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Breast cancer: Role of pharmacogenetics in tamoxifen therapy

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ABSTRACT

The role of pharmacogenetics in the personalization of tamoxifen therapy has relevance in the management of breast cancer. Since tamoxifen is a pro-drug, genetic polymorphism in Phase I and II drug metabolizing enzymes involved in the bioconversion of tamoxifen to therapeutically active metabolites is critical in determining therapeutic efficacy and adverse drug reactions of the therapy in breast cancer patients. In this review, the role of pharmacogenetics in the personalization of tamoxifen therapy has been discussed. Since, metabolism of tamoxifen by cytochrome P450 2D6 is significant in determining the therapeutic efficacy of the drug, most of the clinical evidence on tamoxifen pharmacogenetics have been correlated with cytochrome p450 2D6 genetic polymorphism. However, there is discordance in the clinical data, and one of the reasons is the incomplete analysis of all the alleles of cytochrome p450 2D6. International Tamoxifen Pharmacogenomics Consortium has been formed to assess the discordance. There is also clinical evidence associating genetic polymorphism in cytochrome P450 3A, 2C9, 2C19, Uridine diphosphate - glucuronosyltransferases (UGT) and sulfotransferases (SULT) with clinical outcome of tamoxifen therapy. However, associations of genetic polymorphism in cytochrome P450 3A, 2C9, 2C19, UGTs and SULT with clinical outcome in populations of different ethnicity are unexplored. Evidence on the association of genetic polymorphisms and the clinical outcome has been summarized. Since cost, statistically significant sample population size, labor, and ethical issues are the major concerns of a pharmacogenetic investigation; the significance of bottom-up approach in pharmacogenetics has been discussed.

Key words: Drug metabolism, Drug metabolizing enzymes, Genetic polymorphism, Pro-drug

Introduction

Cancer is one of the leading causes of death. It has a set back on the psychological and socio-economic status of the patient and family. The incidence and mortality rates of cancer are increasing steadily. It has been estimated that in 2012 8.2 million deaths were caused by cancer.[1] The most common cancers include lung, stomach, prostate, colorectal, liver, esophagus, breast, and cervix. Among them, breast cancer is one of the most common cancers in women and is the leading cause of death after lung cancer. The global estimates of incidence and mortality rates due to breast cancer have increased to 20% and 14%, respectively.[1] It has been observed that the death rate in developed nations is lower than less developed countries. However, the incidence of breast cancer is higher in developed countries than less developed nations and more common in young women than old women.[2]

The pathogenesis of breast cancer depends on genetic, environmental, and physiological factors. The formation of DNA adducts is the initial step in the development of cancer. Most of the DNA adducts forms by the activation of the endogenous and exogenous chemicals in the body. The activation and detoxification of the endogenous and exogenous chemicals in the body have been associated with the Phase I and II drug metabolizing enzymes. Most of Phase I and II drug metabolizing enzymes exhibit polymorphism and have altered function. Hence, polymorphisms in drug metabolizing enzymes may influence the frequency of the formation of DNA adducts. Polymorphisms in cytochrome P450 1A1, CYP 1B1, CYP 2A6, CYP 3A4, CYP 3A5, N-acetyltransferase 2, glutathione S-transferase Mu 1, catechol-O-methyltransferase, glutathione S-transferase, uridine diphosphate-glucuronosyltransferase (UGTs), N-acetyltransferase, and epoxide hydrolase have an important role in the clinical manifestation of breast cancer.[3] Besides DNA adducts, free radicals in the body may cause oxidative damage of DNA and significant mutations.

Breast cancer may be classified based on the status of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) into four major subtypes - (a) ER+ and/or PR+ and HER2−; (b) ER+ and/or PR+ and HER2+, or ER+ and/or PR+ and HER2− (luminal-B); (c) ER−, PR−, HER2+; (d) (ER−, PR−, HER2−).[2] The management of breast cancer may depend on the different sub-types.

With the development of sensitive screening techniques and better treatment approaches, the management of breast cancer has improved. Mammography, better screening, and treatment methods have decreased mortality caused by breast cancer. Radiotherapy, chemotherapy, and surgery have been effective in the management of breast cancer. Locoregional management

of breast cancer involving mastectomy has been a preferred approach. Besides, chemotherapeutic agents such as the five drug cooper regimen (1960), doxorubicin (1970), anthracycline-based combinations with cyclophosphamide in metastasis and as adjuvant therapy, combination of cyclophosphamide, adriamycin, and 5-fluorouracil, taxanes for systemic treatment (paclitaxel, docetaxel) anti-estrogen therapies such as tamoxifen, raloxifene, and aromatase inhibitors have shown effective therapeutic efficacy.[4]

Before the discovery and development of anti-estrogens such as tamoxifen, surgical procedures such as oophorectomy, hypophysectomy, or adrenalectomy were performed to reduce the secretion of estrogen and progression of cancer. With the discovery and development of tamoxifen, surgical procedures are less preferred in the management of ER positive breast cancer patients. Tamoxifen has reduced the recurrence of breast cancer and improved the overall survival in ER+ early-stage breast cancer patients. To determine the optimal duration of adjuvant tamoxifen clinical trial unit such as adjuvant tamoxifen longer against shorter (ATLAS) have been established under Clinical Trial Service Unit and Epidemiological Studies Unit. The objective of ATLAS is to establish the benefits between the longer (10 years) and shorter (5 years) duration of tamoxifen therapy in patients.[5]

The application of hybridoma technology to produce monoclonal antibodies as therapeutic agents has emerged as a significant medical intervention. Monoclonal antibodies such as trastuzumab, pertuzumab (anti HER2-HER3 dimerization antibody), and ado-trastuzumab emtansine (antibody drug conjugate) are the current approaches adopted to treat patients' positive for HER2.[4]

In the management of triple negative breast cancer, cetuximab, erlotinib, gefitinib, tyrosine-protein kinase kit (c-KIT), phosphatidylinositol 3-kinase - AKT imatinib, sunitinib, dasatinib, anthracyclines, and mitoxantrone are used. Besides, pharmacological inhibitors of the enzyme poly adenosine diphosphate-ribose polymerase (ParP) designed on the concept of synthetic lethality approach are potential therapeutic agents for the treatment of triple-negative breast cancer.[6]

However, there are several challenges in the clinical management of breast cancer. Some of these challenges include heterogeneous nature of the disease, complex pathogenesis (thus poor prognosis of triple-negative breast cancers) and stages of the breast cancer, diagnosis and therapeutic approaches in different sub-types of breast cancer, recurrence, resistance, menopause status of patient (premenopausal, postmenopausal), polypharmacy, demographic characteristics, drug-drug interactions, herb-drug interactions and socioeconomic factors. Thus, poor prognosis of cancer and mortality in patients is irresistible.

With the emergence of pharmacogenomics and pharmacogenetics, the prognosis of breast cancer is easy, and there is a decline in the death rate. The sequencing of human genome and development of robust, sensitive and high-throughput techniques have resolved the problem of inter-individual variability and adverse drug reactions of a drug. Thus, in the era of translational science, pharmacogenetics has emerged as the state-of-the-art diagnostics to rationalize the concept of personalized medicine successfully. The concept of personalized medicine is foreseen as a paradigm for a sustainable R and D and business model of pharmaceutical industry. In contrast to the notion of "one drug for all," personalized medicine emphasizes the concept of individualization of the drug therapy. Pharmacogenomics is the key component involved in the individualization of the drug therapy.

Pharmacogenetics of cancer therapy has great relevance. Most of the anticancer drugs are pro-drugs and are biotransformed to an active form mediated by enzymes. The genes encoding for the drug metabolizing enzymes exhibit polymorphism. Hence, the catalytic efficiency of enzymes involved in the catalysis of pro-drugs is variable in individuals. To understand the role of polymorphisms in catalysis of drugs, it is the first important to understand the enzyme activity for each genotype.[7] The existence of functional genetic polymorphism in the population is a major factor for inter-individual variation of a drug therapy. Pharmacogenetic studies on polymorphisms in drug metabolizing enzymes, drug transporters, and drug targets have shown correlations to changes in response and toxicity to commonly prescribed chemotherapeutic treatments of breast cancer. However, most of the pharmacogenetic evidence supports the role of polymorphisms in drug metabolizing enzymes. Genetic polymorphism in drug metabolizing enzymes involved in the metabolism of chemotherapeutic agents of breast cancer such as raloxifene, tamoxifen, 6-mercaptopurine, and irinotecan has been investigated.[8] Among them, tamoxifen pharmacogenetics has relevance in breast cancer treatment.

Pharmacogenetics of Tamoxifen

Tamoxifen is a nonsteroidal anti-estrogen and widely used in the management of premenopausal and postmenopausal metastatic breast cancer. Moreover, in early breast cancer, it is used as an adjuvant therapy and chemopreventive agent for high-risk women. It was approved in 1977 by US-Food and Drug Administration.[9] The trans isomer of tamoxifen is active and has antiestrogenic and estrogenic properties. Clinically, it has been found to be less toxic than other anticancer drugs. Therefore, it is used as the first line drug for postmastectomy adjuvant therapy of early breast cancer and in combination with other drugs for advanced stages. It is also utilized in the treatment of hormone-related ovarian and refractory prostate cancers.

Tamoxifen is a pro-drug and on its intake the pro-drug is principally metabolized by cytochrome P450 2D6, 3A, 2C9 and 2C19 of the liver to therapeutically active

metabolites.[10] Evidence on the metabolism of tamoxifen, support the role of cytochrome P450 2D6 as the major enzyme involved in the formation of the therapeutically active metabolites -4-hydroxytamoxifen and endoxifen.[11] However, cytochrome P450 3A, cytochrome P450 2C9, and cytochrome P450 2C19 also contribute in the metabolism of tamoxifen and endoxifen levels in plasma have been associated with clinical outcomes.[12] Both the metabolites 4-hydroxytamoxifen and endoxifen have a higher affinity for the ER than the parent drug. Besides, therapeutically potent metabolites toxic metabolite such as α-tamoxifen, sulfoxy metabolite of tamoxifen (catalyzed by hydoxysteroid sulfotransferases [SULT] 2A1) are also formed. Other Phase I drug metabolizing enzymes such as cytochrome P450 1A1, 1A2, 2B6, flavin-containing monooxygenases (FMO1 and FMO3) also have involvement in the metabolism of tamoxifen. Within the Phase II drug metabolizing enzyme group, UGTs (UGT 1A4, UGT 1A10, UGT 1A8, UGT 2B15, UGT 2B7, and UGT 1A8) and SULTs (hydoxysteroid SULT 2A1, SULT 1A1, SULT 1E1, SULT 1A2) are involved in the metabolism of tamoxifen.[13-15]

Most of Phase I and II drug metabolizing enzymes exhibit genetic polymorphism and it is one of the major reasons for the variation in the clinical outcome of tamoxifen therapy in breast cancer patients.[11] Based on the genotype, breast cancer patients exhibit poor (negligible), intermediate (reduced) and extensive (normal) metabolism of tamoxifen in populations of different ethnicity. Hence, there may be inter-individual variation in therapeutic efficacy and adverse drug reactions such as endometrial cancer and thromboembolic disorders in patients on tamoxifen therapy.[14] The clinical challenge has been investigated by pharmacogenetic studies conducted in populations of different ethnicity. Since cytochrome P450 2D6 is considered to be the principal catalyzing enzyme in tamoxifen metabolism, most of the pharmacogenetic studies explain the significance of cytochrome P450 2D6 polymorphism on tamoxifen therapy in patients with early and advanced stages of breast cancer.[13,16] Breast International Group 1-98 and arimidex, tamoxifen, alone or in combination are randomized clinical trials to assess the role of cytochrome P450 2D6 genotyping in the personalization of tamoxifen therapy.[17] From genotyping studies in populations of different ethnicity, CYP 2D6 extensive metabolizers are homozygous for *1, *2, *33, *35 alleles, poor metabolizers are homozygous for *3, *4, *5, *6, *7, *8, *11-*16, *18-*21, *38, *40, *42, 44, 56, 62 alleles and intermediate metabolizers may be homozygous for *9, *10, *17, *29, *36, *41, 59 alleles and heterozygous involving extensive/intermediate and extensive/poor metabolizer combination of alleles.[9,10]

So far, pharmacogenetic studies on variants of CYP 2D6 (*2, *3, *4, *5, *6, *7, *8, *9, *10, *17, *41), CYP 2C8 (*2, *3, *4), CYP 2C9 (*2, *3), 2C19 (*2, *17), CYP 3A4 (*1B, *3, *17, *22), CYP (*3), SULT1A1 (*2, copy number), SULT1A2 (*2, *3), UGT 1A4 (48Val), UGT1A8 (*3), UGT 2B7 (268Tyr, *2) and UGT 2B15 (*2, 523Lys) have been reported in populations of different ethnicity [Table 1].[11,13,15] The different polymorphisms exhibiting clinically significant outcome such as benefits, disease recurrence, breast cancer specific survival, drug-related hot flashes, and altered *in vitro* metabolism in comparison to wild type is shown in Table 1 (see supporting material). There is also evidence contrary to the hypothesis and role of cytochrome P450 2D6 genetic polymorphism in the clinical outcome of tamoxifen therapy [Table 1] (see supporting material). It has also been observed that there is discordance in the published observations between cytochrome P450 2D6 genetic polymorphism and the outcome of tamoxifen.[16] International Tamoxifen Pharmacogenomics Consortium has been formed to assess the discordance in the clinical data.[18] The discordance in the data may be a consequence of concomitant use of cytochrome P450 2D6 inhibitors, stereoisomers of endoxifen (active and nonactive forms), inaccurate assessment of endoxifen exposure incomplete analysis of all relevant cytochrome P450 2D6 alleles, disobeying Hardy-Weinberg Equilibrium for allele frequencies and potential contributions of variability in cytochrome P450 3A and cytochrome P450 2C9.[13,16,18]

Hence, there is no complete association of cytochrome P450 2D6 genetic polymorphism with interindividual variability of tamoxifen metabolism and clinical outcomes.[9,17,10] Besides, Cytochrome P450 2D6, 3A, 2C9 and 2C19 polymorphism of cytochrome P450 1A2, 2B6, FMOs UGTs and SULT may contribute to differences in tamoxifen plasma concentration in individuals.[14,16,19,20] Therefore, the study of the effect of genetic polymorphism of all the enzymes involved in the metabolism of tamoxifen such as cytochrome P450 2C9, 2D6, 2C19, P450 3A, 1A2, 2B6, FMOs, UGTs, and SULT may be useful in the personalization of tamoxifen therapy.

In general, drug therapy involves the interplay of multiple genes. The lack of genetic polymorphism data of different ethnicity and the gap between clinicians and scientists fail to rationalize the concept of personalized medicine. Therefore, a holistic approach overcoming the limitations of the present pharmacogenetic investigations is required.

Bottom-up Approach in Pharmacogenetics of Tamoxifen Therapy

Computational biology has facilitated a deep and a global understanding of the impact of genetic factors on the biology and management of diseases. In drug discovery and development, the "bottom-up approach" involving modeling and simulation are extensively used to study absorption, distribution, metabolism, and excretion of drugs. Hence, safety and efficacy of drugs may be predicted before administration of drugs in human beings. However, in the current pharmacogenetic paradigm, the implementation of "bottom-up" approach is in early stages.

Several clinical investigations have been performed to study the effect of the genetic polymorphism on tamoxifen therapy using

Table 1: Association of genetic polymorphism with clinical outcome and enzyme activity

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
CYP* 2D6*4 (PM†)	Caucasians/ Rotterdam study	Increased mortality (*4/*4)	(Bijl *et al.* 2009)	CYP 2D6*4	Association with Z-4-hydroxytamoxifen formation rate	(Coller *et al.* 2002)
	South East Health Care Sweden	Better disease free survival (*4/*4)	(Wegman *et al.* 2007)			
	NCCTG USA	Worse relapse free time and disease free survival. Low incidence of hot flashes (*4/*4)	(Goetz et al. 2005)			
	Stockholm Breast Cancer Group Sweden	Better distant recurrence free survival	(Wegman *et al.* 2005)			
	UK Cancer Genetics Center (White Caucasian Ashkenazi Jewish ethnicity)	Reduced overall survival in familial breast cancer patients (*4/*4)	(Newman *et al.* 2008)			
	Brazil	Reduced disease-free survival and worse tumor characteristics not observed	(Martins et al. 2014)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	Increased recurrence, shorter relapse free time and worst event free survival	(Schroth *et al.* 2007)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Worse distant relapse free survival	(Saladores *et al.* 2015)			
	United Kingdom	No association with breast cancer specific survival and overall survival higher risk of disease relapse	(Abraham *et al.* 2010) (Gonzalez-Santiago *et al.* 2007)			
	Daniel den Hoed cancer center for Erasmus MC University Hospital Netherland	Overall survival short	(Lammers *et al.* 2010)			
	Denmark	No association of recurrence with one functional or no functional allele	(Lash *et al.* 2011)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	Austrian Tumor of breast tissue: Incidence, Genetics, and Environmental Risk factors	Statistically insignificant progression free survival between genotypes of *4, shorter mean time to progression (patients on comedication)	(Stingl *et al.* 2010)			
	United Kingdom ATAC Clinical Trial	No association of genotype with recurrence in postmenopausal women	(Rae *et al.* 2012)			
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Regan *et al.* 2012)			

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Table 1: *Continued...*

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	NCCTG	Risk of breast cancer relapse	(Goetz *et al.* 2007)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
	Spain	No clinical outcome recorded	(Zafra-Ceres *et al.* 2013)			
	Germany and USA	Worse event free survival and disease free survival	(Schroth *et al.* 2009)			
	Arkansas Cancer Research Center, UAMS	No association with overall survival	(Nowell *et al.* 2005)			
CYP 2D6*2 EM‡	Korean	No significant difference between the EM and the IM group associated with recurrence free and overall survival	(Park *et al.* 2011)	CYP 2D6*2	Association of Z-4-hydroxytamoxifen formation rate	(Coller et al. 2002)
	United Kingdom ATAC clinical trial	No association of genotype with recurrence in postmenopausal women	(Rae *et al.* 2012)			
	Lombardi Comprehensive Cancer Center at Georgetown University Medical Center, Indiana University Medical Center, and the University of Michigan Comprehensive Cancer Center	Associated with tamoxifen discontinuation	(Rae *et al.* 2009)			
	Asian (Chinese, Malay, Indian)	No clinical association recorded	(Lim *et al.* 2011)			
	Spain	No clinical outcome recorded	(Zafra-Ceres *et al.* 2013)			
CYP2D6*3PM	UK Cancer Genetics Center (White Caucasian Ashkenazi Jewish ethnicity)	Reduced overall survival in familial breast cancer (*3/*3)	(Newman *et al.* 2008)			
	Korean	Poor recurrence-free and overall survival	(Park *et al.* 2011)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Worse distant relapse free survival	(Saladores *et al.* 2015)			
	Daniel den Hoed Cancer Center for Erasmus MC University Hospital Netherland	Overall survival short	(Lammers *et al.* 2010)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			

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Table 1: *Continued...*

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	Germany and USA	Worse event free survival and disease free survival	(Schroth *et al.* 2009)			
	United Kingdom ATAC clinical trial	No association of genotype with recurrence in postmenopausal women	(Rae *et al.* 2012)			
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Rae *et al.* 2012)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
CYP2D6*5PM	UK Cancer Genetics Center (White Caucasian Ashkenazi Jewish ethnicity)	Reduced overall survival in familial breast cancer (*5/*5)	(Newman *et al.* 2008)			
	Korean	Poor recurrence-free and overall survival	(Park *et al.* 2011)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	Increased recurrence , shorter relapse free time and worst event free survival	(Schroth *et al.* 2007)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Worse distant relapse free survival	(Saladores *et al.* 2015)			
	United Kingdom	No association with breast cancer specific survival and overall survival)	(Abraham *et al.* 2010)			
	Daniel den Hoed Cancer Center for Erasmus MC University Hospital, Netherland	Overall survival short	(Lammers *et al.* 2010)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	National Cancer Center, Korea	No association with clinical outcomes in early breast cancer patients	(Park *et al.* 2012)			
	Germany and USA	Worse event free survival and disease free survival	(Schroth *et al.* 2009)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
CYP2D6*6PM	Asians, Middle Eastern Arabs, Caucasian-UK	Worse distant relapse free survival	(Saladores *et al.* 2015)	CYP2D6*6	Association of Z-4-hydroxytamoxifen formation	(Coller *et al.* 2002)
	United Kingdom	Associated with decreased breast cancer specific survival	(Abraham *et al.* 2010)			

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Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	Daniel den Hoed Cancer Center for Erasmus MC University Hospital, Netherland	Overall survival short	(Lammers *et al.* 2010)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	United Kingdom ATAC Clinical Trial	No association of genotype with recurrence in postmenopausal women	(Rae *et al.* 2012)			
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Regan *et al.* 2012)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
CYP2D6*7PM	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Regan *et al.* 2012)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
CYP2D6*8	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
CYP2D6*9 IM[§]	Asians, Middle Eastern Arabs, Caucasian-UK	Low endoxifen formation and short distant relapse free survival	(Saladores *et al.* 2015)	CYP2D6*9	Association of Z-4-hydroxytamoxifen formation	(Coller *et al.* 2002)
	United Kingdom	No association with breast cancer specific survival and overall survival	(Abraham *et al.* 2010)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
	Spain	No clinical outcome recorded	(Zafra-Ceres *et al.* 2013)			
CYP2D6*10 (IM)	Japan	No association with clinical outcome	(Okishiro *et al.* 2009)	CYP2D6*10	Association of Z-4-hydroxytamoxifen formation ,	(Coller et al. 2002)
	Japan	No association with survival (*10/*10)	(Toyama *et al.* 2009)			

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Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	Brazil	Reduced disease-free survival and worse tumor characteristics not observed	(Martins *et al.* 2014)			
	Korean	No significant difference between the EM and the IM group associated with recurrence free and Overall survival	(Martins *et al.* 2014)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	Increased recurrence, shorter relapse free time and worst event free survival	(Schroth *et al.* 2007)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Low endoxifen formation and short distant relapse free survival	(Saladores *et al.* 2015)			
	United Kingdom	No association with breast cancer specific survival and overall survival	(Abraham *et al.* 2010)			
	Daniel den Hoed Cancer Center for Erasmus MC University Hospital, Netherland	Overall survival between extensive and intermediate metabolizers statistically insignificant	(Lammers *et al.* 2010)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	National Cancer Center, Korea	No association with clinical outcomes in early breast cancer patients	(Park *et al.* 2012)			
	Germany and USA	Worse event free survival and disease free survival (heterozygous extensive/inter mediate genotype)	(Schroth *et al.* 2009)			
	Malaysian (Malays, Chinese, Indians)	Risk of recurrence	(Teh *et al.* 2012)			
	China	Worse disease free survival (*10/*10)	(Xu *et al.* 2008)			
	United Kingdom ATAC Clinical Trial	No association of genotype with recurrence in postmenopausal women	(Rae *et al.* 2012)			
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Regan *et al.* 2012)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
	Spain	No clinical association recorded	(Zafra-Ceres *et al.* 2013)			
CYP2D6*17	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			

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Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Regan *et al.* 2012)			
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
	Spain	No clinical outcome recorded	(Zafra-Ceres *et al.* 2013)			
CYP2D6*41 (IM)	U.K. Cancer Genetics Center (White Caucasian Ashkenazi Jewish ethnicity)	No association with clinical outcome	(Newman *et al.* 2008)	CYP2D6*41	Association of Z-4-hydroxytamoxifen formation	(Coller *et al.* 2002)
	Korean	No significant difference between the EM and the IM group associated with recurrence free and Overall survival	(Park *et al.* 2011)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Low endoxifen formation and short distant relapse free survival	(Saladores *et al.* 2015)			
	United Kingdom	No association with breast cancer specific survival and overall survival	(Abraham *et al.* 2010)			
	Daniel den Hoed cancer center for Erasmus MC University Hospital, Netherland	Overall survival between extensive and intermediate metabolizers statistically insignificant	(Lammers *et al.* 2010)			
	The University of Texas MD Anderson Cancer	No significant association with risk of recurrence	(Morrow *et al.* 2012)			
	National Cancer Center, Korea	No association with clinical outcomes in early breast cancer patients	(Park *et al.* 2012)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	Increased recurrence, shorter relapse free time and worst event free survival	(Schroth *et al.* 2007)			
	Germany and USA	Worse event free survival and disease free survival (heterozygous extensive/inter mediate genotype)	(Schroth *et al.* 2009)			
	United Kingdom (ATAC) Clinical Trial	No association of genotype with recurrence in postmenopausal women	(Rae *et al.* 2012)			
	BIG I-trial 98	No association with breast cancer-free interval associated with increased risk of hot flushes	(Regan *et al.* 2012)			

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Table 1: *Continued...*

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	Caucasian (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
	Spain	No clinical outcome recorded	(Zafra-Ceres *et al.* 2013)			
CYP3A5*3	South East Health Care, Sweden	Increased risk of recurrence after 2 years of treatment and improvement in the recurrence free survival after 5 years of treatment	(Wegman *et al.* 2007)	CYP3A5*3	Associated with N-Demethyl tamoxifen formation rate	(Tseng *et al.* 2014)
	NCCTG USA	No association with relapse free time and disease-free survival (*3/*3)	(Goetz *et al.* 2005)		Associated with α-hydroxytamoxifen formation (α-OHT) no significant difference in CL_{int} values (*1/*1, *1/*3, and *3/*3)	(Mugundu *et al.* 2012)
	Asians, Middle Eastern Arabs, Caucasian-UK	No association with N-desmethyltamoxifen formation and Clinical outcome not reported	(Saladores *et al.* 2015)		Associated with N-Desmethyl tamoxifen CL_{int} for *3/*3 was 3-fold lower for *1/*1.	
	Lombardi Comprehensive Cancer Center at Georgetown University Medical Center, Indiana University Medical Center, and the University of Michigan Comprehensive Cancer Center	No association with discontinuation of tamoxifen therapy	(Rae *et al.* 2009)			
	Asian (Chinese, Malay, Indian)	No clinical outcome recorded	(Lim *et al.* 2011)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	No clinical association of genotype with relapse free time and event free survival	(Schroth *et al.* 2007)			
CYP 3A4*1B,*3, *17	Caucasian, (Spain)	No clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
*CYP3A4*22		Associated with endoxifen levels no clinical outcome recorded	(Teft *et al.* 2013)			
		Less likely associated with hot flash	(Baxter *et al.* 2014)			
CYP 2C8 *3,*4	Sweden	Associated with disease free survival	(Jernström *et al.* 2009)			
CYP 2C9*2,*3	Asians, Middle Eastern Arabs, Caucasian-UK	Decreased norendoxifen and (Z)-4-hydroxytamoxifen concentrations clinical outcome not reported	(Saladores *et al.* 2015)	CYP 2C9*2,*3	Decreased intrinsic clearance (Z-4-hydroxytamoxifen)	(Coller *et al.* 2002)

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Table 1: *Continued...*

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	No clinical association of genotype with relapse free time and event free survival	(Schroth *et al.* 2007)			
	Asian (Chinese, Malay, Indian)	Not associated with endoxifen levels no clinical outcome recorded	(Lim *et al.* 2011)			
	Sweden	Associated with disease free survival	(Jernström *et al.* 2009)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Decreased norendoxifen and (Z)-4-hydroxytamoxifen concentrations clinical outcome has not been reported	(Saladores *et al.* 2015)			
	Dutch	Longer time to treatment failure (heterozygotes and homozygotes)	(van Schaik *et al.* 2011)			
	Lombardi Comprehensive Cancer Center at Georgetown University Medical Center, Indiana University Medical Center, and the University of Michigan Comprehensive Cancer Center	No association with discontinuation of tamoxifen therapy	(Rae *et al.* 2009)			
	Asian (Chinese, Malay, Indian)	Not associated with endoxifen levels no clinical outcome recorded (*2,*3)	(Lim *et al.* 2011)			
	Spain	No influence on plasma levels of endoxifen No clinical outcome recorded	(Zafra-Ceres *et al.* 2013)			
	Japan	No association with clinical outcome (*2/*2,*2/*3, *3/*3)	(Zafra-Ceres *et al.* 2013)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	No clinical association of genotype with relapse free time and event free survival	(Schroth *et al.* 2007)			
CYP 2C19*3	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	No clinical association of genotype with relapse free time and event free survival	(Schroth *et al.* 2007)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Decreased norendoxifen and (Z)-4-hydroxytamoxifen concentrations clinical outcome has not been reported	(Saladores *et al.* 2015)			
	Robert-Bosch Hospital Breast Center, Stuttgart, Germany	Reduced breast cancer recurrences, prolonged relapse free time and event free survival	(Schroth *et al.* 2007)			
	Asians, Middle Eastern Arabs, Caucasian-UK	Decreased norendoxifen and (Z)-4-hydroxytamoxifen concentrations clinical outcome has not been reported	(Saladores *et al.* 2015)			

(*Contd...*)

Table 1: *Continued...*

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	Study centre/ population	Clinical outcome		Genetic polymorphism	Enzyme activity	
	Dutch	Associated with a longer disease-free interval (untreated patients)	(van Schaik *et al.* 2011)			
	Lombardi Comprehensive Cancer Center at Georgetown University Medical Center, Indiana University Medical Center, and the University of Michigan Comprehensive Cancer	No association with discontinuation of tamoxifen therapy	(Rae *et al.* 2009)			
	Asian (Chinese, Malay, Indian)	Not associated with endoxifen levels no clinical outcome recorded	(Lim *et al.* 2011)			
	NCCTG	No association with disease free survival	(Moyer *et al.* 2011)			
SULT? 1A1*2	South East Health Care, Sweden	No association between duration of tamoxifen therapy on recurrence free survival and genotype	(Wegman *et al.* 2007)			
	Stockholm Breast Cancer Group Sweden	No association between distant recurrence free survival and genotype	(Wegman *et al.* 2005)			
	Arkansas Cancer Research Center, UAMS	Increased risk of recurrence and poor survival	(Nowell *et al.* 2005)			
	Caucasian (Spain)	No significant difference in metabolite levels no clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
SULT 1A1 copy number	NCCTG	No association with disease free survival	(Moyer *et al.* 2011)			
SULT1A2*2,*3	Caucasian (Spain)	Higher plasma levels of 4-hydroxy-tamoxifen and endoxifen no clinical outcome recorded	(Fernández-Santander *et al.* 2013)			
UGT** 2B15*2	South East Health Care Sweden	No association between duration of tamoxifen therapy on recurrence free survival and genotype	(Wegman *et al.* 2007)			
	Dutch	Associated with disesase free survival (UGT 2B15*2 and estrogen receptor-polymorphism ESR1 PvuII)	(Dezentjé *et al.* 2014)			
	Danish Breast Cancer Cooperative Group	No association with breast cancer recurrence	(Ahern *et al.* 2011)			
	Arkansas Cancer Research Center, UAMS	Increased risk of recurrence and poor survival	(Nowell *et al.* 2005)			
UGT2B15^{523Lys}, UGT1A4^{48Val} UGT2B7^{268Tyr}	Spain	Indicator of effective plasma active tamoxifen metabolite levels no clinical outcome recorded	(Romero-Lorca *et al.* 20 15)	wild type UGT1A4$^{24Pro/48Leu}$ variant UGT1A4$^{24Pro/48Val}$-	Associated with higher rate of formation of 4-hydroxy tamoxifen than wild type	(Sun *et al.* 2006)

(*Contd...*)

Table 1: *Continued...*

Clinical			Reference	*In vitro* drug metabolism		Reference
Genetic polymorphism	**Study centre/ population**	**Clinical outcome**		**Genetic polymorphism**	**Enzyme activity**	
UGT 2B7*2	Danish Breast Cancer Cooperative Group	No association with breast cancer recurrence	(Ahern *et al.* 2011)		Associated with higher *N*-glucuronidation activity in UGT overexpressing cell lines of variant	(Lazarus *et al.* 2009)
UGT1A8*3	Danish Breast Cancer Cooperative Group	No association with breast cancer recurrence	(Ahern *et al.* 2011)	UGT2B7 His268Tyr, Tyr268Tyr, His268His variants	No *N*-glucuronidation activity in human liver microsomes	(Lazarus *et al.* 2009)
				Wild type UGT2B7268His variant UGT2B7268Tyr	Associated with higher O-glucuronidation activity in wild type UGT overexpressing cell lines	
				UGT2B7 (His268Tyr)	Associated with decrease in O-glucuronidation activity in human liver microsomes	
				UGT2B7 (Tyr268Tyr)	Decrease in O-glucuronidation in human liver microsomes	(Lazarus *et al*. 2009) (Blevins-Primeau *et al*. 2009)
				UGT1A10139Lys UGT1A8173Gly/277Cys UGT1A10139Lys	No difference in O-glucuronidation activity compared to wild type in UGT overexpressing cell lines	(Blevins-Primeau *et al*. 2009)
				UGT1A8173Ala/277Tyr	No glucuronidation	

BIG: Breast International Group, UAMS: University of Arkansas for Medical Sciences, NCCTG: North Central Cancer Treatment Group, ATAC: Arimidex (generic name anastrozole) Tamoxifen, alone or in combination, †PM: Poor metabolizers, *CYP: Cytochrome P450, ‡EM, Extensive metabolizers, §IM: Intermediate metabolizer, ?SULT: Sulfotransferases, **UGT: Uridine diphosphate glucuronosyltransferases

"top-down" approach. However, there are limitations of the "top-down" approach in establishing the correlation of genotype with phenotype and genotype with clinical observations in preliminary investigations. Some of the major limitations are inconsistent and bias data, cost, statistically insignificant sample population size to correlate with possible clinical outcomes, labor-intensive and noncompliance of patients enrolled for the study. Thus, in diseases with complex etiology such as cancer, an effective approach is required for the successful implementation of pharmacogenetics in the personalization of medicines.

In the pharmacogenetics of tamoxifen therapy, "bottom-up" approach may be significant in overcoming discordance in clinical observations. Using the approach interactions of tamoxifen with different alleles of cytochrome P450 2D6 and other relevant enzymes involved in the metabolism of tamoxifen may be evaluated. Furthermore, modeling and simulation may be performed using algorithms such as Simcyp (www.simcyp.com), pharsight to predict the behavior of tamoxifen in virtual populations. Subsequently, clinical trials may be performed to test and validate the novel significant interactions of tamoxifen with enzymes in populations of different ethnicity.

Thus, the early adoption of "bottom-up" approach to the pharmacogenetic study of a drug therapy may be a sustainable approach in the personalization of medicines.

Conclusion

In this review, the current role of pharmacogenetics in tamoxifen therapy has been discussed. The evidence supports the role of cytochrome P450 2D6 genetic polymorphism in tamoxifen therapy. Since, there is discordance in the clinical data involving cytochrome P450 2D6 genetic polymorphism, interaction of tamoxifen with other alleles of cytochrome p450 2D6, and other enzymes has been recommended. To accomplish personalization of tamoxifen therapy bottom up approach has been proposed.

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