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CYP2D6*4 polymorphisms and breast cancer risk

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Abstract

CYP2D6 gene plays an important role in detoxification of many drugs and plays a crucial role in the metabolism of tamoxifen, used in endocrine therapy. In the present study, the heterozygous frequency IM (40.8%) was significantly increased in breast cancer patients when compared to controls. When the data on IM and PM were pooled, significant increase in the frequency of pooled genotype in disease group (42.4%) was observed as compared to controls (27.6%). The frequency of IM genotype was found to be increased in women with premenopausal breast cancer patients (43.5%) and patients with familial history of cancer (43.2%). Higher frequency of IM as well as pooled genotypes PM+IM was found in cases with higher BMI and in patients occupied in agriculture (55.6%), patients positive for estrogen receptor (47.8%), progesterone receptor (44.8%), HER2/neu (26.9%) and advanced stage. Our results suggested that the CYP2D6*4 polymorphism plays an important role in the breast cancer etiology and might help in planning hormonal therapy where tamoxifen is used.

Keywords: CYP2D6*4; Polymorphisms; Tamoxifen; Detoxification; Hormonal therapy.

Introduction

CYP2D6 (Debrisoquine hydroxylase) is one of the most thoroughly studied enzymes, since lack of its activity is the basis for adverse events occurring during therapy with some drugs (Eichelbaum et al., 1990). This enzyme has a wide range of activity within human populations, with interindividual variation in the rates of metabolism differing more than 10,000-folds (Nebert, 1997). Most individuals are able to metabolize CYP2D6 substrates extensively (EM). About 5-10% of Caucasians have inactivating mutations in both alleles of the CYP2D6 gene that are termed as poor metabolisers (PM).

CYP2D6 gene is located on chromosome 22q13.1.The most frequent inactivating mutation among Caucasians is the splice site G1934A transition (CYP2D6*4 allele) that cause a truncated protein. G to A transition at the intron3/exon4 boundary of the CYP2D6 gene leads to incorrect splicing of mRNA resulting in a frame shift and premature termination .The G to A transition had been identified as a primary defect at the CYP2D6 locus and is estimated to account for 80-90% of mutant alleles in PM (Gough et al., 1990).

The biotransformation of tamoxifen which is used for the treatment of endocrine related cancer like breast, ovary etc is mediated by CYP2D6 enzymes mainly through demethylation and hydroxylation to form several primary metabolites, principally 4 OH-tamoxifen, α-OH-tamoxifen, N-desmethyl-tamoxifen and 4-OH-N-desmethyl-tamoxifen.4-OH- tamoxifen is considered to be more potent anti-estrogen than the parent substance and is capable of binding the ER with greater affinity (Jin et al., 2005).

CYP2D6 polymorphism can be classified according to one of four levels of activity: poor metabolisers (PMs), intermediate metabolisers (IMs), extensive metabolisers (EMs) and ultra rapid metabolisers (UMs) (Jann et al., 2001). The EM phenotype is expressed by the majority of the population and is therefore considered as normal. PMs inherit two deficient CYP2D6 alleles; as a result, they metabolize drugs at a slower rate. This leads to an accumulation of high levels of unmetabolized drugs. The UM phenotype is caused by the duplication or amplication of active CYP2D6. Individuals with UM genotype will metabolize drugs at an ultra rapid rate, which might lead to a loss of therapeutic efficacy at standard doses. Individuals who were heterozygous for a defective CYP2D6 allele often demonstrate an IM phenotype. This phenotype had a wide spectrum of metabolic activity that can range from marginally better than the PM phenotype, to activity that is close to the EM phenotype (Meyer, 2004).

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CYP2D6 polymorphism had been linked to susceptibility to various diseases, including certain cancers, early onset Parkinson's disease, systemic lupus erthematosus, pituitary ademonas, Balkon nephropathy and ankylosing spondylitis (Lennard, 1990; Mayer et al., 1990). Metabolic activation of a procarcinogens might proceed via CYP2D6 which implies that a patient of extensive metaboliser phenotype forms higher amounts of the active compounds and therefore at a higher risk to develop cancer (Kroemer et al., 1995). The CYP2D6 gene is responsible for the metabolism of known human carcinogens, including nitrosamines and possibly, nicotine. In addition, it was suggested that there might be endogenous substrates for CYP2D6, including tryptamine, a well-known neuroactive amines (Kelsey et al., 1997). However, the influence of CYP2D6 allelic variants in different types of cancer remained controversial. While some studies suggested a role for CYP2D6 in the development of cancer, other studies could not support this (Christensen et al., 1997; Wolf et al., 1994).

CYP2D6 enzyme metabolizes 25% of all medicines, including cytotoxic drugs, tamoxifen and other drugs used in cancer supportive care. Variation in CYP2D6 expression play role in drug-drug interaction, an important implication in oncology practice, since patients are prescribed a number of drugs for symptomatic treatment. Therefore, the study of CYP2D6 polymorphism in breast cancer might throw light on the importance of the gene in the onset and progression of breast cancer.

Materials and Methods

A group of 250 breast cancer patients were selected for study. 250 healthy and age matched women without family history of breast cancer or any other cancers were selected to serve as control group. Cases were chosen from Nizam's Institute of Medical Sciences after confirmed diagnosis. The diagnosis of breast cancer was established by pathological examination, mammography, Fine needle aspiration (FNAC) and biopsy. Epidemiological history such as age at onset of breast caner, diet, socioeconomic status, occupation, reproductive history, family history and consanguinity were taken through personal interview with breast cancer patients using specific proforma. The patients were screened for receptor status of estrogen, progesterone and HER-2/neu by immunohistochemical assay. Clinical history such as size of the tumor, presence of auxiliary nodes, extent of metastasis, stage and type of the breast cancer, chemotherapeutic drugs used and prognosis of the disease was collected with the help of oncologist. Informed consent was taken from all patients and controls included in the study. Five milliliters of blood was collected in an EDTA vaccutainer from patients as well as controls. DNA was isolated (Nuremberg and Lahari, 1991) and used for amplification of CYP2D6*4 gene. PCR-RFLP was done for identification of CYP2D6*4 polymorphism using specific primers (Lemos et al., 1999). The amplified product was digested with 1 unit of BstN1 enzyme (Fermentars) at 37^0C for overnight and electrophoresed on 2% agorose gel. The PM genotype is indicated by the presence of 334 bp fragments, HEM genotype by 334, 230, 104 bp and EM genotype by 230, 104 bp fragments.

Statistical analysis

The results were analyzed using appropriate statistical tests by SPSS Version 14. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. Differences in genotype frequency distribution between disease and control groups were done using 2* $2\chi 2$ and $\chi 2$ test for heterogeneity.

Results and Discussion

In the present study, the heterozygous frequency IM (40.8%) was significantly increased when compared to controls (Table 1). Both disease and controls were deviated from the Hardy-Weinberg equilibrium. Significant elevation in heterozygous frequency in breast cancer indicate heterozygous disadvantage. In general, heterozygous genotypes seem to confer growth-promoting ability and hence favored in disease group. When the data on IM and PM were pooled, significant increase in the frequency of pooled group in disease group (42.4%) was observed as compared to controls (27.6%). Previous studies reported that the frequency of IMs among the melanoma and bladder cancer patients was significantly higher than in controls. Although slight reduction in the proportion of PMs was observed in the teratoma and breast cancer cohorts, there was no significant variation from normal distribution of genotypes and mutant allele frequency in these two cancer groups. The strong association observed between PM genotype and leukemia could be better explained by linkage between

CYP2D6 and a gene involved in the pathogenesis of this disease. It is already known that the C-Sis (Platelet derived growth factor B) protooncogene is located on chromosome 22 (Smith et al., 1992). A slight increase of IM genotype and strong association of PM genotype with drug resistance in cases with CML were observed when compared to controls (Sailaja et al., 2007).

Table 1: CYP2D6 polymorphism and breast cancer.

	PM		IM		EM		Total	Allele Frequency	
	N	%	n	%	n	%		p	q
Disease	4	1.6	102	40.8	144	57.6	250	0.22	0.78
Control	12	4.8	57	22.8	181	72.4	250	0.16	0.84
Total	16		159		325		500		
χ2: 20.94(P=0.00003)*									
Hardy Weinberg disease χ2=8.91* Control χ2=6.42*									
OR (CI 95 %)PM vs IM:0. 1863 (0.0574-0.6045) IM vs EM: 2.2493(1.5212-3.3458) PM vs EM: 0. 419(0.1323-1.3267)									

Goetz et al. (2005) reported that in tamoxifen-treated breast cancer patients, women with CYP2D6 PM genotype tend to have higher risk to disease relapse. As endoxifen is mainly formed by the action of CYP2D6, patients with defective alleles would obtain less benefit from tamoxifen therapy than those carrying functional copies of CYP2D6 gene. The present data lacks data on tamoxifen since quite a number of patients discontinued the drug during course of the study. In general, CYP2D6 is not inducible by drugs. However, it is subjected to inhibition. Some drugs such as Paroxetine inhibit CYP2D6 so strongly that up to 80% of EMs is converted to PMs i.e. markedly reducing ability to metabolize CYP2D6 substrates.

The frequency of IM genotype was found to be increased in women with premenopausal breast cancer patients (43.5%) when compared to postmenopausal cases (38.1%) indicating the risk for early onset of cancer due to accumulation of environmental carcinogens as well as drug toxicity. Equal proportion of pooled genotypes (PM and IM) was observed in both the groups. The frequency of IM genotype was found to be slightly increased in patients with familial history of cancer (43.2%), which suggested the influence of CYP2D6 gene on other familial cancer susceptibility genes. The combined frequencies of PM and IM also showed increased tendency in cases with familial incidence (Table 2, 3).

Table 2: CYP2D6 polymorphism and menopausal status.

	PM		IM		EM		Total	Allele Frequency	
	n	%	N	%	n	%		p	q
Premenopausal	1	0.8	54	43.5	69	55.6	124	0.23	0.77
Postmenopausal	3	2.4	48	38.1	75	59.5	126	0.21	0.79
Total	4		102		144		250		
χ2: 1.587(P=0.45)									
OR (CI 95 %)PM vs IM:0. 2963 (0.0298-2.9446) IM vs EM: 1.2228(0.7358-2.0323) PM vs EM: 0. 3623(0.0368-3.5659)									

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Table 3: CYP2D6 polymorphism and familial incidence.

	PM		IM		EM		Total	Allele Frequency	
	n	%	N	%	n	%		p	q
Familial	2	2.7	32	43.2	40	54.0	74	0.24	0.76
Non-Familial	2	1.1	70	39.8	104	59.1	176	0.21	0.79
Total	4		102		144		250		
$\chi 2$: 1.182 (P=0.554)									
OR (CI 95 %)PM vs IM: 2.1875 (0.2948-16.2304) IM vs EM: 1.1886(0.6824-2.0703) PM vs EM:2.6(0.3541-19.0896)									

Higher frequency of IM as well as pooled genotypes PM+IM was found in cases with higher BMI and in patients occupied in agriculture (55.6%). The IMs cannot completely metabolize the environmental carcinogens present in pesticides and they accumulate in fatty tissue, which increase the risk of developing breast cancer (Table 4, 5).

Table 4: CYP2D6 polymorphism and BMI.

	PM		IM		EM		Total	Allele Frequency	
	n	%	n	%	n	%		p	q
<20	1	7.1	5	35.7	8	57.1	14	0.25	0.75
20-26.4	1	3.7	10	37.0	16	59.3	27	0.22	0.78
26.4-30	1	0.9	45	43.3	58	55.8	104	0.43	0.57
>30	1	2.2	12	26.7	32	71.1	45	0.16	0.84
Total	4		72		114		190		
X2: 6.235(P=0.39)									

Table 5: CYP2D6 polymorphism and occupation.

	PM		IM		EM		Total	Allele Frequency	
	n	%	N	%	n	%		p	q
Housewives	2	1.2	66	38.2	105	60.7	173	0.20	0.80
Agriculture	0	0	15	55.6	12	44.4	27	0.28	0.72
White-collar jobs	2	4.7	17	39.5	24	55.8	43	0.24	0.76
Others	0	0	4	57.1	3	42.9	7	0.29	0.71
Total	4		102		144		250		
$\chi 2$: 1.182(P=0.554)									

The frequency of IM genotype and pooled genotypes (PM and IM) were found to be increased in patients who were positive for estrogen receptor (47.8%), progesterone receptor (44.8%) and also HER2/neu (26.9%) status (Table 6, 7, 8). In general, the breast cancer patients who were positive for hormonal receptors status were advised to take hormonal therapy (tamoxifen), which is mainly metabolized by CYP2D6 enzyme. The IM individuals have a partial defect in the metabolism of this drug, which might cause resistance to tamoxifen in these individuals. It was reported that pooled genotypes of PM and IM was more strongly associated with disease recurrence than EM genotype (Gonzalez-Santiago et al., 2007; Monique et al., 2009; Jean et al., 2010).

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Table 6: CYP2D6 polymorphism and estrogen receptor status.

	PM		IM		EM		Total	Allele Frequency	
	N	%	N	%	n	%		p	q
Positive	2	2.22	43	47.8	45	50.0	90	0.26	0.74
Negative	2	2.0	28	28.6	68	69.4	98	0.16	0.84
Total	4		71		113		188		
X2: 7.524(P=0.023)									
OR (CI 95 %)PM vs IM:0. 6512 (0.0866-4.8941) IM vs EM: 2.3206(1.2647-4.2579) PM vs EM: 1.5111(0.2054-11.1195)									

Table 7: CYP2D6 polymorphism and progesterone receptor status.

	PM		IM		EM		Total	Allele Frequency	
	N	%	N	%	n	%		p	Q
Positive	3	3.4	39	44.8	45	51.7	87	0.26	0.74
Negative	1	0.9	32	31.7	68	67.3	101	0.17	0.83
Total	4		71		113		188		
X2: 5.359(P=0.068)									
OR (CI 95 %)PM vs IM: 2.4615 (0.2441-24.8228) IM vs EM: 1.8417(1.0104-3.357) PM vs EM: 4.5333(0.4571-44.9608)									

Table 8: CYP2D6 polymorphism and HER2/neu.

	PM		IM		EM		Total	Allele Frequency	
	N	%	N	%	n	%		p	Q
Positive	0	0	7	26.9	19	73.1	26	0.13	0.87
Negative	3	11.1	4	14.8	20	74.1	27	0.19	0.81
Total	3		11		39		53		
X2: 3.826(P=0.148)									
OR (CI 95 %) IM vs EM: 1.8421 (0.4636-7.3197)									

When the stage of the disease was observed, the frequency of IM as well as pooled genotypes PM + IM genotype was increased in patients with advanced stage of the disease. In general, the patients were treated with chemotherapeutic drugs, the IM or PM genotype individuals cannot metabolize the drugs properly which leads to drug resistance and further develops aggressive form of the disease.

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Table 9: CYP2D6 polymorphism and stage.

	PM		IM		EM		Total	Allele Frequency	
	n	%	n	%	n	%		p	Q
I	0	0	3	27.3	8	72.7	11	0.14	0.86
II	3	3.1	38	39.6	55	57.3	96	0.23	0.77
III	1	1.4	34	46.6	38	52.1	73	0.25	0.75
IV	0	0	18	37.5	30	62.5	48	0.18	0.81
Total	4		93		131		228		
X2: 4.408(P=0.62)									

The increased trend was observed in the frequency of IM as well as in the combined frequency of IM and PM indicates that reduced or compromised CYP2D6 function might predispose to breast cancer due to inefficient metabolism of toxic compounds and drugs.

Conclusion

Our results suggest that the CYP2D6*4 polymorphism plays an important role in breast cancer etiology as well as in determining the hormonal therapy where tamoxifen is used. This study lacked perfect medical history, therefore, the effect of CYP2D6 inhibitors on outcomes of tamoxifen could not be assessed.

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References

Christensen, Gotzsche PC, Brosen K, 1997. The sparteine/debrisoquine (CYP2D6) oxidation polymorphism and the risk of lung cancer: a meta-analysis. European Journal of Clinical Pharmacology, 51: 389-93.

Eichelbaum M, Gross AS, 1990. The genetic polymorphism of debrisoquine/spartein metabolism-clinical aspects. Pharmacology and Therapeutics, 46: 377-394.

Goetz MP, Rae JM, Suman, 2004. Pharmacogenomic determinants of outcome with tamoxifen therapy: findings from the randomized North Central Cancer Treatment Group adjuvant breast cancer trial 89-30-52. Breast Cancer Research and Treatment, 88: S35.

Gonzalez-Santiago S, Zarate R, Haba-Rodrigues J, Gomez A, Bandres E, Moreno S, Borrege P, Garcia-Foncillas Aranda, E, 2007. CYP2D6*4 polymorphism as blood predictive biomarkers of breast cancer relapse in patients receiving adjuvant tamoxifen. Journal of Clinical Oncology, 25(18): 590.

Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR, 1990. Identification of the primary gene defect at the cytochrome P450 CYP2D locus. Nature, 347: 773-776.

Jann MW, Cohen LJ, 2001. The influence of ethnicity and antidepressant pharmacogenetics in the treatment of depression. Drug Metabolism and Drug Interaction, 16: 39-67.

Jean EA, Mel JM, Kristy ED, Radka P, Bolot K, Caroline B, Craig L, Mitul S, Susan I, David G, Helena ME, Alison MD,Paul DP, Carlos C, 2010. 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 Research, 12: R64.

Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, Skaar T, Storniolo AM, Li L, Araba A, Blanchard R, Nguyen A, Ullmer L, Hayden J, Lemler S, 2005. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. Journal of National Cancer Institute, 97: 30-39.

Kelsey KT, Wrensch M, Zuo ZF, Miike R, Wiencke JK, 1997. A population based study of the CYP2D6 and GSTT1 polymorphisms and malignant brain tumors. Pharmacogenetics, 7: 463-8.

Kroemer HK, Eichelbaum M, 1995. It's the genes, stupid? Molecular basis and clinical consequences of genetic cytochrome P4502D6 polymorphism. Life Sciences, 56: 2285-98.

Lemos MC, Cabrita, Silva HA, Vivan M, Regateiro P, 1999. Genetic polymorphism of CYP2D6, GSTM1 and

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NAT2 and susceptibility to hematological neoplasias. Carcinogenesis, 20(7): 1225-1229.

Lennard MS, 1990. Genetic polymorphism of sparteine/debrisoquine oxidation: A reappraisal. Pharmacology and Toxicology, 67: 273-83.

Mayer UA, Skoda RC, Zanger UM, 1990. The genetic polymorphism of debrisoquine/sparteine metabolism - molecular mechanisms. Pharmacology and Therapeutics, 46: 297-308.

Mayer UA, 2004. Pharmacogenetics - five decades of therapeutic lessons from genetic diversity. Nature Reviews Genetics, 5: 669-676.

Monique JB, Ron HN, van Schaik, Laureen A, Lammers, Hofman A, Arnold GV, Teun van G, Bruno H, Stricker CH, Loes EV, 2009. The CYP2D6*4 polymorphism affects breast cancer survival in tamoxifen users. Breast Cancer Research and Treatment, 118: 122-130.

Nebert DW, 1997. Polymorphism in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? American Journal of Human Genetics, 60: 265-271.

Lahiri DK, Numberger JI, 1991. A rapid non-enzymatic method for the preparation of HMW from blood RFLP studies. Nucleic Acids Research, 19: 5444.

Sailaja K, Vishnupriya S, Surekha D, Nageswara Rao D, Raghunadha Rao D, 2007. Association of CYP2D6*4 polymorphism with chronic myeloid leukemia. Journal of Medical Science Research, 1(1).

Smith CA, Moss E, Gough C, Spurr K, Wolf C, 1992. Molecular genetic analysis of the cytochrome P450-Debrisoquine hydroxylase locus and association with cancer susceptibility. Environmental Health Perspectives, 98: 107-112.

Wolf CR, Smith CAD, Forman D, 1994. Metabolic polymorphisms in carcinogen metabolizing enzymes and cancer susceptibility. British Medical Bulletin, 50: 718-31.

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