ANALYSIS OF GSTM1, GSTT1, GSTP1, AND TP53 POLYMORPHISMS AS GENETIC RISK FACTORS FOR BLADDER CANCER IN THE TURKISH POPULATION

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FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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SEPTEMBER 2001

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ABSTRACT

ANALYSIS OF GSTM1, GSTT1, GSTP1, AND TP53 POLYMORPHISMS AS GENETIC RISK FACTORS FOR BLADDER CANCER IN THE TURKISH POPULATION

Gökçe Altay Törüner

Ph.D in Molecular Biology and Genetics

Supervisor: Assoc. Prof. Tayfun Özçelik

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The effect of the *GSTM1* and *GSTT1* null genotypes, the *GSTP1* Ile105Val, and *TP53* Arg72Pro polymorphism on bladder cancer susceptibility was investigated in a case control study of 121 bladder cancer patients, and 121 age-sex matched controls in the Turkish population. The adjusted odds ratio (for age, sex, and smoking status) for the *GSTM1* null genotype is 1.94 (95% CI 1.15- 3.26) and for the *GSTP1* 105 Ile/Val or Val/Val genotypes is 1.75 (95% CI 1.03- 2.99). *GSTT1*, and *TP53* loci was not shown to be associated with bladder cancer. Combination of the two high risk genotypes, *GSTM1* null and *GSTP1* 105 Ile/Val or Val/Val, revealed that the risk increases by 3.91 times (95% CI 1.88-8.13) when compared with the combination of the low risk genotypes of these loci. In individuals with a combined risk of cigarette smoking and the *GSTM1* null genotype, bladder cancer risk is 2.81 (95% CI 1.23-6.35) relative to persons who do not smoke and carry the *GSTM1* present genotype. The same risk for the *GSTP1* 105 Ile/Val or Val/Val genotypes is 2.38 (95% CI 1.12-4.95). These findings support the role for the *GSTM1* null and the *GSTP1* 105 Ile/Val or Val/Val genotypes in the development of bladder cancer. Furthermore, gene-gene (*GSTM1-GSTP1)* and gene-environment (*GSTM1*-smoking, *GSTP1*-smoking) interactions increase this risk substantially.

ÖZET

GSTM1, GSTT1, GSTP1, AND TP53 GEN POLİMORFİZMLERİNİN TÜRK TOPLUMUNDA MESANE KANSERİ İÇİN GENETİK RİSK FAKTÖRÜ OLARAK İNCELENMESİ

Gökçe Altay Törüner

Moleküler Biyoloji ve Genetik Doktorası

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GSTM1 0/0 ve *GSTT1* 0/0 genotipleri ile, *GSTP1* Ile105Val, ve *TP53* Arg72Pro gen polimorfizmlerinin, Türk toplumunda mesane kanserine yatkınlıkla ilişkisi bir hasta-kontrol çalışması kapsamında incelendi. Çalışma grupları 121 mesane kanseri hastasından ve 121 yaş-cinsiyet açısından uyumlu kontrolden oluşmaktaydı. Yaş, cinsiyet ve sigara öyküsü göz önüne alınarak gerekli istatistiki düzeltmeler yapıldıktan sonra, *GSTM1* 0/0 genotipinin 1.94 (95% GA 1.15- 3.26) ve *GSTP1* 105 Ile/Val+ Val/Val genotiplerinin ise 1.75 (95% GA 1.03-2.99). kat risk artışına neden olduğu gözlendi. Bu risk her iki lokus için, riskli genotipler birlikte incelediğinde 3.91 kat (95% CI 1.88-8.13) olarak saptandı. *GSTT1* ve *TP53* lokusları ile mesane kanseri arasında bir ilişki tesbit edilmedi. Sigara oyküsü ve riskli genotip bir arada bulunduğunda risk *GSTM1* lokusu için 2.81 (95% CI 1.23-6.35), *GSTP1* lokusu içinse 2.38 (95% CI 1.12-4.95) olarak bulundu. Bu bulgular *GSTM1* 0/0 ve *GSTP1* 105 Ile/Val+ Val/Val genotiplerinin mesane kanseri için bir risk faktörü olduğuna işaret etmektedir. Ayrıca gen-gen (*GSTM1- GSTP1)* ve gen-çevre (*GSTM1*-sigara öyküsü, *GSTP1*-sigara öyküsü) etkileşimleri gözlemlenen riski önemli ölçüde artırmaktadır.

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TUBITAK

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ABBREVATIONS

AD	Autosomal Dominant
AHR	Arylcarbon receptor
ALOX5	Arachidonate 5-lipooxygenase
APC	Adenomatous Polyposis of colon
AR	Autosomal Recessive
bp	Base Pair
BRCA1	Breast Cancer Susceptibility gene 1
BRCA2	Breast Cancer Susceptibility gene 2
CASP 10	Caspase 10
CDH1	Cadherin 1
CDKN1C	Cyclin dependent kinase 1C
CDKN2A	Cyclin dependent kinase 2A
CYP1A1	Cytochrome P450 1A1
CYP1A2	Cytochrome P450 1A2
CYP1B1	Cytochrome P450 1B1
CYP2A6	Cytochrome P450 2A6
CYP2C9	Cytochrome P450 2C9
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-complemeting rodent deficiency Complementation group 1
ERCC2	Excision repair cross-complemeting rodent deficiency Complementation group 2
ESRRA	Estrogen-related receptor alpha
EXT1	Exotosin 1
EXT2	Exotosin 2
GRRL1	Glucocorticoid receptor like 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
gr	gram
L	Liter
MADH4	Mothers against decapapenaplegic drosophilia homolog of 4
MCR1	Melanocortin receptor 1
MEN1	Multiple Endocrin Neoplasia 1
MGMT	O-methylguanine-DNA methyltransferase
MLH1	Mut L Homologue 1
ml	milliliter
mM	milimolar
MPO	Myeloperoxidase
MSH2	Mut S Homologue 2
NAT1	N-acetyltransferase 1

NAT2	N-acetyltransferase 2
NF1	Neurofibromatosis 1
NF2	Neurofibromatosis 2
ng	nanogram
NR112	Nuclear receptor subfamily 1, group 1, member 2
PCR	Polymerase chain reaction
pmol	picomol
PKU	Phenylketonuria
PPAR A	Peroxisome proliferative activated receptor, alpha
PPAR G	Peroxisome proliferative activated receptor, gamma
PRPKAR1A	Protein kinase c-AMP depenedent regulatory type 1
POLB	Polymerase beta
PTCH	Patched Drosophilia, homologue of
PTGS1	Prostaglandin-endoperoxidase synthase 1
PTGS2	Prostaglandin-endoperoxidase synthase 2
RB	Retinoblastoma gene
RET	Rearranged during transfection
SDHD	Succinate Dehydyrogenase Complex, Subunit D
SMACB1	SWI/SNF related, Matrix Associated, Actin Dependent regulator of chromatin subfamily 1, Member 1
STK11	Serine/Threonine Protein Kinase 11
SULT1A1	Sulfotransferase 1A1
SULT1A2	Sulfotransferase 1A2
TNF	Tumor necrosing factor
TP53	Tumor protein p53
TSC1	Tubero Sclerosis 1
TSC2	Tubero Sclerosis 1
VDR	Vitamin D receptor

VHL	Von Hipple Landau
XD	X-linked domiant
XR	X-linked Recessive
XRCC1	X-ray complemeting repair in Chinese Hamster Cells 1
XRCC2	X-ray complemeting repair in Chinese Hamster Cells 2
XRCC3	X-ray complemeting repair in Chinese Hamster Cells 3
XRCC4	X-ray complemeting repair in Chinese Hamster Cells 4
XRCC5	X-ray complemeting repair in Chinese Hamster Cells 5
WT1	Wilms Tumor1 gene
µl	microliter

1. Introduction

1.1 Genetic Basis of Human Disease

1.1.1 Mendellian Inheritance

None of the fellow monks in the Augustinian monastery, near Brno (in Czech republic) would have thought the impact of the work of their colleague, Gregor Mendel who likes crossbreeding peas in the garden. His work was published in published in the 1866 issue of the *Verhandlungen des naturforschenden Vereins*, the *Proceedings* of the Natural History Society in Brünn (Ostrer, 1998), and remained dormant until the beginning of 20th century.

Briefly Mendel crossed, parent peas, which has a difference only in one characteristic (i.e. seed shape or seed color). He observed that all the progeny (F1 generation) has one trait, he named this appearing trait as dominant, and the lost trait is recessive. When he crossed the F1 generation, he observed that 25% of the progeny (F2 generation) have the recessive trait that is present in F0, but not F1 generation. The reappearance of the recessive characteristic in F2 generation indicated that recessive genes are neither modified nor lost in F1 generation, but the dominant and recessive genes are independently transmitted, and so are able to segregate independently during the formation of sex cells. This is called Mendel's 1st Law: Principle of Independent Segregation. In his further experiments Mendel crossed the seeds with two traits, pure round yellow, and wrinkled green. He saw that in F1 generation all seeds were dominant round yellow form, in F2 generation wrinkled yellow, and round green forms were also emerged with the ratio of 9 round yellow, 3 round green, 3 wrinkled yellow, and 1 wrinkled green. He concluded that each gene pair was independently to the gamete during sex cell formation.

There is no tendency for genes from the same parent to segregate together. This principle is called as Mendel's second law: Principle of independent assortment (Watson, 1988).

Mendellian diseases are the diseases, which are the result of a single mutant gene that has a large effect on phenotype and that are inherited as simple patterns similar to or identical with those described by Mendel for certain discrete characteristics in garden peas (Gelether, 1998).

In medical genetics, a trait is called dominant, if the individual is heterozygous (i.e. one copy of the mutant allele) for the mutant allele, and exhibits the disease phenotype. A trait is regarded as recessive, if the individual is homozygous. (i.e. two copies of the mutant allele) or compound heterozygote (i.e. two different copies of the mutant allele). If an allele is located on sex chromosome, it is called X-linked or Y-linked, but in other 22 chromosomes (autosomes), the trait is called autosomal. Since genes located on Y chromosome is very rare, for practical purposes there are four patterns of inheritance of monogeneic diseases. Autosomal Dominant (AD), Autosomal Recessive (AR), X-linked Recessive (XR), and X-linked dominant (XD). More than 6500 phenotypes have been reported as Mendellian diseases, and more than 50% are AD, 36% are AR, and less than 10% are X-linked (Gelether, 1998).

1.1.2 Non-Mendellian Inheritance

The Non-Mendellian pattern of inheritance of traits was observed due to two reasons. One is the existence of other mammalian modes of inheritance, which were not envisaged by Mendel laws. The other that is a trait (phenotype) is not necessarily composed of one inheritable unit (i.e. gene), many genes (polygenic) and

additional environmental factors (multifactorial) might be responsible for the phenotype.

Mitochondrial inheritance, and genomic imprinting are the examples for the existence of different modes of inheritance (Ostrer, 1998). In mitochondrial inheritance, only the maternal mitochondria are inherited, therefore only the maternal genes are transmitted. This phenomenon is against the principal of independent segregation, since the concept of independence implicitly refers to existence of more than one alleles, while in this case only the maternal allele is segregating. Imprinting denotes to a case that the gene contributes to the phenotype, not due to whether is dominant or recessive vis-a-vis the other allele, but from which parent it is inherited. It is an exceptional situation where the Mendellian concepts of dominance are totally are meaningless.

When a trait is dependent on more than one genes, or environmental factors, it is regarded as multifactorial, and/or polygenic traits. Although the terms polygenic and multifactorial are often used interchangeably, technically speaking their definitions are different. Polygenic traits are the traits caused by the impact of the many genes, each having only a limited individual impact on phenotype, where as the term multifactorial points out the interaction of genetic susceptibility factors and the environment. (Gelehrter, 1998)

It is impotent to note that most traits of medical importance, such as susceptibility to diabetes, hypertension, cancer, coronary heart disease and infection are inherited as multifactorial and/or polygenic traits (Lander and Schork 1994). Therefore the impact for the population is much more than the impact of Mendellian diseases. However it should be remembered that in complex multifactorial diseases, not the disease by itself but the susceptibility to the disease is determined by genetic

factors. The expression of the disease phenotype on a particular individual is based on the interaction of various genetic and environmental factors.

The current paradigm is that the polygenic traits are usually quantitative rather than qualitative, and frequently distributed continuously in a Gaussian distribution. The phenotype is, however, usually by definition is a discontinuous trait. The threshold model is used for explaining the this phenomenon. According to this model, the phenotype is observed, when the accumulated genetic load passes a threshold.

1.2 Genetic Basis of Cancer

1.2.1 General Information

Cancer is a genetic disease in the sense that mutations must take place for the expression of the phenotype. It is a somatic masochism which are characterized by unscheduled, and uncontrolled cellular proliferation of the affected (Ponder 2001). The other common features of cancer cell phenotype are evading apoptosis, self-sufficiency in growth signals, insensitivity to growth signals, limitless replicate potential, sustained angiogenesis, and tissue invasion and metastasis. (Hanahan ane Weinberg 2000). It is quite striking to see the evolution of a normal behaving cells, to an aggressive cancer cells. The current concept is that all the bunch of cancer cells (neoplastic clone) in a patient is the progeny of a single cell (clonal expansion), and a series of events (genetic or epigenetic alterations) should take place for this transformation (multistep carcinogenesis). These events can be classified as gain of function, and loss of function of events (Ponder 2001).

The genes involving in gain of function events are the proto-oncogenes. They are "activated" in various ways, and this activation gives an evolutionary advantage to the cell on which the "activation" takes place. The oncogenes have the role in transmission of the signals for proliferation (e.g. RAS), in proliferation in itself (e.g. cyclin D), and suppression of apoptosis (e.g. Bcl-2). An important point is that these alterations are dominant in nature, (i.e. an alteration in one allele in cell is enough for the expression of the phenotype).

The tumor suppressor genes are involved in loss of function events, as their name implies their loss is associated with neoplasia. They are recessive in nature, since two of the alleles should be inactivated. These genes are classified into two: Gatekeepers, and caretakers (Kinzler and Vogelstein 1997). Gatekeepers are the genes that control the proliferation (e.g. Rb), where as caretakers are the genes responsible for maintaining the integrity of the genome (e.g. MLH1). The tumor suppressor genes primarily involve in cycle control, apoptosis, and DNA repair. The major genes whose alterations are important in cancer related events are shown in red in Figure 1.

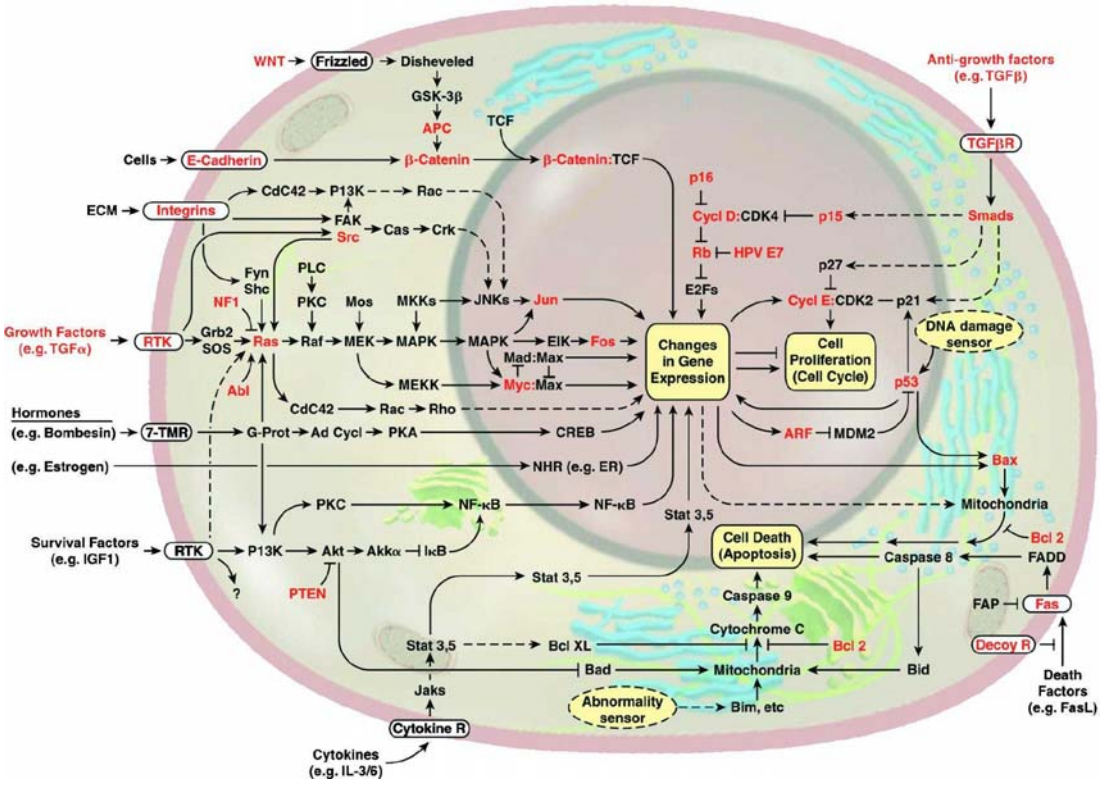

Figure 1. The cellular pathways related to malignant transformation (adopted from Evan and Vousden 2001)

1.2.2 Genetic Predisposition to Cancer

1.2.2.1 Mendellian Inheritance

Importance of familial factors in the pathogenesis of cancer has been appreciated by the medical community, and obtaining a family history from the encountered cancer patient has been routinely conducted. In some rare families cancer has been found to be segregating as an autosomal dominant trait in the family. It was observed that familial history, early age of onset, and neoplasias at multiple sites (either in the same organ or different organs) are the common denominators of these autosomal dominantly segregating familial cancers. In 1971, Alfred Knudson proposed that the germline event in the familial retinablastoma leads to an inactivation of an autosomal tumor suppressor gene in all cells, and a somatic mutation has hit and inactivates the remaining allele, abrogating the total function of the protein, and causes neoplasia. In somatic cancers, however two spontaneous mutations occur in the same cell (Knudson 1971). This model fitted the clinical observations entirely since, it explains the multifocality, and early-age of onset in familial cancers. Knudsons' hypothesis was proven after the cloning of the retinablastoma gene, in 1987 (Lee et al. 1987), and became the central paradigm for familial cancers in many years. The paradigm was challenged by Kinzler and Vogelstein (Kinzler and Vogelstein 1997), by gatekeeper and gatekeeper hypothesis. The reason was that no somatic mutations was found in Hereditary Non-Polyposis colon cancer genes (MLH1, MSH2) which was responsible from DNA repair, and Hereditary Breast Cancer genes (BRCA1, BRCA2) in tumor tissues. Recently however, this observations have been started to be challenged too by the detection of epigenetic silencing of these genes (Bevilacqua and Simpson 2000; Esteller et al.

2000). 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 with the notable exception of RET oncogene. The genes and associated hereditary cancer syndromes are shown in Table 1.

Table 1: List of Familial Cancer Genes and Syndromes

Gene	Locus	Cancer syndrome
APC	5q21	Familial polyposis of colon
BRCA1	17q21	Hereditary Breast/Ovarian Cancer
BRCA2	13q12	Hereditary Breast/Ovarian Cancer
CDH1	16q22.1	Familial gastric carcinoma
CDKN2A	9p21	Cutaneous malignant melanoma
CDKN1C	11p15.5	Beckwith-Wiedeman Syndrome
CYLD	16q12.1	Familial cylindramotosis
EXT1	8q24.11-q24.13	Multiple extoses type 1
EXT2	11p12-p11	Multiple extoses type 2
MADH4	18q21.1	Juvenile Polyposis
MEN1	11q13	Multiple endocrine neoplasia type1
MLH1	3p21	Hereditary non-polyposis colon cancer
MSH2	2p16	Hereditary non-polyposis colon cancer
NF1	17q11.2	Neurofibromatosis type 1
NF2	22q12.2	Neurofibromatosis type
PRKAR1A	17q23-q24	Carney Complex
PTCH	9q22	Nevoid basal cell carcinoma
PTEN	10q23.3	Cowdens Syndrome
RB1	13q14	Familial Retinablastoma
RET	10q11.2	multiple endocrine neoplasia MEN2A, MEN2B and medullar thyroid carcinoma
SDHD	11q23	Familial paraganglioma
SMARCB1	22q11	Rhabdoid predisposition syndrome
TP53	17p13	Li-Fraumeni Syndrome
TSC1	9q34	Tuberous Sclerosis 1
TSC2	16p13.3	Tuberous Sclerosis 1
STK11	19p13.3	Peutz-Jegers syndrome
VHL	3p25	Von Hipple- Lindau Syndrome
WT1	11p13	Familial Wilms Tumor

Adopted from (Futreal et al. 2001), the locus and function information is gathered from GeneCards (http://bioinfo.weizmann.ac.il/cards/)

1.2.2.2 Multifactorial Inheritance

1.2.2.2.1 General Concepts

Cancer pathogenesis is a complex phenomenon. For the pathogenesis, not only what kind of pathway events (i.e. mutations or change in the expression of genes) will occur, but also the factors affecting that probability of the events will occur, and factors that influence the effect of pathway of events are important. (Ponder 2001) (Figure 2). The factors affecting the probability of the events in the cell are actually synonymous, in clinical grounds, with the factors associated with the cancer risk. In cancer syndromes segregating in mendellian fashion, usually part of the pathway of events leading to malignant transformation (e.g. *RB* mutation), or factors affecting the genomic stability in the cell is inherited (e.g. *MLH1* mutation), where as in cancers segregating in non-Mendellian fashion (i.e. so called sporadic cancers), the factors affecting the probability of the events (i.e. mutations) are very important. The main factors are primarily the way the carcinogens are metabolized (Phase I and Phase II drug metabolizing enzymes polymorphisms), and how efficient is the DNA damage is handled (DNA repair enzyme polymorphisms). However the polymorphisms in the genes regulating immune response, hormone regulation, nuclear transcription factors, and cell cycle regulation and apoptosis have been also regarded as important genetic risk factors (see Table 2 for major gene polymorphisms). The impact of these gene polymorphisms for the individual (i.e. their penetrance) is not as dramatic as the genes showing autosomal dominant inheritance.However their impact for the population in terms of public health may be quite important, considering their high frequency in the population.

Table 2 Major gene polymorphisms associated with cancer

Gene	Locus	Protein	Function
CYP1A1	15q22-q24	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP1A2	15q22-qter	Cytochrome P450 1A2	Phase I xenobiotic metabolism
CYP1B1	2p22-p21	Cytochrome P450 1B1	Phase I xenobiotic metabolism
CYP2A6	19q13.2	Cytochrome P450 2A6	Phase I xenobiotic metabolism
CYP2C9	10q24	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP2C19	10q24.1-q24.3	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP2D6	22q13.1	Cytochrome P450 1A1	Phase I xenobiotic metabolism
CYP3A4	7q22.1	Cytochrome P450 1A1	Phase I xenobiotic metabolism
MPO	17q23.1	Myeloperoxidase	Phase I xenobiotic metabolism
DIA4	16q22.1	NAD(P)H: quinone reductase	Phase I xenobiotic metabolism
GSTM1	1p13.3	Glutathione-S-transferase M1	Phase II xenobiotic metabolism
GSTP1	11q13	Glutathione-S-transferase P1	Phase II xenobiotic metabolism
GSTT1	22q11.2	Glutathione-S-transferase T1	Phase II xenobiotic metabolism
NAT1	8p23.1-p21.3	Arylamine N-acetyltransferase type 1	Phase II xenobiotic metabolism
NAT2	8p23.1-p21.3	Arylamine N-acetyltransferase type 1	Phase II xenobiotic metabolism
SULT1A1	16p12.1	Phenol sulfotransferase 1A1	Phase II xenobiotic metabolism
SULT1A2	16p12.1-p11.2	Phenol sulfotransferase 1A1	Phase II xenobiotic metabolism
ERCC1	19q13.2-q13.3	Excision repair cross-complementing rodent repair deficiency, complementation group 1	DNA repair
ERCC2	19q13.2-q13.3	Excision repair cross-complementing rodent repair deficiency, complementation group 2	DNA repair
XRCC1	19q13.2	X-ray repair complementing defective repair in Chinese hamster cells 1	DNA repair
XRRC3	14q32.3	X-ray repair complementing defective repair in Chinese hamster cells 3	DNA repair
XRRC4	16p13.3-p13.13	X-ray repair complementing defective repair in Chinese hamster cells 4	DNA repair
XRCC5	2q35	X-ray repair complementing defective repair in Chinese hamster cells 5	DNA repair
MGMT	10q26	O-6-methylguanine-DNA methyltransferase	DNA repair
POLB	8p11.2	Polymerase (DNA directed), beta	DNA repair
ALOX5	10q11.2	Arachidonate 5-lipoxygenase	Inflammatory and immune response
PTGS1	9q32-q33.3	Prostaglandin-endoperoxide synthase 1	Inflammatory and immune response
PTGS2	1q25.2-q25.3	Prostaglandin-endoperoxide synthase 2	Inflammatory and immune response
CCR2	3p21	Chemokine (C-C motif) receptor 2	Inflammatory and immune response
CCR5	3p21	Chemokine (C-C motif) receptor 5	Inflammatory and immune response
IL1A	2q14	Interleukin-1	Inflammatory and immune response
TNF	6p21.3	TNF (tumor necrosis factor (TNF superfamily, member 2))	Inflammatory and immune response
VDR	12q12-q14	Vitamin D (1,25- dihydroxyvitamin D3) receptor	Hormone regulation
CYP11a	15q23-q24	Cytochrome P450, subfamily Xia	Hormone regulation
CYP17	10q24.3	Cytochrome P450, subfamily XVII	Hormone regulation
CYP19	15q21.1	Cytochrome P450, subfamily XIX	Hormone regulation
ESRRA	11q12	Estrogen-related receptor alpha	Hormone regulation
MC1R	16q24.3	Melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	Hormone regulation
AHR	7p15	Aryl hydrocarbon receptor	Nuclear transcription factor receptor

Gene	Locus	Protein	Function
PPARA	22q13.31	peroxisome proliferative activated receptor, alpha	Nuclear transcription factor receptor
PPARG	3p25	peroxisome proliferative activated receptor, gamma	Nuclear transcription factor receptor
NR1I2	3q12-q13.3	nuclear receptor subfamily 1, group I, member 2	Nuclear transcription factor receptor
TNFRSF6	10q24.1	tumor necrosis factor receptor superfamily, member 6	Cell cycle regulation and apoptosis
TP53	17p13.1	tumor protein p53	Apoptosis, cell cycle regulation,
CASP10	2q33-q34	caspase 10, apoptosis-related cysteine protease	Apoptosis, cell cycle regulation
DFFB	1p36.3	DNA fragmentation factor, 40 kD, beta polypeptide (caspase-activated DNase)	Apoptosis, cell cycle regulation

Partially adopted from Brockmoller et al, 2000, the locus and function information is gathered from GeneCards (http://bioinfo.weizmann.ac.il/cards/)

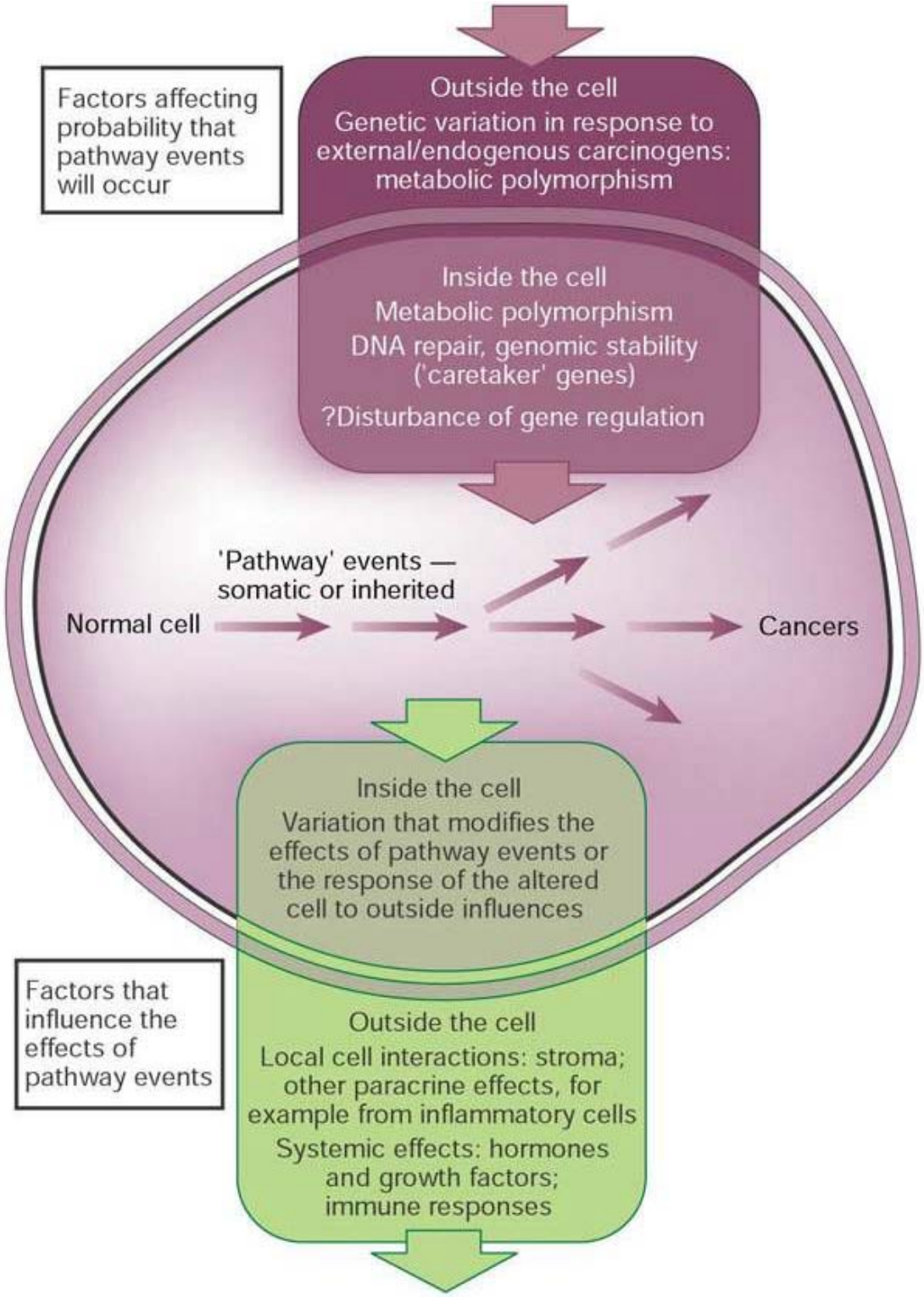

Figure 2. A framework for genetic effects on cancer development.

(adopted from Ponder 2001)

1.2.2.2.2 Glutathione S-Tranferases

Glutahione S-tranferases comprises a super gene family of enzymes of phase 2 enzymes which are responsible of the conjugation of the glutathione to the compounds with a electrophilic, which are activated by cytochrome p450 enzymes (Strange and Fryer 1999). This super family is made of four gene familes (or enzyme classes in a protein oriented perspective), which called are alpha, mu, pi and theta. (there is also a zeta form, which is classified in theta category) (Seidegard and Ekström, 1997; Miller et al. 2000). Each gene family is tandemly located in a particular locus. Alpha is on 6q22, Mu is 1p13, Pi is on 11q13, and Theta is on 22q13.2. Glutathione S-tranferases are dimeric proteins which are located in the cytosol. In addition to these cytosolic enzymes, there are microsamal enzymes which conjugate glutathione. The microsomal enzymes, which are present in outer membrane of microsome mitochondria, do not have a structural similarity to cytosolic GSTs,

Table 3: The Glutathione S-tranferases

Enzyme	Class	Gene	Locus	Compartment
GST A1-1	Alpha	GSTA1	6p12	Cytosol
GSTA2-2	Alpha	GSTA2	6p12	Cytosol
GSTA3-3	Alpha	GSTA4	6p12	Cytosol
GSTA4-4	Alpha	GSTA2	6p12	Cytosol
GSTM1-1	Mu	GSTM1	1p13	Cytosol
GSTM2-2	Mu	GSTM2	1p13	Cytosol
GSTM3-3	Mu	GSTM3	1p13	Cytosol
GSTM4-4	Mu	GSTM4	1p13	Cytosol
GSTM5-5	Mu	GSTM5	1p13	Cytosol
GSTP1-1	Pi	GSTP1	11q13	Cytosol
GSTT1-1	Theta	GSTT1	22q11.2	Cytosol
GSTT1-2	Theta	GSTT2	22q11.2	Cytosol
GSTZ1-1	Theta (zeta?)	GSTZ1	14q24.3	Cytosol
Microsomal gst-1	-	MGST1	1q23	Microsomal membrane
Microsomal gst-2	-	MGST2	4q28-q31	Microsomal membrane
Microsomal gst-3	-	MGST3	12p12.3-p12.1	Microsomal membrane

Partially adopted from (Siegard and Ekström, 1997), the locus information is gathered from GeneCards (http://bioinfo.weizmann.ac.il/cards/)

The range of potential substrates of GSTs is very large, since they can metabolize compounds with an electrophilic center due to high nuclophilicity of the reduced thiol of these enzymes. However, in a biological perspective the substrates of these enzymes can be classified as products of oxidative stress and xenobiotic activation (Strange et al. 1999).

Oxidative products of lipids and DNA can be metabolized by these enzymes. Alpha class of enzymes metabolizes cumene hydoxyoperoxidase, and 4-hydroxyonel, which are products of lipid peroxidation. GSTT2 also catalyze cumene hydoxyoperoxidease. GSTT1 detoxifies oxidative lipid products, and DNA. GSTP1 involves in the detoxification of base propenals (Norppa, 1997)

In addition to metabolizing the products of oxidative stress, these enzymes also catalyze the xenobiotics, which are also environmental carcinogens. One of most important of them is Polycyclic aromatic hydrocarbons. These compounds are activated by cytochrome p450 enzymes. The activated intermediate metabolites actually the carcinogenic form. These epoxide are effective subsrates for mu, and pi class of enzymes. GSTP1-1 enzyme metabolizes the carcinogenic products such as benzo(a)pyrene diol epoxide and acrolein, which are derived from cigarette smoke. GSTT1 enzymes also involve in the metabolism of carcinogenic substances, such as methylating agents, pesticides and industrial solvents. (Seidegard and Ekstöm, 1997; Strange et al, 1999)

It is quite obvious that, the activity of the GSTs is highly critical in the detoxification of the carcinogens. Therefore changes in the activity of these enzymes should have important consequences during the carcinogenic process. The functional consequences of GSTM1 and the GSTT1 null genotypes are clear in terms of enzymatic activity: No gene, no enzyme, no activity. The GSTP1 313 A/G

polymorphism at the nucleotide level leads to an amino acid difference of isoleucine and valinbe at codon 105 in the protein. The valine aminoacid results in decreased enzyme activity (Ali-Osman et al. 1997). Although it is easy to deduce this hypothesis, it is not so easy to prove, which is the main reason that so much controversy exist in the literature about the importance of the genetic polymorphisms and cancer risk.

The data pointing out the significance of these polymorphisms are based on mainly two groups of studies. First group of studies is focused on the association of the polymorphisms and cellular markers showing mutagenic potential. Sister chromatid exchange, Comet assay, and DNA adduct studies are in this group (Norppa, 1997). The second group of studies is case-control and/or case-case type of studies. In these type of studies, genotype frequencies of these polymorphism, and risk factors were assessed.

The association of GSTM1 null genotype with bladder and lung cancer has been replicated in many studies in many ethnic groups. The results of association studies on other cancer sites such as breast, colon, liver, gastric cancer, pituitary adenoma, endometrial cancer, and acute lymphoctic leukemia and larynx are not so replicable. (Table 4).

GSTP1 related data for association studies are largely discordant, though the polymorphisms of this gene might be of importance for neoplasms of breast, prostate, bladder, esophagus and ALL (Table 5).

GSTT1 seems to be associated with cancers of larynx, and skin (basal cell carcinoma), astrocytomas, meningioma, and astrocytomas, ALL and myelodysplastic syndome, but not with cancers of bladder, gastric, liver, endometrium, and ovaries (Table 6).

Table 4. Case-control studies on the association of GSTM1 null genotype and cancer

Reference	Population	Cancer	# of cases	# of controls	Comments
(Chen et al. 1996a)	USA mixed	ALL	197	416	Not associated per se, but interacts with GSTT
(Krajinovic et al. 1999)	French -Canadian	ALL	177	304	Associated
(Saadat and Saadat 2000)	Iranian	ALL	38	75	Associated,
(Chen et al. 1996b)	US Mixed	AML	96	201	Not associated
(Crump et al. 2000)	USA mixed	AML	297	152	No risk
[Chen, 1996 #266]	USA mixed	Anal cancer	71	360	Not associated
(Elexpuru-Camiruaga et al. 1995)	UK Caucasian	Astrocytoma	109	577	Not associated
(Heagerty et al. 1994)	UK Caucasian	BCC	435	153	Associated
(Heagerty et al. 1996)	UK Caucasian	BCC	699	561	Associated
(Marshall et al. 2000a)	UK Mixed	BCC	112	112	Not associated
(Yengi et al. 1996)	UK	BCC	286	300	Not associated
(Aktas et al. 2001)	Turkish	Bladder	102	201	Associated, increase risk of invasion
(Anwar et al. 1996)	Egyptian	Bladder	22	21	Associated, interacts with CYP2D6
(Bell et al. 1993)	USA mixed	Bladder	229	211	Associated, interacts with smoking
(Brockmoller et al. 1996b)	German	Bladder	374	373	Associated
(Georgiou et al. 2000)	Greece	Bladder	89	147	Associated
(Katoh et al. 1998)	Japanese	Bladder	145	145	Associated, interacts with GSTT1
(Kempkes et al. 1996)	German	Bladder	113	170	Associated
(Kim et al. 2000b)	Korea	Bladder	121	222	Associated, interacts with asthma?
(Lin et al. 1994)	USA mixed	Bladder	114	1104	Not associated
(Mungan et al. 2000)	Dutch	Bladder	61	69	Associated
(Okkels et al. 1996)	Danish	Bladder	159	342	Not associated
(Rothman et al. 1996)	Chinese	Bladder	38	43	Not associated
(Salagovic et al. 1999)	Slovakian	Bladder	76	248	Not associated Per se, interacts with GSTT, and smoking1
(Schnakenberg et al. 2000a)	German	Bladder	157	223	Not associated Per se, interacts with NAT2
(Steinhoff et al. 2000)	German	Bladder	135	127	Associated
(Zhong et al. 1993)	UK	Bladder	97	225	Not associated
(Ambrosone et al. 1995)	USA caucasian	Breast	494	439	Not associated

Reference	Population	Cancer	# of cases	# of controls	Comments
(Bailey et al. 1998)	US Mixed	Breast	263	263	Not associated
(Charrier et al. 1999)	French	Breast	361	437	Assocaition with postmenapausal risk
(Curran et al. 2000)	Australia	Breast	129	129	No risk
(Garcia-Closas et al. 1999)	USA mixed	Breast	466	466	Not associated
(Helzlsouer et al. 1998)	US mixed	Breast	110	133	Associated, and interacts with GSTP1
(Maugard et al. 2001)	French	Breast	220	196	Not associated
(Millikan et al. 2000)	US mixed	Breast	688	561	Not associated
(Mitrunen et al. 2001)	Finn	Breast	483	482	Associated in premenaposal woman, interacts with GSTP1, GSTT1
(Park et al. 2000b)	Korea	Breast	189	189	Associated, interacts with GSTT1
(Zhong et al. 1993)	UK	Breast	197	225	Not associated
(Chen and Nirunsuksiri 1999)	USA Caucasian	Cervix	190	206	No risk
(Goodman et al. 2001)	USA Hawai	Cervix	131	180	Not associated
(Abdel-Rahman et al. 1999)	Egyptian	Colon	66	55	No risk
(Butler et al. 2001)	Australian	Colon	219	200	Not associated
(Chenevix-Trench et al. 1995)	Australia	Colon	132	100	Not associated
(Deakin et al. 1996)	UK Caucasian	Colon	252	577	Not associated
(Gawronska-Szklarz et al. 1999)	Poland	Colon	70	145	Associated
(Gertig et al. 1998)	USA mixed	Colon	212	221	Not associated
(Guo et al. 1996)	Chinese	Colon	19	23	Associated
(Inoue et al. 2001)	Japanese	Colon	205	220	Not associated
(Katoh et al. 1996)	Japanese	Colon	103	126	Associated
(Lin et al. 1995)	USA mixed	Colon	446	488	Not associated
(Saadat and Saadat 2001)	Iranian	Colon	42	131	Not associated Per se, interacts with GSTT1
(Welfare et al. 1999)	UK	Colon	178	178	No association
(Zhang et al. 1999)	Swedish	Colon	99	109	No association
(Zhong et al. 1993)	UK	Colon	196	225	Associated
(Esteller et al. 1997)	Spanish	Endometrium	80	60	Not associated
(Tan et al. 2000)	Chinese	Esopahgus	150	146	Associated
(van Lieshout et al. 1999)	Holland	Esopahgus(Barret)	98	247	No association
(Lin et al. 1998b)	China	Esophagus	45	45	Associated, interacts with GSTM1

Reference	Population	Cancer	# of cases	# of controls	Comments
(Morita et al. 1997)	Japanese	Esophagus	53	132	Not associated
(Katoh et al. 1996)	Japanese	Gastric	139	126	Associated
(Baranov et al. 1996)	Russian	GI	37	67	Associated
(McGlynn et al. 1995)	USA Asian	HCC	52	116	Associated
(Omer et al. 2001)	Sudan	HCC	110	189	Associated, interacts with peanut butter
(Yu et al. 1995b)	Taiwan	HCC	30	150	Not associated
(Cheng et al. 1999)	USA mixed	Head and Neck	162	315	Associated
(Kihara et al. 1997)	Japanese	Head and Neck	150	474	Associated, interacts with smoking
(Ko et al. 2001)	German	Head and Neck			Not associated
(Matthias et al. 1999b)	German	Head and Neck	398	216	Not associated
(McWilliams et al. 2000)	US mixed	Head and Neck	160	114	Not associated
(Morita et al. 1999)	Japanese	Head and neck	145	164	Not associated
(Olshan et al. 2000)	US mixed	Head and Neck	182	202	Not associated Per se, but interacts with CYP1A1
(Trizna et al. 1995)	USA	Head and Neck	186	42	Associated
(Hong et al. 2000a)	Korea	Larynx	82	63	Associated, interact with GSTT1
(Jahnke et al. 1996)	UK Caucasian	Larynx	269	216	Associated
[Jourenkova, 1998 #88]	French	Larynx	129	172	Not associated Per se, but interacts with GSTM1
(Jourenkova-Mironova et al. 1999b)	Frecnh	Larynx	129	172	Not associated per se, but interacts with GSTT
(Lemos et al. 1999)	Portugese	Leukemia (mixed)	64	128	Not associated
(Nair et al. 1999)	Indian	Leukoplakia	98	82	Associated,
(Alexandrie et al. 1994)	Swedish	Lung	296	329	Not associated
(Belogubova et al. 2000)	Russian	Lung	58	297	No
(Bennett et al. 1999)	USA Mixed	Lung	106		Smoking, interacts with GSTM1 null hebotype
(Brockmoller et al. 1993)	German	Lung	117	200	Not associated
(Chen et al. 2001)	Chinese	Lung	106	106	Combined risk with CYP1A1 Val allele
(Dresler et al. 2000)	USA mixed	Lung	180	163	Combined risk with CYPA1 for females
(El-Zein et al. 1997)	USA Caucasian	Lung	52	48	Associated
(Ford et al. 2000)	USA Black	Lung	117	120	Associated, interacts with smoking
(Gao and Zhang 1999)	Chinese	Lung	59	132	Associated
(Hirvonen et al. 1993)	Finn	Lung	138	142	Associated
(Hou et al. 2000)	Norwegian	Lung	282	357	Associated, interacts with NAT2
(Kelsey et al. 1997b)	US Mixed	Lung	168	278	No association
(Kihara and Noda 1994)	Japanese	Lung	178	201	Associated, interacts with smoking

Reference	Population	Cancer	# of cases	# of controls	Comments
(Kihara and Noda 1995b)	Japanese	Lung	447	469	Associated, interacts with smoking
(Kihara and Noda 1995a)	Japanese	Lung	118	301	Associated, interacts with smoking, and CYP1A1
(Kihara and Noda 1999)	Japanese	Lung	382	257	Associated, interacts with GSTP1 and smoking
(Lan et al. 2000)	China	Lung	122	122	Associated, interacts with smoky coal
(London et al. 1995)	USA mixed	Lung	342	716	Not associated
(Moreira et al. 1996)	Portugese	Lung	98	84	Not associated
(Persson et al. 1999)	Chinese	Lung	76	122	Not associated
(Ryberg et al. 1997)	Norwegian	Lung	63	177	Associated
(Saarikoski et al. 1998)	Finn	Lung	208	294	Not associated Per se, interacts with GSTM1
(Stucker et al. 2000)	French	Lung	247	254	Associated, interacts with CYP1A1
(To-Figueras et al. 1996)	Spanish	Lung	139	147	Associated, interacts with TP53
(Woodson et al. 1999)	USA mixed	Lung	319	333	No association
(Xue et al. 2001)	Chinese	Lung	112	112	Associated, interacts with CYP1A1
(Baranov et al. 1996)	Russian	Lung,	58	67	Associated
(Deakin et al. 1996)	UK Caucasian	Lung,	108	577	Not associated
(Davies et al. 2000)	USA Caucasian	MDS	232	153	Associated
(Heagerty et al. 1994)	UK Caucasian	Melanoma	64	153	Not associated
(Lafuente et al. 1995)	Spanish	Melanoma	183	147	Associated
(Shanley et al. 1995)	Australia	Melanoma	124	100	Not associated
(Kanetsky et al. 2001)	USA Caucasian	Melanoma	362	271	Not associated Per se, but interacts with hair color
(Elexpuru-Camiruaga et al. 1995)	UK Caucasian	Meningioma	49	577	Not associated
(Hirvonen et al. 1995)	Finn	Mesothelioma	44	270	Associated, interacts with smoking
(Deakin et al. 1996)	UK Caucasian	Oral	40	577	Not associated
(Hung et al. 1997)	Taiwanese	Oral	41	123	Associated, interacts with GSTT1
(Katoh et al. 1999)	Japaneese	Oral	92	147	Associated
(Kietthubthew et al. 2001)	Thailand	Oral	53	53	Assocaited, interacts with smoking
(Park et al. 2000a)	US Black	Oral	63	103	Associated, interacts with smokiing
(Baxter et al. 2001)	Australia	Ovarian	293	219	Associated
(Lallas et al. 2000)	US mixed	Ovarian	80	80	Not associated
(Sarhanis et al. 1996)	UK Caucasian	Ovary	84	312	Not associated
(Spurdle et al. 2001)	Australian	Ovary	285	299	Associated with endometrois, and clear cell Ca
(Liu et al. 2000)	Canada (mixed)	Pancreas	149	149	Not assocaited
(Jourenkova-Mironova et al. 1999a)	French	Pharynx	121	172	Not associated

Reference	Population	Cancer	# of cases	# of controls	Comments
(Fryer et al. 1993)	UK Caucasian	Pituitary adenoma	113	89	Associated
(Autrup et al. 1999)	Danish	Prostate	153	288	Associated
(Gsur et al. 2001)	Austira	Prostate	166	166	Not associated
(Kelada et al. 2000)	USA mixed	Prostate	276	499	Not associated
(Kote-Jarai et al. 2001)	UK Mixed	Prostate	275	280	Not associated
(Murata et al. 2001)	Japanese	Prostate	126	126	Not associated
(Rebbeck et al. 1999)	US Mixed	Prostate	237	239	Not associated
(Bruning et al. 1997)	German	RCC	45	48	Associated
(Longuemaux et al. 1999)	French	RCC	173	211	Not associated Per se, but interacts with GSTP1 and NAT2
(Sweeney et al. 2000)	US Mixed	RCC	130	505	No association
(Heagerty et al. 1994)	UK Caucasian	SCC	85	153	Not associated
(Setiawan et al. 2000)	Chinese	Stoamch	91	429	Not associated
(Kato et al. 1996)	Japanese	Stomach	82	151	Not associated
(Saadat and Saadat 2001)	Iranian	Stomach	46	131	Associated, interacts with GSTT1
(Deakin et al. 1996)	UK Caucasian	Stomach,	136	577	Not associated
(Chen et al. 1999)	USA Mixed	Vulva	137	248	Noı risk

Table 5. Case control studies on the association of GSTP1 Ile105Val polymorphism and cancer

Reference	Population	Cancer	# of cases	# of controls	Comments
(Marshall et al. 2000a)	UK Mixed	BCC	112	112	Val/Val is associated
(Harries et al. 1997)	UK mixed	Bladder	76	155	Not associated
(Steinhoff et al. 2000)	German	Bladder	135	127	Not associated
(Curran et al. 2000)	Australian	Breast	129	129	Not associated
(Helzlsouer et al. 1998)	US mixed	Breast	110	133	Val allele is associated, and interacts with GSTM1
(Krajinovic et al. 2001)	French-Canadian	Breast	149	207	Not associated
(Lavigne et al. 1997)	US Mixed	Breast	112	112	Not associated
(Maugard et al. 2001)	French	Breast	220	196	Ile allele is associated
(Millikan et al. 2000)	US mixed	Breast	688	561	Not associated
(Mitrunen et al. 2001)	Finn	Breast	483	482	Not associated Per se, but interacts with GSTT1, GSTM1
(Harris et al. 1998)	Australian	Colon	131	199	Not associated
(Katoh et al. 1999)	Japanese	Colon	47	122	Not associated
(Welfare et al. 1999)	UK Mixed	Colon	178	178	Not associated
(Yoshioka et al. 1999)	Japanese	Colon	106	100	Not associated Per se, but interacts with GSTM1
(Tan et al. 2000)	Chinese	Esopahgus	150	146	Not associated
(van Lieshout et al. 1999)	Holland	Esopahgus	98	247	Val/Val is associated
(Lee et al. 2000)	Taiwanese	Esophagus	90	254	Ile/Ile is associated, and interacts with smoking
(Lin et al. 1998b)	Chinese	Esophagus	45	45	Not associated
(Morita et al. 1999)	Japanese	Head and neck	145	164	Ile/Ile is associated
(Olshan et al. 2000)	US mixed	Head and Neck	182	202	Not associated
(Jourenkova-Mironova et al. 1999b)	French	Larynx	129	172	Not associated
(Harris et al. 1998)	Australian	Lung	184	199	Not associated
(Katoh et al. 1999)	Japanese	Lung	382	257	Not associated
(Kihara and Noda 1999)	Japanese	Lung	382	257	Not associated Per se, but interacts with GSTM1
(Ryberg et al. 1997)	Norwegian	Lung	135	342	Associated, interacts with GSTM1
(Saarikoski et al. 1998)	Finn	Lung	208	294	Not associated
(To-Figueras et al. 1999)	Spanish	Lung	164	200	Not associated
(Katoh et al. 1999)	Japanese	Oral	83	122	Val/Val is associated

Reference	Population	Cancer	# of cases	# of controls	Comments
(Matthias et al. 1998)	German	Oral/Pharynx	380	180	Val/Val is associated
(Spurdle et al. 2001)	Australian	Ovary	285	299	Not associated
(Jourenkova-Mironova et al. 1999a)	French	Pharynx	121	172	Not associated
(Autrup et al. 1999)	Danish	Prostate	153	288	Not associated
(Gsur et al. 2001)	Austrian	Prostate	166	166	Ile/Ile is associated
(Harries et al. 1997)	UK mixed	Prostate	36	155	Val/Val is associated
(Kote-Jarai et al. 2001)	UK Mixed	Prostate	275	280	Not associated
[Wadelius, 1999 #66]	Swede, Dane	Prostate	425	425	Not associated
(Longuemaux et al. 1999)	French	RCC	173	211	Val allele is associated and interacts with GSTM1
(Sweeney et al. 2000)	US Mixed	RCC	130	505	Not associated
(Katoh et al. 1999)	Japanese	Stomach			Not associated
(Harries et al. 1997)	UK mixed	Testis			Not associated
(Katoh et al. 1999)	Japanese	Urothelial			Not associated

Table 6. Case control studies on the association of GSTT1 null genotype and cancer

Reference	Population	Cancer	# of cases	# of controls	Comments
(Infante-Rivard et al. 1999)	French-Canadian	ALL	491	491	Not associated
(Krajinovic et al. 1999)	French –Canadian	ALL	177	304	Not associated
(Crump et al. 2000)	US Mixed	AML	297	152	Not associated
(Chen et al. 1996a)	USA mixed	Anal cancer	71	360	Not associated
(Elexpuru-Camiruaga et al. 1995)	UK Caucasian	Astrocytoma	109	577	Associated
(van Lieshout et al. 1999)	Holland	Barret's esopahgus	98	247	Not associated
(Heagerty et al. 1996)	UK Caucasian	BCC	699	561	Not associated
(Marshall et al. 2000a)	UK Mixed	BCC	112	112	Not associated
(Yengi et al. 1996)	UK	BCC	286	300	Not associated
(Brockmoller et al. 1996a)	German	Bladder	374	373	Not associated
(Georgiou et al. 2000)	Greek	Bladder	89	147	Not associated
(Katoh et al. 1998)	Japanese	Bladder	145	145	Not associated, but interacts with GSTM1
(Kempkes et al. 1996)	German	Bladder	113	170	Not associated, but interacts with smoking
(Kim et al. 2000b)	Korea	Bladder	121	222	Not associated
(Salagovic et al. 1999)	Slovakian	Bladder	76	248	Associated, interacts with GSTM1, and smoking1
(Schnakenberg et al. 2000b)	German	Bladder	157	223	Not associated
(Steinhoff et al. 2000)	German	Bladder	135	127	Not associated
(Bailey et al. 1998)	US Mixed	Breast	263	263	Not associated
(Charrier et al. 1999)	French	Breast	361	437	Association with postmenopausal risk
(Curran et al. 2000)	Australian	Breast	129	129	Not associated
(Helzlsouer et al. 1998)	US mixed	Breast	110	133	Not associated
(Millikan et al. 2000)	US mixed	Breast	688	561	Not associated
(Mitrunen et al. 2001)	Finn	Breast	483	482	Not associated
(Park et al. 1997)	Korea	Breast	189	189	Associated, interacts with GSTM1
(Goodman et al. 2001)	USA Hawai	Cervix	131	180	Not associated
(Kim et al. 2000a)	Korean	Cervix	181	181	Associated, interacts with GSTM1
(Warwick et al. 1994)	UK	Cervix	175	180	Associated
(Abdel-Rahman et al. 1999)	Egyptian	Colon	66	55	Not associated

Reference	Population	Cancer	# of cases	# of controls	Comments
(Butler et al. 2001)	Australian	Colon	219	200	Not associated
(Chenevix-Trench et al. 1995)	Australia	Colon	132	100	Not associated
(Deakin et al. 1996)	UK Caucasian	Colon	252	577	Associated
(Gertig et al. 1998)	USA mixed	Colon	212	221	Not associated
(Guo et al. 1996)	Chinese	Colon	19	23	Associated
(Inoue et al. 2001)	Japanese	Colon	205	220	Not associated
(Katoh et al. 1996)	Japanese	Colon	103	126	Associated
(Saadat and Saadat 2001)	Iranian	Colon	42	131	Not associated Per se, interacts with GSTM1
(Welfare et al. 1999)	UK	Colon	178	178	No association
(Zhang et al. 1999)	Swedish	Colon	99	109	Associated
(Esteller et al. 1997)	Spanish	Endometrium	80	60	Not associated
(Tan et al. 2000)	Chinese	Esopahgus	150	146	No association
(Lin et al. 1998a)	China	Esophagus	45	45	Associated, interacts with GSTM1
(Katoh et al. 1996)	Japanese	Gastric	139	126	Associated
(Wiencke et al. 1997)	US Caucasian	Glioma	188	166	Associated with oligodendroglioma
(Omer et al. 2001)	Sudan	HCC	110	189	Associated, interacts with peanut butter
(Yu et al. 1995a)	Taiwan	HCC	30	150	Not associated
(Cheng et al. 1999)	US Mixed	Head and Neck	162	315	Associated ,interacts with GSTM1
(Ko et al. 2001)	German	Head and Neck			Not associated
(Matthias et al. 1999a)	German	Head and Neck	398	216	Not associated
(McWilliams et al. 1995)	US mixed	Head and Neck	160	114	Not associated
(Olshan et al. 2000)	US mixed	Head and Neck	182	202	Not associated Per se, but interacts with smoking
(Trizna et al. 1995)	USA	Head and Neck	186	42	Not associated
(Hong et al. 2000b)	Korea	Larynx	82	63	Not associated Per se, but interacts with GSTM1
(Jahnke et al. 1996)	UK Caucasian	Larynx	269	216	Associated
(Jourenkova et al. 1998)	French	Larynx	129	172	Not associated Per se, but interacts with GSTM1
(Jourenkova-Mironova et al. 1999b)	Frecnh	Larynx	129	172	Not associated per se, but interacts with GSTT
(Nair et al. 1999)	Indian	Leukoplakia	98	82	Associated
(Bennett et al. 1999)	USA Mixed	Lung	106		Not associated
(El-Zein et al. 1997)	USA Caucasian	Lung	52	48	Associated
(Kelsey et al. 1997a)	US Mixed	Lung	168	278	Not associated
(Kihara and Noda 1994)	Japanese	Lung	178	201	Associated, interacts with smoking
(Lan et al. 2000)	China	Lung	122	122	Not associated

Reference	Population	Cancer	# of cases	# of controls	Comments
(Saarikoski et al. 1998)	Finn	Lung	208	294	Not associated Per se, interacts with GSTM1
(To-Figueras et al. 1996)	Spanish	Lung	139	147	Not associated
(Xue et al. 2001)	Chinese	Lung	112	112	Associated, interacts with CYP1A1
(Deakin et al. 1996)	UK Caucasian	Lung,	108	577	Not associated
(Chen et al. 1996b)	US Mixed	MDS	96	201	Associated
(Davies et al. 2001)	USA Caucasian	MDS	232	153	Not associated
(Kanetsky et al. 2001)	USA Caucasian	Melanoma	362	271	Not associated Per se, but interacts with hair color
(Shanley et al. 1995)	Australia	Melanoma	124	100	Not associated
(Elexpuru-Camiruaga et al. 1995)	UK Caucasian	Meningioma	49	577	Associated
(Deakin et al. 1996)	UK Caucasian	Oral	40	577	Not associated
(Hung et al. 1997)	Taiwanese	Oral	41	123	Associated, interacts with GSTM1
(Katoh et al. 1999)	Japaneese	Oral	92	147	Not associated
(Kietthubthew et al. 2001)	Thailand	Oral	53	53	Not associated
(Sarhanis et al. 1996)	UK Caucasian	Ovary	84	312	Not associated
(Spurdle et al. 2001)	Australian	Ovary	285	299	Not associated
(Liu et al. 2000)	Canada (mixed)	Pancreas	149	149	Not associated
(Jourenkova-Mironova et al. 1999a)	French	Pharynx	121	172	Associated
(Autrup et al. 1999)	Danish	Prostate	153	288	Not associated, but interacts with GSTM1
(Gsur et al. 2001)	Austira	Prostate	166	166	Not associated
(Kelada et al. 2000)	USA mixed	Prostate	276	499	Associated, interacts with smoking
(Kote-Jarai et al. 2001)	UK Mixed	Prostate	275	280	Not associated
(Murata et al. 2001)	Japanese	Prostate	126	126	Not associated
(Rebbeck et al. 1999)	US Mixed	Prostate	237	239	Associated
(Bruning et al. 1997)	German	RCC	45	48	Associated
(Longuemaux et al. 1999)	French	RCC	173	211	Not associated Per se, but interacts with GSTP1 and NAT2
(Sweeney et al. 2000)	US Mixed	RCC	130	505	Associated
(Setiawan et al. 2000)	Chinese	Stoamch	91	429	Associated
(Kato et al. 1996)	Japanese	Stomach	82	151	Not associated
(Saadat and Saadat 2001)	Iranian	Stomach	46	131	Associated, interacts with GSTM1
(Deakin et al. 1996)	UK Caucasian	Stomach,	136	577	Not associated

1.2.2.2.3 TP53 gene

TP53 is an tumor supressor gene that has been mutated in 50% of all human cancers. It involves the in the cellular functions which are highly related with cancer such as cell cycle regulation, DNA repair, apoptosis and senescence. In addition to mutations, some polymorphisms exist in the coding region of the gene. These polymorphisms are located in codon 21, codon 36, codon 47, codon 72 and codon 213 of the gene. The polymophisms at codon 21, codon 36, and codon 213 gene does not result in an aminoacid change, where as nuclotide change at codon 47 results in Pro-Ser, and at codon 72 results in Arg-Pro change (Table 7)

Table 7. Major exonic polymorphisms of TP53 gene

Codon	Exon	Nucleotide change	Amino Acid Change	Reference
21	2	GAC -> GAT	Asp ->Asp	Ahuja et al, 1990
36	2	CCG -> CCA	Pro ->Pro	Felix et al, 1994
47	4	CCG -> TCG	Pro ->Ser	Felley-Bosco et al, 1993
72	4	CGC -> CCC	Arg ->Pro	Matlasheski et al, 1987
213	6	CGA -> CGG	Arg->Arg	Carbone et al, 1991

The most interesting polymorphism of the TP 53 gene is Arg72Pro polymorphism. It has been known since 1987 (Matlashewski et al. 1987), however its significance as a genetic susceptibility factor for cancer is still a matter of controversy. The association studies on various cancers reveal quite discordant results (see Table 8). The biological consequences of the polymorphism is not clear either. The current models for the biological relevance are as follows: 1. P53 protein encoded by Arg allele is more likely to degraded by a ubiquitin dependent mechanism upon the combination of E6 protein of Human Papilloma Virus (HPV). This model is used for the explanation of the observed susceptibility due to Arg alele in HPV associated cancers, particularly cervix cancer. The other model differences

the between Arg and Pro forms of the p53 protein in binding to p73 protein, and neutrolize p73 induced apoptosis. Arg form binds stronger (Marin et al. 2000)

Table 8. Case control studies on the association of TP53 Arg72Pro polymorphism and cancer

Reference	Population	Cancer	# of cases	# of controls	Comments
(Chen et al. 2000)	Taiwanese	Bladder	59	58	Pro allele is associated with invasiveness
(Papadakis et al. 2000)	Greek	Breast	56	61	Arg/Arg genotype is associated
(Sjalander et al. 1996)	Swedish	Breast			Pro allele is associated
(Agorastos et al. 2000)	Greek	Cervix	88	30	Arg/Arg genotype is associated
(Baek et al. 2000)	Korean	Cervix	52	103	No association
(Kim et al. 2001)	Korean	Cervix	134	100	No association
(Madeleine et al. 2000)	US Mixed	Cervix	111	164	No association
(Minaguchi et al. 1998)	Japanese	Cervix	103	110	No association
(Pegoraro et al. 2000)	Zulu	Cervix	121	251	No association
(Rosenthal et al. 1998)	UK caucasian	Cervix	50	150	No association
(Tenti et al. 2000)	Italian	Cervix	101	140	No association
(Zehbe et al. 1999)	Swedish	Cervix	30	626	Arg/Arg genotype is associated
(Zehbe et al. 1999)	Italian	Cervix	28	40	Arg/Arg genotype is associated
(Murata et al. 1996)	Japansee	Colon	115	152	No association
(Lee et al. 2000)	Taiwanese	Esophagus	90	254	Pro/Pro genotype is associated
(Peixoto Guimaraes et al. 2001)	China	Esophagus	57	32	No association
(Yu et al. 1999)	Taiwanese	HCC	80	328	Not associated Per se, but interacts with GSTM1 and smoking
(Hamel et al. 2000)	French-Canadian	Head and Neck	163	163	No association
(Fan et al. 2000)	US Mixed	Lung	482	510	Pro allele is associated
(Jin et al. 1995)	US Black	Lung	67	74	Pro/Pro genotype is associated
(Jin et al. 1995)	US Mexican	Lung	42	40	Pro/Pro genotype is associated
(Kawajiri et al. 1993)	Japanese	Lung			Pro/Pro genotype is associated
(Murata et al. 1996)	Japanese	Lung	191	152	Arg/Arg genotype is associated
(Pierce et al. 2000)	US Mixed	Lung	334	446	No association
(To-Figueras et al. 1996)	Spanish	Lung	139	147	Not associated Per se, but interacts with GSTM1
(Wang et al. 1999)	Taiwanese	Lung			Pro/Pro genotype is associated

Reference	Population	Cancer	# of cases	# of controls	Comments
(Weston et al. 1994)	US Mixed	Lung	31	39	No association
(Birgander et al. 1996)	Chinese	Nasopahrynx	73	105	Pro allele is associated
(Golovleva et al. 1997)	Chinese	Nasopharynx	64	99	Pro/Pro genotype is associated, and interacts with IFNA17 gene
(Summersgill et al. 2000)	US Mixed	Oral	202	303	No association
(Tandle et al. 2001)	Indian	Oral	72	153	No association
(Rosenthal et al. 1998)	UK caucasian	Ovarian	96	150	No association
(Wu et al. 1995)	Japanese	Prostate	33	56	No association
(Wu et al. 1995)	Japanese	Renal	85	56	No association
(Bastiaens et al. 2001)	Holland	Skin SCC	86	168	No association
(Marshall et al. 2000b)	UK mixed	Skin SCC	55	177	No association
(O'Connor et al. 2001)	Irish	Skin SCC	55	115	No association
(Wu et al. 1995)	Japanese	Testicular	28	56	No association
(Wu et al. 1995)	Japanese	Urothelial	151	56	No association
(Rosenthal et al. 2000)	UK Mixed	Vulva	52	246	Pro allele is associated

1.3 Bladder Cancer

1.3.1 Clinical Information

1.3.1.1 Epidemiology and Etiology

Bladder cancer is the first cancer that an association between environmetal risk factors and the incidence of cancer has been demonstrated. As early as in 1985 Dr. Ludwig Rehn reported on bladder cancer patients who manufactured anniline dyes (Johansson and Cohen, 1997). Although the main cause of bladder cancer is cigarette smoking throughout the world, local conditions also play a role. In the developed countries such as United states, occupational exposure is responsible for 25% of cases. Schistomasis plays an important role in Egypt, Balkan nephropathy is associated with bladder cancer in former Yugoslavia and Bulgaria, and arsenic in drinking water is an important factor in Argentina, Chile and Taiwan. Age, sex and the race is also an important risk determinant. Bladder cancer is more common in males, old persons (more than 55), and Caucasians than females, young persons (less than 55), and Blacks (Johansson and Cohen, 1997).

Bladder cancer is the 3rd most common cancer in males, and the 8th most common cancer in females in the Turkish population (Özsarı and Atasver 1997). These observations are smilar to European Union countries particularly Greece, Italy and Spain (Black et al, 1997). The main etiological agent in Turkey is cigarette smoking (Akdas et al, 1990; Fidaner et al, 2001).

1.3.1.2 Pathology

95 % of bladder cancers are transitional cell carcinoma of the bladder. Squamous cell carcinoma constitutes about remaining 4%. The other rare histological forms are adenocarcinoma, and undifferentiatd carcinoma

The stage is defined as the estimation of extent (size and location) of the cancer at the current time. More specifically, how extensive is the cancer within the bladder and if it has spread to tissues around the bladder, or to other parts of the body. Currently two staging systems are used one is Marshall-Jewett- Strong, which has been developed by Jewett and Strong in 1946, and modified in 1952

the other is TNM system (Tumor, Lymph node, and Metastasis) which has been developed by Union Internationale Contre Le Cancer (UICC). TNM staging is shown in Table 9. In daily paractice, tumors are also classified as superfical and invasive. Superficial tumors are the tumors which did not invade the muscularis propria (i.e lower tha n T2). The patients with superfial tumors has a better prognosis compared to the patiens with invasive tumors.(Lapham et al, 1997).

The tumor grading is based on anaplasia. Grade 1 tumors show mild cytological atypia and rare mitosis; Grade 2 tumors show moderate cytological atypia and the presence of mitotic figures; Grade 3 tumors show severe cytological atypia and frequent mitotic figures.

Table 9. TNM staging of Bladder Cancer

Primary Tumor	
TX	Primary tumor can not be assesed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Ta	Papillary non-invasive carcinoma
T1	Tumor invades subepithelial tissue
T2	Tumor invades superficial muscle
T3	Tumor invades deep muscle
T4	Tumor invades adjacent organs
Regional Lymph Nodes (N)	
NX	Regional lymph nodes can not be assesed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node, less than 2cm.
N2	Metastasis in a single lymph node, more than 2cm, but less than 5cm or multiple lymphnodes
N3	Metastasis in a single lymph node, more than 5cm
Distant metastasis	
MX	Metastasis can not be assesed.
M0	No distant metastasis
M1	Distant metastasis

1.3.2 Genetic predispositon to bladder cancer

The genetic factors have an influence on the risk factor. Broadly speaking there are two patterns of inheritance of bladder cancer. One is the very rare Mendellian pattern, the other is the multifactorial (polygenic) pattern of inheritance. The Mendellian form of bladder cancer has been reported alone (Fraumeni and B. 1967; Capps et al. 1968) or along with other cancers as a syndromic fashion (McCullough et al. 1975; Chan and Pratt 1977; Nagane et al. 1996). No specific gene has been identified yet. Althouhg in a family, a germ line translocation has been reported (Schoenberg et al. 1996), this observation coul not be in larger studies (Aben et al. 2001). Large epidemiological studies have shown that, the first degree relatives appear to have an increased risk for bladder cancer by a factor of 2 compared to general population (Kiemeney and Schoenberg 1996; Dong and

Hemminki 2001) and the interaction of the familial and environmental risk factors have been demonstrated by epidemiological studies(Kunze et al. 1992). The current paradigm is that primarily bladder cancer is a multifactorial disease, in which environmental and genetic factors interact in the predispositon. The association studies between genetic polymorphism and bladder cancer usually points out an association between GSTM1 and NAT2 locus, and bladder cancer. The cytochrome p450 enzyme and H-Ras polymoprphisms does not seem to be risk factor. The polymorphisms of GSTP1, XRRC1, TP53 are emerging hot topics because of initial observed assocaitions (Table 10).

Table 10: Genetic association (case-control) studies on bladder cancer

Reference	Population	Genes	# of cases	# of controls	Comments
(Abdel-Rahman et al. 1998)	Egyptian	GSTM1 GSTT1	37	34	Association for GSTM1 Association for GSTT1 Combined effects of GSTM1, and GSTT1
(Aktas et al. 2001)	Turkish	GSTM1	102	201	Association for GSTM1, increase risk of invasion
(Anwar et al. 1996)	Egyptian	GSTM1 CYP2E1 CYP2D6	22	21	Association for GSTM1 No association for CYP2E1 No association for CYP2D6 Combined effects of GSTM1, and CYP2D6
(Bell et al. 1993)	USA mixed	GSTM1	229	211	Associated, interacts with smoking
(Benitez et al, 1990)	Spanish	CYP2D6	125	556	Association for CYP2D6
(Brockmoller et al. 1996a)	German	GSTM1 GSTT1 NAT2 CYP1A1 CYP2C19 CYP2D6 CYP2E1	374	373	Association for GSTM1 No association for GSTT1 Association for NAT2 No association for CYP1A1 No association for CYP2C19 No association for CYP2D6 No association for CYP2E1
(Chen et al. 2000)	Taiwanese	TP53	57	58	Not associated, but 72 Pro is associated with invasiveness
(Farker et al.1998)	German	CYP2E1	224	304	No association for CYP2E1
(Georgiou et al. 2000)	Greece	GSTM1 GSTT1	89	147	Association for GSTM1, No association for GSTT1
(Hanssen et al, 1985)	German	NAT1-NAT2	105	42	Association for NAT1-NAT2
(Harries et al, 1997)	UK mixed	GSTP1			Association for GSTP1
(Hayward et al, 1988)		H-RAS	35	168	No association for H-RAS
[Hsieh, 1999 #39]	Taiwan	NAT1 NAT2	74	184	No association for NAT1 No association for NAT2

(Inatomi et al. 1999)	Japanese	NAT2	85	146	Association for NAT2 Combined effects with smoking
(Ishikawa et al, 1987)	Japanese	H-RAS	58	58	No association for H-RAS
(Katoh et al. 1995)	Japanese	CYP1A1 GSTM1	83	101	No association for CYP1A1 Association for GSTM1
(Katoh et al. 1998)	Japanese	GSTM1 GSTT1	145	145	Association for GSTM1, No association for GSTT1 Combined effects of GSTM1, T1 and smoking
(Katoh et al, 1999)	Japanese	GSTP1			No association for GSTP1
(Kempkes et al. 1996)	German	GSTM1 GSTT1	113	170	Association for GSTM1, No association for GSTT1
(Kim et al. 2000b)	Korea	NAT2 GSTM1	121	222	No association for NAT2 Association for GSTM1
(Lin et al. 1994)	USA mixed	GSTM1	114	1104	No association for GSTM1
(Mungan et al. 2000)	Dutch	GSTM1	61	69	Association for GSTM1
(Okkels et al. 1996)	Danish	GSTM1	159	342	No association for GSTM1
(Okkels et al. 1997)	Danish	NAT1 NAT2 GSTM1	242	242	No association for NAT1 No association for NAT2 No association for GSTM1
(Risch et al, 1995)	UK Caucasian	NAT2	189	54	Association for NAT2 Combined effects with smoking
(Rothman et al. 1996)	Chinese	GSTM1	38	43	No association for GSTM1
(Salagovic et al. 1999)	Slovakian	GSTM1 GSTT1	76	248	No association for GSTM1 Association for GSTT1 Combined effects of both genes with smoking
Schnakenberg et al, 1998)	German	NAT2	60	154	Association for NAT2

Reference	Population	Genes	# of cases	# of controls	Comments
(Steinhoff et al. 2000)	German	GSTM1 GSTP1 GSTT1	135	127	Association for GSTM1 No association for GSTP1 No association for GSTT1
(Stern et al, 2001)	US Mixed	XRCC1	235	213	Association for XRCC1
(Taylor et al. 1998)	US Mixed	NAT1 NAT2	230	203	Association for NAT1 No association for GSTM1 Combined effects of NAT1, NAT2, and smoking
(Mommsen et al, 1985)	Swedish	NAT1-NAT2	228	100	Association for NAT1-NAT2 (phenotyping study)
(Ladero et al, 1985)	Finn	NAT1-NAT2	157	130	Association for NAT1-NAT2 (phenotyping study)
(Karakaya et al, 1986)	Turkish	NAT1-NAT2	23	109	No association for NAT1-NAT2 (assayed by sulfamethazine metabolism)
(Horai et al, 1989)	Japanese	CYPD6 NAT1-NAT2	51	203	No association for CYPD6 (assayed by metoprolol metabolsim) No association for NAT2 (assayed by dapsone metabolism)
(Hayes et al, 1993)	China	NAT2	38	43	No association for NAT2, when exposed to benzidine
(Kaisary et al,1997)	US Mixed	CYPD6 NAT1-NAT2	98	110	Association for CYPD6 (assayed by debrosquine metabolsm) No association for NAT2 (assayed by dapsone metabolism)
(Zhong et al. 1993)	UK	GSTM1	97	225	No association for GSTM1

1.4 Aim

The purpose of this study is to determine whether GSTM1 null, GSTP1 Ile105Val, GSTT1 null, and TP53 Arg72Pro polymorphisms are genetic susceptibilty factors for the bladder cancer in the Turkey. The questions that this work specifically deals are:

1. Are Glutathione S-tranferase and TP53 polymorphisms genetic risk factors for the bladder cancer in Turkish population?
2. Are Glutathione S-tranferase and TP53 polymorphisms are associated with the invasiveness in bladder cancer?
3. Is there a risk increase due to the interaction of cigarette smoking with Glutathione S-tranferase and TP53 polymorphisms?

The GSTM1 locus was included in this study, since in some populations negative results were reported, and no data about this polymorphism was available for the Turkish population at the beginning of the study.

The GSTP1 locus was studied, because its role as a risk factor for bladder cancer were less established. Actually there were only two studies with opposite conclusions in regard to the association of bladder cancer with this locus. This is the third study adressing this issue, and the first study where cigarette smoking was taken into account in the design of the study.

The GSTT1 locus was analyzed, due to the fact no data was available for the Turkish population in regard to the association with bladder cancer.

TP53 Arg72Pro polymorphism was studied, as data was not available not only for the Turkish population, but also for the Caucasians in general.

2.Materials and Methods

2.1 Materials

2.1.1 Subjects

121 bladder cancers, 121 age-sex matched controls, and 77 random controls were enrolled to the study. Information about the participants were first recorded to the appropriate forms, and this data is stored in computer also in Excel format. 10 ml of venous blood were obtained from all participants, and genomic DNA is isolated as described in section 2.1.2. Informed consent was obtained from all subjects

2.1.1.1 Patient group

121 bladder cancer patients (transitional cell carcinoma, mean age: 60.15, standard deviation: 11.10, age range: 25- 87, % of smokers: 72.0, male-female ratio: 5:1) diagnosed at Hacettepe University Medical School (n=92), and Ankara Numune Hospital (n=29). Information about sex, age of the patient, smoking status and histopathology of the tumor was obtained from medical records.

2.1.1.2 Age-sex matched control group

The age-sex matched control group comprised of 121 individuals from Atatürk Chest Disease Research Hospital (non-cancer patients, mean age: 59.33, standard deviation: 13.58, age range: 23-79, % of smokers: 63.8, male-female ratio: 5:1). Information about sex, age of the patient, and smoking status was obtained from medical records.

2.1.1.3 Random controls

77 randomly selected Bilkent University students were also included in the study. Information age and sex of the patient was obtained by face to face interview during venoupuncture.

2.1.2 Oligonucleotides

The following oligonucleotides, in table were used during the PCR experiments.

Table 11. List of oligonucleotides for PCR experiments

Primer	Sequence	Reference	Target gene	Size
G1 G2	5'-GAA CTC CCT GAA AAG CTA AAG C 5'-GTT GGG CTC AAA TAT ACG GTG G	Anwar et al. *1996*	GSTM1	215 bp
P105-F P105-R	5'-ACC CCA GGG CTC TAT GGG AA 5'-TGA GGG CAC AAG AAG CCC CT	Harries et al. *1997*	GSTP1	176bp
GSTT1-F GSTT1-R	5'-AGG CAG CAG TGG GGG AGG ACC 5'-CTC ACC GGA TCA TGG CCA GCA	Bringuier et al. *1998*	GSTT1	138bp
CYP2E1-F CYP2E1-R	5'-CCA GTC GAG TCT ACA TTG TCA 5'-TTC ATT CTG TCT TCT AAC TGG	Anwar et al. *1996*	CYP2E1	412bp
P53+ P53-	5'-TCC CCC CTT GCC GTC CCA A 5'-CGT GCA AGT CAC AGA CTT'	Storey et al, 1998	TP53	279bp

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
Isoamyl alcohol	Carlo Erba, Milano, Italy
NuSieve 3:1 Agarose	Basica LE, EU
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

Gene Amp PCR system 9600	Perkin Elmer, CA, USA
Taq polymerase (5U/µl)	MBI Fermentas Inc., NY, USA
10X PCR buffer	
(100 mM Tris-HCl (pH 8.8 at 25 °C),	
500 mM KCl, 0.8% Nonidet P40)	MBI Fermentas Inc., NY, USA
25 mM $MgCl_2$	MBI Fermentas Inc., NY, USA
10 mM dNTP mix	MBI Fermentas Inc., NY, USA
Thermowell™ (0.2 ml) tubes	Corning Costar Corp.,MA, USA

2.1.5 Restriction enzymes

Alw261	MBI Fermentas Inc., NY, USA
BstU1	MBI Fermentas Inc., NY, USA

2.1.6 Standard solutions

Agarose gel loading buffer (6X)

15 % ficoll

0.05 % bromphenol blue

0.05 % xylene cyanol

Extraction buffer

10 mM Tris HCl, pH 8.0

10 mM EDTA, pH 8.0

0.5 % SDS

Proteinase K 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

2.2 Methods

2.2.1 DNA isolation

Blood samples can be stored at 4 ^{0} C for a maximum of five days before aliquoting and freezing. Before starting DNA isolation, blood was frozen in 700 µl aliquots at - 80 ^{0} C for at least one day. Blood was thawed and 800 µl of 1X SSC was added and the content was mixed by vortexing. Then, it was centrifuged in a microfuge (Heraeus instruments, Biofuge, Osterode, Germany) at 13000 rpm for 1 minute. The supernatant was removed and discarded into the disinfectant. It is important not to disturb the cell pellet during this step. 1.4 ml 1X SSC was added, the tube was vortexed briefly to resuspend the cell pellet, and was centrifuged at 13000 rpm for a minute. The supernatant was removed again. The washing proceedure with 1XSSC can be repeated for several times if necessary. 800 µl extraction buffer (10 mM TrisHCl ph 8.0, 10 mM EDTA pH 8.0, 0.5 % SDS) and 10 µl proteinase K (20 g/ml ddH_2O) were added to the tube, and the cell pellet was resuspended. The suspension was incubated at 56 ^{0} C for at least 4 hours. If the cell pellet were dissolved, overnight incubation was done. When the cell pellet was dissolved completely 400 µl phenol/chloroform/isoamyl alcohol (25:24:1) was added, then the tube was vortexed for 60 seconds. This step must be carried out in the fume hood. Afterwards the tube was centrifuged in a microfuge for 5 minutes at 13,000 rpm. The upper aqueous layer (~ 700 µl) (the part containing DNA) was removed and placed in a new tube. If DNA supernatant was sticky or if the interface was not clear after this step, the supernatant was not removed, and the extraction was repeated until the interface is clear. The recovered supernatant was separated into two tubes (350 µl per tube) The DNA was then precipitated from the suspension by adding 35 µl NaOAc (3M, pH 5.2) and 700 µl ice-cold absolute ethanol (EtOH) are

added to each tube, mixing by inversion and placing at - 20 ^{0} C for 30 minutes. The tubes were spun in a microfuge for 15 minutes at 13,000 rpm. After removing the absolute ethanol, the pellet was washed with 1.0 ml room temperature 70 % ethanol. Then the tubes were centifuged in a microfuge for 5 minutes at 13,000 rpm. All the alcohol was removed with a micropipette and the tubes were left open on the bench (~30 min) to allow the ethanol to evaporate. The isolated DNA was solubilized in 200 µl TE (pH 8.0) by incubating at 56 ^{0} C for at least 1 hour. Overnight incubation was done the pellet was not in solution. The DNA was then stored at - 20 ^{0} C.

2.2.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a technique, which is used to in the analysis of specific nucleotide sequences. PCR amplification involves two oligonucleotide primers that flank the target DNA and repeated cycles of amplification. There are three distinct events in PCR cycle : Template denaturation, primer annealing and DNA synthesis. After denaturation (i.e seperation of DNA doublestrands), the primers anneal to their complementary single-stranded target sequences. The last step is the extension of the oligonucleotide primer by the heat stable Thermus aquaticus (Taq) tpolymerase. Each cycle causes an exponential increase of the target DNA fragment, about 2^{n} where n is the number of the cycles. Initial denaturation or final elongation steps can be added to before and after of PCR cycles for better yield.

2.2.3 Agarose Gel Elcetrophoresis

Agarose gel electrophoresis is a commonly used method for DNA analysis. The method is based on the mobility of DNA molecules in the pores of agarose, which is an algue derrived polymer. DNA runs in the agarose (from cathode to anode) during the electrophoresis, since it has a negative charge due to phosphate groups in the backbone. The rate of migration is a function of the size of the pores (i.e concentration of the agarose), the magnitude of the applied current, and the weight and the shape of the DNA molecule.

For the purpose of the analysis of PCR amplification products, and TP53 fragments after digestion, 2% (gr/ml) agarose gels were used. Agarose gels were preapered with 1XTBE. They contain 1 μl of Ethidium Bromide solution (20mg/ml). 5 μl of PCR products was loaded in 1 μl 6X loading buffer to the gel. Runs were performed with 1XTBE at 100 V for 30 minutes.

GSTP1 digestion products were analyzed in 3% 3:1 NuSieve gel. 20 μl of digested PCR product was loaded 4 μl 6X loading buffer to the gel. Runs were performed with 1XTBE at 60 V for 2 hours . The gel was stained in a container with with Ethidium Bromide solution (1 mg/ml)

2.2.4 Analysis with restriction endonucleases

Restriction enzyme digestion of PCR products were performed in 25 μl reaction volumes. Reactions are carried out using the reaction buffer and conditions recommended by the manufacturer. Two unist of enzyme is used to digest the PCR products. PCR samples were run on agarose gel before the digestion. The incubation temperature was 37 ^{0} C for all of the enzymes. After digestion, heat inactivation was performed at 65 ^{0} C. After incubation the cut and uncut PCR fragments were

analysed by agarose gel electrophoresis. DNA size markers are used to calculate the sizes of the bands.

2.2.5 Genotyping of DNA samples

2.2.5.1 GSTM1 genotyping

GSTM1 genotyping was done by simultaneous amplification of GSTM1 primers with CYP2E1 primers (Table 2) in the same polymerase chain reaction (PCR) tube.. PCR products were electrophoresed in 2% agarose gels, and visualized by ethidium bromide staining. Null genotype was scored by the presence of a 412-bp *CYP2E1* band in the absence of a 215 bp *GSTM1* fragment. (See Figure 3 for schematic description.)

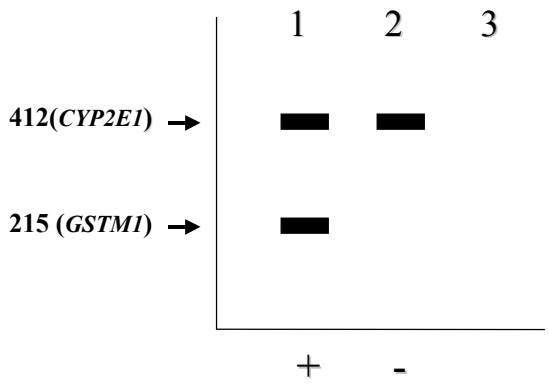

Figure 3. Schematic description of GSTM1 genotyping

2.2.5.2 GSTP1 genotyping

Ile105Val polymorphism in *GSTP1* was analyzed by this method. Amplification was carried out using primers p105F, and p1051R (Table 2). 176 bp amplified product was digested with 2 U *Alw*261 at 37^{o}C for 4 hours. The digested fragments were electrophoresed in 3% NuSieve gel. Presence of the restriction site resulted in two fragments of 91 bp and 85 bp which was indicative of the Val allele. (See Figure 4 for schematic description.)

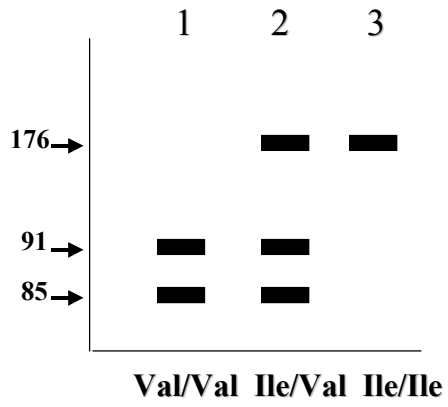

Figure 4. Schematic description of GSTP1 genotyping

2.2.5.3 GSTT1 genotyping

GSTT1 genotype was determined by using the previously described primers GSTT1F, and GSTT1R in combination with the above mentioned *GSTP1* primers. A *GSTT1* specific 138 bp fragment was observed in positive individuals. Null genotype was scored after confirming with at least two independent experiments. (See Figure 5 for schematic description.)

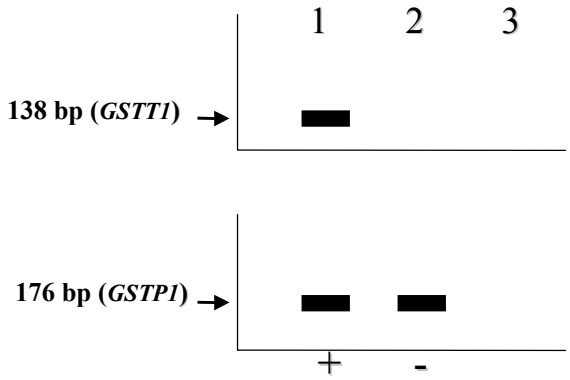

Figure 5. Schematic description of GSTT1 genotyping

2.2.5.4 TP53 genotyping

P53 Arg72Pro polymorphism was determined by a PCR-RFLP method. Amplification was carried out using primers P53+ and , P53-. 279 bp amplified product was digested with *Bst*U1 enzyme at 37^{o}C for 4 hours and electrophoresed in 2% agarose gels. Presence of the restriction site resulted in two fragments of 160 bp, and 129 bp which was indicative of the Arg allele.

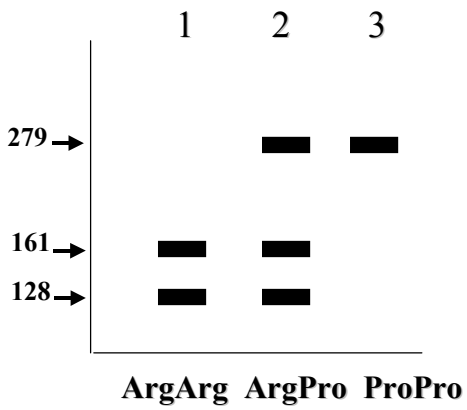

Figure 6. Schematic description of TP53 codon 72 genotyping

2.2.6 Statistics

2.2.6.1 Chi-square test

The chi-square test is a technique for the analysis of counts and frequency data. It is primarily used for evaluation of categorical variables, (e.g GSTP1 genotype, whose values are Ile/Ile, Ile/Val, and Val/Val). The quantative data employed in the computation of the statistic, are the frequncies associated with each category of the one or more variables under study. There are two type of frequecies: Observed, and Expected. Observed frequencies are the number of the subjects that fall into varios categories of the variable of interest (e.g variable: GSTP1, categories: Ile/Ile, Ile/Val, and Val/Val). Expected frequencies are the number of subjects which are expected to be observed, if null hypothesis is about the variable is true. (Null hypothesis is the hypothesis to be tested , which is also called as a hypothesis of no difference). The test statistic for chi-squre test is

$$X^2 = \Sigma \left[\frac{(Oi - Ei)}{Ei} \right]$$

Where the null hypothesis is true, X^2 is distributed approxiamately as X^2 with k-r degrees of freedom. In determining the 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 columns represent the various level of a second criterion, degrees of freedom are calculated as (r-1)(c-1)=df

The quantity X^2 will be small if the observed and expected frequencies are close and will be large if the differences are large. The calculated X^2 value is compared to the tabulated (in a X^2 table or stored in a computer) X^2 value with the appropriate degrees of freedom. Null hypothesis rejected if the calculated X^2 is

larger or equal to the tabulated X^2 for a chosen α (α value denotes type I error which is rejecting the the probability of rejecting the true null hypothesis -i.e. stating that the difference is meaningful, where as it is not-. The α values below 0.05 are accepted as statistically significant by convention).

2.2.6.2 Odds ratio calculation

There are two types of observational studies. One is prospective, the other is reterospective. Prospective study is related with future. The subjects are stratified according to whether they have the risk factor or not. Then after a certain time of follow up, the outcome was evaluated. (e.g GST genotyping now, follow up for 30 years to see who will have bladder cancer). Retrospective study, retrospective literally means looking back, is related with past. The persons with the outcome, consitutes the study group, and the subjects were determined whether they have the risk factor or not (e.g take a bladder cancer group, and control group, determine they smoked or not, and then GST genotyping). In general prospective study is more expensive, and difficult to carry out, but the information is more valuable, since it resembles an experiment. The term relative risk is used for the risk estimation obtained from prospective studies. It is actually the ratio of the risk of devoloing a disease among subjects with the risk factor to the risk of developing the disease among subjects without the risk factor.

The relative risk estimation is not meaningful in a retrospective study. In a retrospoective studies odds ratio is used. Odds ratio can be a estimation of the relative risk if the disease in a given population if the studied disease is a rare disease. (e.g cancer is OK, but not common cold). A value greater than 1 indicates increaes odds of having disease among subjects in whom the risk factor is present.

An odds ratio value greater than 1 is statistically significant, if the lower border of 95% confidince interval is greater than 1 (Daniel 1995).

Odds ratios (OR) and 95 %confidence intervals (CI) were calculated according to these formulas. (Daniel 1995)

$$OR=AD/BC$$

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

Table 12. Sample 2x2 Table for OR analysis

	Control	Case
Risky genotype	A	C
Non-Risky genotype	B	D

A: # of controls bearing the risky genotype
B: # of controls bearing the non-risky genotype
C: # of cases bearing the risky genotype
D: # of cases bearing non risky genotype

2.6.2.3 Analysis of Gene-Gene Inteaction

The analysis was by a model adopted from (Yang and Khoury). While using this method, both cases and controls are stratified accoding to the genotypes, then the odds ratios were calculated by comparing the reference (the stratum the individuals inheriting no risk genotypes) to the other strata respectively. (Table 10). The odds ratio for the reference group (i.e 00 individuals) is 1, since odds ratio for this group is calculated by comparing the reference group by itself.

Table 13. A simple gene-gene interaction model for case- control studies

Gene X	Gene Y	Cases	Controls	Odds ratio
0	0	A_{00}	B_{00}	1
0	1	A_{01}	B_{01}	$R_X=A_{01}\ B_{00/}\ A_{00}\ B_{01}$
1	0	A_{10}	B_{10}	$R_Y=A_{10}\ B_{00/}\ A_{00}\ B_{10}$
1	1	A_{11}	B_{11}	$R_{XY}=A_{11}\ B_{00/}\ A_{00}\ B_{11}$

0= risk allele absent; 1=risky allele present

$R_{X=}$ Relative risk caused by risky alelle of Gene X
$R_{Y=}$ Relative risk caused by risky alelle of Gene Y
$R_{XY=}$ Relative risk caused by risky alelles of Gene X and Gene Y

3. Results

3.1 Glutathone S-transferases

The genotype frequencies of the *GSTM1*, *GSTP1* and *GSTT1* polymorphisms in the patient, and the age-sex matched control groups is summarized in Table 14. The adjusted relative risk (for age, sex, and smoking status) conferred by the *GSTM1* null genotype for bladder cancer is 1.94 (95% CI 1.15-3.26). Since the *GSTP1* 313 Val/Val genotype frequency was too low in our population, *GSTP1* 105 Ile/Val and Val/Val genotypes were combined for cancer risk estimation. The risk figure is 1.75 (95% CI 1.03- 2.99). Finally, *GSTT1* null genotype was not found to be a significant risk factor (OR 1.27; 95% CI 0.66-2.47) for bladder cancer.

Table 14. Distribution of the *GSTM1*, *GSTP1* and *GSTT1* genotypes in the age-sex matched controls and bladder cancer patients

Locus	Genotype	Case n=121 (%)	Control n=121 (%)	Crude OR *(95% CI)*	Adjusted OR[1] (95%CI)	p
GSTM1	Present	46 (38.02)	66 (54.55)			
	Null	75 (61.98)	55 (45.45)			
				1.96 (1.18-3.22)	1.94 (1.15-3.26)	0.010
GSTP1	Ile/Ile	67 (55.37)	83 (68.60)			
	Ile/Val	42 (34.71)	33 (27.27)			
	Val/Val	12 (9.92)	5 (4.13)			
	Ile/Val or Val/Val	54 (44.63)	38 (31.40)			
				1.76 (1.04-2.94)	1.75 (1.03-2.99)	0.034
GSTT1	Present	97 (80.17)	100 (82.64)			
	Null	24 (19.83)	21 (17.36)			
				1.17 (0.61-2.22)	1.27 (0.66-2.47)	0.620

A group of randomly selected university students (n=77) was also genotyped to compare with the age-sex matched control group. In the randomly selected group, the *GSTM1* null genotype is 46.7% (p=0.858), the *GSTT1* null genotype is 17.25% (p=0.936), and the *GSTP1* genotype frequencies are 67.53% (Ile/Ile), 31.16% (Ile/Val) and 1.31% (Val/Val) (p=0.820). These results reveal that the genotype frequencies for the age-sex matched control group, and the randomly selected group is not significantly different. This indicates absence of bias of ascertainement during the selection of the age-sex matched control group. Distrubition of GST genotypes were in Hardy-Weinberg equilibrium in all three groups.

Combination of the two high risk genotypes, *GSTM1* null and *GSTP1* 105 Ile/Val or Val/Val, revealed that the risk increases by 3.91 times (95% CI 1.88-8.13) when compared with the combination of the low risk genotypes of these loci (Table 15).

Table 15. Combination of the *GSTM1* null with *GSTP1* 105 Ile/Val or Val/Val genotypes and bladder cancer risk

Genotype at risk	*GSTM1*	*GSTP1*	Case n=(121)	Control (n=121)	Crude OR (95%CI)	Adjusted OR[2] (95%CI)
None[1]	Present	Ile/Ile	24	41	1.00 (referral)	1.00 (referral)
One	Null	Ile/Val	43	42	1.75 (0.94-3.25)	2.07 (1.00-4.30
	Present	Ile/Val, Val/Val	22	25	1.50 (0.69-3.74)	1.89 (0.91-3.93)
Two	Null	Ile/Val, Val/Val	32	13	4.20 (1.85-9.58)	3.91 (1.88-8.13)

[1]The group that includes the combination of no-risk genotypes "None" 'is used as a reference group for relative risk analysis.
[2]Adjusted for age, sex and smoking status.

The risk associated with the combination of the risky genotypes of all three GST loci was further investigated (Table 16), even though the *GSTT1* null genotype alone does not appear to be a significant risk factor for bladder cancer in the Turkish

population. Individuals with all three putative low risk genotypes, that is the presence of *GSTM1* and *GSTT1* genotypes and the homozygous Ile/Ile genotype for *GSTP1* is designated as the reference group. The relative risk conferred by the three high-risk genotypes versus no high-risk genotype is 8.00 (95% CI 1.52-287.10).

Table 16. GST genotype distribution and risk associated with genotype combinations

High-risk Genotypes	*GSTM1*	*GSTP1*	*GSTT1*	*Cases (n=121)*	*Controls* (n=121)	OR (95% CI)
Three	Null	Ile/Val or Val/Val	Null	8	2	8.00 (1.52-287.10)
Two	Null	Ile/Val or Val/Val	Present	24	11	4.36 (1.75-10.80)
	Null	A/A	Null	7	8	1.75 (0.54-5.52)
	Present	Ile/Val or Val/Val	Null	2	4	1.00 (0.16-5.58)
One	Null	A/A	Present	36	34	2.11 (1.06-4.41)
	Present	Ile/Val or Val/Val	Present	20	21	1.90 (0.84-1.69)
	Present	A/A	Null	7	7	2.00 (0.60-6.61)
No	Present	A/A	Positive	17	34	1.00

The risk of bladder cancer from GST genotypes was also evaluated by smoking status (Table 17). Among non smokers, a slight but not statistically significant increased risk of bladder cancer which was associated with the *GSTM1* null (OR 1.95; 95% CI 0.74-5.05), the *GSTP1* Ile/Val or Val/Val (OR 1.78; 95% CI 0.65-4.80), and the *GSTT1* (OR 1.53; 95% CI 0.51-4.52) genotypes was observed. Among smokers a significantly elevated risk of bladder cancer which was associated with the *GSTM1* null genotype was detected (OR 2.02; 95% CI 1.04-3.93). An association was not observed for either *GSTP1* or *GSTT1*.

The effect of the combined contributions of genotype and smoking status to bladder cancer risk is displayed in Table 18. Individuals with the *GSTM1* null genotype who smoke have an increased risk of 2.81 (95% CI 1.23-6.35) compared to the individuals with the *GSTM1* present genotype who do not smoke. With respect

to the *GSTP1* locus, this risk figure is 2.38 (1.12-4.95). An association with the *GSTT1* locus was not found.

The patients were also grouped according to the stage of the disease to determine whether GST genotypes are associated with the invasiveness of the tumor (Table 19). Although statistically significant results could not be obtained, the *GSTP1* 105 Ile/Val+ Val/Val genotypes appear to be a risk factor for invasiveness either alone (OR: 2.06, 95% CI 0.91- 4.6) or in combination with the *GSTM1* null genotype (OR: 3.42, 95% CI 0.96- 12.2).

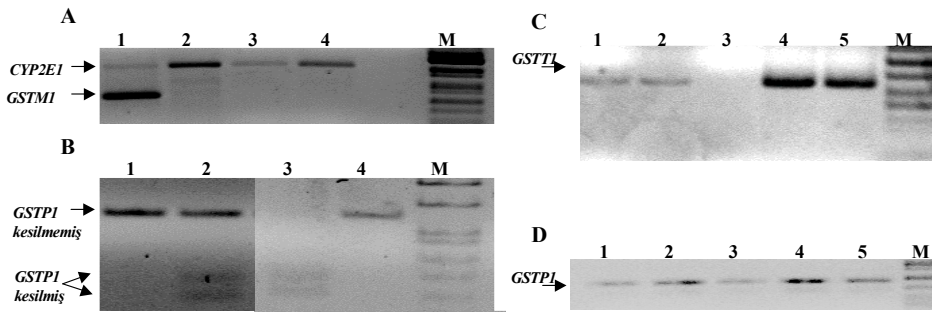

Figure 3. Genotyping of Glutathione S-Tranferase genes

A. *GSTM1* primers generate a 215 bp product, and the internal control *CYP2E1* yields a 412 bp product. Sample 00-58 in lane 1 is positive, and samples 00-59, 00-60, 00-61 in lanes 2, 3, and 4 respectively are negative (null genotype). *CYP2E1* is positive in all lanes; B. Amplified 176 bp *GSTP1* fragment is digested with Alw261. In the presence of the restriction site two fragments of 91 and 85 bp are observed. Individuals homozygous for the 313 AA allele have only the undigested fragment (97-121 in lane 1 and 97-584 in lane 4), heterozygous for the 313 AG alleles have both the undigested and the digested fragments (97-133 in lane 2), and homozygous for the 313 GG alleles have only the digested fragments (97-603 in lane 3); C. *GSTT1* primers generate a 138 bp product. Samples 97-533, B4, B59 and B85 in lanes 1, 2, 4, 5 respectively are positive, and B32 in lane 3 is negative (null genotype); D. *GSTP1* is simutaneously analyzed as control for *GSTT1* genotyping

Table 17. Distribution of GST genotypes stratified according to smoking status in cases and controls

		Non smokers			Smokers		
Locus	*Genotype*	Case	Control	OR (95%CI)	Case	Control	OR (95%CI)
GSTM1	Present	12 (38.70)	21 (55.20)		27 (33.75)	34 (50.70)	
GSTM1	Null	19 (61.30)	17 (44.80)		53 (66.25)	33 (49.30)	
				1.95 (0.74-5.05)			2.02 (1.04-3.93)
GSTP1	Ile/Ile	18 (58.06)	27 (71.05)		45 (56.25)	45 (67.10)	
GSTP1	Ile/Val or Val/Val	13 (41.94)	11 (28.95)		35 (43.75)	22 (32.90)	
				1.78 (0.65-4.80)			1.59 (0.83-3.03)
GSTT1	Present	22 (70.90)	30 (78.90)		66 (82.50)	56 (83.50)	
GSTT1	Null	9 (29.10)	8 (21.10)		14 (17.50)	11 (16.50)	
				1.53 (0.51-4.52)			1.08 (0.42-2.51)

Table 18. Combined risk of bladder cancer associated with smoking and GST genotypes

	OR (95% CI)					
	GSTM1		*GSTP1*		*GSTT1*	
Smoking status	Present	Null	Ile/Ile	Ile/Val or Val/Val	Present	Null
No	1	1.95 (0.74-5.06)	1	1.77 (0.65-4.75	1	1.53 (0.53-4.34)
Yes	1.38 (0.73-2.58)	2.81 (1.23-6.35)	1.50 (0.72-3.06)	2.38 (1.12-4.95)	1.60 (0.83-3.06)	1.73 (0.77-3.74)

Table 19. Distribution of the *GSTM1*, *GSTP1* and *GSTT1* genotypes in invasive and superficial bladder tumors.

Locus	Genotype	Invasive[1] tumors n=33 (%)	Superficial tumors n=88 (%)	OR (95%CI)	p
GSTM1	Present	10 (30.30)	36 (40.91)		
	Null	23 (69.70)	52 (59.09)		
				1.59 (0.68-3.75)	0.28
GSTP1	A/A	14 (42.42)	53 (60.23)		
	A/G	17 (51.52)	25 (28.41)		
	G/G	2 (6.06)	10 (11.36)		
				2.06 (0.91-4.63)	0.07
GSTT1	Present	29 (87.88)	68 (77.27)		
	Null	4 (12.12)	20 (22.73)		
				0.47 (0.15-0.49)	0.19

[1] "Invasive" denotes to at least muscle invasion (≥T2 stage)

3.2 TP53 codon 72

The distribution of the p53 Arg72Pro genotypes in the patient, and the control groups is shown in Table 20. A significant difference between the two groups was not found (p=0.878).

Table 20. Distribution of the *TP53*genotypes in the age-sex matched controls and bladder cancer patients

Locus	Genotype	Case n=121 (%)	Control n=114 (%)	Crude OR (95% CI)	Adjusted OR[1] (95%CI)	p
TP53	Arg/Arg	43 (35.54)	42 (36.84)			
	Arg/Pro	57 (47.11)	55 (48.25)			
	Pro/Pro	21 (17.35)	17 (14.91)			
	Arg/Pro or Pro/Pro	78 (64.46)	72 (63.16)			
				1.06 (0.63-1.73)	1.07 (0.64-1.75)	0.878

[1]Adjusted for age, sex and smoking status.

In the randomly selected group, the p53 genotype frequencies are 42.85% (Arg/Arg), 45.45% (Arg/Pro) and 11.70% (Pro/Pro) (p=0.820). These results reveal that the genotype frequencies for the age-sex matched control group, and the randomly selected group is not significantly different. This indicates absence of bias of ascertainement during the selection of the age-sex matched control group. Distrubition of TP53 genotypes were in Hardy-Weinberg equilibrium in all three groups.

The risk of bladder cancer from *TP53* genotypes was also evaluated by smoking status. An increased risk due to TP53 Arg72Pro polymorphism was observed in neither non-smoker, nor smoker groups.

When the tumors were stratified as superficial and invasive according to their pathological stage (Table 21), no statistically significant difference was observed (X^2= 2.542, df: 2, p=0.281).

Table 21. Distribution of the *TP53* genotypes in invasive and superficial bladder tumors

Locus	Genotype	Invasive[1] tumors n=33 (%)	Superficial tumors n=88 (%)	OR (95%CI)	p
TP53	Arg/Arg	13 (39.40)	30 (34.09)		
	Arg/Pro	12 (36.36)	45 (51.14)		
	Pro/Pro	8 (24.24)	13 (14.77)		
	Arg/Pro or Pro/Pro	20 (60.60)	58 (65.91)	1.08	0.281

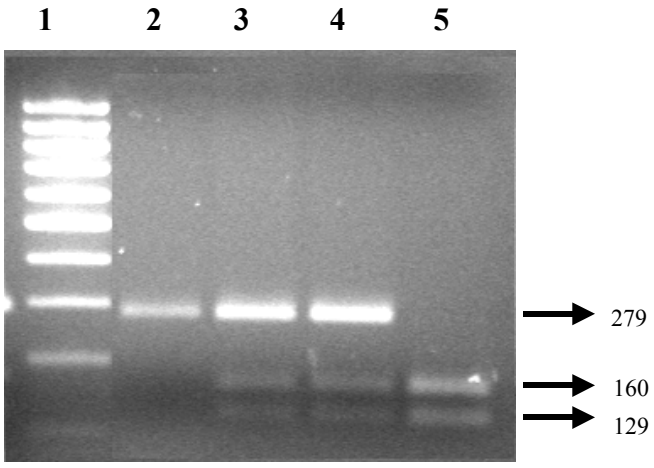

Figure 8. Genotyping of TP53 gene

Amplified 279 bp *TP53* fragment is digested with BstU1. In the presence of the restriction site two fragments of 160 and 119 bp are observed. Individuals homozygous for the 72 Pro/Pro allele have only the undigested fragment (B-26 in lane 2), heterozygous for the 72 Arg/Pro alleles have both the undigested and the digested fragments (B-33 in lane 3, and B-34 in lane 4), and homozygous for the 72 Arg/Arg alleles have only the digested fragments (97-603 in lane 5). PUC Mix DNA ladder (MBI Fermentas) in lane 1.

4. Discussion

GSTM1, *GSTP1*, and *GSTT1* polymorphisms were analyzed in 121 bladder cancer patients, and 121 age-sex matched controls. When the two groups were compared, the relative risk conferred by the *GSTM1* null genotype is 1.94, and *GSTP1* 105 Ile/Val or Val/Val genotypes is 1.75. The *GSTT1* null genotype was not found to be associated with a significantly increased bladder cancer risk (Table 14). When the genotype frequencies of the patient and the control groups were compared, non of the p53 Arg72Pro genotypes were found to be associated with a significantly increased bladder cancer risk (Table 20). Our odds ratio figure for the *GSTM1* null genotype is in agreement with a recent meta-analysis study pointing out a slightly increased relative risk of the *GSTM1* null genotype for bladder cancer, though our risk figure of 1.94 is higher than the reported risk of 1.5 in the meta-analysis (Johns and Houlston 2000). Association of the *GSTP1* 105 Ile/Val and Val/Val genotypes with bladder cancer in the Turkish population is in concordance with the British (Harries et al. *1997*), but not with the Japanese (Katoh et al. *1999*) or the German (Steinhoff et al. 2000) populations, and the lack of association between bladder cancer and the *GSTT1* locus is in agreement with the studies in the Greek (Georgiou et al. 2000)and the German (Kempkes et al. 1996; Steinhoff et al. 2000) populations, but not the Slovaks (Salagovic et al. 1999). The lack of association of TP53 locus with susceptibility to bladder cancer is in agreement with the two previous bladder cancer studies (Wu et al. 1995; Chen et al. 2000)

The patients were also grouped according to the stage of the disease to determine whether GST genotypes are associated with the invasiveness of the tumor (Table 19). Although statistically significant results could not be obtained, the *GST105* Ile/Val+ Val/Val genotypes appear to be a risk factor for invasiveness either

alone (OR: 2.06, 95% CI 0.91- 4.6) or in combination with the *GSTM1* null genotype (OR: 3.42, 95% CI 0.96- 12.2). GSTT1 and TP53 loci are not associated with invasiveness of bladder cancer.

Bladder cancer is a malignancy in which gene-environment interactions are thought to play an important role in addition to the genetic status of the individual. Smoking is one of the important environmental risk factors. Since GSTs are involved in the metabolism of smoking related carcinogens such as epoxides and polycylic aromatic hyrocarbons,the risk of bladder cancer was anlyzed from GST genotypes by smoking status (Table 17), and the combined risk of bladder cancer associated with smoking and GST genotypes (Table 18). In order to examine the genetic risk independently by eliminating the contribution of smoking to bladder cancer risk, we stratified the subjects by smoking status. An association was observed only in individuals who smoke and carry the *GSTM1* null genotype (OR 2.02; 95% CI 1.04-3.93). However, it should be noted that the stratification process which reduced the analyzed number of samples may have resulted in statistically insignificant confidence intervals in the remaining groups. Combined analyses of the smoking status and GST genotypes indicates an interaction between smoking and the *GSTM1* null genotype as well as the *GSTP1* Ile/Val + Val/Val genotypes. The risk figure is 2.81 for the former and 2.38 for the latter. This observation is in accordance with the results of the U.S. (Bell et al. 1993) but not the Dutch (Mungan et al. 2000), and the Korean (Kim et al. 2000b) studies. No data was available for *GSTP1* locus in the literature for bladder cancer. Neither stratification of the subjects according to their smoking status nor combined analysis revealed a significant association or interaction between smoking, and TP53 locus in terms of bladder cancer risk.

The combination of the *GSTM1* null and the *GSTP1* 105 Ile/Val or ValVal genotypes leads approximately to a four times increased cancer risk when compared with the combination of the low risk genotypes of these loci (Table 17). This observation suggests that gene-gene interactions may contribute to genetic susceptibility in bladder cancer. Simultaneous analysis of the *GSTM1* and *GSTP1* loci was conducted for bladder cancer in only one study from Germany (Steinhoff et al. *2000*) where an increased risk was not observed. On the other hand in a Japanese lung cancer (Kihara and Noda 1999), and a U.S. breast cancer (Helzlsouer et al. 1998) study where the high risk genotypes of the *GSTM1* and *GSTP1* loci were analyzed simultaneously, a risk increase for combination of risky genotypes was detected.

Population admixture is an important concern, particularly in countries like Turkey, having a genetically heterogenous population. In order to avoid that problem, an independent random control group was also genotyped. It was observed that the genotype distributions in the random group, and the age-sex matched control group are very similar for all genotyping experiments. In addition the genotype frequencies of GSTM1, and GSTT1 genotypes in our control group (no data was present for GSTP1 locus) resemble the frequency figures from prior Turkish studies (Aktas et al, 2001; Oke et al, 1998). Besides in a Turkish study an association for GSTM1 null genotype was observed (Aktas et al. 2001). Therefore it is very unlikely that that the observed differences in age-sex matched control group, and bladder cancer group are not genuine.

Conclusion and Future Perspectives

In this case- control study, the following observations are made

1. GSTM1 null, and GSTP1 Ile105Val polymorphism, but not GSTT1 null, and TP53 Arg72Pro polymorphism is a genetic susceptibility factor for bladder cancer. In addition the combination of the risky genotypes of GSTM1, and GSTP1 loci causes a substantial risk.
2. GSTM1 null, and GSTP1 Ile105Val polymorphism, but not GSTT1 null, and TP53 Arg72Pro polymorphism is "marginally" (not statistically significant) associated with the invasiveness bladder cancer.
3. The combined analysis of smoking and analyzed genes revealed that GSTT1 null, and TP53 Arg72Pro polymorphisms do not interact with smoking. However the smokers who bear GSTM1 null or GSTP1 105 Ile/Val+Val/Val genotypes are under considerable risk compared to the non- smoking individuals who dont have these risky genotypes.

As a future persrpective the followings can be done.

1. This study should be replicated with a different cohort from the Turkish population. Although it is unlikely, due to the reasons which are explained in the previous sections, population admixture is still a possibilty, which can not be totally ruled out, for the observed positive findings in this study.
2. The marginal association of GSTM1, and GSTP1 loci with the invasiveness is quite interesting, additional patients can be enrolled to test that whether it is actual or an artifact.

3. Additional loci, which is thought to be involved in bladder cancer pathogenesis, (Table) can be analyzed with this study group. In my opinion XRCC1 is the first gene to study, since there is only one published paper about this gene in the litreature. In the long run, multiple gene testing (e.g all the relevant polymorphisms) can be done by utilizing microarray technology.

5.References

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