POLYMORPHISMS OF GLUTATHIONE S- TRANSFERASE GENES (*GSTM1*, *GSTP1*, AND *GSTT1*) AND BREAST CANCER SUSCEPTIBILITY IN THE TURKISH POPULATION

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THE DEGREE OF MASTER OF SCIENCE

BY
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AUGUST, 2002

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ABSTRACT

POLYMORPHISMS OF GLUTATHIONE S- TRANSFERASE GENES (*GSTM1*, *GSTP1*, AND *GSTT1*) AND BREAST CANCER SUSCEPTIBILITY IN THE TURKISH POPULATION

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Ms. in Molecular Biology and Genetics

Supervisor: Asst.Prof.Dr.Işık G. YULUĞ

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The potential association between the Glutathione S- transferase genes *GSTM1*, *GSTT1*, *GSTP1* and breast cancer susceptibility was investigated in a case control study of 264 female patients and 233 age-matched controls in the Turkish population. The combined *GSTP1* 105 Ile/Val or Val/Val genotypes was significantly associated with breast cancer risk in all women (odds ratio OR=1.64, 95% confidence interval CI=1.09-2.47 and in premenopausal women is OR= 2.01, 95% CI=1.06-3.83). Neither *GSTM1* nor *GSTT1* was found to be associated with breast cancer. Distribution of *GSTP1* genotypes was stratified according to body mass index (BMI), age, age at menarche, age at full-term pregnancy, number of full-term pregnancies, and family history of breast cancer. The association of the combined *GSTP1* 105 Ile/Val or Val/Val genotypes with breast cancer risk was further exacerbated in women with high BMI (OR=2.12, 95% CI=1.35-3.62), but not with a low BMI (OR=0.78, 95% CI=0.45-1.34). These findings support the role for the combined *GSTP1* 105 Ile/Val or Val/Val genotypes in the development of breast cancer, particularly with a high BMI.

ÖZET

TÜRK TOPLUMUNDA GLUTATYON S-TRANSFERAZ GENLERİNİN (*GSTM1*, *GSTT1*,*GSTP1*) POLİMORFİZMLERİ VE MEME KANSERİ İLE İLİŞKİSİ

Ebru DEMİR
Moleküler Biyoloji ve Genetik Yüksek Lisansı
Tez Yöneticisi: Yrd.Doç.Dr.Işık G. YULUĞ
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GSTM1, *GSTT1* ve *GSTP1* Glutatyon S-Transferaz genleri ile meme kanserine yatkınlık arasındaki olası ilişki Türk toplumunda 264 kadın hasta ve 233 yaş bakımından eşleştirilmiş kontrol bireyinde incelendi. Kombine *GSTP1* 105 Ile/Val veya Val/Val genotipleri tüm kadınlarda (olasılık oranı OR=1.64, %95 güven aralığı GA=1.09-2.47) ve premenopozal kadınlarda (OR=2.01, %95 GA=1.06-3.83) (belirgin şekilde artmış olarak) meme kanseri riskiyle ilişkiliydi. Ne *GSTM1* ne de *GSTT1* meme kanseri ile ilişkili bulunmadı. *GSTP1* genotiplerinin dağılımı vücut kütle oranı (VKO), yaş, menarş yaşı, miyadında doğum yaşı, miyadında doğum sayısı ve ailede meme kanseri öyküsüne göre gruplandırıldı. Kombine *GSTP1* 105 Ile/Val veya Val/Val genotiplerinin meme kanseri riski ile ilişkisi yüksek VKO'lu hastalarda (OR=2.12, %95 GA=1.35-3.62) daha da belirgindi, ama düşük VKO'lu hastalarda değildi (OR=0.78, %95 GA=0.45-1.34). Bu bulgular meme kanseri gelişiminde, özellikle yüksek VKO'lu kadınlarda kombine *GSTP1* 105 Ile/Val veya Val/Val genotiplerinin rolü olduğu düşüncesini desteklemektedir.

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ABBREVIATIONS

APC	Adenomatous Polyposis of the Colon
BRCA1	Breast Cancer Susceptibility Gene 1
BRCA2	Breast Cancer Susceptibility Gene 2
CASP10	Caspase 10
CDH1	Cadherin 1
CDKN1C	Cyclin dependent kinase 1C
CDKN2A	Cylin Dependent kinase 2A
CI	Confidence Interval
CYP1A1	Cytochrome P450 1A1
CYP1A2	Cytochrome P450 1A2
CYP1B1	Cytochrome P450 1B1
CYP2A6	Cytochrome P450 2A6
CYP2C19	Cytochrome P450 2C19
CYP2D6	Cytochrome P450 2D6
CYP3A4	Cytochrome P450 3A4
CYP11a	Cytochrome P450, subfamily Xia
CYP17	Cytochrome P450, subfamily XVII
CYP19	Cytochrome P450, subfamily XIX
DNA	Deoxyribonucleic acid
DIA4	Diaphorase 4
dNTP	Deoxynucleotide triphosphate
ERCC1	Excision repair cross-complementing rodent deficiency complementation group 1
ERCC2	Excision repair cross-complementing rodent deficiency complementation group 2
ESRRA	Estrogen-related receptor alpha
EXT1	Exostosin 1
EXT1	Exostosin 1
GSTM1	Glutathione S-Transferase mu 1
GSTM2	Glutathione S-Transferase mu 2
GSTM3	Glutathione S-Transferase mu 3

GSTM4	Glutathione S-Transferase mu 4
GSTM5	Glutathione S-Transferase mu 5
MADH4	Mothers against decapapenaplegic Drosophila Homolog of 4
MEN1	Multiple Endocrine Neoplasia type1
MLH1	Mut L Homolog 1
ml	milliliter
mM	milimolar
µl	microliter
MPO	Myeloperoxidase
MSH2	Mut S Homolog 2
NAT1	N-Acetyl Trransferase Type 1
NAT2	N-Acetyl Transferase Type 2
NF1	Neurofibromatosis 1
NF2	Neurofibromatosis 2
ng	nanogram
OR	odds ratio
pmol	picomol
PPARA	Peroxisome Proliferative Activated Receptor, Alpha
PPARG	Peroxisome Proliferative Activated Receptor,Gamma
PRKAR1A	Protein kinase,c-AMP dependent regulatory,type 1
POLB	Polymerase Beta
PTGS1	Prostaglandin-Endoperoxide Synthase 1
PTGS2	Prostaglandin-EndoperoxideSynthase 2
RB	Retinoblastoma gene
RET	Rearranged during Transfection
SDHD	Succinate Dehydrogenase Complex, Subunit D
SMARCB1	SWI/SNF-related, Matrix-Associated, Actin-Dependent regulator of chromatin Subfamily1, Member 1
SULT1A1	Sulphotransferase 1A1
SULT1A2	Sulphotransferase 1A2
TNF	Tumor Necrosing Factor
TP53	Tumor Protein p53

TSC1	Tuberous Sclerosis 1
TSC2	Tuberous Sclerosis 2
VDR	Vitamin D Receptor
VHL	Von Hipple-Lindau
XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1
X^2	Chi-square
WT1	Wilm's Tumor 1 gene

1. Introduction

1.1 Genetic Basis of Human Cancer

All cancers are caused by abnormalities in DNA sequence. Throughout life, the DNA in human cells is exposed to mutagens which causes errors in replication. This process results in progressive, subtle changes in the DNA sequence of each cell (Futreal PA. *et al*. 2001). Occasionally, one of these somatic mutations alters the function of a critical gene, providing a growth advantage to the cell in which it has occurred and resulting in the emergence of an expanded clone derived from this cell. Additional mutations in the relevant target genes and consequent waves of clonal expansion produce cells that invade surrounding tissues and metastasize. Cancer is the most common genetic disease: one in three people in the western world develop cancer, and one in five die from it (Higgison J. *et al* 1992).

Self-sufficiency in growth signals, insensitivity to growth-inhibitory (anti-growth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis are six capabilities that are shared in common by almost all types of human tumors (Hanahan D. and Weinberg AR. 2000).

1.1.1 Cancer and Related Genes

Initiation and progression of cancer and the major genes, which take part in these processes, are shown in Figure 1.

1.1.1.1. Genetic Events in Cancer, Gain-of-function

Oncogenes are altered forms of normal cellular genes called proto-oncogenes. In human cancers, proto-oncogenes are frequently located adjacent to chromosomal breakpoints and are targets for mutation. The products of proto-oncogenes regulate several events of cell cycle, cell division and differentiation. In a cancer cell, one or more of the components of these pathways are altered. Oncogenes exhibit a dominant phenotype at the cellular level and gain-of-function occurs when one copy of an oncogene is activated. Oncogenes may be transmitted from generation to generation

when the proto-oncogene mutates in the germ-line. A good example of an oncogene is *ERBB2*, which codes for a receptor for epidermal growth factor and is involved in glioblastoma, brain cancer and breast cancer. Another example is *Bcl-1* coding for cyclin D1, which is a component of the cell cycle clock and is involved in breast, head and neck cancers. Other examples include C-*Myc*, N-*Myc* and L-*Myc* which are transcription factors that activate growth promoting genes and are involved in leukemia, neuroblastoma, and breast, lung and stomach cancers.

1.1.1.2 Genetic Events in Cancer, Loss-of-function

Tumor suppressor genes encode proteins that function in growth regulatory or differentiation pathways and if altered contribute to cancer formation. Tumor supressor genes exhibit a recessive phenotype and require inactivation of both alleles. They are divided into two categories: Gatekeepers and Caretakers (Kinzler KW. and Vogelstein B. 1997). Genes whose mutation or altered expression distrupts the cell-cyle control and cell division, death or lifespan, promoting the outgrowth of cancer cells (e.g. *Rb*) are termed `Gatekeepers` and those whose change causes genomic instability, increasing the frequency of alteration in gatekeeper genes are defined as `Caretakers` (e.g. *MLH1*, *BRCA1*).

1.1.1.3 Patterns of Tumorigenic Events

Four to seven rate-limiting genetic events are needed for the development of the common epithelial cancers (Renan MJ. e*t al*. 1993). The precise pattern of genetic alteration differs between cancers of different types and even of the same type. However, the patterns are not random (Liotta L. *et al*. 2000 and Suzuki S. *et al*. 2000). The molecular profiling of tumors by genomic alterations or expression changes will reflect the possible mechanisms of tumor evolution, which may provide information of clinical value.

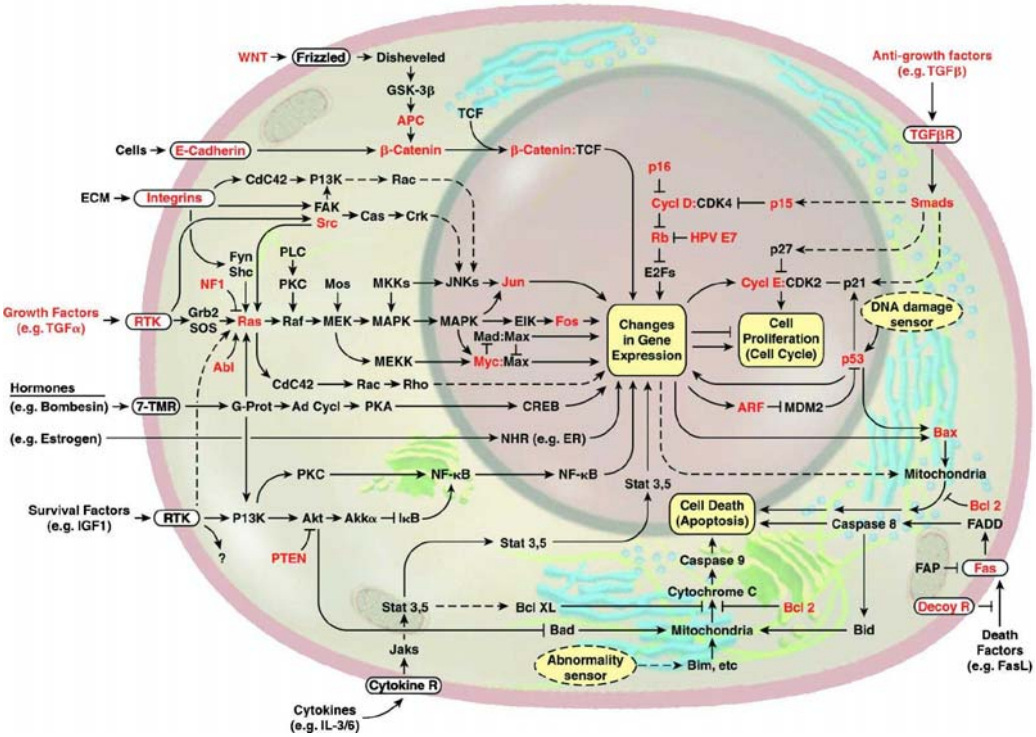

Figure 1. The cellular pathways in cancer (Adopted from Evan GI. and Vousden KH 2001).

1.1.2 Inherited Predisposition

Genetic factors are involved in varying degrees in carcinogenesis. Germ-line mutations in *BRCA1* or *BRCA2* genes confer a high breast cancer risk to the individual; however, such strong predispositions are rare in a population. At the other end of the spectrum are the weak genetic effects (predisposition without evident family-history) that confer a low risk to the individual, even though they may be common in a population.

1.1.2.1 Strong Predisposition

Familial adenomatous polyposis was described at the beginning of 20th century. At that time hereditary cancer syndromes were thought to be very rare until a case-control study showed that a positive family history of stomach or colon cancer meant a three-fold increased risk for those cancers in family members (Brose MS *et al*. 2000).

In 1960's, family studies suggested an autosomal dominant mode of genetic transmission of certain clusters of carcinoma of the breast, ovary and colon (Brose MS *et al*. 2000). In the 1980's, the gene for familial adenomatous polyposis was linked to 5q and then mapped to 5q21 (Brose MS *et al*. 2000). There are now more then 40 germ-line mutations known to be responsible for cancer susceptibility (Table 1).

With the notable exception of *RET* oncogene, the germ-line mutations in hereditary cancers are usually on the tumor suppressor genes which are responsible for regulation of cell cycle and DNA repair. When the entire human genome mapping is completed, more cancer susceptibility genes may be found. The researchers will not be able to match so many genes to hereditary disorders without examining family histories.

General features of hereditary cancer syndromes include the following:

- Vertical transmission of cancer predisposition. This refers to the presence of a genetic predisposition in sequential generations. To have the cancer predisposition a person must inherit it from a parent.

- The mutant gene can be passed on to both male and female children. In the case of breast cancer, the women are at higher risk. Males develop breast cancer rarely. A male who inherits a cancer predisposition and shows no evidence of it can pass the altered gene on to his children.
- When a parent carries an autosomal dominant predisposition, each child has a 50% chance of inheriting the predisposition.
- Clinical characteristics. Patients with an autosomal dominant predisposition are diagnosed at an earlier age than in sporadic cases. Most known mutations that increase breast cancer risk also increase risk of ovarian cancer. In addition, two or more primary cancers such as multiple primary cancers of the same type (e.g. bilateral breast cancer) or primary cancers of different types (e.g. breast and ovarian cancer) can occur in the same individual.

Table 1: List of Familial Cancer Genes and Syndromes

Gene	Cancer syndrome
APC	Familial polyposis of colon
BRCA1	Hereditary Breast/Ovarian Cancer
BRCA2	Hereditary Breast/Ovarian Cancer
CDH1	Familial gastric carcinoma
CDKN2A	Cutaneous malignant melanoma
CDKN1C	Beckwith-Wiedeman Syndrome
CYLD	Familial cylindramotosis
EXT1	Multiple exostoses type 1
EXT2	Multiple exostoses type 2
MADH4	Juvenile Polyposis
MEN1	Multiple endocrine neoplasia type1
MLH1	Hereditary non-polyposis colon cancer
MSH2	Hereditary non-polyposis colon cancer
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type 2
PRKAR1A	Carney Complex
PTCH	Nevoid basal cell carcinoma
PTEN	Cowdens` Syndrome
RB1	Familial Retinoblastoma
RET	Multiple endocrine neoplasia MEN2A, MEN2B and medullary thyroid carcinoma
SDHD	Familial paraganglioma
SMARCB1	Rhabdoid predisposition syndrome
TP53	Li-Fraumeni Syndrome
TSC1	Tuberous Sclerosis 1
TSC2	Tuberous Sclerosis 1
STK11	Peutz-Jegers Syndrome
VHL	Von Hipple-Lindau Syndrome
WT1	Familial Wilms` Tumor

1.1.2.2 Weak Predisposition

Weak predisposition to cancer may result from genetic variations in cancer pathways and low penetrance genes. Subtle sequence variants or polymorphisms may be associated with a small to moderately increased risk for cancer. In sporadic cancers, such factors affecting the probability of the events are very important. Low penetrance gene candidates are found in many pathways such as environmental carcinogen detoxification, steroid hormone metabolism and DNA damage repair. However, polymorphisms in the genes regulating immune response, hormone regulation and apoptosis are also regarded as important genetic factors (Table 2) (Brockmoller J. *et al.* 2000). Identification of these genes will be greatly accelerated by the data from the Human Genome Project (Chakravarti A. 2001).

The search for candidate genes relies on cataloguing the DNA sequence variation within the population and showing that particular variants are significantly associated either with disease susceptibility or with some other aspects of the disease phenotype such as treatment response or survival (Cardon LR. and Bell JI. 2001). The most readily assayed form of genomic variation is a single nucleotide polymorphism (SNP). 2,84 million SNPs have been identified so far and are available from genomic databases (The Interval SNP Map Working Group, 2001). Although SNPs are mostly biallelic and less informative than microsatellite markers, they are more stable mutations. This enables more suitable association studies in which linkage disequilibrium (LD) between markers and an unknown variant is used to map disease-causing mutations. Since SNPs have only two alleles, which can be genotyped by a simple assay, this makes them more suitable to automated analysis. When identifying genes involved in determining complex traits, association studies are better suited for detecting genetic effects of low penetrance with higher resolution. For such studies, many more markers will be required in addition to better statistical tools and high-throughput low-cost genotyping technology to analyze large marker sets in many samples. The performance of numerous analyses on the small surface of oligonucleotide micro-arrays is one of the most promising approaches for large-scale SNP genotyping (Tillib SV. *et al* 2001)

Table 2: Major gene polymorphisms associated with cancer.

Gene	Protein	Function
CYP1A1	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP1A2	Cytochrome P450 1A2	Phase I xenobiotic metabolism
CYP1B1	Cytochrome P450 1B1	Phase I xenobiotic metabolism
CYP2A6	Cytochrome P450 2A6	Phase I xenobiotic metabolism
CYP2C9	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP2C19	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP2D6	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP3A4	Cytochrome P450 1A1	Phase I xenobiotic metabolism
MPO	Myeloperoxidase	Phase I xenobiotic metabolism
DIA4	NAD(P)H: quinone reductase	Phase I xenobiotic metabolism
GSTM1	Glutathione-S-transferase M1	Phase II xenobiotic metabolism
GSTP1	Glutathione-S-transferase P1	Phase II xenobiotic metabolism
GSTT1	Glutathione-S-transferase T1	Phase II xenobiotic metabolism
NAT1	Arylamine N-acetyltransferase type 1	Phase II xenobiotic metabolism
NAT2	Arylamine N-acetyltransferase type 1	Phase II xenobiotic metabolism
SULT1A1	Phenol sulfotransferase 1A1	Phase II xenobiotic metabolism
SULT1A2	Phenol sulfotransferase 1A1	Phase II xenobiotic metabolism
ERCC1	Excision repair cross-complementing rodent repair deficiency, complementation group 1	DNA repair
ERCC2	Excision repair cross-complementing rodent repair deficiency, complementation group 2	DNA repair
XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1	DNA repair
XRRC3	X-ray repair complementing defective repair in Chinese hamster cells 3	DNA repair
XRRC4	X-ray repair complementing defective repair in Chinese hamster cells 4	DNA repair
XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5	DNA repair
MGMT	O-6-methylguanine-DNA methyltransferase	DNA repair

Gene	Protein	Function
POLB	Polymerase (DNA directed), beta	DNA repair
ALOX5	Arachidonate 5-lipoxygenase	Inflammatory and immune response
PTGS1	Prostaglandin-endoperoxide synthase 1	Inflammatory and immune response
PTGS2	Prostaglandin-endoperoxide synthase 2	Inflammatory and immune response
CCR2	Chemokine (C-C motif) receptor 2	Inflammatory and immune response
CCR5	Chemokine (C-C motif) receptor 5	Inflammatory and immune response
IL1A	Interleukin-1	Inflammatory and immune response
TNF	TNF (tumor necrosis factor (TNF superfamily, member 2))	Inflammatory and immune response
VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor	Hormone regulation
CYP11a	Cytochrome P450, subfamily Xia	Hormone regulation
CYP17	Cytochrome P450, subfamily XVII	Hormone regulation
CYP19	Cytochrome P450, subfamily XIX	Hormone regulation
ESRRA	Estrogen-related receptor alpha	Hormone regulation
MC1R	Melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	Hormone regulation
AHR	Aryl hydrocarbon receptor	Nuclear transcription factor receptor
PPARA	peroxisome proliferative activated receptor, alpha	Nuclear transcription factor receptor
PPARG	peroxisome proliferative activated receptor, gamma	Nuclear transcription factor receptor
NR1I2	nuclear receptor subfamily 1, group I, member 2	Nuclear transcription factor receptor
TNFRSF6	tumor necrosis factor receptor superfamily, member 6	Cell cycle regulation and apoptosis
TP53	tumor protein p53	Apoptosis, cell cycle regulation,
CASP10	caspase 10, apoptosis-related cysteine protease	Apoptosis, cell cycle regulation
DFFB	DNA fragmentation factor, 40 kD, beta polypeptide (caspase-activated DNase)	Apoptosis, cell cycle regulation

(Adapted from Brockmoller J. *et al.* 2001)

1.1.2.2.1 Glutathione S-Transferases (*GST*s)

Living organisms are continuously exposed to non-nutritional foreign chemical species. These xenobiotics may harm the organism, causing toxic and sometimes carcinogenic effects. Naturally occurring toxic compounds include plant and fungal toxins (e.g. plant phenols and aflatoxins) and reactive oxygen species (e.g. the superoxide radical and hydrogen peroxide). The enzymatic detoxification of xenobiotics such as polycyclic aromatic hydrocarbons (PAH) has been classified into three distinct phases. Phase I and II involve the conversion of a lipophilic, non-polar xenobiotic into a more water-soluble and therefore less toxic metabolite, which can then be eliminated more easily from the cell (phase III) (Figure 2).

Phase I is catalyzed mainly by the cytochrome P450 system. Phase II enzymes catalyze the conjugation of activated xenobiotics to endogenous water-soluble substrates, such as reduced glutathione (GSH), UDP-glucuronic acid or glycine. In many species, conjugation to reduced glutathione catalyzed by *GST*s is the major phase II reaction. *GST*s can catalyze reactions resulting in the formation of GSH conjugates such as Micheal addition reactions which involve the addition of an enolate ion in a conjugate fashion to α, β-unsaturated ketones, nucleophilic aromatic substitutions, and epoxide ring-opening reactions. The reduction of hydroperoxides is also catalyzed by *GST*s and results in the formation of oxidized glutathione (GSSG) (Hayes JD. and McLellan LI. 1999).

The GSH-xenobiotic conjugate is too hydrophilic to diffuse freely from the cell and must be pumped out actively by a transmembrane ATPase such as the GS-X pump (Ishikawa T. 1992) (Figure 2).

*GST*s are dimeric and mainly cytosolic. In addition to their catalytic role in detoxification, they have extensive ligand binding properties (Barycki JJ. and Colman RF. 1997). Quite distinct from the cytosolic enzymes, a separate microsomal class of *GST*s exists. The microsomal class of *GST*s is designated as `membrane-associated protein in eicosanoid and glutathione` metabolism (MAPEG) (Jakobsson PJ. *et al* 1999).

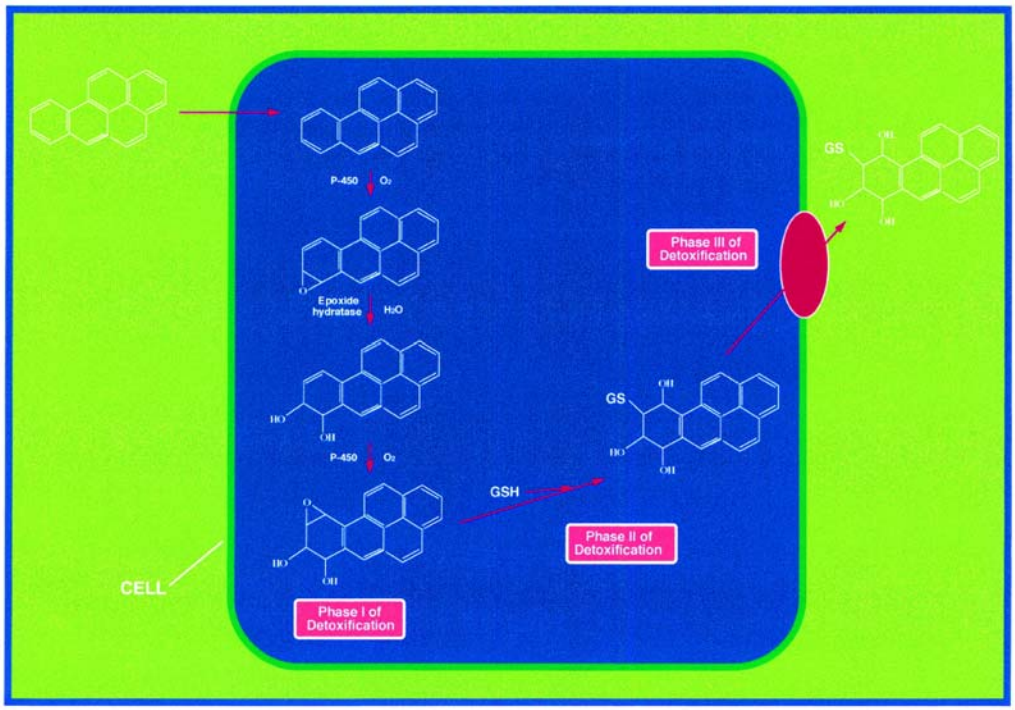

Figure 2: Overview of enzymatic detoxification (adopted from Sheehan D. *et al*. 2001)

The *GST*s comprise a complex and widespread enzyme super-family that has been subdivided into a number of classes by the amino acid/nucleotide sequence, and immunological kinetic and tertiary/quaternary structural properties. Human *GST*s are a family of isozymes that includes at least eight distinct classes: alpha (A), mu (M), pi (P), sigma (S), theta (T), kappa (K), zeta (Z), and omega (O) (Strange CR. *et al*. 2001) (Figure3).

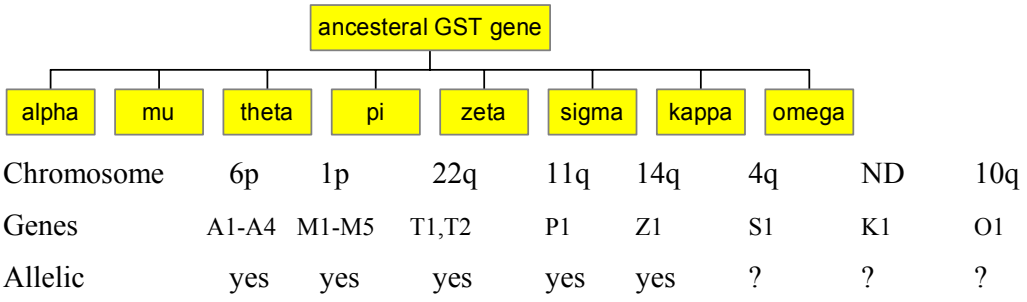

Figure 3: The glutathione S-tranferase super-gene family (Adopted from Strange CR *et al*. 2001).

Several enzymes have been recognized as belonging to the Alpha and Mu classes. While the Pi class originally contained only one protein, *GSTP1,* at least five distinct Mu-class subunits (M1, M2, M3, M4 and M5) have been identified in humans with homologous gene loci (Strange CR *et al*. 2001).

Alpha-class *GST*s comprises 4 types of subunits (A1, A2, A3, and A4) with homologous gene loci in humans. The identification of subgroups within the Alpha class was carried out by comparison of substrate preferences and sequence similarities. The A4 subunit has particularly high activity with ethacrynic acid, lipid hydroperoxides, and 4-hydroxyalkenals (Hubatsch I. e*t al*. 1998).

GSTP1 is involved in the detoxification of base propenals (Norppa H. 1997), and metabolizes carcinogenic products such as benzo-(a)-pyrene dial epoxide, and acrolein, which are derived from cigarette smoke (Seidegard J. and Ekstrom G.1997).

Theta-class enzymes have unique substrate specificity in that they lack activity with 1-chloro-2,4-dinitrobenzene (CDNB), the `universal` *GST* substrate. Two distinct homodimers (*GST1-1* and *GST2-2*) have been identified in humans with the T1 and T2 subunits (Pemble SE. *et al* 1994, and Schroder KR. *et al* 1996).

Human *GSTP1-l* has been shown to catalyze the isomerization of 13-*cis*-retinoic acid to all-*trans*-retinoic acid (Chen H, and Juchau MR 1998). This is an example of an endogenous non-detoxification function for *GST*s. In addition to their isomerization and GSH-conjugation activities, these enzymes contribute to defense against oxidative stress by their role as inhibitors of the Jun N-terminal kinase (Pi class) and their role in selenium-independent GSH peroxidase activities (Alpha class) (Zhao TJ. *et al*. 1998). These activities protects cells against the harmful effects of hydrogen peroxide including cell death (Adler V. *et al* 1999, and Yin Z. *et al* 2000).

GSTT1 detoxifies oxidative products of lipids and DNA. *GSTT2* catalyzes cumene hydroxyoperoxidease (Norpha H. 1997). *GSTT1* enzymes are also involved in the metabolism of carcinogenic substrates, such as methylating agents, pesticides and industrial solvents (Sheehan D. *et al* 2001).

Zeta-class is classified in the theta category (Miller MC. *et al* 2001).

Omega class enzyme shows high activity with CDNB (7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole), p-nitrophenyl acetate and thiol transferase (Sheehan D. *et al* 2001). Omega class *GST*s may act as a GSH-dependent thiol transferase removing S-thiol adducts which some proteins form with GSH and cysteine in response to oxidative stress (Board PG. *et al*. 2000). A novel possible role for Omega

class *GST*s is protecting cells form apoptosis induced by Ca^{2+} mobilization from intracellular stores (Dulhunty A. *et al* 2001).

Polymorphisms in thee genes coding for enzymes involved in protection against oxidative stress have been implicated in predisposition to cancer (Forsberg L. *et al*. 2001).

It is obvious that the activity of *GST*s is highly critical in the detoxification of carcinogens. Alterations in the structure, function or level of expression of *GST* genes or polymorphisms could alter the ability of the cell to inactivate carcinogens and mutagenes, thereby modifying cancer risk. The *GSTM1* and the *GSTT1* genes both exhibit deletion polymorphisms. Homozygous deletions of these genes, called *GSTM1* and *GSTT1* null genotyping, results in lack of enzyme activity (Gudmundsdottir K. *et al*. 2000). An A to G polymorphism at nucleotide 313 in the *GSTP1* gene results in an amino acid substitution (Ile105Val). This residue lies in the substrate-binding site of the enzyme and the polymorphism has been shown to affect enzyme activity (Gudmundsdottir K. *et al*. 2000). A decrease in the *GSTP1* enzyme activity will result in inefficient detoxification of carcinogens and an increase in cancer risk.

The association of *GSTM1* null genotype with cancer was observed mostly in bladder and lung cancers. However, in some studies, *GSTM1* null genotype was found to be associated with breast cancer risk (Table 3).

The results of association studies between *GSTP1* genotype and many cancers including breast cancer are discordant in different populations (Table 4).

The *GSTT1* null genotype seems to be associated with cancers of the larynx, skin, astrocytomas, meningioma, and the myelodysplastic syndrome, but not with cancers of the bladder, stomach, liver, ovary or endometrium (Table 5).

Table 3: The association of GSTM1 null genotype and cancer in case control studies.

Reference	Population	Cancer	# cases	# controls	Comments
(Chen C. *et al.* 1996)	USA mixed	ALL	197	416	Not associated *per se*, but interacts with *GSTT*
(Krajinovic M. *et al.* 1999)	French Canadian	ALL	177	304	Associated
(Saadat I. and Saadat M. 2000)	Iranian	ALL	38	75	Associated
(Chen C. *et al.* 1996)	USA Mixed	AML	96	201	Not associated
(Crump C. *et al.* 2000)	USA mixed	AML	297	152	No risk
(Chen C. *et al.* 1996)	USA mixed	Anal cancer	71	360	Not associated
(Elexpuru-Camiruaga J. *et al.* 1995)	UK Caucasian	Astrocytoma	109	577	Not associated
(Heagerty A. *et al.* 1994)	UK Caucasian	BCC	435	153	Associated
(Heagerty A. *et al.* 1996)	UK Caucasian	BCC	699	561	Associated
(Marshall SE. *et al.* 2000)	UK Mixed	BCC	112	112	Not associated
(Yengi L. *et al.* 1996)	UK	BCC	286	300	Not associated
(Aktas D. *et al.* 2001)	Turkish	Bladder	102	201	Associated, increase risk of invasion
(Anwar WA. *et al.* 1996)	Egyptian	Bladder	22	21	Associated, interacts with *CYP1A1*
(Bell DA. *et al.* 1993)	USA mixed	Bladder	229	211	Associated, interacts with smoking
(Brockmoller J. *et al.* 1996)	German	Bladder	374	373	Associated
(Georgiou I. *et al.* 2000)	Greece	Bladder	89	147	Associated
(Katoh T. *et al.* 1998)	Japanese	Bladder	145	145	Associated, interacts with *GSTT*
(Kempkes M. *et al.* 1996)	German	Bladder	113	170	Associated
(Kim JW. *et al.* 2000)	Korea	Bladder	121	222	Associated, interacts with asthma
(Lin HJ. *et al.* 1994)	USA mixed	Bladder	114	1104	Not associated

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(Heagerty A. *et al.* 1996)	UK Caucasian	BCC	699	561	Associated
(Marshall SE. *et al.* 2000)	UK Mixed	BCC	112	112	Not associated
(Yengi L. *et al.* 1996)	UK	BCC	286	300	Not associated
(Aktas D. *et al.* 2001)	Turkish	Bladder	102	201	Associated, increase risk of invasion
(Anwar WA. *et al.* 1996)	Egyptian	Bladder	22	21	Associated, interacts with *CYP1A1*
(Bell DA. *et al.* 1993)	USA mixed	Bladder	229	211	Associated, interacts with smoking
(Brockmoller J. *et al.* 1996)	German	Bladder	374	373	Associated
(Georgiou I. *et al.* 2000)	Greece	Bladder	89	147	Associated
(Katoh T. *et al.* 1998)	Japanese	Bladder	145	145	Associated, interacts with *GSTT1*
(Kempkes M. *et al.* 1996)	German	Bladder	113	170	Associated
(Kim JW. *et al.* 2000)	Korea	Bladder	121	222	Associated, interacts with asthma
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(Crump C. *et al.* 2000)	USA mixed	AML	297	152	No risk
(Chen C. *et al.* 1996)	USA mixed	Anal cancer	71	360	Not associated
(Elexpuru-Camiruaga J. *et al.* 1995)	UK Caucasian	Astrocytoma	109	577	Not associated
(Heagerty A. *et al.* 1994)	UK Caucasian	BCC	435	153	Associated
(Heagerty A. *et al.* 1996)	UK Caucasian	BCC	699	561	Associated
(Marshall SE. *et al.* 2000)	UK Mixed	BCC	112	112	Not associated
(Yengi L. *et al.* 1996)	UK	BCC	286	300	Not associated
(Aktas D. *et al.* 2001)	Turkish	Bladder	102	201	Associated, increase risk of invasion
(Anwar WA. *et al.* 1996)	Egyptian	Bladder	22	21	Associated, interacts with *CYP2D6*
(Bell DA. *et al.* 1993)	USA mixed	Bladder	229	211	Associated, interacts with smoking
(Brockmoller J. *et al.* 1996)	German	Bladder	374	373	Associated
(Georgiou I. *et al.* 2000)	Greece	Bladder	89	147	Associated
(Katoh T. *et al.* 1998)	Japanese	Bladder	145	145	Associated, interacts with *GSTT1*
(Kempkes M. *et al.* 1996)	German	Bladder	113	170	Associated
(Kim JW. *et al.* 2000)	Korea	Bladder	121	222	Associated, interacts with asthma
(Lin HJ. *et al.* 1994)	USA mixed	Bladder	114	1104	Not associated

Reference	Population	Cancer	#cases	#controls	Comments
(Baranov VS. *et al.* 1996)	Russian	GI	37	67	Associated
(McGlynn KA. *et al.* 1995)	USA Asian	HCC	52	116	Associated
(Omer RE. *et al.* 2001)	Sudan	HCC	110	189	Associated, interacts with peanut butter
(Yu MW. *et al.* 1995)	Taiwan	HCC	30	150	Not associated
(Cheng L. *et al.* 1999)	USA mixed	Head and Neck	162	315	Associated
(Kihara M. *et al.* 1997)	Japanese	Head and Neck	150	474	Associated, interacts with smoking
(Ko Y. *et al.* 2001)	German	Head and Neck			Not associated
(Matthias C. *et al.* 1999)	German	Head and Neck	398	216	Not associated
(McWilliams JE. *et al.* 2000)	USA mixed	Head and Neck	160	114	Not associated
(Morita S. *et al.* 1999)	Japanese	Head and neck	145	164	Not associated
(Olshan AF. *et al.* 2000)	USA mixed	Head and Neck	182	202	Not associated *pwise*, but interacts with *GSTM1*
(Trizna Z. *et al.* 1995)	USA	Head and Neck	186	42	Associated
(Cabelguenne A. *et al.* 2001)	French	Larynx	162	264	Associated
(Hong YC. *et al.* 2000)	Korea	Larynx	82	63	Associated, interact with *GSTT1*
(Jahnke V. *et al.* 1996)	UK Caucasian	Larynx	269	216	Associated
(Jourenkova-Mironova N. *et al.* 1999)	French	Larynx	129	172	Not associated *pwise*, but interacts with *GSTT1*
(Jourenkova, N. 1998)	French	Larynx	129	172	Not associated *pwise*, but interacts with *GSTM1*
(Yuille M. *et al.* 2002)	UK	Leukemia	138	280	Associated
(Lemos MC. *et al.* 1999)	Portuguese	Leukemia (mixed)	64	128	Not associated
(Nair UJ. *et al.* 1999)	Indian	Leukoplakia	98	82	Associated
(Alexandrie AK. *et al.* 1994)	Swedish	Lung	296	329	Not associated
(Bolgubova EV. *et al.* 2000)	Russian	Lung	58	297	No risk

Reference	Population	Cancer	# cases	# controls	Comments
(Bennett W P. *et al.* 1999)	USA Mixed	Lung	106		Smoking, interacts with *GSTM1* null hebotype
(Brockmoller J. *et al.* 1993)	German	Lung	117	200	Not associated
(Chen C. *et al.* 2001)	Chinese	Lung	106	106	Combined risk with *CYP1A1* Valallele
(Dresler C M. *et al.* 2000)	USA mixed	Lung	180	163	Combined risk with *CYP1A1* for females
(El-Zein RA. *et al.* 1997)	USA Caucasian	Lung	52	48	Associated
(Ford JG. *et al.* 2000)	USA Black	Lung	117	120	Associated, interacts with smoking
(Gao Y. and Zhang Q. 1999)	Chinese	Lung	59	132	Associated
(Hirvonen A. *et al.* 1993)	Finn	Lung	138	142	Associated
(Hou SM. *et al.* 2000)	Norwegian	Lung	282	357	Associated, interacts with *NAT2*
(Kelsey KT. *et al.* 1997)	USA mixed	Lung	168	278	No association
(Kihara M. and Noda K. 1994)	Japanese	Lung	178	201	Associated, interacts with smoking
(Kihara M. and Noda K. 1995)	Japanese	Lung	447	469	Associated, interacts with smoking
(Kihara M. and Noda K. 1995)	Japanese	Lung	118	301	Associated, interacts with smoking, and *CYP1A1*
(Kihara M. and Noda K. 1999)	Japanese	Lung	382	257	Associated, interacts with *GSTT1* and smoking
(Lan Q. *et al.* 2000)	China	Lung	122	122	Associated, interacts with charcoal smoke
(Lewis JS. *et al.* 2002)	UK	Lung	94	165	No risk
(London SJ. *et al.* 1995)	USA mixed	Lung	342	716	Not associated
(Miller PD. *et al.* 2002)	USA mixed	Lung	767	927	Not associated
(Moreira A. *et al.* 1996)	Portuguese	Lung	98	84	Not associated
(Persson I. *et al.* 1999)	Chinese	Lung	76	122	Not associated
(Ryberg D. *et al.* 1997)	Norwegian	Lung	63	177	Associated
(Saarikoski ST. *et al.* 1998)	Finn	Lung	208	294	Not associated *per se*, interacts with *GSTM1*

Reference	Population	Cancer	# cases	# controls	Comments
(Stucker I. *et al.* 2000)	French	Lung	247	254	Associated, interacts with *CYP1A1*
(To-Figueras J. *et al.* 1996)	Spanish	Lung	139	147	Associated, interacts with *TPMT*
(Woodson K. *et al.* 1999)	USA mixed	Lung	319	333	No association
(Xue K. *et al.* 2001)	Chinese	Lung	112	112	Associated, interacts with *CYP1A1*
(Baranov VS. *et al.* 1996)	Russian	Lung,	58	67	Associated
(Deakin M. *et al.* 1996)	UK Caucasian	Lung,	108	577	Not associated
(Davies SM. *et al.* 2000)	USA Caucasian	MDS	232	153	Associated
(Heagerty AH. *et al.* 1994)	UK Caucasian	Melanoma	64	153	Not associated
(Kanetsky PA. *et al.* 2001)	USA Caucasian	Melanoma	362	271	Not associated *per se*, but interacts with hair color
(Lafuente A. *et al.* 1995)	Spanish	Melanoma	183	147	Associated
(Shanley SM. *et al.* 1995)	Australia	Melanoma	124	100	Not associated
(Elexpuru-Camiruaga J. *et al.* 1995)	UK Caucasian	Meningioma	49	577	Not associated
(Hirvonen A. *et al.* 1995)	Finn	Mesothelioma	44	270	Associated, interacts with smoking
(Buch SC. *et al.* 2002)	Indian	Oral	297	450	Associated
(Deakin M. *et al.* 1996)	UK Caucasian	Oral	40	577	Not associated
(Hahn M. *et al.* 2002)	German	Oral	94	92	Not associated
(Hung HC. *et al.* 1997)	Taiwanese	Oral	41	123	Associated, interacts with *GSTT1*
(Katoh T. *et al.* 1999)	Japanese	Oral	92	147	Associated
(Kietthubthew S. *et al.* 2001)	Thailand	Oral	53	53	Associated, interacts with smoking
(Park LY. *et al.* 2000)	USA Black	Oral	63	103	Associated, interacts with smoking
(Baxter SW. *et al.* 2001)	Australia	Ovarian	293	219	Associated
(Lallas TA. *et al.* 2000)	USA mixed	Ovarian	80	80	Not associated

Reference	Population	Cancer	# cases	# controls	Comments
(Sarhanis P. *et al.* 1996)	UK Caucasian	Ovary	84	312	Not associated
(Spurdle A.B. *et al.* 2001)	Australian	Ovary	285	299	Associated with endometriosis, and clear cell Ca
(Liu G. *et al.* 2000)	Canada (mixed)	Pancreas	149	149	Not associated
(Jourenkova-Mironova N. *et al.* 1999)	French	Pharynx	121	172	Not associated
(Fryer AA. *et al.* 1993)	UK Caucasian	Pituitary adenoma	113	89	Associated
(Autrup JL. *et al.* 1999)	Danish	Prostate	153	288	Associated
(Gsur A. *et al.* 2001)	Austrian	Prostate	166	166	Not associated
(Kelada SN. *et al.* 2000)	USA mixed	Prostate	276	499	Not associated
(Kote-Jarai Z. *et al.* 2001)	UK Mixed	Prostate	275	280	Not associated
(Murata M. *et al.* 2001)	Japanese	Prostate	126	126	Not associated
(Rebbeck TR. *et al.* 1999)	USA Mixed	Prostate	237	239	Not associated
(Bruning T. *et al.* 1997)	German	RCC	45	48	Associated
(Longuemaux S. *et al.* 1999)	French	RCC	173	211	Not associated *per se*, but interacts with *GSTT1* and *M1T1*
(Sweeney C. *et al.* 2000)	USA Mixed	RCC	130	505	No association
(Heagerty A.H. *et al.* 1994)	UK Caucasian	SCC	85	153	Not associated
(Setiawan VW. *et al.* 2000)	Chinese	Stomach	91	429	Not associated
(Kato S. *et al.* 1996)	Japanese	Stomach	82	151	Not associated
(Saadat I. and Saadat M. 2001)	Iranian	Stomach	46	131	Associated, interacts with *GSTT1*
(Deakin M. *et al.* 1996)	UK Caucasian	Stomach	136	577	Not associated
(Chen C. *et al.* 1999)	USA Mixed	Vulva	137	248	No risk

Table 4: The association of *GSTP1* Ile 105 Val polymorphism and cancer in case control studies.

Reference	Population	Cancer	# case	# control	Comments
(Marshall SE. *et al.* 2000)	UK Mixed	BCC	112	112	Val/Val is associated
(Harries LW. *et al.* 1997)	UK mixed	Bladder	76	155	Not associated
(Steinhoff C. *et al.* 2000)	German	Bladder	135	127	Not associated
(Tornuer GA. *et al.* 2001)	Turkish	Bladder	121	121	*GSTP1* Ile/Val or Val/Val is associated in combination with *GSTM1*
(Curran JE. *et al.* 2000)	Australian	Breast	129	129	Not associated
(Helzlsouer KJ. *et al.* 1998)	USA mixed	Breast	110	133	Val allele is associated, and interacts with *GSTM1*
(Krajinovic M. *et al.* 2001)	French-Canadian	Breast	149	207	Not associated
(Lavigne JA. *et al.* 1997)	USA mixed	Breast	112	112	Not associated
(Maugard CM. *et al.* 2001)	French	Breast	220	196	Ile allele is associated
(Millikan R. *et al.* 2000)	USA mixed	Breast	688	561	Not associated
(Mitrunen K. *et al.* 2001)	Finn	Breast	483	482	Not associated *per se*, but interacts with *GSTT1*, *GSTM1*
(Harris MJ. *et al.* 1998)	Australian	Colon	131	199	Not associated
(Katoh T. *et al.* 1999)	Japanese	Colon	47	122	Not associated
(Welfare M. *et al.* 1999)	UK Mixed	Colon	178	178	Not associated
(Yoshioka M. *et al.* 1999)	Japanese	Colon	106	100	Not associated *per se*, but interacts with *GSTM1*
(Loktionov A. *et al.* 2001)	UK	Colorectal	206	355	Not associated
(Tan W. *et al.* 2000)	Chinese	Esophagus	150	146	Not associated
(van Lieshout EM. *et al.* 1999)	Holland	Esophagus	98	247	Val/Val is associated

Reference	Population	Cancer	#cases	#controls	Comments
(Lee JM. *et al.* 2000)	Taiwanese	Esophagus	90	254	Ile/Ile is associated, and interacts with smoking
(Lin DX. *et al.* 1998)	Chinese	Esophagus	45	45	Not associated
(Steiwan WW. *et al.* 2001)	Chinese	Gastric	133	433	Not associated
(Morita S. *et al.* 1999)	Japanese	Head and neck	145	164	Ile/Ile is associated
(Olshan AF. *et al.* 2000)	USA mixed	Head and Neck	182	202	Not associated
(Jourenkova-Mironova N. *et al.* 1999)	French	Larynx	129	172	Not associated
(Cabelguenne A. *et al.* 2001)	French	Larynx	162	264	Val/Val is associated
(Yuille M. *et al.* 2002)	UK	Leukemia	138	280	Ile/Ile is associated
(Harris MJ. *et al.* 1998)	Australian	Lung	184	199	Not associated
(Katoh T. *et al.* 1999)	Japanese	Lung	382	257	Not associated
(Kihara M. and Noda K. 1999)	Japanese	Lung	382	257	Not associated *per se*, but interacts with *GSTM1*
(Ryberg D. *et al.* 1997)	Norwegian	Lung	135	342	Associated, interacts with *GSTM1*
(Saarikoski ST. *et al.* 1998)	Finn	Lung	208	294	Not associated
(To-Figueras J. *et al.* 1999)	Spanish	Lung	164	200	Not associated
(Lewis JS. *et al.* 2002)	UK	Lung	94	165	Not associated
(Miller PD. *et al.* 2002)	USA mixed	Lung	767	927	Not associated
(Katoh T. *et al.* 1999)	Japanese	Oral	83	122	Val/Val is associated
(Matthias C. *et al.* 1998)	German	Oral/Pharynx	380	180	Val/Val is associated
(Spurdle AB. *et al.* 2001)	Australian	Ovary	285	299	Not associated

Reference	Population	Cancer	#cases	#controls	Comments
(Jourenkova-Mironova N. *et al.* 1999)	French	Pharynx	121	172	Not associated
(Autrup JL. *et al.* 1999)	Danish	Prostate	153	288	Not associated
(Gsur A. *et al.* 2001)	Austrian	Prostate	166	166	Ile/Ile is associated
(Harries LW. *et al.* 1997)	UK mixed	Prostate	36	155	Val/Val is associated
(Kote-Jarai Z. *et al.* 2001)	UK Mixed	Prostate	275	280	Not associated
(Wadelius M. *et al.* 1999)	Sweden, Danish	Prostate	425	425	Not associated
(Jeronimo C. *et al.* 2002)	USA mixed	Prostate	105	141	Not associated
(Longuemaux S. *et al.* 1999)	French	RCC	173	211	Val allele is associated and interacts with <GSTM1
(Sweeney C. *et al.* 2000)	USA mixed	RCC	130	505	Not associated
(Katoh T. *et al.* 1999)	Japanese	Stomach			Not associated
(Harries LW. *et al.* 1997)	UK mixed	Testis			Not associated
(Katoh T. *et al.* 1999)	Japanese	Urothelial			Not associated

Table 5: The association of *GSTT1* null genotype and cancer in case control studies.

Reference	Population	Cancer	# cases	# controls	Comments
(Infante-Rivard C. *et al.* 1999)	French-Canadian	ALL	491	491	Not associated
(Krajinovic M. *et al.* 1999)	French-Canadian	ALL	177	304	Not associated
(Crump C. *et al.* 2000)	USA mixed	AML	297	152	Not associated
(Chen C. *et al.* 1996)	USA mixed	Anal cancer	71	360	Not associated
(Elexpuru-Camiruaga J. *et al.* 1995)	UK Caucasian	Astrocytoma	109	577	Associated
(van Lieshout EM. *et al.* 1999)	Holland	Barret's esophagus	98	247	Not associated
(Heagerty A. *et al.* 1996)	UK Caucasian	BCC	699	561	Not associated
(Marshall SE. *et al.* 2000)	UK Mixed	BCC	112	112	Not associated
(Yengi L. *et al.* 1996)	UK	BCC	286	300	Not associated
(Broekmoller J. *et al.* 1996)	German	Bladder	374	373	Not associated
(Georgiou I. *et al.* 2000)	Greek	Bladder	89	147	Not associated
(Katoh T. *et al.* 1998)	Japanese	Bladder	145	145	Not associated, but interacts with *GSTM1*
(Kempkes M. *et al.* 1996)	German	Bladder	113	170	Not associated, but interacts with smoking
(Kim W J. *et al.* 2000)	Korea	Bladder	121	222	Not associated
(Sahgovic J. *et al.* 1999)	Slovakian	Bladder	76	248	Associated, interacts with *GSTM1*, and smoking
(Schmakenberg E. *et al.* 2000)	German	Bladder	157	223	Not associated
(Steinhoff C. *et al.* 2000)	German	Bladder	135	127	Not associated
(Bailey LR. *et al.* 1998)	USA mixed	Breast	263	263	Not associated

Reference	Population	Cancer	# cases	# controls	Comments
(Curran JE. *et al.* 2000)	Australian	Breast	129	129	Not associated
(Cherrier J. *et al.* 1999)	French	Breast	361	437	Association with postmenopausal risk
(Helzlsouer KJ. *et al.* 1998)	USA mixed	Breast	110	133	Not associated
(Millikan R.. *et al.* 2000)	USA mixed	Breast	688	561	Not associated
(Mitrunen K. *et al.* 2001)	Finn	Breast	483	482	Not associated
(Park JY. *et al.* 1997)	Korea	Breast	189	189	Associated, interacts with *GSTM1*
(Goodman MT. *et al.* 2001)	USA Hawaii	Cervix	131	180	Not associated
(Kim WJ. *et al.* 2000)	Korean	Cervix	181	181	Associated, interacts with *GSTM1*
(Warwick AP. *et al.* 1994)	UK	Cervix	175	180	Associated
(Abdel-Rahman SZ. *et al.* 1999)	Egyptian	Colon	66	55	Not associated
(Butler WJ. *et al.* 2001)	Australian	Colon	219	200	Not associated
(Chenevix-Trench G. *et al.* 1995)	Australian	Colon	132	100	Not associated
(Deakin M. *et al.* 1996)	UK Caucasian	Colon	252	577	Associated
(Gertig D M. *et al.* 1998)	USA mixed	Colon	212	221	Not associated
(Guo JY. *et al.* 1996)	Chinese	Colon	19	23	Associated
(Inoue K. *et al.* 2001)	Japanese	Colon	205	220	Not associated
(Katoh T. *et al.* 1996)	Japanese	Colon	103	126	Associated
(Saadat I. and Saadat M. 2001)	Iranian	Colon	42	131	Not associated *p=.ns*, interacts with *GSTM1*
(Welfare M. *et al.* 1999)	UK	Colon	178	178	No association
(Zhang H. *et al.* 1999)	Swedish	Colon	99	109	Associated
(Loktionov A. *et al.* 2001)	UK	Colorectal	206	355	Not associated

Reference	Population	Cancer	#cases	#controls	Comments
(Tan W. *et al.* 2000)	Chinese	Esophagus	150	146	No association
(Lin DX. *et al.* 1998)	Chinese	Esophagus	45	45	Associated, interacts with *GSTT1*
(Esteller M. *et al.* 1997)	Spanish	Endometrium	80	60	Not associated
(Katoh T. *et al.* 1996)	Japanese	Gastric	139	126	Associated
(Wiencke JK. *et al.* 1997)	USA Caucasian	Glioma	188	166	Associated with oligodendroglioma
(Omer RE. *et al.* 2001)	Sudan	HCC	110	189	Associated, interacts with peanut butter
(Yu MC. *et al.* 1995)	Taiwan	HCC	30	150	Not associated
(Cheng L. *et al.* 1999)	USA mixed	Head and Neck	162	315	Associated ,interacts with *GSTT1*
(Ko Y. *et al.* 2001)	German	Head and Neck			Not associated
(Matthias C. *et al.* 1999)	German	Head and Neck	398	216	Not associated
(McWilliams JE. *et al.* 1995)	USA mixed	Head and Neck	160	114	Not associated
(Olshan AF. *et al.* 2000)	USA mixed	Head and Neck	182	202	Not associated *per se*, but interacts with smoking
(Trizna Z. *et al.* 1995)	USA	Head and Neck	186	42	Not associated
(Hong YJ. *et al.* 2000)	Korea	Larynx	82	63	Not associated *per se*, but interacts with *GSTT1*
(Jahuke V. *et al.* 1996)	UK Caucasian	Larynx	269	216	Associated
(Jourenkova N. *et al.* 1998)	French	Larynx	129	172	Not associated *per se*, but interacts with *GSTT1*
(Jourenkova-Mironova N. *et al.* 1999)	French	Larynx	129	172	Not associated *per se* but interacts with *GSTT1*
(Cabelguenne A. *et al.* 2001)	French	Larynx	162	264	Associated
(Yuille M. *et al.* 2002)	UK	Leukemia	138	280	Associated
(Nair UJ. *et al.* 1999)	Indian	Leukoplakia	98	82	Associated

Reference	Population	Cancer	#cases	#controls	Comments
(El-Zein RA. *et al.* 1997)	USA Caucasian	Lung	52	48	Associated
(Kelsey KT. *et al.* 1997)	USA mixed	Lung	168	278	Not associated
(Bennett WP. *et al.* 1999)	USA mixed	Lung	106		Not associated
(Kihara M. and Noda K.1994)	Japanese	Lung	178	201	Associated, interacts with smoking
(Lan Q. *et al.* 2000)	Chinese	Lung	122	122	Not associated
(Saarikoski ST. *et al.* 1998)	Finnish	Lung	208	294	Not associated *per se*, interacts with *GSTM1*
(To-Figueras J. *et al.* 1996)	Spanish	Lung	139	147	Not associated
(Xue K. *et al.* 2001)	Chinese	Lung	112	112	Associated, interacts with *CYP1A1*
(Lewis JS. *et al.* 2002)	UK	Lung	94	165	Not associated
(Deakin M. *et al.* 1996)	UK Caucasian	Lung,	108	577	Not associated
(Chen C. *et al.* 1996)	USA mixed	MDS	96	201	Associated
(Davies SM. *et al.* 2001)	USA Caucasian	MDS	232	153	Not associated
(Kanetsky PA. *et al.* 2001)	USA Caucasian	Melanoma	362	271	Not associated *per se*, but interacts with hair color
(Shanley SM. *et al.* 1995)	Australian	Melanoma	124	100	Not associated
(Elexpuru-Camiruaga J. *et al.* 1995)	UK Caucasian	Meningioma	49	577	Associated
(Deakin M. *et al.* 1996)	UK Caucasian	Oral	40	577	Not associated
(Hung HC. *et al.* 1997)	Taiwanese	Oral	41	123	Associated, interacts with *GSTM1*
(Katoh T. *et al.* 1999)	Japanese	Oral	92	147	Not associated
(Kietthubthew S. *et al.* 2001)	Thailand	Oral	53	53	Not associated
(Buch SC. *et al.*2002)	Indian	Oral	297	450	Not associated but interacts with *GSTM1*
(Sarlanis P. *et al.* 1996)	UK Caucasian	Ovary	84	312	Not associated

Reference	Population	Cancer	# cases	# controls	Comments
(Liu G. *et al.* 2000)	Canada (mixed)	Pancreas	149	149	Not associated
(Jourenkova-Mironova N. *et al.* 1999)	French	Pharynx	121	172	Associated
(Spurdle A.B. *et al.* 2001)	Australian	Ovary	285	299	Not associated
(Autrup JL. *et al.* 1999)	Danish	Prostate	153	288	Not associated, but interacts with *GSTM1*
(Gsur A. *et al.* 2001)	Australian	Prostate	166	166	Not associated
(Kelada SN. *et al.* 2000)	USA mixed	Prostate	276	499	Associated, interacts with smoking
(Kote-Jarai Z. *et al.* 2001)	UK Mixed	Prostate	275	280	Not associated
(Murata M. *et al.* 2001)	Japanese	Prostate	126	126	Not associated
(Rebbeck TR. *et al.* 1999)	US Mixed	Prostate	237	239	Associated
(Bruning T. *et al.* 1997)	German	RCC	45	48	Associated
(Longuemaux S. *et al.* 1999)	French	RCC	173	211	Not associated *per se*, but interacts with *GSTT1* and *MET*
(Sweeney C. *et al.* 2000)	USA Mixed	RCC	130	505	Associated
(Setiawan VW. *et al.* 2000)	Chinese	Stomach	91	429	Associated
(Kato S. *et al.* 1996)	Japanese	Stomach	82	151	Not associated
(Saadat I. and Saadat M. 2001)	Iranian	Stomach	46	131	Associated, interacts with *GSTT1*
(Deakin M. *et al.* 1996)	UK Caucasian	Stomach	136	577	Not associated

1.1.3 Genetic Events Outside the Cancer Pathway

Genetic variations may determine the outcome of interactions between exogenous carcinogens and the cell. Such gene-environment interaction between exposure to certain chemicals and genetic variations may increase cancer risk. Although variations may account for large and important differences in cancer susceptibility in the population, information on the gene-environment interaction may show us ways of reducing these risks. Tissue specific expressions of genes may indicate the relation between the tissue specific genes and exposures (Willams JA. 2001).

Variations in the circulating levels of growth factors or hormones increase cancer risk. It has been shown that prolonged exposure to estrogen is associated with an increased risk of developing breast cancer. Therefore, factors that increase the number of menstrual cycles such as early age at menarche, nulliparity, and the late onset of menopause increase the probability of breast cancer (Michels B. *et al*. 2001)

Several factors influence the evolution of cancer (Figure 4).

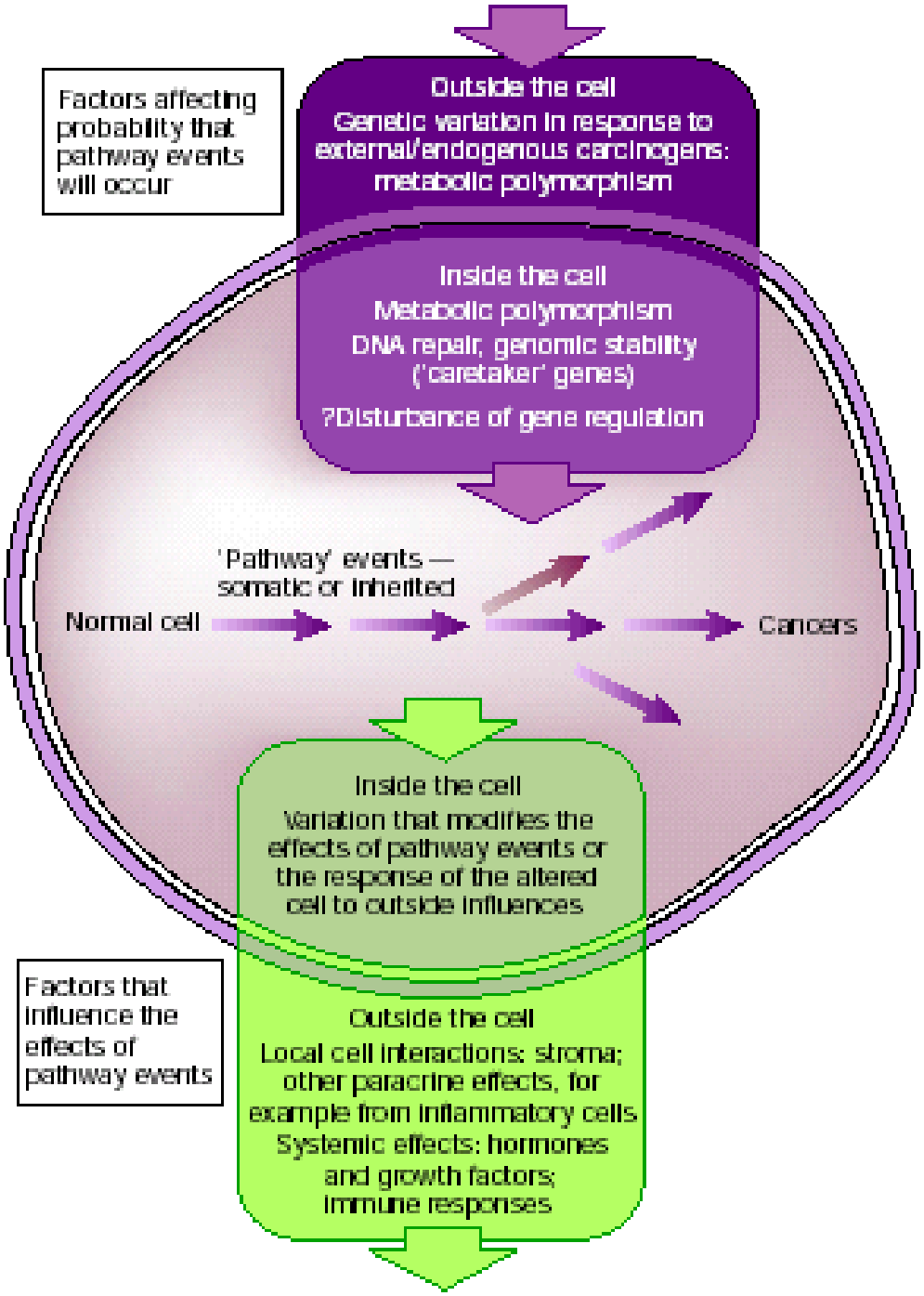

Factors affecting probability that pathway events will occur
Outside the cell
Genetic variation in response to external/endogenous carcinogens: metabolic polymorphism
Inside the cell
Metabolic polymorphism
DNA repair, genomic stability ('caretaker' genes)
?Disturbance of gene regulation
'Pathway' events — somatic or inherited
Normal cell
Cancers
Inside the cell
Variation that modifies the effects of pathway events or the response of the altered cell to outside influences
Factors that influence the effects of pathway events
Outside the cell
Local cell interactions: stroma; other paracrine effects, for example from inflammatory cells
Systemic effects: hormones and growth factors; immune responses

Figure 4: A framework for genetic events related to cancer development (adopted from Ponder BAJ. 2001).

1.2 Breast Cancer

1.2.1 Clinical Information

1.2.1.1 Epidemiology and Etiology

Breast cancer is the most commonly diagnosed cancer among women, after nonmelanoma skin cancer. Breast cancer is the second leading cause of cancer deaths after lung cancer. In 2002, an estimated 205,000 new cases will be diagnosed and 40,000 deaths from breast cancer will occur in USA (Atlanta GA. 2002).

Breast cancer is a complex, multifactorial disease where both genetic and environmental factors have important contributions. The cumulative risk of breast cancer increases with age with most breast cancers occurring after the age of 50 (Feuer EJ. *et al*. 1993). Breast cancer occurs at an earlier age in women with a genetic susceptibility. Breast cancer risk increases with early menarche and late menopause, and is reduced by early first full term pregnancy. It is reported that these factors influence breast cancer risk only among women who did not have a mother or sister with breast cancer (Colditz GA. *et al*. 1996). However, a protective effect has been seen with early age at first live birth, and also with parity of 3 or more, in women with known mutations of the *BRCA1* gene (Norad S. *et al*. 1993, and Norad SA. *et al*. 1995). The effect of reproductive history can only be explained by the contribution of other factors to breast cancer. Several lifestyle factors such as weight gain, obesity, fat intake, and level of physical activity are also associated with breast cancer risk. Overweight women are most commonly observed to be at increased risk of postmenopausal breast cancer and at reduced risk of premenopausal breast cancer that is thought to be estrogen related. However, these factors have not been well evaluated in women with a positive family history of breast cancer or in carriers of cancer-predisposing mutations. Similarly, alcohol consumption and a high-fat diet may be associated with an increased risk. Other risk factors may be important in subgroups of women defined according to genotype. For example,

polymorphisms of *NAT* gene have been observed to influence female smokers' risk for breast cancer (Ambrosone CB. *et al*. 1996).

Breast cancer is the most common cancer in females in Turkey (Ozsari H. and Atasever L. 1997). The life-time prevalence of the disease ranges between 1 in 8 to 1 in 12 in Western populations (Pharoah PD. and Mackay JF. 1998, and National Cancer Institute 1999).

1.2.2. Genetic Predisposition to Breast Cancer

Genetic factors influence the development of breast cancer. Females with germ-line mutations in *BRCA1* or *BRCA2* genes have an extremely high risk of developing breast cancer, but such strong predispositions are rare. Approximately 10-15% of breast cancer cases have a family history of the disease. Germ-line *BRCA1* and *BRCA2* mutations have been identified in approximately 5% of women diagnosed with breast cancer (Claus EB. *et al*. 1996, and Ozdag H. *et al*. 2000). Somatic mutations are absent in *BRCA1* and a very low frequency of *BRCA2* mutations exist in breast cancer cases. Mutations in *BRCA1* and *BRCA2* interacting proteins may affect their function. Another gene causing predisposition to very rare breast cancer susceptibility is *TP53* (Borresen AL. *et al*. 1992). The most interesting polymorphism of the *TP53* gene is Arg72Pro polymorphism. Studies on this polymorphism in various cancers reveal quite discordant results. The interaction of *p53* with *p73* is influenced by this polymorphism.

Other genetic variations confer a low risk to the individual, but are common in a population. Weak predisposition to breast cancer may result from genetic variations in cancer pathways and low penetrance genes. These polymorphically expressed low penetrance genes code for the enzymes that may have a role in the metabolism of estrogens or detoxification of drugs and environmental carcinogens. Although the clinical significance in breast cancer is unclear, genetic polymorphisms may account for the individual differences in sensitivity to carcinogens such as estrogen metabolites.

Molecular epidemiology studies of breast cancer have found associations with P450 cytochrome genotypes such as *CYP1A1*, *CYP2D6*, and *CYP17* (Table 7). Studies of the *NAT2* genotype and breast cancer susceptibility have shown inconsistent results (Table 6).

Individuals with a polymorphism in the *GSTM1*, *GSTT1* or *GSTP1* genes may have a higher risk of breast cancer because of their impaired ability to metabolize and eliminate carcinogens. Carcinogens such as PAHs, are lipophilic and stored in adipose tissues, including breast tissue (Wu F. *et al*. 2002). The most extensively studied polymorphisms in human breast cancer are associated with carcinogen-metabolism (Table 6, and Table 7).

The results of association studies between *GST* genotypes and breast cancer are discordant in different populations (Rebbeck TR. *et al*. 1997, Helzlsouer KJ. *et al*. 1998, Ambrosone CB. *et al*. 1999, and Maugard CM. *et al*. 2001) despite this neat theoretical framework.

Table 6: Genetic association (case control) studies in breast cancer.

Reference	Population	Genes	# cases	# controls	Comments
(Zheng W. *et al*2000)	Chinese	*CYP1B1*	186	200	Association for *CYP1B1*
(Amorim E. *et al*2002)	Brazilian	*CYP1A1* *GSTM1* *GSTT1*	128	256	Association for *CYP1A1* No Association for *GSTM1* No Association for *GSTT1* Combined effects of *GSTM1* and *GSTT1*
(Matheson C M. *et al*2002)	Australian	*GSTT1* *GSTM1* *NAT2*	157	157	Association for *GSTT1* No Association for *GSTM1* No Association for *NAT2*
(Mitrunen K. *et al*2002)	Finnish	*CYP1B1* *GSTP1* *GSTT1* *GSTM1*	483	482	Association for *CYP1B1* interacts with hormone replacement therapy Association for *GSTP1* interacts with hormone replacement therapy Association for *GSTT1* interacts with hormone replacement therapy No Association for *GSTM1* interacts with hormone replacement therapy Combined effects of *GSTM1* and *CYP1B1*
(Wu P.Y. *et al*2002)	Taiwan	*CYP1A1* *NAT2* *GSTM1* *GSTT1*	60	60	No Association for *CYP1A1* No Association for *NAT2* No Association for *GSTM1* No Association for *GSTT1*
(Gudmundsdottirk K. *et al*2002)	Icelandic	*GSTM1* *GSTT1*	500	395	No Association for *GSTM1* No Association for *GSTT1*

Reference	Population	Genes	#cases	#controls	Comments
(Gudmundsdottirk K. *et al*2002)	Icelandic	*GSTT1*			No Association for *GSTT1*
(Lavigne J.A. *et al*1997)	USA mixed	*CYP1B1* *GSTM1* *GSTT1*	112	112	Association for *CYP1B1* in postmenopausal women No Association for *GSTM1* No Association for *GSTT1*
					Combines effects of *CYP1B1*, *GSTM1* and *GSTT1* in postmenopausal women
(Maugard M.C. *et al*2001)	Caucasian	*GSTT1* *GSTM1*	220	196	Association for *GSTT1* No Association for *GSTM1*
(Deitz C.A. *et al*2000)	Caucasian	*NAT2*	174	387	Association for *NAT2* among postmenopausal
(Millikan R. *et al*2000)	USA mixed	*GSTM1* *GSTT1* *GSTT1*	688	663	No Association for *GSTM1* No Association for *GSTT1* No Association for *GSTT1*
(Curran E.J. *et al*2001)	Australian	*ESR*	125	125	Association for *ESR*
		GRL			Association for *GRL*
(Krajinovic M. *et al*2001)	French-Canadian	*CYP1A1* *CYP2D6* *GSTM1* *GSTT1* *GSTT1* *NAT1* *NAT2*	414	429	Association for *CYP1A1* No Association for *CYP2D6* No Association for *GSTM1* No Association for *GSTT1* No Association for *GSTT1* Association for *NAT1* Association for *NAT2*
(Helzlsouer J.K. *et al*1998)	USA mixed	*GSTM1*	115	115	Association for *GSTM1*

Reference	Population	Gene	# cases	# controls	Comments
(Helzlsouer J.K. *et al*/1998)	USA mixed	*GSTT1*			Association for *GSTT1*
(Helzlsouer J.K. *et al*/1998)	USA mixed	*GSTP1*			Association for *GSTP1*
					Combined effect of *GSTM1*, *GSTT1*, and *GSTP1*
(Zhao M. *et al*/2001)	USA mixed	*GSTP1*	273	657	No Association for *GSTP1*
(Ambrosone C B *et al*/1999)	USA mixed	*GSTM1*	279	340	No Association for *GSTM1*
(Garcia-Closas M. *et al*/1999)	USA mixed	*GSTM1*	466	466	No Association for *GSTM1*
		GSTT1			No Association for *GSTT1*
(Zhong S. *et al*/1993)	Dundee	*GSTM1*	197	225	No Association for *GSTM1*
(Dialyna I.A. *et al*/2001)	Greek	*CYP1A1*	207	171	No Association for *CYP1A1*
		GSTM1			No Association for *GSTM1*
		GSTP1			No Association for *GSTP1*
		GSTT1			No Association for *GSTT1*
(Ambrosone C B. *et al*/1995)	USA mixed	*CYP1A1*	216	282	Association for *CYP1A1* interacts with smoking
		GSTM1			Association for *GSTM1* in young postmenopaousal women

Table 7. *CYPs* and Breast Cancer in women.

Reference	Population	Gene	# cases	# controls	Comments
(Rebbeck *et al.*1994)	Caucasian	*CYP1A1*	96	126	No Association for *CYP1A1*
(Taioli *et al.*1995)	Caucasian	*CYP1A1*	30	183	Association for *CYP1A1*
(Bailey *et al.*1998)	Caucasian	*CYP1A1*	164	164	No Association for *CYP1A1*
(Ishibe *et al.*1998)	USA mixed	*CYP1A1*	466	466	No Association for *CYP1A1*
(Moysich *et al.*1999)	USA mixed	*CYP1A1*	154	192	No Association for *CYP1A1*
(Ambrosone C A. *et al.*1995)	USA mixed	*CYP1A1*	216	282	No Association for *CYP1A1* in postmenopausal women
(Bailey *et al.*1998)	Caucasian	*CYP1B1*	16	164	No Association for *CYP1B1*
(Buchert *et al.*1996)	USA mixed	*CYP2D6*	167	114	No Association for *CYP2D6*
(Ladona *et al.*1996)	Spanish	*CYP2D6*	151	187	Association for *CYP2D6*
(Shields *et al.*1996)	Caucasian	*CYP2E1*	166	221	No Association for *CYP2E1*
(Freigelson *et al.*1997)	USA mixed	*CYP17*	174	285	No Association for *CYP17*
(Dunning *et al.*1998)	Anglia	*CYP17*	835	591	No Association for *CYP17*
(Weston *et al.*1998)	USA mixed	*CYP17*	123	240	No Association for *CYP17*
(Helzlsouer J.K. *et al.*1996)	Americans of Europe	*CYP17*	109	113	No Association for *CYP17*
(Haiman *et al.*1999)	USA mixed	*CYP17*	463	618	No Association for *CYP17*
(Kristensen *et al.*1998)	Swedish and Norwegian	*CYP19*	367	252	Association for *CYP19*
(Siegelmann D. and Buetow 1999)	USA mixed	*CYP19*	348	145	Association for *CYP19*

1.3. Aim

The purpose of this study is to determine whether *GSTM1* null, *GSTP1* Ile105Val, *GSTT1* null genotypes are genetic susceptibility factors for breast cancer in the Turkish population.

This study deals with the following questions:

1. Are Glutathione S-tranferase gene polymorphisms genetic risk factors for breast cancer in the Turkish population?
2. Are Glutathione S-tranferase polymorphisms associated with the established risk factors for breast cancer?

The *GSTM1* locus was included in this study, since negative results have been reported in some populations, and no data about *GSTM1* polymorphism was available for the Turkish population.

The *GSTP1* locus was studied because its role was less established as a breast cancer risk factor.

The *GSTT1* and *GSTP1* loci were analyzed because no data was available for the Turkish population in regard to their association with breast cancer.

2. Materials and Methods

2.1. Materials

2.1.1 Subject:

Our study population consisted of 264 females previously diagnosed with breast cancer, 233 age-matched females and 77 random controls as a control group with no history of cancer. Cases and controls consented to participate in this study by giving blood samples and personal information. At the time of blood donation, each individual completed a standardized questionnaire including data on age, weight, height, menstrual and reproductive histories, family history of breast and other cancers (first degree relatives; only mother, sister or daughters) and smoking status.

A blood sample was collected from each volunteer and DNA extracted using a standard procedure as described in section 2.1.2.

2.1.1.1 Patients:

264 breast cancer patients were included in the study (Table 8). All patients were diagnosed at Hacettepe University Medical School, Ankara, Numune Hospital, and SSK Ankara Oncology Hospital, which are located in Ankara and predominantly serve patients from central Anatolia.

Information about age, weight and height of the patient, age at menarche, age at full term pregnancy, number of full term pregnancies, family history of breast cancer, and smoking history were obtained from standardized questionnaire forms. Information about the histopathology of the tumors, estrogen receptor status, and progesterone receptor status were obtained from the medical records (See; questionnaire form)

Table 8. Selected characteristics of breast cancer patients (n=264) and age-matched control subjects (n=233).

Characteristics	Case	Control
Age, year, mean (standard deviation)	49.28 (13.83)	46.15 (14.11)
Age, year, range	20-80	15-83
Age, year at first birth, mean (standard deviation)	21.78 (4.73)	20.52 (3.93)
Age, year at menarche, mean (standard deviation)	13.65 (1.44)	13.86 (1.42)
Number of children, mean (standard deviation)	2.95 (2.16)	3.03 (2.12)
Body mass index (kg/m2), mean (standard deviation)	24.48 (4.72)	26.96 (4.92)
Menopausal status at blood donation:		
Premenopausal	103 (39.46%)	122(52.36%)
Postmenopausal	158(60.54%)	111(47.64%)
Family history of breast cancer in mother, sister or daughter:		
No	237(90.76%)	227(97.39%)
Yes	24(9.24%)	6(2.61%)

1. Adı Soyadı:
2. Yaşı:
3. Medeni Hali:
4. Yaşadığı şehir ve süresi:
5. Ağırlığı (kg):
6. Boyu (cm):
7. Mesleği:
8. İlk menstürasyon periyodunun başlama yaşı:
9. Menapozal durumu:
   Premenapozal ise; son menstürasyon periyodunun kaç gün önce olduğu:
   Postmenapozal ise; son menstürasyon periyodunun kaç gün önce olduğu:
10. Tanı konulduğu zamanki menapozal durumu:
11. Tanının ne zaman konulduğu:
12. Uygulanan tedavi:
13. Daha önce hormon tedavisi gördü mü? Ne tip?
14. Oral kontraseptif kullandı mı? Nedir?
15. Kaç çocuğu var?
    a. İlk doğumunu yaptığı yaş:
    b. Son doğumunu yaptığı yaş:
16. Daha önce meme ile ilgili operasyon geçirdi mi?
17. Ooferektomi (yumurtalıkların alınması) yapıldı mı? Yapıldı ise kaç yıl önce?
18. Sigara içme alışkanlığı:
    Hiç içmedim () Eskiden içerdim ()
    1-10 sigara /gün () 11-20 sigara /gün () 20 ve daha fazla/gün ()
    1 yıldır içiyorum () 2-5 yıldır içiyorum () 5-10 yıldır içiyorum ()
    10-15 yıldır içiyorum() 15-20 yıldır içiyorum () 20 ve daha fazla yıldır içiyorum ()

17. Sigara içilen ortamda sıkça bulunuyormusunuz?
    (a) Evet (b) Hayır
18. Alkol kullanıyormusunuz?
    (a) Evet (b) Hayır
    Nadiren Haftada 1 kez Haftada 2-3 kez Haftada 4-5 kez Haftada 6-7 kez
19. Beslenme alışkanlığınızda size en fazla uyan tanım aşağıdakilerden hangisidir?
    (a) Kızartma ağırlıklı yağlı diyet
    (b) Sebze ağırlıklı yağsız diyet
    (c) Dengeli beslenme
20. Radyasyona maruz kaldınız mı? Hangi sıklıkla?
    (a) Evet (b) Hayır
21. Tiroid ile ilgili bir rahatsızlığınız var mı?
    (a) Evet (b) Hayır
    Hipertiroidizm () Hipotiroidizm ()
22. Aile bireylerinde ve sizde genetik bir rahatsızlık var mı? Tipi.
    (a) Evet (b) Hayır
23. Ailenizde meme kanserli başka bireyler var mı? (Anne, kardeş, anneanne, vb.)
24. Tümörün histopatolojisi
25. Tümör grade
26. Tümör stage
27. Östrojen reseptör durumu (+) veya (-)
28. Progesteron reseptör durumu (+) veya (-)

2.1.1.2 Age-matched Control Group:

233 women from Ankara Numune Hospital and SSK Ankara Oncology Hospital (Table 8) were included. Information about the age, weight, height, age at menarche, age at full term pregnancy, number of full term pregnancies, family history of breast cancer, and smoking history were obtained from standardized questionnaire forms.

2.1.1.3 Random Control Group

The random control group consisted of 77 students from Bilkent University. Information about age and sex were obtained from each individual.

2.1.2 Oligonucleotides:

The oligonucleotides used in PCR experiments are given in Table 9.

Table 9. List of primers for gene specific amplification.

Primer	Sequence (5' – 3')	Target gene	Size	Reference
GSTM1-F	GAA CTC CCT GAA AAG CTA AGC	*GSTM1*	215 bp	Anwar WA. *et al*
GSTM1-R	GTT GGG CTC AAA TAT ACG TGG			1996
GSTT1-F	ACC CCA GGG CTC TAT GGG AA	*GSTT1*	176 bp	Harries LW. *et al*
GSTT1-R	TGA GGG CAC AAG AAG CCC CT			1997.
GSTP1-F	AGG CAG CAG TGG GGG AGG CC	*GSTP1*	138 bp	Bringuier PP. *et al*
GSTP1-R	CTC ACC GGA TCA TGG CCA GCA			1998
CYP2E1-F	CCA GTC GAG TCT ACA TTG TCA	*CYP2E1*	412 bp	Anwar WA. *et al*
CYP2E1-R	TTC ATT CTG TCT TCT AAC TGG			1996

2.1.3 Chemical and Reagents

Agarose	Basica LE, EU
Boric acid	Sigma, St.Louis, MO, USA
Bromophenol blue	Sigma, St.Louis, MO, USA
Chloroform	Carlo Erba, Milano, Italy
Ethanol	Merck, Frankfurt, Germany
Ethidium bromide	Sigma, St.Louis, MO, USA
Ficoll Type 400	Sigma, St.Louis, MO, USA
Gamma Micropor Agarose	Prona LE, EU
Isoamyl alcohol	Carlo Erba, Milano, Italy
Phenol	Carlo Erba, Milano, Italy
Proteinase K	Appligene-Oncor, USA
pUC Mix Marker, 8	MBI Fermentas Inc., NY, USA
Sodium acetate	Carlo Erba, Milano, Italy
Sodium dodecyl sulfate(SDS)	Sigma, St.Louis, MO, USA
TrisHCl	Sigma, St.Louis, MO, USA
Trisodium citrate	Sigma, St.Louis, MO, USA
Xylene cyanol	Sigma, St.Louis, MO, USA

2.1.4 PCR Materials

Taq polymerase (5U/µl), 10X PCR buffer (100 mM Tris-HCl, pH 8.8 at 25 ^{O}C, 500 mM KCl, 0.8% Nonidet P40), 25 mM $MgCl_2$, 10 mM dNTP mix were obtained from MBI Fermentas Inc., NY, USA.

2.1.5. Restriction Endonucleases

*Alw*261 restriction endonuclease enzyme was obtained from MBI Fermentas Inc., NY, USA.

2.1.6 Standard Solutions

Agarose gel loading buffer (6X)

15 % ficoll
0.05 % bromophenol blue
0.05 % xylene cyanol

DNA Extraction buffer

10 mM Tris HCl, pH 8.0
10 mM EDTA, pH 8.0
0.5 % SDS

Proteinase K (stock); 20 mg/ml

SSC (20X)

3 M NaCl
0.3 M trisodium citrate, pH 7.0

TE Buffer

10 mM Tris HCl pH 8.0
1 mM EDTA

Tris-boric acid-EDTA (TBE) (10 X) (1L)

108 g Tris HCl

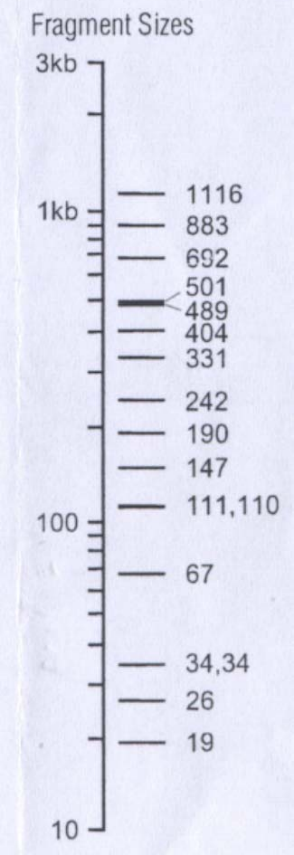
55 g boric acid

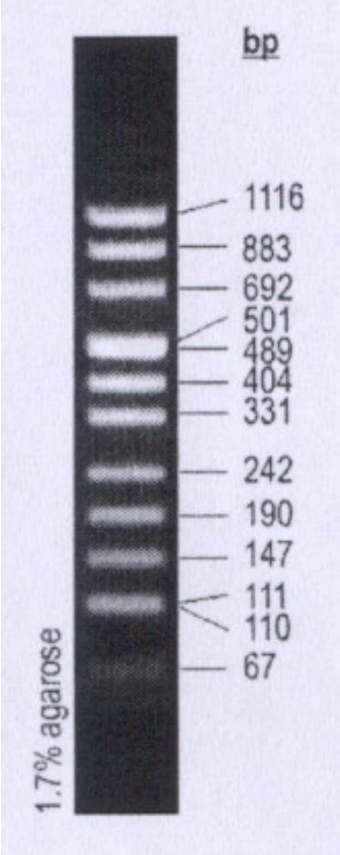
20 ml 0.5 M EDTA

Complete final volume to 1 L with ddH_2O

Standard DNA size markers

PUC Mix Marker, 8 (MBI, Fermentas)





2.2 Methods

2.2.1 DNA Isolation:

Peripheral blood was collected in EDTA-containing tubes and stored at 4 ^{o}C for a period of five days. The blood was then divided into 800 µl aliquots and stored at -20 ^{o}C. These 800 µl blood samples were used for DNA extraction by standard proteinase K/SDS digestion and phenol-chloroform extraction. The blood samples were washed before proteinase K/SDS digestion. After the aliquots were thawed 800µl l x SCC was added and mixed by vortexing. The samples were then centrifuged at 13,000 rpm for 1 minute. The supernatant was carefully removed and discarded into the chloros. The cell pellet was resuspended in 1.4 ml l x SSC and centrifuged at 13,000 rpm for 1 minute. This washing step was repeated until the pellet became white. The pellet was then resuspended in 800µl DNA extraction buffer containing 20µl proteinase K (20 mg/ml) solution. The samples were incubated at 56 ^{o}C for 4 hours, and were briefly mixed every 20 minutes. If the cell pellet was not dissolved completely at the end of this incubation period, the tubes were left overnight at 56 ^{o}C.

After the cell pellet was completely dissolved, the phenol/chloroform step was carried out in the fume-hood. 400µl phenol/chloroform/isoamylalcohol (25:24:1) was added and the tube was vortexed vigorously. The tube was then centrifuged at 13,000 rpm for 5 minutes. The upper aqueous DNA-containing layer (~700 µl) was transferred into a new tube. If the DNA supernatant was sticky and not resuspended completely or if interface was not clear the extraction step was repeated by adding 350µl phenol/chloroform/isoamylalcohol (25:24:1). Then 35µl NaOAc (3mM, pH=5.2) and 700µl ice-cold absolute ethanol (EtOH) were added to the upper aqueous layer to precipitate the DNA, mixed by inversion and incubated at -20 ^{o}C for a duration of 30 minutes to overnight. The tubes were then centrifuged at 13,000 rpm for 15 minutes. Afterwards, ethanol was discarded and the pellet air-dried. The pellet was solubilized in 200 µl TE (pH 8.0) or in sterile ddH_2O by incubation at 56 ^{o}C for 1 hour. If the pellet was not dissolved completely, overnight incubation at 56 ^{o}C was carried out. The DNA samples were stored at 4 ºC up to 2 months or at -20 ^{o}C for long-term.

2.2.2 Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a method for oligonucleotide primer directed enzymatic amplification of a specific DNA sequence of interest.

All amplification reactions were carried out on a Perkin Elmer 9600 PCR machine.

2.2.3 Restriction Endonuclease Digestion :

Amplified *GSTP1* products were subjected to digestion to analyze A3136 polymorphism in *GSTP1*. Enzyme digestion reaction was carried out using 10 µl PCR product, 10 x buffer Y^+/TANGO (MBI Fermantas) (33 mM Tris-acetate, 10 mM Magnesium acetate, 66 mM Potassium acetate, 0.1 mg/ml BSA pH=7.9 at 37 ^oC), 3 units of *Alw*26I (MBI, Fermentas) in 30 µl reaction volume and the samples were incubated at 37 ^oC for 4 hours.

2.2.4 Agarose Gel Electrophoresis :

Agarose gel electrophoresis was used to analyze the PCR products. 2% (w/v) agarose gels were prepared in 1xTAE buffer and 1µl of ethidium bromide solution from 10mg/ml stock was added to the buffer. 8µl PCR product was mixed with 1.5µl 6x loading buffer and the mix was loaded onto the gel. The products were run at 90 volts for 45 minutes. The gel was then analyzed under the transilluminator and photographs were taken.

To analyze the restriction fragments, 3% 1:1 ratio of Agarose: Gamma micropore was used. 20µl of digested products were mixed with 4µl of 6x loading buffer and the mix was loaded onto the gel. Electrophoresis was performed at 90 volts for 30-45 minutes. The gel was photographed under UV light. pUCmix8 (MBI Fermentas) was used as the DNA size marker.

2.2.5 Genotyping of Individuals :

The *GSTP1* polymorphism was analyzed by PCR and restriction enzyme digestion for genotyping. *GSTT1* and *GSTM1* genotypes were analyzed by PCR. The genotypes of each individual were scored by two independent researchers to eliminate uncertainty.

2.2.5.1 *GSTP1* Genotyping

Ile 105 Val polymorphism in *GSTP1* was analyzed by PCR and restriction digestion. For *GSTP1* PCR amplification, 50-100ng genomic DNA was used in a total of 25µl reaction volume containing 10pmol each of *GSTP1* primers, 200µM of dNTP mix, 10xPCR buffer, 1.5mM $MgCl_2$, 1U DNA Taq polymerase. The amplification conditions were as follows; initial denaturing step at 94 ^{o}C for 5 minutes, followed by 30 cycles of denaturing for 30 seconds at 94 ^{o}C, annealing for 30 seconds at 57 ^{o}C, extension for 30 seconds at 72 ^{o}C. The reaction was completed with a final extension at 72 ^{o}C for 7 minutes. The expected amplification product, 176bp, was digested with 3 U *Alw*26I at 37 ^{o}C for 4 hours. The digested fragments were electrophoresed in 3% 1:1 ratio of Agarose: Gamma Micropore. The presence of 91bp and 85bp restriction fragments indicate the presence of Val allele (see Figure 5 for schematic representation).

2.2.5.2. *GSTT1* Genotyping

GSTT1 genotyping was determined by PCR using *GSTT1* gene specific primers. *GSTP1* primers were also included in the PCR mixture as a control to see the independent amplification of each sample. For *GSTT1* PCR genotyping, 50-100ng genomic DNA was used in a total volume of 25 µl containing 10 pmol of each *GSTT1* primers, 200µM of dNTP, 10xPCR buffer, 2.0mM MgCl2, and 1U of DNA Taq polymerase. The amplification conditions were as follows: initial denaturing step at 94 ^{o}C for 5 minutes, followed by 30 cycles of denaturing for 30 seconds at 94 ^{o}C, annealing for 30 seconds at 60^{o}C, extension for 30 seconds at 72 ^{o}C. The reaction was completed with a final extension at 72 ^{o}C for 7

minutes. The expected amplification product was 138bp in *GSTT1* positive individuals. For *GSTP1* genotyping, reaction conditions were carried out as described previously in Section 2.2.4.1. Null genotypes were scored after *GSTP1* amplifications were confirmed (see Figure 6 for schematic representation).

2.2.5.3 *GSTM1* Genotyping

GSTM1 genotype was determined by *GSTM1* amplification and by CYP2E1 amplification as an internal control reaction. CYP2E1 primers were also included in the PCR mixture as a control to see the independent amplification of each sample. Both reactions were carried out in the same reaction tube. *GSTM1* PCR genotyping experiments were performed by using 50-100ng genomic DNA, 10xPCR buffer, 10 pmol of each *GSTM1* primers, 20 pmol of each CYP2E1 primers, 200µM dNTP, 1.5 mM $MgCl_2$ in a total volume of 25µl. The amplifications were carried out by the following conditions; 94 ^{o}C initial denaturation for 5 minutes followed by 35 cycles of denaturation for 30 seconds at 94 ^{o}C, annealing for 30 seconds at 55 ^{o}C and extension for 45 seconds at 72 ^{o}C, with a final extension at 72 ^{o}C for 7 minutes. The expected amplification product was 215 bp in *GSTM1* positive individuals. The 412 bp product size for CYP2E1 was expected to be amplified in all samples (see Figure 7 for schematic representation).

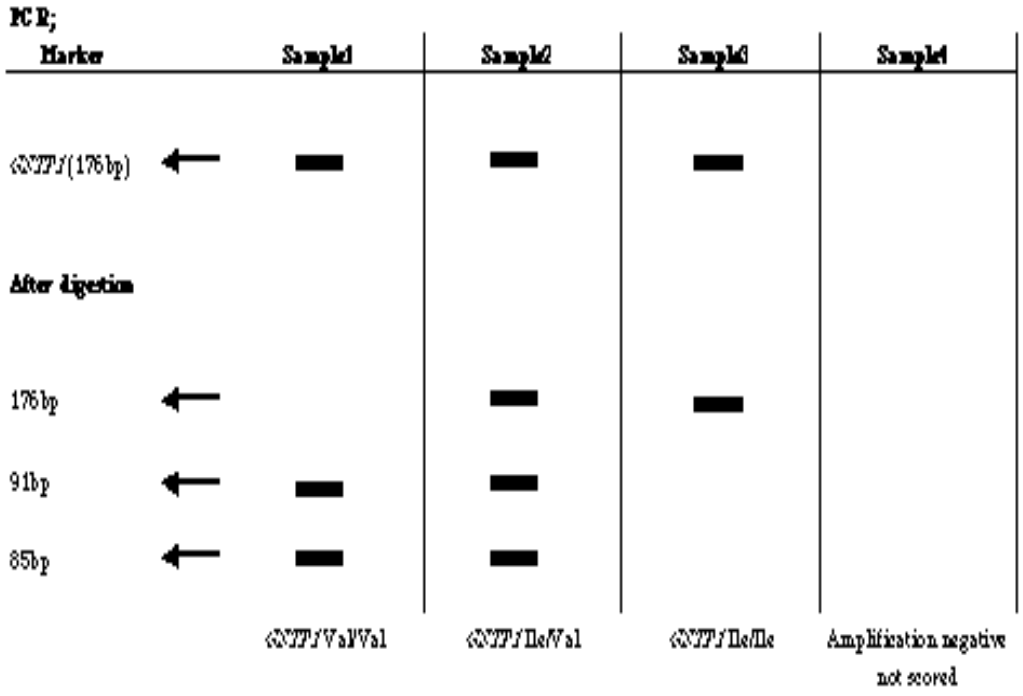


Figure 5. Schematic representation of *CNTF1* genotyping.

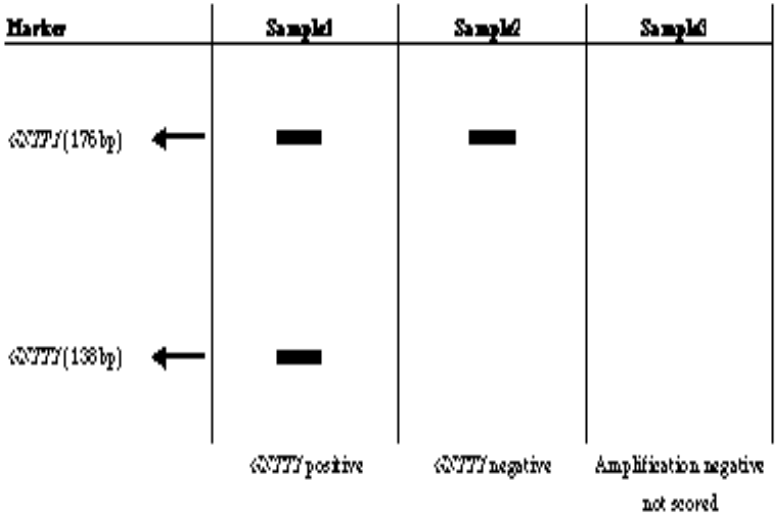


Figure 6. Schematic representation of *GNTT1* genotyping.

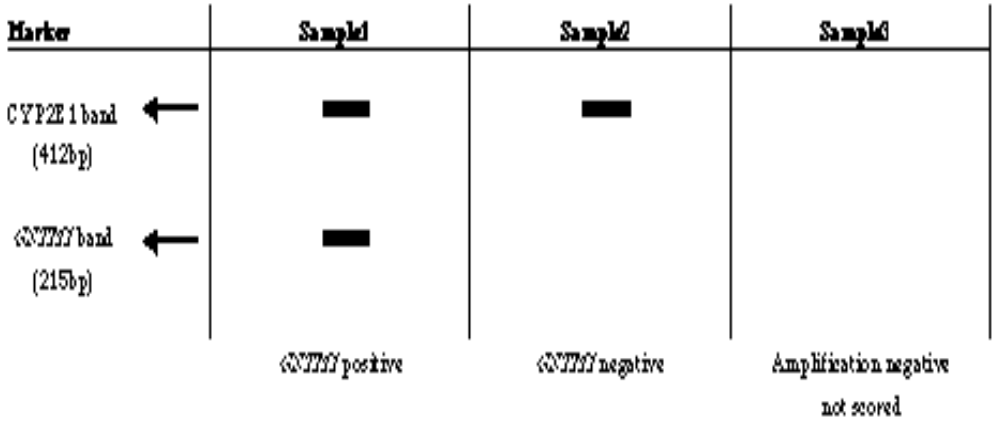


Figure 7. Schematic representation of *CYP2E1* genotyping.

2.2.6 Statistical Analyses

Statistical analyses were carried out with the Minitab 13.1 software program.

2.2.6.1 Chi-square Test

There are basically two types of random variables yielding two types of data: numerical (e.g. number of children) and categorical (e.g. *GSTP1* genotype, whose values are Ile/Ile, Ile/Val, Val/Val). A chi-square (X^2) statistic is used to investigate whether distributions of categorical variables differ from one another. The chi-square test is also a test of independence; it provides little information about the strength (e.g. strong, weak, perfect) or form (e.g. positive, negative) of association between two variables (Daniel WW. 1995). It is a series of mathematical formulas which compare the actual observed frequencies (e.g. variable: *GSTP1*, categories: Ile/Ile, Ile/Val, and Val/Val) with the expected frequencies. That is, the chi-square analysis tests observes results against the null hypothesis (null hypothesis is the hypothesis to be tested) and assesses whether the actual results are different from the expected ones (Daniel WW. 1995). The requirements for the test are:

- The sample must be randomly drawn from the population.
- Data must be reported in raw frequencies (not percentages).
- Any observations must fall into only one category or value on each variable.
- This test should only be used when observations are independent (e.g. no category or response is dependent upon or influenced by another).
- Observed frequencies can not be too small. For instance, the *GSTP1* 105 Val/Val genotype frequency was too low in our population (8.43% in cases and 8.58% in controls). So, the *GSTP1* 105 Ile/Val and Val/Val genotypes were combined in our study.

The chi-square test is one of the methods of calculating a P value. The P value shows us whether a result is statistically significant. In other situations, to make a decision based on a single comparison, the steps of statistical hypothesis testing must be followed:

- A threshold P value must first be settled. The threshold value is traditionally usually set as 0.05.

- The null hypothesis must be defined. If two means are being compared, the null hypothesis is that the two populations have the same mean.
- The chi-square test must be carried out to compute the P value.
- The P value must be compared to the preset threshold value.
- If the P value is less than the threshold, the null hypothesis is rejected and the difference is statistically significant.
- If the P value is greater than the threshold, the null hypothesis is not rejected and the difference is not statistically significant, and there sufficient evidence is not present to reject the null hypothesis.

The P value is a probability, with a value ranging from zero to one. If the P value is small, it is concluded that the difference is quite unlikely to be caused by random sampling, and the populations have different means.

If a result is statistically significant, there are two possible explanations: The populations are identical, so there really is no difference. By chance, larger values in one group and smaller values in the other are obtained. Finding a statistically significant result when the populations are identical is called making a Type I error. If statistically significant is defined to mean "$P<0.05$", then a Type I error is made in 5% of experiments where there really is no difference. The other explanation is that the populations are really different and that the conclusion is correct (Pagano M. and Gauvreau K. 1992).

If a result is not statistically significant, it is also possible that the study missed a small effect due to small sample size and/or large scatter. In this case, a Type II error has been made concluding that there is no difference when in fact there is a difference (Pagano M. and Gauvreau K. 1992).

Statistical calculations combine sample size and variability (standard deviation) to generate a confidence interval (CI) for the population mean. Intervals can be calculated for any desired degree of confidence, but 95% confidence intervals are used most commonly. If many 95% CI from many data sets are generated, the CI is expected to include the true population mean in 95% of the cases and not to include the true mean value in the other 5%.

The other most frequent use of chi-square distribution is to test the null hypothesis that two criteria of classification are independent when applied to the same set of entries. According to two criteria, a table in which the rows (r) represent the various levels of one criterion of classification and the columns (c) represent the various levels of the second criterion is prepared. Such a table is generally called a contingency table.

Where the null hypothesis is true, chi-square is distributed approximately with k-r degrees of freedom. In determining the degrees of freedom, k is the number of the groups for which observed and expected frequencies are available, and r is the number of the restrictions or constraints imposed on the given comparison. For the analysis of the contingency tables, in which r rows represent the various levels of one criterion, and the c columns represent the various level of a second criterion, degrees of freedom are calculated as (r-1)(c-1)=df (Pagano M. and Gauvreau K. 1992).

2.2.6.2 Odds Ratio Calculation

There are two types of observational studies: prospective and retrospective case-control studies. The primary difference between the two is the sampling scheme. When sampling is based upon the response variable, the study is called a retrospective study. When sampling is based upon the stimulus variable, the study is called a prospective study. A prospective study is related to the future. The subjects are stratified according to whether they have the risk factor or not. The outcome is evaluated after a certain follow-up period has passed (e.g. after *GST* genotyping follow-up for 30 years to observe the individuals that will develop breast cancer). A retrospective study is related to past. The persons with the outcome constitute the study group, and whether these subjects have the risk factor or not is determined (e.g. find a breast cancer group and control group, determine if they are postmenopausal or premenopausal, and then carry out *GST* genotyping). The retrospective or case history studies are relatively quick and inexpensive, easily repeatable and enable a larger number of individuals to be examined (Slome C. 1982). The characteristics of the disease under study plays a role in determining whether a prospective or retrospective study should be employed. The rarer the disease or the longer the

interval between the suspected cause and the condition, the more difficult is the cohort study. The term relative risk is used for the risk estimation obtained from prospective studies. It is actually the ratio of the risk of developing a disease among subjects with the risk factor to the risk among subjects without the risk factor. If the data are from a retrospective study, relative risk is not a meaningful measure for comparing the two groups. The appropriate test for comparing cases and controls in a retrospective study is the odds ratio (Rim AA. 1981). In any event, for rare diseases the odds ratio is a close approximation of the relative risk.

The odds ratio can assume a value between zero and infinity. A value of zero is the indicator of no association between the risk factor and disease status. A value greater than 1 indicates a higher risk among cases when compared to controls. The odds ratio takes a value somewhere between the lower and upper limits of the confidence intervals. An odds ratio value greater than 1 is statistically significant, if the lower limit of 95% confidence intervals is greater than 1 (Daniel WW. 1995).

Table 10. Sample 2x2 Table for OR analysis

Risk factor	**Control**	**Case**
Present	a	c
Absent	b	d

a: number of controls with the risk factor

b: number of controls without the risk factor

c: number of cases with the risk factor

d: number of cases without the risk factor

The following formulas are used for odds ratio calculations, and confidence intervals:

OR=ad/bc

95% CI= e $^{\ln [OR] \pm 1.96 \text{ times square root of } (1/A+1/B+1/C+1/D)}$

2.2.6.3. Multivariate Adjusted Odds Ratio Calculation

To measure the relationship between one interval dependent variable (e.g. *GSTP1* genotype) and several independent variables (e.g. age, age at menarche, age at first full-term pregnancy, number of children, family history of breast cancer) the multiple regression test is used. In this analysis, the independent variables can predict the dependent variables, but the dependent variables can not be used to predict the independent variables. Independent variables should be justified theoretically. The selected independent variables should have strong correlations with the dependent variable but only weak correlations with other independent variables. Each independent variable should have the same relationship with the dependent variable at each value of other independent variables. Multiple regression modeling is used to determine what variables contribute to the explanation of the dependent variable and to what degree. A theoretically well-defined model when applied to analysis, the adjusted odds ratio is a valuable statistical tool.

2.2.6.4. Gene-environment, Gene-gene Interaction Analyses

If cases or controls that are being compared differ in any characteristic that is related to the disease (in this instance breast cancer) and to the exposure (or potential risk factor or cause), then these differences must be taken into account when making these comparisons (Dunning MA. *et al*. 1999).

A case control study group is designed to investigate the presence of an interaction between a genetic and environmental factor. The environmental (E=e) and genetic factors (G=g) are binary variables that take values of 1 for exposed (e.

high BMI) or susceptible (e.g. the combination of *GSTP1* 105 Ile/Val or Val/Val genotypes), and 0 for unexposed (e.g. low BMI) or not susceptible (e.g. *GSTP1* Ile/Ile). Disease status (D=d) takes a value of 1 for affected (breast cancer patients) and 0 for the unaffected (age-matched control) (Garcia-Closas M. *et al*. 1999). The odds ratio OR_{eg} is the measure of association between disease and environmental and genetic factors.

The multiplicative interaction parameter is Ψ. In the absence of a multiplicative interaction, Ψ=1 (Table 11).

The additive interaction parameter is Φ. In the absence of an additive interaction Φ=1 (Table 11).

The odds ratio for the reference group (e.g. 00 individuals) is 1, since the odds ratio for this group is calculated by comparing the reference group by itself. The odds ratios were calculated by comparing the reference group (the individuals inheriting no risk genotypes) to the others respectively.

For gene-gene interaction (the combined effects of studied genes) analysis, the same method can be used. However, that time the environmental (E=e) factor is replaced with the genetic factor. These binary variables take values of 1 for both susceptible (e.g. *GSTM1* null genotype or *GSTT1* null genotype), and 0 for both not susceptible cases (e.g. *GSTM1* positive or *GSTT1* positive).

Table 11: Definition of ORs (OR_{01}, OR_{10}, OR_{11}) and interaction parameters (Ψ^a,Φ^a) for the relations of two dichotomous environmental and genetic factors and cancer.

		Genetic factor (G)	
		G = 0	***G = 1***
Environmental factor	E = 0	**1.0**[a]	OR_{01}
	E = 1	OR_{10}	OR_{11}

$$\Psi = \frac{OR_{11}}{OR_{10} \cdot OR_{01}}$$

$$\Phi = \frac{(OR11 - 1)}{(OR_{10} - 1) + (OR_{01} - 1)}$$

R[a]eference category

3. RESULTS:

We examined associations for gluthathione S-transferases M1 (*GSTM1*), T1 (*GSTT1*), and P1 (*GSTP1*) genotypes and breast cancer risk in the Turkish population. Genotyping for G*ST*s was conducted on 264 breast cancer cases and 233 age-matched controls. A group of randomly selected university students (n=77) was also genotyped to compare with the age-matched control group.

The nucleotide polymorphisms were identified by PCR assays for *GSTM1* and *GSTT1* genes. The examples of PCR analysis for *GSTM1* and *GSTT1* genotyping are shown in Figures 8 and 10. *GSTP1* polymorphism was identified by restriction enzyme site digestion of the *GSTP1* PCR product. An example of the result of this genotyping analysis is shown in Figure 9.

All 264 breast cancer patients and 233 control groups were subjected to genotyping analysis, the results were scored and the frequencies of the *GSTM1*, *GSTT1,* and *GSTP1* genotypes were compared. The characteristics of the participants in this study have been described in Table 12. The mean age was 49.29 (SD: 13.83, range: 20-80) for cases and 46.15 years (SD: 14.11, range: 15-83) for controls, contributing to a higher proportion of cases (60.54%) than controls (47.64%) being postmenopausal. The mean age was 13.65 (SD: 1.44) at menarche, and 21.78 (SD: 4.73) at first birth while the mean number of children was 2.95 (SD: 2.16) for the cases. For the control group, the mean age was 13.86 (SD: 1.42) at menarche and 20.52 (SD: 3.93) at first birth while the mean of number of children was 3.03 (SD: 2.12). The mean BMI was 24.48 (SD: 4.72) for the cases and 26.96 (SD: 4.92) for the controls. The risk of breast cancer was higher for women who had a BMI $\geq$ 26.96 (the mean BMI of controls) (OR= 1.76; 95% CI= 1.23-2.52). The breast cancer risk was also higher for postmenopausal cases (OR= 1.69; 95% CI=1.18-2.42). The risk of breast cancer was slightly increased for women whose age at menarche was $\leq$ 12 (OR= 1.33; 95% CI=0.81-2.18). The risk of breast cancer was 3.80 times higher for women who had first-degree relatives with breast cancer (OR= 3.80; 95% CI=1.51-9.55). There was a slight increased case-control difference in the association between high BMI and postmenopausal status in the Turkish population for breast cancer (OR= 1.26; 95 % CI=0.77-2.05) (Table 12).

The distribution of *GSTM1*, *GSTP1*, and *GSTT1* genotypes in the breast cancer patients and age-matched controls by menopausal status, and multivariate

adjusted OR stratified according to age, age at menarche, age at full-term pregnancy, number of full-term pregnancies, and family history of breast cancer are summarized in Table 13. Since the *GSTP1* 105 Val/Val genotype frequency was too low in our population to analyze statistically, *GSTP1* 105 Ile/Val and Val/Val genotypes were combined for cancer risk estimation (Katoh T. *et al.* 1999).

The crude odds ratios were 1.07 (95% CI=0.75-1.52) for the *GSTM1* null genotype, 1.36 (95% CI=0.95-1.94) for the combined *GSTP1* 105 Ile/Val and Val/Val genotypes and 1.03 (95% C=0.66-1.60) for the *GSTT1* null genotypes for all subjects. In the premenopausal breast cancer group crude odds ratios were 1.27 (95% CI=0.75-2.15) for the *GSTM1* null genotype, 1.31 (95% CI=0.77-2.23) for the combined *GSTP1* 105 Ile/Val and Val/Val genotypes, and 1.51 (95% CI=0.75-3.05) for the *GSTT1* null genotypes. The crude odds ratio of postmenopausal subjects were 0.92 (95% CI=0.56-1.49) for *GSTM1* null genotypes, 1.47 (95% CI=0.89-2.41) for the combined *GSTP1* 105 Ile/Val and Val/Val genotypes, and 0.85 (95% CI=0.46-1.56) for the *GSTT1* null genotype.

The adjusted odds ratios were 1.03 (95% CI=0.69-1.55) for the *GSTM1* null genotype, 1.64 (95% CI=1.09-2.47) for the combined *GSTP1* 105 Ile/Val and Val/Val genotypes, and 1.09 (95% CI=0.65-1.85) for the *GSTT1* null genotype when premenopausal and postmenopausal breast cancer patients were considered together. In the premenopausal breast cancer group adjusted odds ratios were 1.20 (95% CI=0.64-2.27) for the *GSTM1* null genotype, 2.01 (95% CI=1.06-3.83) for the combined *GSTP1* 105 Ile/Val and Val/Val genotypes, and 1.62 (95% CI=0.66-4.00) for the *GSTT1* null genotype. Finally, in the postmenopausal breast cancer group adjusted odds ratios were 0.75 (95% CI=0.42-1.33) for the *GSTM1* null genotype, 1.50 (95% CI=0.85-2.65) for the combined *GSTP1* 105 Ile/Val and Val/Val genotypes, and 1.04 (95% CI =0.50-2.15) for the *GSTT1* null genotype.

The odds ratio for all subjects and the premenopausal subjects with the combined *GSTP1* 105 Ile/Val and Val/Val genotypes was increased when the multivariate adjustment model was carried out. The multivariate logistic regression model stratified odds ratios according to age, age at menarche, age at full-term pregnancy, number of full-term pregnancies, and family history of breast cancer. According to the model, the combined *GSTP1* 105 Ile/Val and Val/Val genotypes in the premenopausal status were two times or more risky for breast cancer and also the

combined *GSTP1* 105 Ile/Val and Val/Val genotypes for all subjects was found to be a significant risk factor for breast cancer.

To compare the age–matched control group, randomly selected 77 Bilkent University students were genotyped. In the random control group, *GSTM1* null genotype was 46% (p=0.51), and the *GSTT1* null genotype was 17.25% (P=0.57), GSTP1 genotype was 67% (Ile/Ile), 31.16% (Ile/Val) and 1.31% (Val/Val) (P=0.27) and combined *GSTP1* 105 Ile/Val and Val/Val genotype was 32.47%. These results pointed out that there was no significant difference between the genotype frequencies of the age-matched control group and the randomly selected group, so the selected age-matched controls were appropriate for the study. The distribution of *GST* genotypes was in Hardy-Weinberg equilibrium in all three groups.

The risk of breast cancer from *GST* genotypes was evaluated by body mass index (kg/m^2) that is summarized in Table 14. BMI was dichotomized based on the median values (>26.96 kg/m^2) for controls (Mitrunen K. *et al*. 2001). Among women with a high BMI, it was shown that a significantly increased risk of breast cancer was associated with the combined *GSTP1* 105 Ile/Val or Val/Val genotypes (OR=2.12; 95% CI=1.35-3.62). There was also a significantly increased risk present among premenopausal women with the combined *GSTP1* 105 Ile/Val and Val/Val genotypes (OR=2.14; 95% CI=0.97-4.70) and the postmenopausal women with the *GSTP1* 105 Ile/Val and Val/Val genotypes (OR=2.16; 95% CI=1.14-4.09).

Although the combined *GSTP1* 105 Ile/Val and Val/Val genotypes was shown to be a significant risk factor for breast cancer, when the two genotypes' relative risks were combined (combined analysis of *GSTT1* null genotypes with the combined *GSTP1* 105 Ile/Val or Val/Val genotypes) the results indicated that there was no increase of risk (OR=0.69; 95% CI=0.35-1.38) (Table 15). The combined analysis of *GSTM1* null genotype and the *GSTP1* 105 Ile/Val or Val/Val genotypes was also carried out. Table 16 reveals that the risk for breast cancer did not increase by combination of the relative risks of both genotypes (OR =1.39; 95 % CI=0.85-2.28).

The risk association for the combination of three *GST* risk genotypes was then analyzed. The reference group was designated as *GSTM1* and *GSTT1* present genotypes and the *GSTP1* Ile105Ile genotype. Combinations of three risk genotypes did not reveal a significant relative risk (OR=0.95; 95 % CI=0.37-2.43) (Table 17).

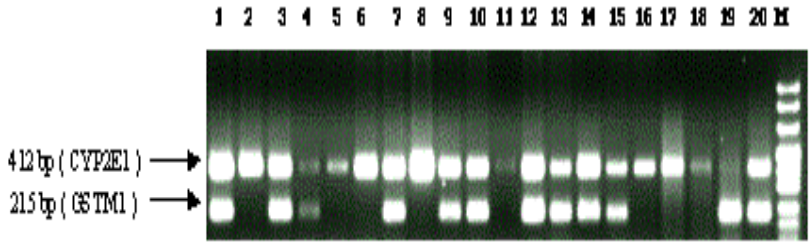


Figure 8. Genotyping of *GSTM1*. *GSTM1* primers generated a 215 bp product and the internal control CYP2E1 yielded a 412 bp product. Samples MCK004, MCK025, MCK028, MCK031, MCK032, MCK036, MCK030, MCK069, NK018, NK032, NK033, NK065 in lanes 1, 3, 4, 7, 9, 10, 12, 13, 14, 15, 19, 20 were positive for *GSTM1* amplifications and samples MCK011, MCK013, MCK021, MCK027, NK004, NK014, NK015 in lanes 2, 5, 6, 8, 16, 17, 18 were negative (null genotypes). M is the DNA size marker; pUC mix 8. All individuals that are scored for *GSTM1* genotyping were positive for CYP2E1 PCR product.

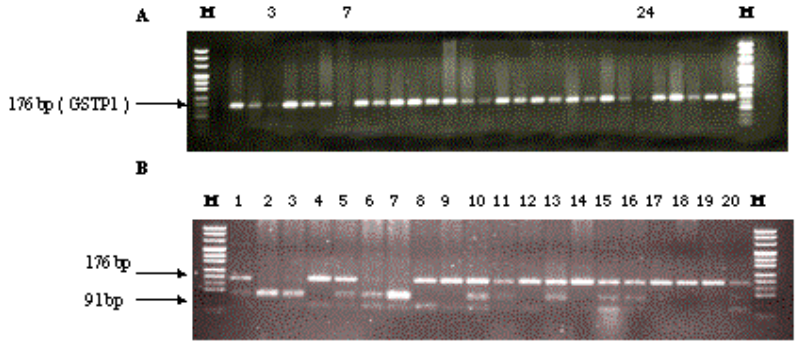


Figure 9. Genotyping of *GSTP1* gene. **A.** *GSTP1* primers generated a 176 bp product in all samples except the ones in lanes 3, 7 and 24. **B.** Amplified 176 bp *GSTP1* fragments were digested with *Alw*26I. In the presents of restriction site two fragments of 91 and 85 bp were observed. Individuals homozygous for the Ile105Ile allele had the undigested fragment (samples MCK002, MCK007, MCK009, MCK014, MCK022, MCK032, NK053, NK055 in lanes 4, 8, 9, 12, 14, 17, 18, 19), heterozygous for the 105Ile/Val alleles had both the undigested and the digested fragments (samples MCK012, MCK015, MCK018, MCK020, MCK023, NK003, NK005, NK013 in lanes 1, 5, 10, 11, 13, 15, 16, 20), and homozygous for Val105Val alleles had only the digested fragments (samples MCK064, MCK001, NK023 in lanes 2, 3, 6, 7). M is the DNA size marker; pUC mix 8.

138 bp

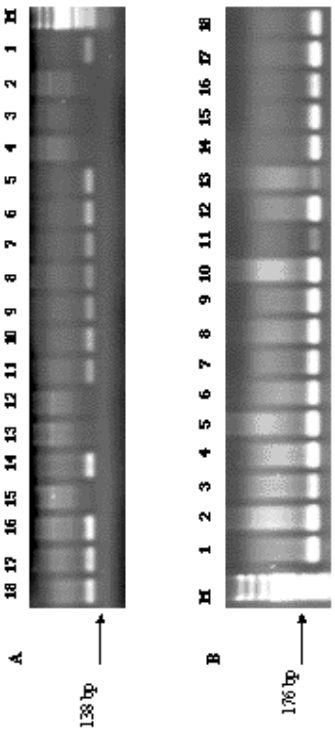


Figure 10: A. Genotyping of *GSTT1*. *GSTT1* primers generated a 138 bp product. Samples MCK139, MCK141, MCK142, MCK143, MCK144, NK023, MCK100, NK151, NK071, MCK081, NK072, NK073 in lanes 1, 5, 6, 7, 8, 9, 10, 11, 14, 16, 17, 18 were respectively positive for *GSTT1* amplification. Samples MCK136, MCK137, NK126, NK111, MCK059, MCK063 in lanes 2, 3, 4, 12, 13, 15 were negative for *GSTT1* amplification. **B.** *GSTT1* PCR was also performed as a control for the amplification of all the samples. M is the size DNA marker; pUC mix8.

Table 12:Characteristics of participants in this study

	Cases n=261	Controls n=233	OR (95% CI)
Age (yr), mean	49.29	46.15	
1st degree relative with breast cancer	9.24%	2.61%	OR=3.80 (1.51-9.55)
Body mass index, mean	24.48	26.96	
Age at first live birth, mean	21.78	20.52	
Number of children, mean	2.95	3.03	
Age at menopause, mean	13.65	13.86	
Premenopausal	39.46%	52.36%	
Postmenopausal	60.54%	47.64%	OR=1.69 (1.18-2.42)
BMI ≥ 26.96	62.83%	48.93%	OR=1.76 (1.23-2.52)
Premenopausal	39.85%	28.33%	
Postmenopausal	22.99%	20.60%	OR=1.26 (0.77-2.05)
Age at men ≤ 12	80.53%	84.72%	OR=1.33 (0.81-2.18)

Table 13. Distribution of the *GSTM1*, *GSTP1* and *GSTT1* genotypes in the age matched controls and breast cancer patients

Menopause	Locus	Genotype	Case n=264 (%)	Control n=233 (%)	Crude OR (95% CI)	Adjusted OR* (95%CI)
All	*GSTM1*	Present	125 (47.35)	114 (48.93)		
		Null	139 (52.65)	119 (46.12)	1,07 (0,75-1,52)	1,03 (0,69-1,55)
	GSTP1	A/A	132 (50.57)	141 (60.52)		
		A/G	101 (38.70)	72 (30.90)		
		G/G	22 (8.43)	20 (8.58)		
		A/G or G/G	123 (47.13)	92 (39.48)	1,36 (0,95-1,94)	1,64 (1,09-2,47)
	GSTT1	Present	212 (81.23)	187 (80.26)		
		Null	49 (18.77)	46 (19.74)	1,03 (0,66-1,60)	1,09 (0,65-1,85)
Pre	*GSTM1*	Present	47 (45.63)	63 (51.84)		
		Null	56 (54.37)	59 (48.46)	1,27 (0,75-2,13)	1,20 (0,64-2,27)
	GSTP1	A/A	53 (51.46)	71 (58.20)		
		A/G	40 (38.83)	43 (35.25)		
		G/G	10 (9.71)	8 (6.56)		
		A/G or G/G	50 (48.54)	51 (41.80)	1,31 (0,77-2,23)	2,01 (1,06-3,83)
	GSTT1	Present	88 (85.44)	97 (79.51)		
		Null	15 (14.56)	25 (20.49)	1,51 (0,75-3,03)	1,62 (0,66-4,00)
Post	*GSTM1*	Present	78 (48.10)	51 (45.95)		
		Null	82 (51.90)	60 (54.05)	0,92 (0,56-1,49)	0,75 (0,42-1,33)
	GSTP1	A/A	85 (53.80)	70 (63.06)		
		A/G	61 (38.61)	29 (26.13)		
		G/G	12 (7.59)	12 (10.81)		
		A/G or G/G	73 (46.20)	41 (36.94)	1,47 (0,89-2,41)	1,50 (0,85-2,65)
	GSTT1	Present	124 (78.48)	90 (81.08)		
		Null	34 (21.52)	21 (18.92)	0,85 (0,46-1,56)	1,04 (0,50-2,13)

(* Adjustment of the model is according to age, age at menarche, age at full-term pregnancy, number of full-term pregnancies, and family history of the breast cancer.)

Table 14 : Distribution of GST genotypes stratified according to BMI in cases and controls.

			Low BMI			High BMI		
Menopause	Locus	Genotype	Case	Control	OR(95% CI)	Case	Control	OR(95% CI)
ALL	*GSTM1*	Present	43(49.38)	36(47.06)		77(46.11)	33(30.88)	
	GSTM1	Null	49(30.32)	63(32.94)	0.91(0.33-1.36)	90(33.89)	36(49.12)	1.21(0.73-1.94)
	GSTP1	AA	39(60.32)	63(34.62)		81(48.30)	76(66.67)	
	GSTP1	AG/GG	38(39.18)	34(43.33)	0.78(0.43-1.34)	86(31.30)	33(33.33)	2.12(1.33-3.62)
	GSTT1	Present	78(80.41)	93(78.13)		133(80.84)	94(82.46)	
	GSTT1	Null	19(19.39)	26(21.83)	0.87(0.43-1.70)	32(19.16)	20(17.54)	1.11(0.60-2.06)
PRE	*GSTM1*	Present	21(48.84)	37(30.00)		26(43.33)	26(34.17)	
	GSTM1	Null	22(31.16)	37(30.00)	1.04(0.30-2.21)	34(36.67)	22(43.83)	1.33(0.72-3.33)
	GSTP1	AA	24(33.31)	39(32.70)		29(48.33)	32(66.67)	
	GSTP1	AG/GG	19(44.19)	33(47.30)	0.88(0.41-1.87)	31(31.67)	16(33.33)	2.14(0.97-4.70)
	GSTT1	Present	37(86.03)	39(79.73)		31(83.00)	38(79.17)	
	GSTT1	Null	6(13.93)	13(20.27)	0.64(0.23-1.80)	9(13.00)	10(20.83)	0.67(0.23-1.81)
POST	*GSTM1*	Present	27(30.00)	19(42.22)		49(47.12)	32(48.48)	
	GSTM1	Null	27(30.00)	26(37.78)	0.73(0.33-1.62)	33(32.88)	34(31.32)	1.06(0.57-1.97)
	GSTP1	AA	33(64.31)	26(37.78)		30(48.08)	44(66.67)	
	GSTP1	AG/GG	19(33.19)	19(42.22)	0.74(0.33-1.67)	34(31.92)	22(33.33)	2.16(1.14-4.09)
	GSTT1	Present	41(73.93)	34(73.36)		33(79.81)	36(84.83)	
	GSTT1	Null	13(24.07)	11(24.44)	0.98(0.39-2.47)	21(20.19)	10(13.13)	1.42(0.62-3.24)

Table 15: Combination of the *GSTT1* null genotype with the *GSTP1* 105 Ile/Val or Val/Val genotype for breast cancer risk.

Genotype at risk	*GSTT1*	*GSTP1*	Case (n=264)[%]	Control (n=233)[%]	Crude OR (95% CI)
None*	Present	Ile/Ile	104 (39.39)	120 (51.50)	1.00 (referral)
One	Null	Ile/Val	36 (13.64)	21 (9.01)	1.98 (1.09-3.60)
	Present	Ile/Val, Val/Val	109 (41.29)	67 (28.76)	1.88 (1.26-2.81)
Two	Null	Ile/Val, Val/Val	15 (5.68)	25 (10.73)	0.69 (0.35-1.38)

(* None group is used as a reference group for relative risk analysis.)

Table 16: Combination of the GSTT1null genotype with the GSTP1 105 Ile/Val or Val/Val genotype for breast cancer risk.

Genotype at risk	GSTT1	GSTP1	Cases (n=264)(%)	Control (n=233)	Crude OR (95% CI)
None*	Present	Ile/Ile	64 (24.24)	75 (32.19)	1.00 (referral)
One	Null	Ile/Val	76 (28.79)	66 (28.33)	1.35 (0.84-2.16)
	Present	Ile/Val, Val/Val	61 (23.11)	39 (16.74)	1.83 (1.09-3.08)
Two	Null	Ile/Val, Val/Val	63 (23.86)	53 (22.75)	1.39 (0.85-2.28)

(* None group is used as a reference group for relative risk analysis.)

Table 17: Combination of the *GSTT1* and *GSTM1* null genotypes with the *GSTP1* 105 Ile/Val or Val/Val genotype for breast cancer risk.

High-risk Genotypes	*GSTM1*	*GSTP1*	*GSTT1*	*Cases* (n=264)(%)	*Controls* (n=233)(%)	OR (95% CI)
Three	Null	Ile/Val or Val/Val	Null	9 (3.41)	12 (5.15)	0.95 (0.37-2.43)
Two	Null	Ile/Val or Val/Val	Present	54 (20.45)	41 (17.60)	1.67 (0.97-2.88)
	Null	Ile/Ile	Null	24 (9.09)	12 (5.15)	2.54 (1.16-5.55)
	Present	Ile/Val or Val/Val	Null	6 (2.27)	13 (5.58)	0.59 (0.21-1.66)
One	Null	Ile/Ile	Present	52 (19.70)	54 (23.18)	1.22 (0.72-2.07)
	Present	Ile/Val or Val/Val	Present	55 (20.83)	26 (11.16)	2.68 (1.48-4.84)
	Present	Ile/Ile	Null	12 (4.55)	9 (3.86)	1.69 (0.66-4.32)
None *	Present	Ile/Ile	Positive	52 (19.70)	66 (28.33)	1.00 (referral)

(* None group is used as a reference group for relative risk analysis.)

4.DISCUSSION:

It has been suggested that up to 80% of human cancers arise as a consequence of environmental exposure (Doll R. *et al*. 1981). The first line of defense against cancer is provided by the ability of the organism to metabolize and detoxify endogenous toxins (Smith G. *et al*. 1995). Therefore, inherited capacity for these metabolic activation and/or detoxification reactions may regulate individual susceptibility to environmentally induced diseases such as cancer. *GST*s are a super-family of enzymes that are potentially important in regulating susceptibility to cancer because of their ability to metabolize reactive electrophilic intermediates to usually less reactive and more water soluble glutathione conjugates (Hayes JD. *et al*. 1995). It has been postulated that polymorphisms in enzymes involved in carcinogen metabolism increase the risk of cancer in some individuals. The *GSTM1* and *GSTT1* genes both exhibit deletion polymorphisms, and homozygous deletions of these genes, called *GSTM1* and *GSTT1* null genotypes, result in a lack of enzyme activity (Pemble S. *et al*. 1994, and Seidegard J. *et al*. 1988). An A to G polymorphism at codon 105 in the *GSTP1* gene results in an amino acid substitution (Ile105Val). This residue lies in the substrate binding site of the enzyme and the polymorphism has been shown to affect enzyme activity (Gudmundsdottir K. *et al*. 1997). A decrease in *GST* enzyme activity could result in inefficient detoxification of carcinogens which could lead to genetic damage and increased cancer risk.

It is not yet clear whether the *GST* polymorphisms affect breast cancer risk. To observe the effects of those polymorphisms on breast cancer, *GSTM1*, *GSTP1* and *GSTT1* polymorphisms were analyzed in 264 female breast cancer patients and 233 age-matched controls. When the cases and the controls were compared a statistically significant association was observed only for the *GSTP1* 105 Ile/Val or Val/Val genotypes (OR= 1.64; 95% CI=1.09–2.47) for all women, and for the premenopausal breast cancer patients (OR=2.01, 95% CI=1.06–3.83), which means that premenopausal cases with the *GSTP1* 105 Ile/Val or Val/Val genotype had two or more times risk for breast cancer. The significant association of *GSTP1* 105 Ile/Val or Val/Val genotypes with a high BMI (OR= 2.12, 95% CI=1.35–3.62) was shown in this study, but not with a low BMI (OR= 0.78; 95% CI= 0.45–1.34) and also the same significant association was observed when the women were grouped as

premenopausal (OR=2.14; 95% CI=0.98–4.70) or postmenopausal (OR=2.16; 95% CI=1.14–4.09). The analysis of the *GSTM1* null genotype and the *GSTP1* 105 Ile/Val or Val/Val genotype interaction and also the *GSTT1* null genotype and the *GSTP1* 105 Ile/Val or Val/Val genotype interaction revealed that no possible statistically significant interaction is present for these genes (OR=1.39; 95% CI=0.85-2.28 for *GSTM1* and *GSTP1* combined effect) and (OR= 0.69; 95% CI= 0.35-1.38 for *GSTT1* and *GSTP1* combined effect).

The risk association with the combined risk genotypes of all three *GST* genes was investigated. There was no statistically significant association for the three high risk genotypes, *GSTM1* null genotype, *GSTP1* 105 Ile/Val or Val/Val ge notype, and the *GSTT1* null genotype, (OR= 0.95; 95% CI= 0.37-2.43).

Our observation of the lack of association between breast cancer and *GSTM1* or *GSTT1* null genotypes is in parallel with studies conducted on Australian (Curran JE *et al*. 2000), French (Maugard CM. *et al*. 2001), US Caucasian (Ambrosone CB. *et al*. 1995) and US mixed (Bailey LR. *et al*. 1998) populations. However, our observation contradicts the positive results that have been observed in French (Charrier J. *et al*. 1999), US mixed (Helzlouser KJ. *et al*. 1998), Korean (Park SK. *et al*. 1993) and Finn (Mitrunen K. *et al*. 2001) populations. In our study, we found a positive association between the combined *GSTP1* 105 Ile/Val or Val/Val genotypes in all women and particularly in premenopausal women and breast cancer in the Turkish population. This result appears to be unique except for a US mixed population study (Helzlsouer KJ. *et al*. 1998) in which postmenopausal breast cancer patients were found to be at higher risk in the presence of the *GSTP1* 105 Ile/Val or Val/Val genotypes.

The combination of the *GSTM1* null and the *GSTP1* 105 Ile/Val or Val/Val genotypes and also the combination of the *GSTT1* null genotype and the *GSTP1* 105 Ile/Val or Val/Val genotypes does not lead to any increased risk for breast cancer when compared with the combination of the lower risk genotypes of these genes (Table 13 and Table 14). However, the analysis of a Japanese population for lung cancer (Kihara M. and Noda K. 1999) and a USA population for breast cancer (Helzlouser KJ. *et al*. 1998) showed an increased risk for the combination of the high risk genotypes of the *GSTM1* and the *GSTP1* genes. The analysis of the *GSTM1* and *GSTP1* loci, in a study from Germany for bladder cancer, found no significant association for an increased risk (Steinhoff C. *et al*. 2000).

The risk associated with the combination of the risky genotypes of all three loci was further analyzed and no statistically significant increased risk association was observed. However, the analysis of a Finnish population for breast cancer showed an increased risk for combination of high risk genotypes of the *GSTP1*, *GSTM1* and *GSTT1* genes (Mitrunen K. *et al*. 2001).

The estimation of joint effects for *GST* genotypes and *BRCA1* or *BRCA2* status was not carried out because of the predicted small number of *BRCA1* carriers in the population, and the prediction of *BRCA1* carrier number was due to family history of breast cancer of the cohort, that information was supplied by the questionnaire forms. The increased for breast cancer risk was observed (3.8 times or more) when stratification according to family history of breast cancer was carried out in our study population (OR= 3.80; 95% CI= 1.51-9.55),(Table 12).

The differences in the outcomes of the studies conducted may partly be due to differences in the populations studied and of differences in their exposures to the agents that are relevant to the development of breast cancer. Population heterogeneity is an important issue for the Turkish population and an independent random control cohort was genotyped to test for that issue. It was shown that genotype distributions of the age-matched control group and the randomly selected group were not statistically different. The genotype distributions of the age-matched and the randomly selected controls were compared with the previously reported Turkish population results (Oke B. *et al*. 1998, Toruner GA. *et al*. 2001) by employing homogeneity test (Daniel WW. 1995), and it was shown that none of the *GST* loci differ significantly.

It is well understood that one of the most important risk factors for developing breast cancer is a family history of the disease. However, many non-genetic risk factors contribute to disease etiology. They can be categorized as hormonal and nonhormonal risk factors. As for the environmental exposures, smoking history did not modify the effect of *GST* genotypes as a risk for breast cancer. The information about smoking history of our cohort was missing, however, stratification with the smoking status of known subjects gave no risk assessment related to smoking for breast cancer in consistency with most of the earlier studies (Helzlsouer KJ. *et al*. 1998, Kelsey KT. *et al*. 1997, and Garcia – Closas *et al*. 1999). Non-hormonal risk factors include exposure to ionizing radiation, alcohol consumption and certain dietary factors such as high dietary fat and “well-done”

meat (Wynder EL. *et al*. 1997 and Zheng W. *et al*. 1998). Evidence for non-hormonal risk factors for developing breast cancer is controversial due to study bias, discrepant data and the inherent difficulties associated with obtaining dietary exposure histories (Martin AM. and Weber BL. 2000). A history of alcohol consumption or exposure to ionizing radiation data were not available for our study group.

Estrogen exposure is a well-documented risk factor for breast cancer. A prolonged or increased exposure such as early age at menarche, nulliparity, and late onset of menopause is associated with increased risk. In our study, the cohort was analyzed for established breast cancer risk factors. Compared to controls, cases were slightly older and more likely to have a family history of breast cancer among first-degree relatives. Cases had slightly earlier age at menarche, later age at first live birth, less number of children, and most of the cases were postmenopausal.

There is an association between obesity and increased risk for breast cancer (Ursin G. *et al*. 1997). The major source of estrogen in postmenopausal women is from the conversion of androstenedione to estrone by adipose tissue, thus obesity is associated with a long-term increase in estrogen exposure. According to our analysis, the risk of breast cancer was increased for women who had a high BMI ($\geq$ 26.96) (OR= 1.76; 95% CI= 1.23-2.52). There was a slight increased case-control difference between high body mass index and postmenopausal state in the Turkish population for breast cancer (OR= 1.26; 95% CI= 0.77- 2.05). These observations are consistent with premenopausal observations and the direct association of body mass index with the increased breast cancer risk of postmenopausal women (Chu SY. *et al*. 1991, Brinton LA. *et al*. 1992, Radiner K. *et al*. 1993, and Franceschi S. *et al*. 1996). Interestingly, in our study, it was shown that high body mass index contributed to higher breast cancer risk in relationship to the combined *GSTP1* 105 Ile/Val or Val/Val genotype regardless of the menopausal status. The women with more fat tissue might be exposed to a continuous source of carcinogens, since adipose tissue stores toxins, and stored toxins might serve as a continuous source of carcinogens (Kohlmeier L. *et al*. 1995). The *GSTP1* Ile105Val substitution is located near the substrate binding site of the enzyme and the polymorphism has been shown to affect the enzyme's activity (Gudmundsdottir K. *et al*. 1997). A decrease in *GSTP1* enzyme activity might result in inefficient detoxification of high amounts of carcinogens

deposited in adipose tissues of women with high body mass index which could lead to genetic damage and increased breast cancer risk.

To our knowledge, this is the first genetic study on the associations of *GSTs* with breast cancer in the Turkish population. Our findings support the role for the *GSTP1* 105 Ile/Val or Val/Val genotypes in the development of breast cancer in women, especially in premenopausal women and women with high BMI.

5. Conclusion and Future Perspectives

Our study provided the following data:

1. *GSTP1* Ile105Val polymorphism but not *GSTM1* null and *GSTT1* null is a genetic susceptibility factor for breast cancer, especially for premenopausal cases. However, the combination of the studied polymorphisms of *GSTM1* and *GSTP1*; or *GSTT1* and *GSTP1*; and all three loci do not cause a substantial risk.
2. Traditionally important risk factors for developing breast cancer such as family history of breast cancer, earlier age at menarche, high body mass index, and postmenopausal state contributed to a higher breast cancer risk in the Turkish population.
3. The combined analysis of high body mass index and the studied genes revealed that *GSTT1* and *GSTM1* null genotypes do not interact with a high body mass index. However, if individuals with a high body mass index carry the combined *GSTP1* 105 Ile/Val or Val/Val genotypes their relative risk compared to lean individuals is considerably increased.

The unmeasured genetic and enviromental factors that interact with *GSTs* could also contribute to differences in results across epidemiological studies.

Further studies, including more genotyping, mutation screening and gene expression studies may give us a better understanding of the effects of these genetic variations.

Studies on better defined groups can evaluate the relationship between *GST* polymorphisms and breast cancer pathological staging. Polymorphisms in other genes, which may have important roles in the cellular pathways can also be studied and the combined effect of their interaction with the *GST* genes and with each other on an individual's breast cancer risk can be determined. The analysis of a large number of DNA variations (polymorphisms and mutations) on a genome-wide scale can be carried out with oligonucleotide microarray-based technologies.

The possible effect of GST polymorphisms on DNA damage and the frequency of mutation in cancer-related genes can be analyzed in relation to other factors, most notably the possible modifying effects on the risk associated with germ-line mutations in the BRCA genes.

6. References

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