PHARMACOKINETICS AND DISPOSITION

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Allele and genotype frequencies of polymorphic DCP1, CETP, ADRB2, and HTR2A in the Egyptian population

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Abstract Objective: The goal of this study was to determine the frequencies of important allelic variants of two drug targets, dipeptidyl carboxypeptidase (DCP1) and cholesteryl ester transfer protein (CETP), and two other drug receptors, beta-2 adrenergic receptor (ADRB2) and 5-hydroxytryptamine 2A receptor (HTR2A), in the Egyptian population and compare them with the frequencies in other ethnic populations.

Methods: A sensitive real-time polymerase chain reaction assay was developed and successfully applied for genotyping of the consensus (wild-type) alleles plus five variants of four genes: DCP1 [the insertion allele (I) versus the deletion allele (D)]: CETP* TaqI (B1 versus B2), ADRB2* R16G, ADRB2* Q27E, and HTR2A* 102T>C. This study was carried out in 242 unrelated Egyptian subjects and is the first to describe these allelic variants in the Egyptian population.

Results: The frequencies of the tested alleles were found as: DCP1 (I:D, 0.32:0.68), CETP* TaqI (B1:B2, 0.65:0.35), ADRB2* R16G (Arg16: Gly16, 0.57:0.43), ADRB2* Q27E (Glu27:Gly27, 0.76:0.24), and HTR2A* 102T>C (T102:C102, 0.53:0.47). The common Arabian ancestors of the Egyptians, Spanish, Saudi, and Emirate had created a common pattern of distribution of some allelic variants (DCP1 and CETP). However, in the genotyping of ADRB2, the frequency of the polymorphism at codon 16 was found to be similar to the Chinese population, whereas that at codon 27 was similar to African-Americans with significant differences than other Caucasian populations. The frequency of the HTR2A* 102T>C variant appeared to be similar to many Caucasian populations and African-Americans.

Conclusions: We have explored the frequencies of important allelic variants DCP1, CETP, ADRB2, and HTR2A among the Egyptian population focusing on the ethnic diversity in the distribution of the tested mutant alleles. Our results may help in better understanding the observed ethnic variation in angiotensin-converting enzyme inhibition and atherosclerosis therapy. It also may contribute to better characterization of interethnic differences in isoprenaline and clozapine response, which will have implications for the cost effective and rational prescribing of these drugs.

Keywords Pharmacogenetics · Drug targets · Receptors

Introduction

Genetic polymorphisms modulate pharmacological and toxicological reactions in individuals upon exposure to drugs [1, 2]. Kinetic variations in absorption, distribution, metabolism, and excretion of therapeutic agents are well known and have been studied extensively during the past two decades [3]. More recently, pharmacodynamic variations, including drug target, receptor, and transporter polymorphisms, have been shown to cause individual variations in drug responses [2].

Pharmacogenetic studies have revealed that many of the genes encoding drug targets and receptors exhibit genetic polymorphism, which in many cases alters their sensitivity to specific medications. Such examples of polymorphisms in drug targets include a 287-bp Alu repeat sequence in intron 6 of dipeptidyl carboxypeptidase (DCP1) and its sensitivity to angiotensin-converting enzyme (ACE) inhibitors [4]. Moreover a silent base change affecting the first intron of the cholesteryl ester transfer protein (CETP) gene referred to TaqI B has been associated with efficacy of pravastatin therapy in patients with coronary atherosclerosis [5]. The allele carrying the TaqI site is called B1, whereas the one in which the TaqI site is missing is known as B2.
Alterations of drug response have been also associated with polymorphisms in the drug receptors. Such examples include the polymorphic beta-2 adrenergic receptor (β2-AR) and its relation to β2-AR agonists in asthmatics. Two common mutations have been identified in the gene coding for β2-AR (ADRB2) [6], which are 46A to G and 79C to G. These mutations result in changes in the N terminus of the receptor at amino acid positions: 16 arginine to glycine (ADRB2*R16G) and 27 glutamine to glutamic acid (ADRB2*Q27E).

The Gly16 variant shows markedly enhanced agonist-promoted downregulation of receptor expression in vitro after prolonged exposure to the β2-AR agonist compared with the wild-type Arg16 receptor, irrespective of the co-expressed Glu27/Glu27 polymorphism [7]. In contrast, the Glu27 variant was found to be resistant to agonist-induced downregulation in vitro when co-expressed with the Arg16 [8] and to be associated with lower bronchial hyperreactivity [9] and greater vaso-dilation in vivo [10].

Another example of polymorphic receptors is 5-hydroxy tryptamine 2A receptor (5-HT2AR), which plays an important role in the mechanism of action of clozapine and other atypical antipsychotics. Arranz et al. [11] first reported an association between a silent polymorphism in exon 1 of the gene coding for 5-HT2AR (HTR2A*102T>C) and response to clozapine. To date, initial studies on clozapine response showed disparate results in different populations. Addressing HTR2A*102T>C allele frequencies across a range of ethnic groups may help in clarifying the relationship between this mutation and response to neuroleptics such as clozapine.

Contrasting the remarkably rich literature on ethnic variation of structure and function of polymorphic drug-metabolizing enzymes, relatively few population studies have investigated the frequencies of drug response-related polymorphisms in different ethnic groups. In addition, no data have been available on the response-related polymorphisms in different ethnic groups. Addressing HTR2A*102T>C results in different populations. Addressing HTR2A*102T>C allele frequencies across a range of ethnic groups may help in clarifying the relationship between this mutation and response to neuroleptics such as clozapine.

Oligonucleotides

The primers were synthesized by Nihon Gene Research Laboratories, Inc. (Sendai, Japan). The TaqMan probes were synthesized by Applied Biosystems, contained 6-carboxyfluorescein (FAM) at the 5’ end and 6-carboxytetr methylrhodamine (TAMRA) with a phosphate molecule at the 3’ end. Sequences for the TaqMan probes and allele-specific polymerase chain reaction (PCR) primers used in this study are given in Table 1.

Allele-specific fluorogenic 5’ nuclelease chain reaction assay

Allele-specific fluorogenic 5’ nuclease chain reaction assay was performed using the method described previously by Hiratsuka et al. [12]. All assays proceeded in 30 μl TaqMan Universal PCR Master Mix (Applied Biosystems) containing 0.4 μM forward primers (wild or mutant) and reverse primers, 0.1 μM TaqMan

<table>
<thead>
<tr>
<th>Allele</th>
<th>WT</th>
<th>MT</th>
<th>TM</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCP1</td>
<td>GCTGGGATTACAGGCGGTATAC</td>
<td>TTTCTTCAGACCCCCCTGCTGTATAC</td>
<td>AGCTCCCCCTACAAAGACA-GAGGTGACCTAAG</td>
<td>GGTGTAATGCGTACCCCTCA</td>
</tr>
<tr>
<td>CTP</td>
<td>CCAGAATCCTGGGGTTTCG</td>
<td>CCAGAATCACGGGGTTCG</td>
<td>AGGTGACAGGAGGTCCTACCTGACTG</td>
<td>AAGAGACTGAGGCGGAGAGAG</td>
</tr>
<tr>
<td>ADRB2A</td>
<td>A46G, Arg16Gly</td>
<td>CTTCCTGCGCCACCCCAAG</td>
<td>CTTCCTGCGGCGAACC</td>
<td>CTTCCTGCGGCAGG</td>
</tr>
<tr>
<td>ADRB2B</td>
<td>C79G, Gly27Glu</td>
<td>ACCAGGAAGTCAGCAGC</td>
<td>ACCAGGCTCTCAATGAGTTA</td>
<td>ACCAGGCTCTCAATGAGTTA</td>
</tr>
<tr>
<td>HTR2A</td>
<td>102T&gt;C</td>
<td>ACCAGGCTCTCAATGAGTTA</td>
<td>ACCAGGCTCTCAATGAGTTA</td>
<td>ACCAGGCTCTCAATGAGTTA</td>
</tr>
</tbody>
</table>

The mismatched nucleotide sites at position 2 are underlined.

Methods

Subjects

Two hundred forty-two unrelated Egyptian subjects participated in this study. The Egyptian population is divided into several cultural groups: Bedouins, Nubians, Berbers, Peasants, and Urbanites. The subjects who participated in our study were students and staff at Cairo University, thereby considered as Urbanites living in Cairo or other surrounding cities. Each subject gave a sample of about 1 ml saliva after detailed explanation of the purpose of the study; a signed written consent was also obtained from each subject. Genomic DNA was isolated from the saliva using a QIAamp DNA Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. Sample collection and DNA isolation were performed under the supervision and ethics approval of the dean of the faculty of pharmacy of Cairo University. The isolated DNA samples were sent to our laboratory in Japan, and the genotyping protocol was approved by the institutional ethics committee of Tohoku University School of Medicine, Sendai, Japan and Cairo University Faculty of Pharmacy, Cairo, Egypt.

The summary of primer pairs [wild-type (WT) or mutant (MT) and common (CM) primers] and TaqMan probe (TM) sequences (5’–3’). DCP1 dipetidyl carboxypeptidase, CTEP cholesterl ester transfer protein, ADRB2 beta-2 adrenergic receptor, HTR2A 5-hydroxy tryptamine 2A receptor, I insertion allele, D deletion allele.