



A Review of GENETIC Polymorphism of GSTs (Glutathione –s Transferase) Genes in Breast Cancer

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Authors' contributions

This work was carried out in collaboration among all authors. Author GG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MS and AM managed the analyses of the study. Author NA managed the literature searches. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

In this review article, several researches about genetic polymorphisms of Glutathione –s transferase (GST) enzymes that may have an etiological role in breast cancer have been reviewed. Breast carcinoma is the most frequent malignancy in women and represents the second leading cause of cancer death among women and accounts for about one-fourth of female cancer cases all over the world. GSTs are a family of Phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a wide variety of xenobiotic. The incorporation of glutathione increases the molecule's water solubility. This detoxification ability to become Glutathione S-transferases play an important role in drug, carcinogen, and reactive oxygen species detoxication and act both as peroxidases and as catalysts of glutathione transfer to hydrophobic electrophiles. It is evaluated in Iran and other countries that the annual incidence of cancer in the world will increase to 25 million in 2030, of which more than 70 percent occurs in developing countries. It is the second cause of death among Iranian women and one million new cases diagnosed worldwide every year.

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ABBREVIATION

GST : *Glutathione –S transferase;*
CE-Q–GSH: *quinines glutathione conjugated;*
MAPEG : *'Membrane associated proteins in eicosanoid and glutathione metabolism;*
GSTA : *Glutathione S-transferase Alpha;*
GSTO : *Glutathione S-transferase Omega;*
GSTZ : *Glutathione S-transferase Zeta;*
GSTM : *Glutathione S-transferase Mu;*
GSTT : *Glutathione S-transferase Theta;*
GSTP : *Glutathione S-transferase Pi;*
GSEC : *The Genetic Susceptibility to Environmental Carcinogen;*

1. INTRODUCTION

The National Cancer Institute estimates that the cost of breast cancer care will reach \$20 billion in the U.S. in 2020. It is evaluated in Iran and several other countries that the annual incidence of cancer in the world will rise from 18 million in 2018 to 25 million in 2030, of which more than 70 percent occurs especially in developing countries [1]. However, Breast carcinoma is still a major health problem in many developed countries. Breast carcinoma incidence rates increased rapidly predominantly in women 50 and older. In the 2000s due to the increased detection of smaller, earlier-stage cancer with the widespread adoption of the screening of mammography among asymptomatic women, counts for 21–30% of malignant tumors in women with an annual incidence of about 900–1100 cases [2].

Several studies have addressed GST polymorphisms and breast cancer. Current evidence suggests that the metabolic by-products of estrogen in the body would act as initiators of cellular alterations. Xeno-estrogens that include pesticides, dyes, pollutants, plasticizers and food preservatives and also have estrogen-like effects, have been suggested to have an important role in the etiology of breast cancer Xeno-estrogens. It also called endocrine disrupters because they interfere with the actions of endogenous estrogens. For example, catechol metabolites of polychlorinated biphenyls (PCBs) have been proposed to alter estrogen metabolism by inhibiting the inactivation of carcinogenic estrogen metabolites [3].

2. ETIOLOGY OF BREAST CANCER

It is suggested that the effect of low penetrance cancer susceptibility genes is mostly to account

for most of sporadic breast carcinoma cases modulated by environmental exposure and lifestyle factors. GSTs are also included in detoxifying reactive compounds generated during estrogen metabolism. In premenopausal non-pregnant women, mostly all estrogen has ovarian origin, while after menopause most estrogen is formed by aromatization of androstenedione to estrogen in peripheral adipose tissue [4,5]. In this study, several researches about genetic polymorphisms of Glutathione –s transferase (GST) enzymes that may have an etiological role in breast cancer have been reviewed.

3. INCIDENCE AND PREVALENCE RATE

Breast cancer is cancer that forms in the cells of the breasts. The high rates of breast cancer incidence and mortality is recorded in industrialized Western nations and lower rates for less industrialized and Asian nations, disparate breast cancer incidence rates among American Caucasian. Increased estrogen levels are a known risk factor for breast cancer. According to the World Health Organization's recommendations complementing national cancer control programs, assessment of the magnitude of the cancer problem (i.e., incidence, prevalence, and mortality) is the first step in this process. There are many published studies about breast cancer in Iran, but the epidemiological aspects of Iranian breast cancer are uncertain. They can result from variations in the enzymatic machinery responsible for estrogen metabolism, a reason why functional polymorphisms of enzymes involved in estrogen biosynthesis and catabolism may contribute to this risk [6]. Breast cancer is by far the most common female cancer, comprising about 21% of all new cancers in women. The highest age-adjusted incidence rate is reported for North America, being 87 per 100 thousand women per year, while the lowest rate reported in China. Breast cancer follows a steeply increasing age gradient up to 40 years of age, after which the rate of increase slows down [7]. Even though, there are three times as many new cases diagnosed annually as in the late 1980s, breast cancer mortality has remained largely unchanged. This may at least partly be explained by earlier detection of the disease due to effective screening programs and availability of improved therapies. The highest annual mortality rates for breast cancer are reported for the UK, The

Netherlands and Denmark, being over 25 per 100 thousand in these countries. So far, conflicting results have been reported from association studies [8].

4. INCIDENCE AND PREVALENCE RATE IN IRAN

Breast cancer affects Iranian women at least one decade younger than their counterparts in developed countries. The mortality rate of breast cancer was 5.8 per 100 thousand women in Tehran in 2008, 2.5 per 100,000 for female population, and 13236 life lost in the 18 provinces of Iran in 2011. The mean lesion size was 2.3 (1.32) centimeters. The age, side of lesion, and gender were related to malignancy ($P=0.01$) and size was not related to type of lesion ($P>0.05$). According to the obtained results in this study it may be concluded that general characteristics and related factors of benign lesions in Iranian patients are similar to other worldwide reports. It is reported 298 patients with breast cancer in 2014 and 382 cases in 2016 in Mazandaran province. Crude incidence rate of breast cancer among the women in Mazandaran province was 17.45 and 16.32 per thousand in 2014 and 2016, respectively. Developing countries hope to be on the threshold of eliminating breast cancer as a major public health threat. Early detection of breast cancer remains an important challenge to health professionals [9].

In a descriptive cross-sectional study, four-year period medical existing data related to pathology center in Tehran were assessed. Data were extracted and clinical and other variables were compared between benign and malignant lesions. Results showed that the mean (\pm standard deviation) age was 42 years. In 400 cases (36%) the lesions were malignant and in 700 cases (64%) those were benign. The most common types of benign lesions were fibrocystic changes (43%) and Adenofibroma (28%) and the most common malignancy was invasive ductal carcinoma (88%)(8). The lesions were left-sided, right-sided, and bilateral in 51%, 43%, and 6%, respectively. Breast cancer accounted for 26% of all female cancers with a crude incidence rate of 23% in 100 thousand in Tehran in 2010. Two screening programs and a cross-sectional study showed the prevalence rate of breast cancer was 352 per 100 thousand women aged 30–65 in Bushehr in 2010, 660 per 100 thousand for women 35 years and older in Shiraz in 2011, and 150 per 100 thousand for

women aged 30 years and over in Northwest of Tabriz incidence rates in Iran, as in other Asian countries, during last four decades, increasing its incidence rate has made breast cancer one of the most frequent malignancies among Iranian women. The ASR rates of breast cancer was 27 and 25 per 100 thousand in the studied periods, respectively. Most cases occurred in women aged 50-56 years (22%, annual rate). The most common type of breast cancer morphology in 2014 and 2016 was ductal carcinoma [10] In this study, a comprehensive investigation carried out into the expression of a wide range of GSTs in breast cancer.

5. GSTs FUNCTION AND CLASSIFICATION

GSTs are a family of Phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a wide variety of xenobiotic. The incorporation of glutathione increases the molecule's water solubility and excretability. This detoxification ability plays a vital role in Glutathione S-transferase to become drug, carcinogen, and reactive oxygen species detoxication and act both as peroxidases and as catalysts of glutathione transfer to hydrophobic electrophiles. The soluble GSTs exist as dimeric proteins of nearly 25 kDa, and the sequences and the known three-dimensional (3D) structures suggest that these proteins share a common ancestry, though the precise details of their evolution remain obscure [11]. They are suggested at high levels in mammalian liver constituting up to 4% of the total soluble proteins. cellular protection from environmental and oxidative stress, yet is also implicated in cellular resistance to drugs.

Human GSTs are divided into three main families: cytosolic, mitochondrial and membrane-bound microsomal. Microsomal GSTs are designated as 'membrane associated proteins in eicosanoid and glutathione metabolism' (MAPEGs) [12]. MAPEGs are structurally distinct from cytosolic GSTs but are functionally similar in the ability to catalyze the conjugation of GSH to electrophilic compounds.

The mammalian cytosolic family of GSTs exist as monomers and are catalytically active in a homo- or hetero dimeric state. The cytosolic family is further divided into seven classes: Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta. Classification is based on sharing greater than 60% identity within a class and focuses mainly

on the more highly conserved N-terminal domain that contains a catalytically active tyrosine, cysteine or serine residue. At least 20 isoenzymatic forms of GST have been identified, and many of them show genetically based individual variability of enzyme activity [13]. The catalytic residue interacts with the thiol group of GSH (G-site), and crystal structure has confirmed as substrate binding site (H-site) that facilitates catalysis and is in proximity to the G-site. They have an important part of the cellular detoxification system and, maybe, evolved to protect cells against reactive oxygen metabolites. These proteins were found in all eukaryotic and prokaryotic systems, in the cytoplasm, in the microtomes and in mitochondria. Allelic variation has been found in genes encoding for these GSTs. Inherited differences in the capacity of xenobiotic metabolizing enzymes might be an important factor of genetic susceptibility to make cancer [14]. The active site of a GST molecule is composed of two subunits the glutathione-binding site and the hydrophobic substrate-binding site. The residues forming the glutathione-binding site are conserved in the different classes, while those forming the substrate-binding site vary considerably, leading to a wide substrate specificity. In estrogen metabolism, GSTs play a vital role in the catalysis of GSH conjugation of catechol estrogen quinines, the reactive intermediates of estrogen metabolism capable of bind to DNA. There are no reports in the formation of CE-Q-GSH conjugates or detoxification of reactive oxidant damage, regarding the specific GST isoforms. Several environmental risk factors have been previously associated with increased susceptibility to breast cancer.

Several environmental risk factors have been previously associated with increased susceptibility to breast cancer. Western blot analysis of glutathione S-transferase proteins in breast tissues from 14 individuals showed expression of 'p' and 'm' as the major isoforms, although glutathione S-transferase was detectable at lower levels. Critical function of Glutathione S-transferase (GST) is protection against electrophiles and the products of oxidative stress (Table 1) [15].

The GSTs are included in the metabolism of many xenobiotic, including an array of environmental carcinogens and chemotherapeutic agents and endogenous

derived reactive oxygen species. GSTs are widely distributed in nature and are found in essentially all eukaryotic species. Variation in the mammary expression of glutathione S-transferase isoforms may be relevant to protecting against the genotoxic effects of PHAs (Polycyclic aromatic hydrocarbons) in the breast; glutathione S-transferase a (A1-1) detoxifies N-acetoxy-PhIP (N-Acetoxy-PhIP) (2-Acetoxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine) (thereby preventing DNA binding) in the presence of glutathione. Although a meta-analysis of studies published before 2017 has suggested a slight risk increase in carriers. Besides, glutathione transferases may have a role in the metabolism of lipid and DNA products of oxidative stress and also in the resistance to cancer chemotherapeutic agents [16].

GSTs detoxify a variety of carcinogens and cytotoxic drugs [e.g., benzo(a) pyrene; mono halothanes such as methyl chloride, ethylene oxide, and pesticides; and solvents used in industry by catalyzing the conjugation of a glutathione moiety to the substrate. However, two enzymes have glutathione peroxidase activity, one selenium-dependent and one selenium-independent, the former was found to make up the majority of glutathione peroxidase activity in the breast. The selenium-dependent enzyme can reduce both hydrogen peroxide and organic hydro peroxides. The selenium-independent form will only metabolize organic hydro peroxides, although other xenobiotic substrates have not yet been investigated [17].

5.1 GSTs Classification

5.1.1 Alpha class

GSTa isoform is expressed mainly in the liver and is encoded by a gene cluster localized on chromosome 6p12 (Table 2). This cluster contains five genes encoding proteins belonging to GSTA1-A5. Human tissues widely express transcripts for GSTA1, A2 and A4, whereas expression of GSTA3 is rare and GSTA5 has yet to be detected in human tissues. Epidemiological results show that aberrant expression of GSTa has been linked to an increased risk in colorectal cancer, ovarian cancer and clear cell renal cell carcinoma. A genetic polymorphism of GSTA1 is characterized by two alleles, GSTA1*A and GSTA1*B (Table 3). This may be due to substitution of the highly conserved Promotor region. GSTA3-3 is selectively expressed in steroidogenic tissues and plays important role in

Table 1. GSTs functions

GST Class	Organisms	Function
Alpha	Eukaryotes	Detoxification, peroxide reduction
Beta	Bacteria	Haloalkane conjugation
Delta	Insects	Detoxification, insecticide resistance
Epsilon	Insects	Detoxification, insecticide resistance
Zeta	Bacteria and eukaryotes	Methylacetoacetate and methylpyruvate isomerases tetrachloro hydroquinone dehalogenase
Theta	Bacteria and eukaryotes	Detoxification, peroxide reduction, haloalkane conjugation
Mu	Eukaryotes	Detoxification
Pi	Eukaryotes	Detoxification
Sigma	Insects and animals	Detoxification, prostaglandin D synthesis, lens crystallins in cephalopods
Tau	Plants	Anthocyanin synthesis
Phi	Plants	Anthocyanin synthesis
Omega	Bacteria and eukaryotes	Thiol transferase, dehydroascorbate reduction

steroid hormone biosynthesis. Polymorphisms in GSTA3 may affect steroidogenesis through altered protein levels or function, and it has recently been hypothesized that alterations in genes involved in steroidogenesis and sex steroid metabolism could potentiate risk factors for the development of ovarian cancer. Although, as with many such published accounts, other groups have recently reported conflicting evidence finding no association between GST expression and susceptibility to colorectal cancer GSTA2 has not been extensively studied, but it is known to have several variants (GSTA2*A–E) [18]. Single nucleotide polymorphisms for GSTA2 are summarized in Table 3. The catalytic properties of GSTA2 variants A–D do not seem to differ; however, the novel variant GSTA2E shows reduced rates of catalysis when compared to A–D.

5.1.2 Omega class

The Omega class of GSTs contains two members and a pseudogene: GSTO1 and GSTO2 and GSTO3p. GSTO1 is a single gene located on chromosome 10 that codes for proteins expressed abundantly in the liver, macrophages, glial and endocrine cells. Recently, four polymorphisms have been identified, GSTO1*A–D (Table 3). GSTO2, like GSTO1, is ubiquitously expressed and shares GSH-dependent dehydrate ascorbate reductase activity. However, GSTO2 has a high catalytic activity toward CDNB, and its overexpression induced apoptosis, suggesting a possible role in cell signaling. This allele was first described as the human monomethyl arsenic acid reductase, (MMA[V]), and is the rate-limit in Genzyme of

inorganic arsenic metabolism [19]. Thioltransferase activity differs among the GSTO1*A–C polymorphisms and may contribute to an individual's ability to metabolize arsenic. GSTO2, while separated from GSTO1 by 7.5 kb on chromosome 10, shares 64% amino-acid identity. Among the Australian, African and Chinese populations, GSTO1*A was the most prevalent haplotype with a frequency ranging from 0.61 to 0.91; whereas GSTO1*B*A was the least common, with frequency of 0.01–0.05. GSTO1* demonstrated as a GSH-dependent reduction of dehydrate ascorbate, a function characteristic of glutaredoxins rather than GSTs.

5.1.3 Zeta class

The Zeta class (GSTZ1) is a single 10.9 kb gene which located on chromosome 14 that codes for a 29 kDa protein (Table 1). GSTZ1 was independently characterized as methylacetoacetate isomerase (MAAI) and plays a putative isomerase role in the catabolic pathway of phenylalanine and tyrosine, besides of GSH-dependent transformation of ahalogenated acids. Whereas the clinical data for GSTZ1 are insufficient to deduce a role for GSTZ1 in inherited genetic disease, it is plausible that a perturbation in GSTZ1-mediated tyrosine metabolism is contributory to the described pathology. The isozyme GSTZ1*A has the highest catalytic activity toward dichloroacetic acid, an investigational drug for certain metabolic disorders and a nephrotoxic metabolite of industrial solvents [19]. In contrast, GSTZ1*D (p. T82M) has a reduced catalytic activity and has been associated with in born errors in tyrosine metabolism, although the disorders have also

been attributed to mutations in other enzymes. GSTZ1 is preferentially expressed in hepatocytes and renal proximal tubule cells where phenylalanine and tyrosine are catabolized. While the literature is limiting for this class, GSTZ1 polymorphisms have been identified (GSTZ1*A–D) (Table 3). Rodent models deficient for GSTZ1 provide insight into its role in metabolic deficiencies. GSTZ1/MAAI converts maleylacetoacetate to fumarylacetoacetate (FAA). GSTZ1-deficient mice have an elevated urinary excretion of FAA and were subject to renal injury following phenylalanine and tyrosine overload. Four families have been identified that have GSTZ1-deficient members and died within the first year of life. Whereas the clinical data for GSTZ1 are insufficient to deduce a role for GSTZ1 in inherited genetic disease, it is plausible that a perturbation in GSTZ1-mediated tyrosine metabolism is contributory to the described pathology [20].

Table 2. Cytosolic GSTs

Class	Gene	Chromosome location
Alpha (α)	GSTA1	6p12
	GSTA2	6p12.2
	GSTA3	6p12
	GSTA4	6p12
	GSTA5	6p12.1
Mu (μ)	GSTM1	1p13.3
	GSTM2	1p13
	GSTM3	1p13.3
	GSTM4	1p13.3
	GSTM5	1p13.3
Omega (ω)	GSTO1	10q25.1
	GSTO2	10q25.1
Pi (π)	GSTP1	11q13qter
Theta (θ)	GSTP1	22q11.2
	GSTP2	22q11.2
Zeta (ξ)	GSTZ1	14q24.3

5.1.4 Mu class

Five GST isoforms have been described which belonging to the mu class (GSTM1-5). A gene cluster located on chromosome 1 encodes for GSTM1-5 (Table 2). The elimination of the gene has been proposed to have occurred by unequal crossing over. The frequency of the null genotype is around 50% in Caucasians and Asians, but only 27% in Africans. The GSTM1 gene contains four different alleles allowing for several M1 class polymorphisms. GSTM1*A and GSTM1*B are functionally identical and only differ by one p.K173N amino-acid substitution

(Table 3). These two polymorphisms can combine to form four different isoforms. The p.W147 variant seems to exhibit decreased catalytic and specific activity, whereas the p.I224 variant has shown increased catalytic and specific activity that introduces a recognition motif for the transcript. The presence of the GSTM1*A allele has been associated with a decreased risk of bladder cancer with the implication that detoxification of possible bladder specific carcinogens may occur in these individuals [21]. GSTM3*AA was proposed to occur more frequently in patients with multiple cutaneous basal cell carcinoma than GSTM3*BB. Now a days, two new polymorphisms have been identified in the GSTM3 gene, a rare p.G147W substitution and a more common p.V224I substitution. One breast cancer study has reported on a potential interaction between GSTM1 null genotype and PAH–DNA adducts [22].

5.1.5 Theta class

The Theta class of GSTs includes two different subfamilies: GSTT1 and GSTT2. Genes encoding both proteins are localized on chromosome 22 and are separated by 50 kb. Polymorphisms exist within both genes including a null phenotype (GSTT1*0) that exhibits decreased catalytic activity and has been associated an increased risk of cancers of the head, neck and oral cavity. The presence of a G to A transition at intron 2 has given rise to a pseudogene (GSTT2P) of GSTT2. Evidence proved transcription of this gene, although the gene product is thought to be inactive. An allele identified in GSTT2 involves a rare p.M139L substitution. A phenotype related to this substitution has yet to be identified, although there is nuclear indication that this substitution affects enzyme function. A single nucleotide polymorphism in exon 3 results in two different variants, GSTT1*A and GSTT1*B (Table 3). GST genotypes have also been investigated in relation to the age at which breast cancer was diagnosed in women with appositive family history of breast cancer. Rebecca et al. studied 185 cases of breast cancer ascertained through hereditary breast cancer clinics in the United States and found no association between GSTM1 genotypes and risk of breast cancer. GSTT1*A has a threonine at residue 104, which is substituted to a proline in the GSTT1*B allele. The GSTT1*B allele shows a decreased catalytic activity when compared to the GSTT1*A allele, which could be attributed to the

conformational change induced by the praline substitution [23]. Three recent studies found no association between the GSTT1 null genotype and the breast cancer risk but one study suggested a remarkably lower risk for premenopausal women lacking the GSTT1 gene. These inconsistent results across studies would be explained by the limited number of subjects available for some analyses or by population differences in other risk factors for breast cancer. Specifically, GSTM1 and GSTT1 phenotypes were examined in patients with AML, and it was shown that patients null for GSTT1 exhibited an increased toxicity and reduced survival rate following chemotherapeutic treatment. In contrast to this lower survival rate, patients null for GSTM1 or GSTT1 showed a twofold reduction in cancer relapse during remission [24].

5.2 GST Pi

The GST Pi class is encoded by a single gene spanning approximately 3 kb and located on chromosome 11. Four active, functionally different polymorphisms (GSTP1*A–D) have been identified. In both variant alleles, a point mutation at nucleotide 313 results in a single amino acid change from isoleucine (Ile) to valine (Val) at codon 105. This residue lies in close proximity to the hydrophobic binding site for electrophilic substrates, and the Val105 variant allele has been demonstrated to exhibit altered specific activity and affinity for electrophilic substrates. The GSTP1 genotype has been associated with differences in chemotherapeutic response and cancer susceptibility and is over expressed in a wide variety of tumors including ovarian, NSCLC, breast, pancreas and lymphoma and colon. GSTP1*A has been reported to play a role in the acquisition of resistance to cisplatin via formation of platinum–GSH conjugates [25]. Patients expressing homozygosity for the GSTP1*B allele have a diminished capacity to detoxify platinum-based anticancer agents, thus making this phenotype favorable for response rates. Besides, the GST-mediated conjugation of GSH to a number of anticancer drug substrates has long been linked to anticancer drug resistance in a variety of tumors. A disparity of this is that GST has a weak affinity for the majority of anticancer drugs, although its increased expression is highly correlated with multidrug resistance. In addition, our lab has found that cells deficient in GSTP1-1 and/or GSTP2-2 have a reduced capacity to respond to

oxidative or nitrosative stress by enacting glutathionylation of a select group of target proteins. These findings imply that GSTp may play a direct role in control of post translational glutathionylation reactions [26]. This study illustrates that GSTP1 is important for human colon cancer cell survival and proliferation, and results show that GSTP1 plays a critical role in protection from cell cycle arrest and oxidative stress under growth-limiting conditions. The GSTP1 is the major GST expressed consistently in both normal and tumor breast tissue. It has been speculated that the absence or decrease in expression of GSTp results in a reduced detoxification of possible carcinogens that may be causal to malignant transformation and disease progression.

5.3 GST null Phenotype and Cancer Prognosis

It's totally understanding from the capacity of GSTs to regulate kinase-dependent proliferation pathways, especially in the case of GSTp, may be of more consequence than its catalytic properties alone. GSTs play a regulatory role in cellular signaling by forming protein: protein interactions with critical kinases involved in controlling stress response, apoptosis and proliferation. The ligand-binding capacity of GST results in the negative regulation of signaling pathways through sequestration of signaling kinases. Although, results were contrary for ovarian cancer patients who were double null for GSTM1 and GSTT1. When compared to patients with the wild-type GSTM1 or GSTT1 allele, the null genotype patients exhibited a diminished response to chemotherapy resulting in both a poorer prognosis as well as a decrease in remission rates. Deletion of the GSTM1 and GSTT1 genes results in a 'null' genotype characterized by a general deficit in enzymatic activity [27]. Individuals homozygous for these deletions are thought to be at increased risk for malignancies as a consequence of a decreased capacity to detoxify possible carcinogens. Where this phenotype has been studied as a predictive factor for cancer prognosis or response to therapy, the results have been contingent upon tumor type. Patients with GSTM1*0 or GSTT1*0 showed a better survival rate after chemotherapeutic treatment for invasive ovarian cancer compared to other patients.

5.4 Other Finding About GSTs and their Polymorphisms

The Genetic Susceptibility to Environmental Carcinogen(GSEC) Study - an international collaborative project - has been initiated to evaluate the relationships of polymorphisms in genes that metabolize environmental carcinogens and cancers at different sites. The deletion mutations in GSTM1 and GSTT1 and the105V variant in GSTP1 have been evaluated for associations with breast cancer in a large number of studies. Several studies reported about a 20–50% elevated risk associated with the nullGSTM1 genotype, and a stronger association was reported in two other studies. However, the majority of studies reported no relation or even a possible inverse association of the null GSTM1 variant with breast cancer. The

established database offered the opportunity for investigating the association of polymorphisms in the GSTM1, GSTT1, and GSTP1 and breast cancer using individual data of several studies and taking into account the potential modifying effect of reproductive factors and tobacco consumptions. Of particular interest, GSTM1 and GSTP1 can detoxify carcinogenic polycyclic aromatic hydrocarbons, such as absence of specific isoenzymes affects the tolerance of organisms to chemical challenges and may result in increased somatic mutation rate and there by higher susceptibility to malignancies.

absence of specific isoenzymes affects the tolerance of organisms to chemical challenges and may result in increased somatic mutation rate and there by higher susceptibility to malignancies. benzo[a] pyrene and the

Table 3. Genetic variations of GSTs

Allele	Nucleotid variability
GSTA1*A	Wild-type
GSTA1*B	Promotor point mutation
GSTA2*A	Pro110; Ser112; Lys 196; Glu210
GSTA2*B	Pro110; Ser112; Lys 196; Ala210
GSTA2*C	Pro110; Ser112; Lys 196; Glu210
GSTA2*D	Pro110; Ser112; Asn 196; Glu210
GSTA2*E	Pro110; Ser112; Lys 196; Glu210
GSTO1*A	Ala 140; Glu155
GSTO1*B	Ala 140; Glu155 deletion
GSTO1*C	Asp 140; Glu155
GSTO1*D	Asp 140; Glu155 deletion
GSTO2*A	Asn 142
GSTO2*B	Asp 142
GSTZ1*A	Lys32; Arg42; Thr82
GSTZ1*B	Lys32; Gly42; Thr82
GSTZ1*C	Glu32; Gly42; Thr82
GSTZ1*D	Glu32; Gly42; Met82
GSTM1*A	Lys173
GSTM1*B	Asn173
GSTM1*0	Deletion
GSTM1*1x2	Duplication
GSTM3*A	Wild type
GSTM3*B	3 base deletion, intron 6
GSTM4*A	Tyr2517
GSTM4*B	Cyt2517
GSTT1*A	Thr 104
GSTT1*B	Pro 104
GSTT1*0	Deletion
GSTT2*A	Mer 139
GSTT2*B	Ile 139
GSTP1*A	Ile 105; Ala 114
GSTP1*B	Val 105; Ala 114
GSTP1*C	Val 105; Val 114
GSTP1*D	Ile 105; Val 114

mycotoxinaflatoxin, while GSTT1 can detoxify smaller reactive hydrocarbons, such as ethylene oxide and diepoxybutane [28].

The ability of many tumors to exhibit increased levels of intracellular GST expression has been linked to mechanisms of chemotherapeutic drug resistance. Human tissues show differential expression of the multiple forms of GSTs. The absence of specific isoenzymes affects the tolerance of organisms to chemical challenges and may result in increased somatic mutation rate and there by higher susceptibility to malignancies.

Enzymes belonging to the GST classes A, k, and u are involved in the detoxification of benzo (a) pyrene and other polycyclic aromatic hydrocarbons found in tobacco smoke and have received considerable attention in relation to smoking. A coding sequence polymorphism, A313G (changing codon 105 from Ile to Val), in the GSTP1 gene was identified several years ago, but little information has been published about how it affects GSTP1 phenotype in human tissues or its association with cancer risk.

This anomaly may be explained by the ability of GSTs to act as ligand-binding proteins in the regulation of cell cycle components such as mitogen-activated protein kinases (MAPK) and extracellular-regulated kinases (ERK). A recent case-control study suggested an increased risk of breast cancer in relation to polycyclic aromatic hydrocarbon-DNA adducts. Although tobacco smoking does not seem to be a risk factor of breast cancer in studies of unselected populations, the possibility of an increased risk in genetically predisposed groups remains. An association between the GST null phenotype and cancer susceptibility was described for lung cancer, colorectal cancer, and bladder cancer. Polycyclic aromatic hydrocarbons induced mammary tumors in animal models, and polycyclic aromatic hydrocarbon-DNA adducts have been identified in human mammary epithelial cells. Chemotherapeutic-resistant tumor cell lines have been shown to over express GST isozymes. This over expression leads to an accelerated detoxification of drug substrates and thus an acquired resistance. Although, drug resistance is exhibited in cells expressing certain isoforms of GSTs even when that specific selecting drug is not an enzyme substrate.

6. SUMMARY AND CONCLUSIONS

It's not possible to explain the etiology of breast cancer by allelic variability at a single locus. Instead, the main burden of breast cancer in the population probably results from complex interactions among several genetic and environmental factors over time. Cumulative lifetime exposure to estrogen, metabolites, and other physiological factors, as well as environmental exposures, could play an important role in the etiology of breast cancer in genetically predisposed women. Most of the risk factors for breast cancer relate to the increased or prolonged exposure to estrogen. In estrogen metabolism GSTs play a role in the catalysis of GSH conjugation of catechol estrogen quinines. It may be due to substitution of the highly conserved Pro residue, GSTA3-3 is selectively expressed in steroidogenic tissues and plays a role in steroid hormone biosynthesis intermediates of estrogen metabolism capable of bind to DNA. The main effect of estrogens is thought to be via stimulation of breast-cell proliferations, thereby increasing the chances that a cell bearing a potentially cancer-causing mutation will multiply. Estrogen in vitro activate MAPK and NFκB, driving the expression of the antioxidant enzymes SOD and GSH-Px. Another evidence provided on skeletal muscle of oophorectomized mice reported that estrogen replacement increased anti oxidant gene expression via Estrogen Receptor α. Data definitively support such observation. Very interestingly, we did not observe any variation in the expression of GST, which is considered a phase II enzyme, catalyzing conjugation reactions that convert the highly reactive species produced during phase I metabolism to less reactive products. It has been demonstrated that E2 may inhibit phase II enzymes expression via a pathway involving NRF2 and the antioxidant response element [29]. An improved conception of the interplay of xenobiotic exposures, endogenous physiology and genetic variability at multiple loci may help to identify women who are at increased risk for breast cancer. The genetic polymorphisms that may be linked to breast cancer are so high. germline mutations - called high-penetrance cancer susceptibility genes, BRCA1 or BRCA2, In hereditary breast carcinoma, mutations in highly penetrant genes such as confer a relatively high risk for developing breast carcinoma, though this risk accounts only for about 6 to 12% of all breast carcinoma cases and these mutations are found in up to 40% of breast cancers Therefore,

relatively common genes acting together with endogenous lifestyle risk factors (low-penetrance genes), are likely to account for a much higher portion of the breast cancer cases together with yet unidentified high-penetrance gene. Several classes of compounds have been identified as mammary carcinogens in animals [e.g., aromatic amino/nitro compounds and epoxide-forming chemicals, Aromatic DNA adducts have been found in higher levels in breast tissue from cancer patients than in breast tissue from control subjects without cancer. It is biologically plausible that low-activity-level genotypes would be associated with an enhanced susceptibility to cancer. The cytosolic family of GSTs is further divided into seven classes: Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta Classification. The glutathione transferases are a family of Phase II detoxification enzymes that catalyze the conjugation of glutathione to a wide variety of xenobiotic, also potential carcinogenic compounds and the isozymes have distinct but overlapping substrate specificity. These substrates include polycyclic aromatic hydrocarbon diol-epoxides, organic epoxides, peroxides, N-acetyl benzoquinoneimine, hydroxyalkyl-arenes, and steroids. In the studies done about GSTs classes as reviewed, Among the Australian, African and Chinese populations, GSTO1*A was the most prevalent haplotype with a frequency ranging from 0.6 to 0.9; whereas GSTO1*B*A was the least common, with a frequency of 0.01–0.05. Rodent models deficient for GSTZ1 provide insight into its role in metabolic deficiencies. GSTZ1/MAAI converts maleylacetoacetate to fumarylacetoacetate (FAA). GSTZ1-deficient mice have an elevated urinary excretion of FAA and were subject to renal injury following phenylalanine and tyrosine overload. There are also two active allelic variants termed GSTM1*A and GSTM1*B but the two gene products, which differ by an Asn or Lys, respectively, at residue 172, appear to be catalytically similar overall association was seen, women carrying the GSTM3 *B allele appeared to be protected against breast cancer if they had never used alcohol. An interaction was also seen with the GSTP1 gene. A trend toward increased risk was also observed for valine/valine homozygosity in GSTP1 and for the homozygous deletion (or null) genotype in GSTT1. The OR for the combined GSTM1 null, GSTT1 null, and one or two copies of GSTP1 valine (i.e. the higher-risk genotypes) was 3.77 (95% CI, 1.10 –12.88). Suggested a trend for increasing risk with higher numbers of GSTP1 Val105 alleles. GSTP1*C, an allelic variant that is

predominant in malignant glioma cells, differs from other GSTP1 variants by two transitions resulting in p. I104V and p. A113V. Individuals positive for GSTP1*C seem to have a lower incidence of breast cancer. GSTs play a regulatory role in cellular signaling by forming protein: protein interactions with critical kinases involved in controlling stress response, apoptosis and proliferation. There is different studying about polymorphism of GSTs genes classes that will be reviewed. The GSTM1 and GSTT1 polymorphisms are of more important in toxicology than drug metabolism. A positive relationship of the GSTP1 Val/Val genotype with breast cancer was reported initially although this finding which was not replicated in most subsequent studies. GST μ and GST θ are important in the detoxification of carcinogens implicated in breast cancer absence of these enzymes may increase the risk of this common malignancy it can be inferred that the capacity of GSTs to regulate kinase-dependent proliferation. At the ways, especially in the case of GSTp, may be of more consequence than its catalytic properties alone.

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COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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