ORIGINAL ARTICLE

# Frequency of CYP2D6*10 genotypes in Pakistani breast cancer patients taking adjuvant tamoxifen

Nusrat Nazir,[1] Akbar Waheed,[2] Kulsoom Farhat,[3] Muhammad Ismail,[4] Qaisar Mansoor[5]


## Abstract

**Objective:** To investigate the frequency of cytochrome P2D6*10 in breast cancer patients.

**Methods:** This retrospective study was conducted at the Nuclear Medicine, Oncology and Radiotherapy Institute, Islamabad, and the Combined Military Hospital, Rawalpindi, Pakistan, and comprised medical records of breast cancer patients from January 2000 to September 2013. Pre- and post-menopausal women with diagnosed breast cancer who were advised 20mg/day of tamoxifen as adjuvant therapy were included. The frequency of the cytochrome was determined using polymerase chain reaction-restriction fragment length polymorphism analysis.

**Results:** Of the 232 participants, 25(10.8%) had stage I disease, 127(54.7%) had stage II and 80(34.5%) had stage III disease. The overall mean age was 46.9±9.9 years. The allele frequency of cytochrome CYP2D6*1 was 431(93%) and that of CYP2D6*10 was 33(7 %). Pakistanis differed significantly from the Asian populations and other ethnic groups in the distribution of the allele cytochrome, but its frequency was comparable to South Indians.

**Conclusion:** The frequency of CYP2D6*10 allele in Pakistani breast cancer women was comparable to the South Indians, but was significantly lower than other populations.


**Keywords:** CYP2D6*10, Breast neoplasm, Pakistani. (JPMA 66: 1554; 2016)

## Introduction

Tamoxifen is the most frequently prescribed and extensively used treatment and adjuvant therapy drug for pre- and post-menopausal women who are oestrogen receptor/progesterone receptor (ER/PR) positive breast cancer patients.[1,2] Nevertheless around 30%-50% of ER-positive breast cancer patients do not show response to tamoxifen therapy and have reappearance of the disease.[3] One of the most important drug metabolising enzymes which is concerned in the biotransformation of many clinically significant drugs is cytochrome P450 2D6 (CYP2D6), and is known to be genetically polymorphic resulting in a high degree of inter-individual disparity in drug therapy.[4] CYP2D6*10 is the most frequent polymorphism of CYP2D6 in Asian women and is linked with the production of an unstable enzyme having reduced CYP2D6 activity[5] affecting the efficacy and adverse reactions of a drug. Quite a few research studies have shown that breast cancer patients treated with adjuvant tamoxifen and have reduced CYP2D6 enzymatic activity due to genetic polymorphisms or drug interactions may have an increased risk of recurrence and shortened disease-free survival.[6]

[1-3]Department of Pharmacology & Therapeutics Army Medical College (National University of Sciences and Technology), [4,5]Institute of Biomedical and Genetic Engineering, Islamabad.

**Correspondence:** Nusrat Nazir. Email: njafery@hotmail.com

Depending upon the activity of the enzyme and the combinations of the alleles, individuals are classified as poor metabolisers (PMs), intermediate metabolisers (IMs), extensive metabolisers (EMs) and ultra-extensive metabolisers (UMs) of CYP2D6 substrates. PMs are individuals who are homozygous for one deficient allele or heterozygous for two variant deficient alleles; IMs are heterozygous for one deficient allele or are carriers of two alleles with reduced enzyme activity; EMs have two wild-type alleles; and UMs are those who have multiple functional copies of the gene.[7] Comparison between different polymorphism studies[5,8,9] of the CYP2D6*10 reveals a variation in frequencies of the allele among different populations. In Pakistan, there is no such pharmacogenomics information regarding frequency of CYP2D6 genotypes and, to our knowledge, their comparison with other ethnic populations has never been reported. The current study was planned to investigate the frequency of CYP2D6*10 in Pakistani breast cancer patients for the first time and also compare it with other populations. We hope this study will serve as important baseline information for subsequent studies on cancer patients.

## Materials and Methods

This retrospective study was conducted in Nuclear Medicine, Oncology and Radiotherapy Institute (NORI), Islamabad, and Combined Military Hospital (CMH), Rawalpindi, Pakistan, and comprised medical records of breast cancer patients from January 2000 to

September 2013. The study was carried out in accordance with the current good clinical practices (GCP) according to the Helsinki Declaration.[10] The protocol of the study was approved by the ethical committee of the Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi.

Written informed consent was obtained from all patients. After going through various research studies,[11,12] pre- and post-menopausal females aged 18 years or above with diagnosed breast cancer stage I, II or III were included. These patients were receiving 20mg/day of adjuvant tamoxifen from breast cancer clinics at NORI and CMH and belonged to different regions of Pakistan. Breast cancer patients who were pregnant or lactating or undergoing hormonal treatment other than tamoxifen or taking enzyme inhibitors or inducers of CYP2D6 or having hepatic or renal disease were excluded.

The analytical procedures were carried out at the Institute of Biomedical and Genetic Engineering (IBGE), Islamabad. A 5ml blood sample was taken from all the participants.

Genomic deoxyribonucleic acid (DNA) was isolated from the peripheral blood by the standard organic phenol-chloroform method.[13] Genotypes were determined by using polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (PCR-RFLP) method. The PCR for 100C>T single nucleotide polymorphism (SNP) genotyping was carried out by using forward 5'_GTGCTGAGAGTGTCCTGCC_3' and reverse 5'_CACCCACCATCCATGTTTGC_3 primers and 20ng genomic DNA. The final volume was adjusted to 25uL with PCR water (Table-2). The PCR reaction was carried out under the following thermal and cycling profile: (i) initial denaturation at 95°C for 1 minute; (ii) 35 cycles each of denaturation at 95°C for 45 seconds, primer annealing at 56°C for 45 seconds and extension at 72°C for 45 seconds; and (iii) final extension at 72°C for 08 minutes.

A total volume of 10 to 15 µL containing the PCR product was used in restriction enzyme digestion reactions. The enzyme reaction buffer, bovine serum albumin (BSA) and 5 U of restriction enzyme were used. The volume was made up with double distilled water (dH2O). In order to ensure meticulous and complete digestion of PCR products, the digestion reactions were incubated overnight at their required incubation temperatures. After the PCR of target DNA region containing the *10, RFLP was performed and the amplified DNA fragment was digested with the haemophilus parahaemolyticus (HphI) restriction enzyme (Fermentas UAB, Vilnius, Lithuania) at 37°C for 16 hours. The digested products were analysed on 2.5% weight per volume (w/v) agarose gel through electrophoresis (Figure). After digestion, the homozygous individuals for wild type had two fragments of 263bp and 62bp. The heterozygous containing both the wild and mutant allele yielded fragments of 263bp, 183bp, 80bp and 62bp. The mutant homozygous individuals, who produce fragments of 183bp, 80bp and 62bp, were absent in our patients.

Genotype frequencies were calculated by direct counting and then were tested for Hardy-Weinberg equilibrium (HWE). Chi-square test was used to compare the observed genotype frequencies to published data for other populations. A p-value of less than 0.05 was considered significant. Demographic data was determined and presented as mean, median, percentage or frequency where suitable for qualitative or quantitative variables.

## Results

Of the 232 participants, 25(10.8%) had stage I disease, 127(54.7%) had stage II and 80(34.5%) had stage III disease. The overall mean age was 46.9±9.9 years while the mean values for height and weight were 155.9±5.58cm and 66.1±11.7kg, respectively. Moreover, there were 133(57.3%) pre-menopausal and 99(42.7%) post-menopausal patients (Table-1).

**Table-1:** Demographic data of the patients.

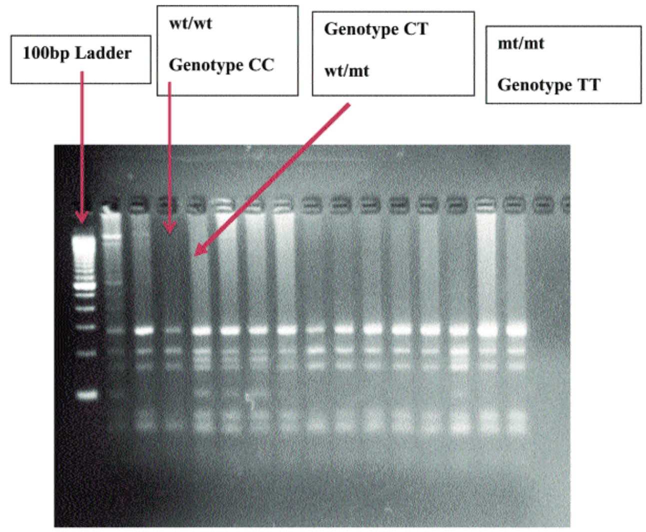
| **Descriptive data** | **N** | **%** |
|---|---|---|
| Number of patients | 232 | 100 |
| Mean Age (years) | 46.9 ± 9.9 | |
| Mean Height (cm) | 155.9 ± 5.58 | |
| Mean Weight (Kg) | 66.1± 11.7 | |
| Body mass index | | |
| ≥ 28 | 98 | 42.25 |
| < 28 | 134 | 57.75 |
| **Menopausal status** | | |
| Premenopausal | 133 | 57.3 |
| Post menopausal | 99 | 42.7 |
| **Stage** | | |
| 1 | 25 | 10.8 |
| II | 127 | 54.7 |
| III | 80 | 34.5 |
| **Marital status** | | |
| Married | 223 | 96.1 |
| Unmarried | 9 | 3.9 |

SD: Standard Deviation.

**Table-2:** Master mix for each assay.

| PCR Reagents | Stock Conc. | Final Conc. | Vol. in uL |
|---|---|---|---|
| PCR buffer | 10X | 1X | 2.5 |
| MgCl2 | 25mM | 1.25mM | 1 |
| dNTPs | 2mM | 0.1mM | 1 |
| Forward Primer (SNP) | 100mM | 1nM | 1 |
| Reverse Primer (SNP) | 100mM | 1nM | 1 |
| Taq DNA Polymerase | 5U/µL | 0.05U | 0.2 |
| Template DNA | 20ng | 3ng | 1.5 |
| Adjust vol. with dH2O | Upto 25 µl- | Upto 25 µl- | 16.8 |
| Final Vol. | 25 | 25 | 25 |

PCR: Polymerase chain reaction
dNTPs: Deoxynucleotides
SNP: Single nucleotide polymorphism
DNA: Deoxyribonucleic acid
dH2O: Distilled water




**Homozygous wild type CC allele (263, 62bp); heterozygous CT (263bp, 183bp, 80bp, 62bp)**
**PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism.**
**CYP2D6: Cytochrome P450 2D6.**

**Figure:** Electrophoresis patterns for CYP2D6*10 (100C>T) alleles analysed by PCR-RFLP.

**Table-3:** Comparison of CYP2D6*10 Allele in Pakistani Population with Major Ethnic Groups.

| Countries | Allele*10 frequency % |
|---|---|
| Pakistani ( n=232) | 07 |
| South Indians (n=447)19 | 10.2 |
| Japanese (n=162)8 | 38.6* |
| Chinese (n=223)20 | 51.6* |
| Koreans (n=400)9 | 45* |
| Malaysian (n=95)21 | 48.9* |
| Thai (n= 67)22 | 51* |
| Caucasian (n=204)5 | 2.0* |
| African Americans (n=191)5 | 3.9 |

CYP2D6: Cytochrome P450 2D6
n= No of subjects, * p< 0.05 compared with the Pakistani population.

Amongst the three genotypes group, none of the patients was CYP2D6 homozygous variant genotype (CYP2D6*10/*10), 33(14.2%) were CYP2D6 heterozygous genotype (CYP2D6*1/*10) and 199(85.8%) were CYP2D6 homozygous wild type genotype (CYP2D6*1/*1). The allele frequency of CYP2D6*1 was 431(93%) and CYP2D6*10 was 33(7%) (Table-2).

In contrast, the allele frequency of CYP2D6*10 was 447(10.2%) among South Indians, 162(38.6%) among Japanese, 233(51.6%) among Chinese, 400(45%) among Koreans, 95(48.9%) among Malaysians, 67(51%) among Thais, 204(2%) among Caucasians and 191(3.9%) among African Americans (Table-3). Allelic frequencies of CYP2D6 genotypes were in HWE (p=0.2435) (Table-4).

Therefore, we accepted the null hypothesis that the observed and expected values were not significantly different, and that our population was indeed in Hardy-Weinberg equilibrium.

## Discussion

In oncology, the use of pharmacogenomic indicator is critical because of the usually narrow therapeutic index

**Table-4:** Frequency of CYP2D6*10 genotypes.

| | 232 patients X 2= 464 Alleles (N) | | | Observed | | |
|---|---|---|---|---|---|---|
| Alleles | N= 464 | % | Genotypes | N= 232 | % | (HWE) |
| *1 | 431 | 93 | *1 / *1 | 199 | 85.7 | 200 |
| *10 | 33 | 07 | *1 / *10 | 33 | 14.3 | 31 |
| | | | *10 / *10 | 0 | 0 | 1 |

Chi-square =1.36; p = 0.2435
HWE: Hardy-Weinberg Equilibrium
CYP2D6: Cytochrome P450 2D6.

of available drugs, the significant requirement for a positive drug response and the potentially life-threatening cost of drug toxicity.[14] Almost a quarter or more of the drugs available in the market are metabolised by the CYP2D6 gene.[15] The burden of the disparity in the metabolism of tamoxifen, which has been the regular treatment for ER+ve breast cancer for more than three decades, is now placed on genetic polymorphisms of the CYP2D6 gene.[16,17] N-desmethyl tamoxifen, the main metabolite found in the serum of treated patients, undergoes secondary metabolism to 4-hydroxy-N-desmethyl tamoxifen (endoxifen).[18] Variety in the CYP2D6 gene is high amongst diverse populations and amid individuals in the same population.[5] Asians have a high incidence of the intermediate metaboliser (IM) allele, CYP2D6*10, which encodes an enzyme with reduced activity.[19] According to some research studies carried out among Asians, patients who were homozygous for *10 alleles are associated with a poorer survival outcomes.[20,21] Genetic polymorphisms of CYP2D6 are known to be quite different among ethnic groups. The CYP2D6*10 allele frequency determined in this study is compared to the previously established frequencies in other ethnic populations. The allele frequency of CYP2D6*10 in volunteers from our study was 7%, which is less than that in South Indians[22] (10.2 %), but is comparable. The frequency of CYP2D6*10 allele in our study subjects is significantly less than the Chinese[23] as well as population from the Far Eastern countries of Malaysia,[12] Thailand[11] and Korea.[9] The Caucasians and African Americans[5] have CYP2D6*10 allele frequencies ranging from 2% to 4%.

The current study is the first major study reporting the CYP2D6 genotype distribution among Pakistanis, and presented an outline for further studies regarding the role of polymorphic substitutes of CYP2D6 gene in the origin of various diseases or designing future pharmacogenetic and pharmacokinetic research studies conducted with the CYP2D6 substrates in the Pakistani population. It will allow increasing potency of the studies together with reduced economic costs and ethical risks for the participating individuals. Genotype data presented here may be used for comparisons between different populations as a reference for understanding CYP2D6 enzyme activity in this population, and may be used in disease association studies.

## Conclusion

The frequency of CYP2D6*10 allele among Pakistani breast cancer women was comparable to the south Indians, but was significantly lower as compared to other populations, including those of China, Malaysia, Thailand and Korea.

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**Conflict of Interest:** None.

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## References

1. Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst. 2005; 97:1652-62.
2. Colleoni M, Gelber S, Goldhirsch A, Aebi S, Castiglione-Gertsch M, Price KN. et al Tamoxifen after adjuvant chemotherapy for premenopausal women with lymph node-positive breast cancer: Int Breast Cancer Study Group Trial 13-93. J Clin Oncol. 2006; 24:1332-41.
3. Osborne CK. Tamoxifen in the treatment of breast cancer. N Engl J Med. 1998; 339:1609-18.
4. Ingelman-Sundberg M. Genetic polymorphisms of Cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 2005; 5:6-13.
5. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics J. 2002; 3:229-43.
6. Gaston C, Kolesat J.Clinical significance of CYP2D6 polymorphisms and Tamoxifen in women with breast cancer. Clin Adv Hematol Oncol. 2008; 6:825-33.
7. Goetz MP, Kamal A, Ames MM. Tamoxifen pharmacogenomics; the role of CYP2D6 as a predictor of drug response. Clin Phamacol Ther. 2008; 83:160-6.
8. Kubota T, Yamaura Y, Ohkawa N, Hara H, Chiba K. Frequencies of CYP2D6 mutant alleles in a normal Japanese population and metabolic activity of dextromethorphan O-demethylation in different CYP2D6 genotypes. Br J Clin Pharmacol. 2000; 50:31-4.
9. Lee SY, Sohn KM, Ryu JY, Yoon YR, Shin JG, Kim JW, et al. Sequence-based CYP2D6 Genotyping in the Korean Population. Ther Drug Monit. 2006; 28:382-7.
10. World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013; 310:2191-4.
11. Rungwanonchai P, Na Ayudhya DP, Areepium N, Voravud N, Satthaporn S. Prevalence of CYP2D6*10 Genotype in Thai Breast Cancer Patients. R Thai Army Med J. 2012; 65:113-7.
12. Teh LK, Mohamed NI, Salleh MZ, Rohaizak M.Shahru NS, Saladina JJ, et al. The risk of recurrence in breast cancer patients treated with tamoxifen: polymorphisms of CYP2D6 and ABCB1. AAPS J. 2012; 14:52-9.
13. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual 2d ed. New york: Cold Spring Harbor Laboratory Press, 1989.
14. O'Donnell PH, Ratain MJ. Germline pharmacogenomics in

oncology:decoding the patient for targeting therapy. Mol Oncol. 2012;6: 251-9.
15. Bernard S, Neville KA, Nguyen AT, Flockhart DA. Interethnic differences igenetic polymorphism of CYP2D6 in the U.S. population: clinical implications. Oncologist. 2006; 11:126-35.
16. Teh LK, Bertilsson L. Pharmacogenomics of CYP2D6: Molecular Genetics, interethnic differences and clinical importance. Drug Metab Pharmacokinet. 2012; 27:55-67.
17. Kelly CM, Pritchard KI. CYP2D6 genotype as a marker for benefit of adjuvant tamoxifen in postmenopausal women: lessons learned. J Natl Cancer Inst. 2012; 104:427-8.
18. Abraham JE, Maranian MJ, Driver KE, Platte R, Kalmyrzaev B, Baynes C, et al. CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. Breast Cancer Res. 2010; 12:R64.
19. Ramamoorthy Y, Tyndale RF, Sellers EM. Cytochrome P450 2D6.1and cytochrome P450 2D6*10 differs in catalytic activity for multiple substrates. Pharmacogenetics and Genomics. 2001; 11:477-87.
20. Xu Y, Sun Y, Yao L, Shi L, Wu Y, Ouyang T, et al.Association between CYP2D6 *10 genotype and survival of breast cancer patients receiving tamoxifen treatment. Ann Oncol. 2008; 19:1423-9.
21. Kiyotani K, Mushiroda T, Imamura CK, Hosono N, Tsunoda T, Kubo M, et al. Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. J Clin Oncol. 2010; 28:1287-93.
22. Naveen AT, Adithan C, Soya SS, Gerard N, Krishnamoorthy R. CYP2D6 genetic polymorphism in South Indian populations. Biol Pharm Bull. 2006; 29:1655-8.
23. Ji, L, Pan S, Marti-Jaun, J Hanseler E ,Rentsch K, Hersberger M. Single-Step Assays to Analyze CYP2D6 Gene Polymorphisms in Asians: Allele Frequencies and a Novel *14B Allele in Mainland Chinese. Clin Chem. 2002; 48:983-8.