorphisms, respectively [2]. It has been shown that the B2AR Gly16-variant displays enhanced agonist-promoted down-

regulation, whilst the Arg16 form represents the wild type [3]. On the other hand, the Glu27 variant is resistant to downregulation, but only when this variant is in linkage-disequilibrium with Arg16 [3]. The third mutation is at amino acid 164 and leads to a threonine to isoleucine (Thr164Ile) exchange. Although this polymorphism is less frequent than the Arg16Gly and Gln27Glu polymorphisms, agonist binding and coupling to adenylyl cyclase was altered significantly by this polymorphism [4]. The last amino acid exchange involves a valine to methionine (Val34Met) interchange at codon 34. However, besides its very rare occurrence, it has been shown that this substitution does not alter receptor function [4].

The distribution of B2AR mutations were mostly investigated in patients suffering of asthma and compared with healthy reference subjects [2, 5–10]. Taking into account that B2AR is an important target of many drugs and endogenous substances, interethnic differences in this

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receptor may explain differences in drug response and disease susceptibility. Therefore, we aimed to investigate the frequencies of the main mutations of the B2AR gene in Turks. Since we consider PCR-RFLP analysis as one of the currently most reliable methods for population genotyping, the respective methods for the B2AR were adopted or newly developed.

Methods

Subjects and blood samples

A group of 104 unrelated healthy Turkish volunteers (40 female and 64 male), with a median age of 25 years (range 18–60 years), were studied after their informed and written consent. All individuals were born in Turkey with Turkish parents. Genomic DNA was extracted from leucocytes by standard 3-step phenol/chloroform extraction.

Genotyping

The three mutation sites of the B2AR gene were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR reactions were carried out in a total volume of 25 μ l containing specified amounts of DNA as template, 0.2 μ mol l^{-1} of each primer (TIB Molbiol), 0.5 units *Taq*-DNA-polymerase (Life Technologies), 0.2 mmol l^{-1} of each dNTP (Roche), 10 mmol l^{-1} Tris-HCl (pH 8.8), 50 mmol l^{-1} KCl, and 1.5 mmol l^{-1} $MgCl_2$. A 242-bp fragment including both the polymorphic sites at codon 16 and 27, was amplified using primers 5'-GAACGG CAGCGCCTTCTTGCTGGCACCCCAT (sense, AB3) and 5'-CTGCCAGGCCCATGACCAGATCAG (antisense, AB2). Compared with the original sequence of the B2AR, the underlined nucleotide was artificially exchanged from A by C in order to generate a polymorphism specific restriction site. PCR conditions were as follows: 2 min of initial denaturation at 94° C, then 35 cycles of amplification (denaturation 94° C, 30 s; primer annealing 64° C, 45 s; polymerization 72° C, 1 min) followed by final elongation (7 min 72° C) using a thermal cycler (Perkin Elmer). For detection of the Arg16Gly polymorphism, overnight digestion at 37° C with 10 U *Eco130I* (Fermentas) was performed. The Gln27Glu polymorphism was identified in the other half of the PCR product using 10 U *Fnu4HI* (New England BioLabs). For evaluation the codon 164 mutation, a second PCR procedure was performed generating a 280-bp fragment with primers 5'-GTGATCGCAG-TGGATCGCTACT (sense, AB4) and 5'-AGACGA-AGACCATGATCACCAG (antisense, AB5) under the same conditions described above, except 58° C for primer annealing. Again 10 μ l of the PCR product was digested

by 10 U *MnII* (New England BioLabs). All RFLP fragments were separated on a 3% 3:1 NuSieve-agarose gel and visualized with ethidium bromide staining. The restriction patterns as found and as expected from the nucleotide sequence are summarized in Table 1.

Statistical analysis

Expected genotype frequencies were derived by the Hardy-Weinberg equation from single allele frequencies. Comparison of the B2AR genotypes or alleles between different populations was performed using Fisher's exact test.

Results and discussion

For detection of the amino acid polymorphisms in the B2AR gene, different approaches such as allele-specific PCR, allele-specific hybridization, PCR-RFLP, and direct sequencing have been applied previously [2, 5–8]. We have developed a simple PCR-RFLP assay to determine the three mutation sites of the B2AR gene within a population. No useful site of this type could be included in the other assays, thereby external controls with known mutations for the different genotypes were included in each assay.

The B2AR-genotype frequencies Arg16Gly, Glu27Gln, and Thr164Leu in our sample were in Hardy-Weinberg equilibrium as tested by comparison of the frequencies found *vs* the frequencies expected from the found proportion of the more frequent allele. The frequencies of Gly16 and Glu27 variants were found as 59.6% and 31.7%, respectively (Table 2). The Thr164Ile polymorphism was found only in one subject, who was heterozygous for the isoleucine variant.

Arginine at codon 16 occurred not together with glutamic acid at codon 27 by comparing the found genotypes with the values expected from random segregation of Arg16 and Glu27 ($P < 0.0001$ by chi-square test). Obviously, there are at least three alleles, namely Arg16Gln27, Gly16Gln27, and Gly16Glu27. There was no subject homozygous for both Arg16 and Glu27 and there was also no heterozygous carrier of Arg16Gly combined with the homozygote Glu27Glu genotype in our sample.

We have found that the genotypic and allelic frequencies of Gln27Glu polymorphism of the B2AR gene in our sample are significantly different from various Caucasian populations ($P < 0.001$) [6, 7, 9]. A similar finding as in this study was reported for Hispanic children, who carried the Gln27 allele significantly more frequently than Caucasian children [8]. However, the number of the other two polymorphisms, Arg16Gly and Thr164Leu, were not different.

Table 1 Diagnostic RFLP patterns for the common B2AR amino acid polymorphisms.

Polymorphic amino acid	Nucleotide*	Primers†	Enzyme	RFLP-pattern
Arg16	46A	AB2 + AB3	<i>Eco</i> 130I (<i>Sty</i> I‡)	242
Gly16	46G			214 + 28
Gln27	79C		<i>Fnu</i> 4HI	181 + 55 + 6
Glu27	79G			236 + 6
Ile164	491T	AB4 + AB5	<i>Mn</i> II	230 + 50
Thr164	491C			116 + 114 + 50

*Counted from the adenine of the start codon.

†See text for nucleotide sequence.

‡*Eco*130I and *Sty*I are isoschizomers.**Table 2** Observed and expected genotype distributions of B2AR polymorphisms and B2AR alleles detected among 104 Turkish subjects.

B2AR genotype	n	Observed (%)	Expected* 95%CL (%)	(%)
Arg16Arg	16	15.4	9.1–23.8	16.3
Arg16Gly	52	50.0	40.0–60.0	48.2
Gly16Gly	36	34.6	25.6–44.6	35.5
Gln27Gln	49	47.1	37.3–57.2	46.6
Gln27Glu	44	42.3	32.7–52.4	43.3
Glu27Glu	11	10.6	5.4–18.1	10.1
Thr164Thr	103	99.0	94.8–99.9	99.0
Thr164Ile	1	1.0	0.2–5.2	1.0
Ile164Ile	0	0.0	0.0–3.5	0.0
Allele frequency (%)				
Arg16/Gly16	40.4/59.6			
Gln27/Glu27	68.3/31.7			
Thr164/Ile164	99.5/0.5			

*Expected genotype frequencies were calculated from the frequencies of single allele.

In recent studies it has been shown that relatively higher activities exist for certain drug metabolizing enzymes, such as CYP2C19 and CYP2D6, in Turks [11] compared with Northern Europeans and there was a close similarity to Hispanic populations. Thus, there is an obvious parallelism in the B2AR and drug metabolizing enzymes when we compare Turkish, Spanish and Northern European populations, probably explained by common Arabian ancestors of Turkish and Spanish populations.

In conclusion, the present study indicates that the Glu27 variant of the B2AR in Turks appeared very rare compared with various Caucasian populations, but much the same as Hispanics, suggesting that interethnic differences may exist for receptor polymorphisms, like for enzymes responsible for foreign compound metabolism. Our results should provide a basis for clinical and

molecular-epidemiological studies of B2AR and other receptor polymorphisms in the Turkish population and also understanding of interethnic differences in the distribution of polymorphic targets important in drug response.

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