Key Words: CYP2D6, pharmacogenetics, PCR-RFLP

Investigation of CYP2D6 Gene Polymorphisms in Turkish Population

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ABSTRACT *~ Pharmacogenetics is interested in the variable response to drugs depending on the genetic constitution of an individual. Depending on the genetic variation in individuals known as polymorphism; leads to differences in the types of proteins, enzymes or receptors that play a role in the elimination of drugs. Investigation of the correlation between the genotype with phenotype changes in drug metabolism is among the most important topics of today. CYP2D6 gene polymorphisms show clinical efficiency in the use of especially antidepressants, neuroleptics, antiarrhythmic, antihypertensive, beta blocker, and morphine derivatives. Poor metabolizers have been shown to demonstrate adverse drug reactions to these drugs. The plasma concentrations tend to increase inducing side effects after using a standard dose in poor metabolizers. The ratio of poor metabolizers in Caucasians is 5–10%, whereas 3.4–3.8% of the Turkish population. The allele frequencies of CYP2D6 *2, *3, *4 and *10 were found in 35%, 6%, 10% and 26% respectively in 200 healthy controls. The ratio of poor metabolizers in our population revealed as 1%. Genotyping of CYP2D6 is very important for determining a better genotype-phenotype relation.* Psychopharmacology Bulletin. *2016;46(1):67–72.*

Introduction

Genetic variation in drug metabolism has been evaluated to understand the predictors of response and toxicity of drugs are known as Pharmacogenomics. There are differences have been observed in the responses to the same drug at the same amounts between similar individuals. The reason is due to the genotypes between different people.[1]

The CYP2D6 gene has been localized to 22q13.1 with the CYP2D7 and CYP2D8 pseudogenes.[2] The alleles of CYP2D6 gene have been associated with normal, reduced, increased enzyme activity with and non-functional activity.[3] The most common CYP2D6 alleles seen in Caucasians are reported to be *2, *3, *4, *5, *6, *10 and *41. The *2 and *17 are common in Africans whereas *10 and *36 alleles are frequently seen in Asians.[2]

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CYP2D6 allele distribution based on ethnic characteristics vary and some drugs used in together (such as CYP2D6 inhibitors) may affect the activity of the enzyme.[4] CYP2D6 gene polymorphisms lead to variation in the clinical effects of the therapeutic use of antidepressants, neuroleptic, antihypertensive, antiarrhythmic, beta blockers and morphine derivatives. An increase in the concentration of such drugs in the plasma and side effects have been reported in poor metabolizers after usage of the standard dose.[5]

The ratio of poor metabolizers has been reported as 5–10% in Caucasians and 3.4–3.8% of the Turkish population.[2,5] The poor metabolizer (PM) alleles in both in Caucasians and Africans are *3, *4, *5, *6 and *10.[6]

According to the studies with CYP2D6 in the Turkish population; the allele frequencies for CYP2D6*1, *2, *4, *5, *10 and *17 have been reported to be 37%, 35%, 11%, 1%, 6% and 1%, respectively. The poor metabolizer (PM) rate in Turkey has been reported as 1.49%.[7] The allele frequency of CYP2D6*3 has been reported to be 25%, whereas the allele frequency of CYP2D6*4 has been reported as 13.4–21% in the Turkish population.[8,9] Besides the allele frequencies of CYP2D6*5 and *6 reported as 1.49% and 0.5%, respectively, are less frequent compared to *3 and *4 alleles.[9, 10]

In this study, we genotyped the *2, *3, *4 and *10 alleles of CYP2D6 gene in 200 healthy people in order to determine the frequency of these alleles with PM in the Turkish population.

Material and Methods

Subjects

In this study, 200 healthy controls (100 male, 100 female) were included. This study had been accepted by the Local, Ethical Committee of Gazi University Faculty of Medicine with a number of 220 on June 13, 2012.

Molecular Genetics Studies

Five ml of blood samples was taken from all of the participants. DNA isolation was performed by the high salt concentration method. PCR-RFLP technique was used for the polymorphism analyses.

Regarding CYP2D6*2 polymorphism 5′-CTGACAGGT GCAGAATTGGAG-3′ and 5′-CATCCCGGCAGAGAACAG-3′ primers; for CYP2D6*3 polymorphism 5′-GGATGAGCTGCTA ACTGAGCTC-3′ and 5′-GCCTCCCCTCATTCCTCCT-3′ primers; for CYP2D6*4 polymorphism 5′-GTGGGTGATGGGCAGAAG-3′

and 5′-GAGGGAGGCGATCACGTT primers and finally for CYP2D6*10 polymorphism, CAACGCTGGGCTGCACGgT-3′ and 5′-GGCCCTTGCCCTACTCTTCCTTGG-3′ primers were used for PCR.

In order to genotype the amplified PCR products, restriction enzymes FspI, Bpu10I, BstNI and Acc65I were used for detecting CYP2D6*2, *3, *4 and *10 polymorphisms.

Statistical Analysis

The categorical variables regarding the genotypes of CYP2D6*2, *3, *4 and *10 alleles were examined with Pearson's chi-square test. A p value lower than 0.05 was considered to be statistically significant.

Results

The allele frequency revealed 35% (female = 34%, male 36%) regarding CYP2D6*2 (rs16947) allele. The *2/*2 genotype was found at 10.5%. No statistical significance was found between the female and male controls (p = 0.905).

The allele frequency of CYP2D6*3 allele (rs35742686), revealed 6% (female = 3%, male = 9%). There was a statistical significance between wild type and heterozygous genotypes (p = 0.035), also we found corelation between wild type genotypes with respect to heterozygous plus mutant genotypes (p = 0.0238) (Table 1). Only one control revealed to have a *3/*3 genotype.

The allele frequency revealed 1% (female = male = 1%) for the CYP2D6*4 allele (rs3892097). We did not find *4/*4 genotype and also, no statistical significance was found (p = 0.858).

Finally, the allele frequency revealed 26% (female = 27.5%, male = 25%) for CYP2D6*10 allele (rs1065852). No statistical significance was found (p = 0.07) and six controls revealed *10/*10 genotype.

TABLE 1

The Allele Frequency of CYP2D6*3 (rs35742686) Allele in Female And Male Controls

CONTROLS	WILD	HETEROZYGOUS	MUTANT	P
Female (100)	94	6	0	0.035*
Male (100)	84	15	1	0.0238**
Total (100)	178	21	1	

Notes: *A p value regarding wild types with respect to heterozygous genotypes. **A p value regarding wild types with respect to heterozygous plus mutant genotypes.

Discussion

The cytochrome P450 is known to be involved in the metabolism of up to 25% of the drugs that are in common use in the clinic.[11] The use of standard doses of antidepressants, neuroleptic, antihypertensive, antiarrhythmic, beta blockers and morphine derivates, causes an increase in the plasma concentrations of these drugs with side effects in poor metabolizers (PM).[2,5,12] The PM phenotype is more common in Caucasians than Asians, so this will cause more risk regarding the adverse drug reactions.[2]

CYP2D6*2 allele has been reported to have reduced function when compared with CYP2D6*1.[13] The CYP2D6*2 allele has also been reported to be the most common allele after *1 allele.[14] The allele frequency of *2 alleles has been studied in different populations and revealed 10% in Korean; 14.6% in Japan; 32.4% in German; and 5–28% in Mexican populations.[15–17] In this study, we found the *2 allele frequency as 35% that is compatible with the previous studies.[7]

The CYP2D6*3 allele has been reported to be a non-functional allele.[18] CYP2D6*3 has been reported to be one of the several CYP2D6 haplotypes that can contribute to the phenotypic observation of a PM.[19] The allele frequency of *3 alleles has also been studied in different populations, revealing a 0 % in African, Chinese, Japan and Mexican populations; and 1.5–2% in German populations.[15,17,20,21] Two studies in Turkish population revealed 1% and 2.5% for this allele.[8,22] Our finding was 6% for *3 alleles.

CYP2D6*4 has also been reported to be a non-functional haplotype contributing to the majority of PMs seen in Caucasian populations.[23] The allele frequency of *4 alleles has been reported in different populations, including; 0% in African, Chinese, Japan and Mexican populations; and 0.6% in Japan, 17.5–20.7% in German populations.[15,16,20,21,24] This study revealed 10% for this allele that was compatible with two other Turkish population studies revealing 10% and 11% for this allele, respectively.[7, 22]

CYP2D6*10 has been reported to be a reduced function haplotype of CYP2D6.[25] The *10/*10 genotypes are reported to be common, and resulting in the intermediate phenotype.[26] Finally, the allele frequency of *10 allele has been studied in different populations resulting 0% in Mexican; 1.5% in Japan; 6% in African; 43% in Japan; and 47–70 in Chinese populations.[3,15,17,20] Our findings for *10 alleles revealed 26% that were high from other Turkish studies indicating between 6–14.5%.[7,22]

The reason for the unavailability of routine CYP2D6 genotyping in clinical evaluation depends on finding many mutant alleles and having

no definite genotype-phenotype correlation.[15] Three major mutant alleles, are termed as CYP2D6*3, *4 and *5 associated with the PM phenotype in Caucasians.[27] In this study, we established one patient for *3/3 genotypes and one for *3/4 genotype revealing a 1% for PM in our population. Our results are also compatible with previous Turkish studies revealing 0.95%, 1.49% and, 3.4–3.8%.[2,5,7,28]

Conclusion

CYP2D6 gene polymorphisms result in adverse drug reactions, including antidepressants, neuroleptic, antiarrhythmic, antihypertensive, beta blockers and morphine derivatives. For this reason, it is important to know the genotypes. The ratio of PM in Caucasians is reported to be 5–10%, whereas in Turkish population this is between 3.4–10%. However, our results 1% for PM.[5,7,29]

Genetic polymorphism differs between populations as well as individuals. Therefore, it is important to determine the genotypes of them. In this study the frequency of the CYP2D6 alleles revealed 35% for *2 alleles; 6% for *3 alleles; 10% for *4 alleles and 26% for *10 allele. This study both enabled us to learn the allele frequencies of CYP2D6 gene with the ratio of poor metabolizers in the Turkish population in order to establish a better genotype-phenotype relation. ✤

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