ASSOCIATION OF CYP2E1, NQO1 AND GST GENETIC POLYMORPHISMS WITH RISK OF ACUTE LYMPHOBLASTIC LEUKEMIA IN TURKISH CHILDREN

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

GÜLEN ULUSOY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOCHEMISTRY

FEBRUARY 2009

Approval of the thesis:

ASSOCIATION OF CYP2E1, NQO1 AND GST GENETIC POLYMORPHISMS WITH RISK OF ACUTE LYMPHOBLASTIC LEUKEMIA IN TURKISH CHILDREN

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ABSTRACT

ASSOCIATION OF CYP2E1, NQO1 AND GST GENETIC POLYMORPHISMS WITH RISK OF ACUTE LYMPHOBLASTIC LEUKEMIA IN TURKISH CHILDREN

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February 2009, 259 pages

Acute lymphoblastic leukemia (ALL) is the most common type of cancer affecting children in the world and in our country. The exact molecular etiology of the disease still remains to be elucidated. This study hypothesized that four genes, namely *CYP2E1*5B, *6,* and **7B, NQO1*2* SNPs*, GSTM1* null and *GSTT1* null, alone or in combination, could contribute to the risk of development of childhood ALL. Also interactions of these polymorphisms with non-genetic risk factors were investigated.

The genotyping of these polymorphisms were done on 209 healthy subjects, and 185 patients with childhood ALL, in Turkish population. Venous blood samples were collected and genomic DNA was isolated from these samples. Genotyping was done by PCR-RFLP techniques.

In the case-control analyses for the risk of development of childhood ALL, only *GSTT1* null was found to be associated with the development of disease (OR= 1.8, p=0.01). *CYP2E1*5B* and **6* combination showed an increased risk of 2.7 fold (p= 0.04). Also co-presence of *CYP2E1*6-GSTT1* and *CYP2E1*7B-GSTT1* polymorphisms increased the risk significantly above 4.0 fold. The risk increased more to 7.6 fold, when *CYP2E1*5B,*6* and *GSTT1* null were considered together, with borderline significance (p=0.04). When interaction of exposure to cigarette smoke and genetic polymorphisms were investigated, *NQO1*2* and *GSTM1* null were turned out to be significant risk factors for the development of disease when the parental or child's postnatal exposure to cigarette smoke was considered.

This study presented several new findings to the literature in terms of genetic epidemiology of childhood ALL. The present work would also contribute to public health in determining the susceptibility of the Turkish population to childhood ALL.

Key words: Childhood acute lymphoblastic leukemia; Turkish population; CYP2E1**5B, *6, *7B* genetic polymorphisms; *NQO1*2* genetic polymorphism, *GSTM1/T1* null.

Öz

TÜRK ÇOCUKLARINDA CYP2E1, NQO1 VE GST GENETİK POLİMORFİZMLERİNİN AKUT LENFOBLASTİK LÖSEMİ RİSKİ İLE İLİŞKİSİ

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Akut lenfoblastik lösemi (ALL), dünyada ve ülkemizde çocukları en fazla etkileyen kanser türüdür. Hastalığın tam moleküler etiyolojisi hala belirlenememiştir. Bu çalışmanın hipotezi, dört genin, *CYP2E1*5B, *6,* ve **7B, NQO1*2, GSTM1* ve *GSTT1* genetik polimorfizmlerinin tek başlarına veya kombinasyonlar halinde, çocukluk ALL'sini arttıran risk faktörleri olabilecekleridir. Ayrıca bu genetik polimorfizmlerin diğer genetik olmayan risk faktörleriyle ilişkisi de incelenmiştir.

Bahsedilen polimorfizmlerin genotiplenmesi Türk popülasyonundan 209 sağlıklı gönüllü ve 185 çocukluk ALL hastası üzerinde yapılmıştır. Genomik DNA, deneklerden toplanan kanlardan izole edilmiş ve genotipleme PCR-RFLP teknikleriyle yapılmıştır.

Çocukluk ALL'si riski üzerine yapılan hasta-kontrol analizinde, tek başına sadece *GSTT1* polimorfizminin çocukluk ALL riski ile anlamlı derecede ilişkili olduğu saptanmıştır (OR= 1.8, p=0.01). *CYP2E1*5B* ve **6* kombinasyonu 2.7 kat (p= 0.04) riski arttırmıştır. Ayrıca *CYP2E1*6-GSTT1* ve *CYP2E1*7B-GSTT1* polimorfizm kombinasyonları da riski anlamı derecede 4.0 katın üzerinde arttırmıştır. Risk, *CYP2E1*5B,*6* ve *GSTT1* polimorfizm kombinasyonu ele alındığında daha da fazla, 7.6 kat artmıştır, ancak anlamlılık düzeyi sınırdadır (p=0.04). Sigara dumanına maruziyet ve genetik polimorfizmlerin ilişkisi incelendiğinde, *NQO1*2* ve *GSTM1* polimorfizmleri, annenin, babanın ve çocuğun postnatal sigara maruziyeti ele alındığında, anlamlı derecede risk arttıran faktörler olarak bulunmuşlardır.

Bu çalışma literatüre bir çok yeni bulgular sunmuştur. Ayrıca bu çalışma Türk popülasyonunun çocukluk ALL yatkınlığını belirleyerek halk sağlığına da katkıda bulunmuştur.

Anahtar kelimeler: Çocukluk akut lenfoblastik lösemisi; Türk popülasyonu; *CYP2E1*5B, *6, *7B* genetik polimorfizmleri; *NQO1*2* genetik polimorfizmi, *GSTM1/T1* nul.

Dedicated to my parents,

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor Prof. Dr. Orhan ADALI for his valuable guidiance, critical discussions, and continued advice throughout the course of my Ph.D. studies.

I am deeply grateful to my co-supervisor Prof. Dr. Emel ARINÇ for her valuable guidiance, critical discussions, and continued advice throughout this study, as well for her encouragement and support.

I would like to thank to my examining committee members Prof Dr. Meral YÜCEL, Prof. Dr. Mesude İŞCAN, Prof. Dr. Alaattin ŞEN and Assist. Prof. Dr. Azra BOZCARMUTLU for their valuable suggestions and constructive comments.

I would like to thank to Prof. Dr. Mahir ULUSOY for his valuable guidiance and suggestions in statistical analyses.

I am grateful to Dr. Gürses ŞAHİN, Prof. Dr. Sevgi GÖZDAŞOĞLU, and nurses of Dr. Sami Ulus Children's Hospital and Ankara University, Pediatric Hematology Department for their collaboration and help in collecting patient blood samples. My thanks are extended to Dr. Gürses Şahin for providing smoking status questionnairres. I also wish to thank to the patients and their parents for participating in the study.

I am also grateful to Dr. Nusret TAHERİ and Dr. Sibel YILDIZ, as well to Pelin KAYA and nurses of METU Health Center's Biochemistry

Laboratory for their help in collecting blood samples for the control population of this study. I also thank to all the volunteers that gave blood samples to be used in this study.

I wish to thank to all my labmates Mine NUYAN, Aysun TÜRKANOĞLU, Çiğdem KALIN, Esra ŞAHİN, Birsen CAN DEMİRDÖĞEN, Serdar KARAKURT for their friendship and support. My special thanks extend to Duygu YILMAZ and Cihan TANRIKUT for their friendship and help throughout this study.

I have special thanks to my labmates Tuğba BOYUNEĞMEZ TUMER, Şevki ARSLAN and Haydar ÇELİK, whom I would call sister and brothers, for their invaluable friendship, encouragement and support. My special thanks extend to Tuğba BOYUNEĞMEZ TÜMER for her valuable critics and ideas throughout this study.

I also wish to thank to Bilsev KOYUNCU, Emel SAĞLAR and Pembegül UYAR for their joyfull friendship and support, and members of UÇUŞ KULÜBÜ for their friendship and making me feel freedom in a different sense.

I would like to thank to all members of Department of Biological Sciences, especially to students who made my assistantship a salutary joy.

Finally, I would like to express my sincere gratitude to my parents Filiz ULUSOY and Mahir ULUSOY, whom I was inspired of their scientific thought, for their help, permanent support and encouragement throughout this study. I would also like to express my gratitude to my brother İnan ULUSOY, and his wife Ayşe ULUSOY, who supported and encouraged me throughout this study.

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χ^2	Chi-Square
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
ANNL	Acute Non-Lymphoblastic Leukemia
CLL	Chronic Lymphoblastic Leukemia
CML	Chronic Myeloid Leukemia
COR	Case-only Odds Ratio
CYP	Cytochrome P450
EH	Epoxide hydrolase
FMO	Flavin containing monooxygenase
GST	Glutathione S-tranferase
MTHFR	Methylenetetrahydrofolate-transferase
NAT	N-acetly transferase
NQO	NAD(P)H quinone oxidoreductase
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Lenght Polymorphism
SNP	Single Nucleotide Polymorphism
TMPT	Thiopurine methyl-transferase
UGT	UDP-glucuronosyl-transferase

CHAPTER 1

INTRODUCTION

Cancer is a serious disease of today's world, being the second leading cause of death after cardiovascular diseases in the world and in our country (http://www.gata.edu.tr/dahilibilimler/onkoloji/kanser_ epidemiyolojisi.htm, GATA Tıbbi Onkoloji Bilim Dalı). It is now well known that cancer arises from the accumulation of mutations that alter the genome or gene expression and result in loss of cell cycle control. Because mutation is the underlying cause of cancer, there will always be a baseline rate of cancer, as there is a background rate of spontaneous mutation. Over and above this baseline rate, environmental factors that promote mutation in the genome play an important role in the development of cancer (Klug and Cummings, 1997). Ionizing radiation, occupational exposure to physical and chemical agents, exposure to sunlight, viruses, personal behavior such as tobacco use are included in these environmental factors, and almost all of them act by creating mutations (Bunin, 2004). Besides, it is now known that individuals possess genetic polymorphisms which may increase the toxic effects of chemical agents, therefore making the individual more prone to carcinogenic effects of exposure. Polymorphisms occurring in xenobiotic metabolizing enzymes and DNA repair enzymes that have roles in the repair of mutations that occur in DNA, are currently an important area of scientific research dealing with the etiology of cancer.

In general, the cells have protection systems against mutations, and several mutations have to accumulate in a cell in order to convert it to a cancerous one. In order for the mutations that develop cancer to accumulate, usually years must pass and the individual should be exposed to mutation creating environmental factors. Therefore, cancer usually develops as the individual is aged. However, there are also cancer types that develop in childhood and even in infancy. A child is supposed to be exposed to chemicals and other environmental factors for a lesser period of time when compared to adults, hence the carcinogenic effect of chemicals is also different in children; therefore etiology of childhood cancers should be investigated separately from adult cancers. As the xenobiotic metabolizing systems are not yet fully developed in childhood, the effect of genetic polymorphisms should also be considered accordingly.

There are various types of childhood cancers, but acute lymphoblastic leukemia (ALL) is the most common form of cancer seen in children under the age of 15, and it comprises 30% of all the childhood cancers (http://www.cancer.gov/cancertopics/types/leukemia, National Cancer Institute). Although remarkable improvements have been achieved especially in the treatment of this disease, the molecular etiology has not been elucidated completely yet. The role of the environmental factors and the effect of genetic polymorphisms in the risk of development of ALL are still ongoing research areas and clarification of these effects would prove useful in elucidating mechanisms underlying the development of ALL.

1.1 Leukemia and Acute Lymphoblastic Leukemia (ALL)

Leukemia is a heterogeneous disease, characterized by the uncontrolled proliferation of blood precursor cells of myeloid or lymphoid origin which eventually invade bone marrow, peripheral blood and other organs. It can be classified as acute (low level of differentiation) or chronic (high level of differentiation) (Smith *et al*., 2005; Sinnet *et al.*, 2006). The four major types are acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia (CLL), acute myeloid leukemia (AML) - which is also described as acute nonlymphoblastic leukemia (ANLL), and chronic myeloid leukemia (CML) (http://www.cancer.gov/cancertopics/types/leukemia, National Cancer Institute).

Most leukemia cases occur in older adults; the median patient age at diagnosis is 67 years (http://www.leukemia-lymphoma.org/all_page.adp?Item_id=9346, The Leukemia and Lymphoma Society). Although leukemia affects approximately 10 times more adults than children, it is the most common cancer among children comprising 30% of all childhood cancers in U.S. (Kim *et al.*, 2006). In the U.S. there are approximately 3,250 children diagnosed each year with leukemia and 2,400 with acute lymphoblastic leukemia (Ries *et al.,* 1999). The most frequently observed leukemia type in children is acute lymphoblastic leukemia, accounting for 78% of all childhood leukemia cases (Kim *et al.*, 2006). It should also be mentioned that the peak incidence of childhood ALL occurs between 2 and 5 years of age (Chan, 2002). In Turkey, leukemia is also the most frequently observed childhood cancer accounting for 23% of all childhood cancers (Table 1.1), and among leukemia cases, ALL has the highest incidence rate with 81% (Kutluk and Yeşilipek, 2008; personal communication with Tezer Kutluk).

Table 1.1 Incidence of cancer types observed in children between 2002 and 2006 according to the Pediatric Tumor Records of Türk Pediyatrik Onkoloji Grubu (TPOG) / Türk Pediyatrik Hematoloji Derneği (TPHD). Table presented by the permission of Tezer Kutluk and Akif Yeşilipek.

Histopathological Diagnosis	**N**	**%**
Leukemia	1769	23,05
Lymphomas & RES	1513	19,72
CNS & Intracranial and Intraspinal Neoplasm	1129	14,71
Sympathetic Nervous System Tumors	593	7,73
Soft-Tissue Sarcomas	499	6,50
Retinoblastoma	297	3,87
Renal Tumors	463	6,03
Hepatic Tumors	114	1,49
Malignant Bone Tumors	460	6,00
Germ Cell, Trophoblastic and Other Gonadal Tumors	365	4,76
Carcinomas and Other Malignant Epithelial Tumors	231	3,01
Other and Unspecified Malignant Neoplasms	138	1,80
LCH	102	1,33
Total	7673	100,00

Fortunately, the improvements in the treatment of ALL increased the overall survival of children with ALL up to 80%, which was below 5% in the early 1960s. However, it should be noted that the survival is very dependent on the age at diagnosis, with the most favorable outcome observed for children diagnosed between 1 and 10 years of age (Ries *et al.,* 1999).

1.1.1 Prognosis of Acute Lymphoblastic Leukemia

The current approach for the treatment of ALL is to assign risk groups to each patient at the diagnosis and alter the intensity of therapy according to these risk groups and to the respond of patient to the induction chemotherapy (Chan, 2002).

There is no international consensus about risk assignment, and various groups have used different and overlapping risk classification schema for treatment decisions. The U.S. National Cancer Institute (NCI) sponsored a risk classification workshop in 1993, and an agreement was reached to use age and WBC count at diagnosis as the starting point for risk assignment (Smith *et al. 1996*). Children of ages 1 through 9 and with WBC count less than 50,000/μL are considered to be at standard risk. This constitutes about two thirds of all cases and has an estimated event-free survival rate of more than 80%. The rest of the patients (those younger than 1 year or older than 10 years of age or with a WBC count more than 50,000/μL) belong to the high-risk group (Chan, 2002).

Further risk assignment is done according to the genetic alterations or immunophenotypic characteristics of the patient. Gross chromosomal changes such as hyperdiploidy or translocations which create fusion genes are found in many ALL cases. Such genetic alterations are usually present in the high-risk group which is defined above (Chan, 2002; Lightfoot and Roman, 2004; Kim *et al.* 2006). Besides, ALL have subtypes according to the lineage-associated antigens of the single progenitor cell which turned into the cancerous cell. Immunophenotypically, 85% of ALL cases belong to the B-cell lineage, and 15% to T-cell lineage (Sinnet *et al,* 2006). T-cell ALL is generally considered to be high- or very high-risk, depending on the patients'

response to induction therapy. It should also be noted that male sex is generally associated with poor prognosis (Pui *et al.,* 2004).

1.1.2 Treatment of Acute Lymphoblastic Leukemia

Although different protocols are being used for treatment of ALL, all protocols basically include remission induction, prevention of leukemic infiltration of the central nervous system, intensification and prolonged maintenance therapy (for 2-3 years). Induction stage comprise of glucocorticoid, vincristine, and L-asparaginase treatment and results in bone marrow remission in vast majority of patients. Irradiation or intrathecal administration of chemotherapeutic agents reduces the incidence of reoccurrence of leukemia in central nervous system. Intensification is one or more courses of rotational, multiagent chemotherapy during the first 6 to 8 months from diagnosis. There is wide variation among protocols in the choice of drugs, the dose intensity, and the timing of intensification treatments. After intensification, maintenance therapy starts and lasts for 2 years for girls and 3 years for boys (Chan, 2002).

Induction treatment may be the same for all risk groups in different protocols; however, the intensity of intensification stage differs according to the risk groups. Patients at high risk are typically treated with intensified postremission chemotherapy, and patients at very high risk are considered as candidates for hematopoietic stem cell transplantation (Pui *et al.,* 2004).

As is the case for other chemotherapies, treatment of ALL also involves use of multiple drugs. Hence, the response of the patient to chemotherapy can be affected by the polymorphisms in the drug metabolizing enzymes. A striking example is the polymorphisms of

thiopurine methyltransferase (TPMT). It is a phase II drug metabolizing enzyme which metabolizes 6-Mercaptopurine, an essential anticancer drug used in the maintenance phase of childhood ALL treatment (Spire-Vayron de la Moureyre *et al.,* 1998). Some genetic polymorphisms of TPMT result in low activity of the enzyme, and there are studies reporting that the individuals bearing defective TPMT alleles suffered from toxicity when the drug 6-mercaptopurine was administered (McLeod *et al.*, 1999; Boyunegmez Tumer *et al.*, 2007). Therefore, in 2002, FDA has added TPMT genetic information to the azathiopurine and mercaptopurine package insert information, detailing a genetic risk for neutropenia (http://www.fda.gov/medwatch/SAFETY/2003/03DEC_PI/Tabloid_PI.pdf, Med Watch) and suggesting dose adjustment according to TPMT genotype to cure the patient without observing toxic effects (Spire-Vayron de la Moureyre *et al.,* 1998; Relling *et al.,* 1999). TPMT is an important example revealing the clinical significance of pharmacogenetics.

Pharmacogenetics is important not only in the chemotherapy but also in the risk of development of cancer because some of the xenobiotic metabolizing enzymes that activate procarcinogens are also polymorphic. In order to clarify the importance of these polymorphisms for the risk of ALL, the mechanisms underlying the development of disease are explained in proceeding pages.

1.1.3 Development of Acute Lymphoblastic Leukemia

As mentioned previously, several mutations are required to convert a normal cell to a cancerous one. Multi-stage carcinogenesis models have been considered in the following general terms: initiation of normal cells to pre-neoplastic ones in a specific organ, promotion of pre-neoplastic cells to neoplastic ones, and progression to a metastatic tumor. Genetic alterations are thought to be key components of such stage transitions. A

multi-stage model also has been hypothesized for childhood ALL by Greaves (1996) where he postulated that ALL results from at least two mutations, primary event occurring prenatally and the second occurring postnatally after the exposure of infant to infectious agents.

There is now compelling evidence that chromosome translocations (especially the TEL and AML fusion genes) are often the first or initiating events in leukemia, occurring prenatally during fetal development. This evidence comes from two sources -identical twin infants (Ford *et al.,* 1993; Wiemels *et al.*, 1999b) and retrospective investigation of neonatal blood spots (Gale *et al.,* 1997; Wiemels *et al.,* 1999a). The studies on monozygotic twins who both developed ALL showed that both twins share the same fusion gene breakpoint. Given that the breakpoints of the same fusion gene observed among ALL patients are highly variable, the detection of the same breakpoint in each twin pair at diagnosis is explained by prenatal origin of the leukemias (Ford *et al.,* 1993; Greaves, 2003; Greaves *et al.*, 2003). Another evidence comes from the investigation of blood spots from the children with ALL taken at the time of diagnosis with that taken neonatally. The comparison of the PCR-based detection fusion genes from both samples revealed the same fusion gene sequences which supports the hypothesis that leukemia is initiated prenatally (Gale *et al.,* 1997; Wiemels *et al.,* 1999a). It is assumed that at the neonatal stage, a clinically silent preleukemic clone is generated by the gene translocations that create fusion genes (Greaves, 2002).

However, these translocations are present at birth at 100 times the leukemia rate; besides ALL typically develops (generally 2 to 5) years after birth. So, it seems like additional chromosomal changes is a stringent requirement for the development of the disease (Greaves, 2002; Smith *et al.,* 2005). Greaves (1996) have hypothesized that after the first prenatal event creating the preleukemic cells, a postnatal second

event is required for disease manifestation. The mutli-stage model for ALL development suggests that there are three periods –preconceptional, prenatal, and postnatal– that are potentially critical windows during which exposure to exogenous agents could influence leukemogenesis (Figure 1.1). Exposure of both parents before conception or exposure of mother during pregnancy may influence the generation of fusion genes that create preleukemic cells. Exposure of breast-feeding mother or exposure of the children after birth is critical in the creation of second event that develops leukemia (Kim *et al.,* 2006).

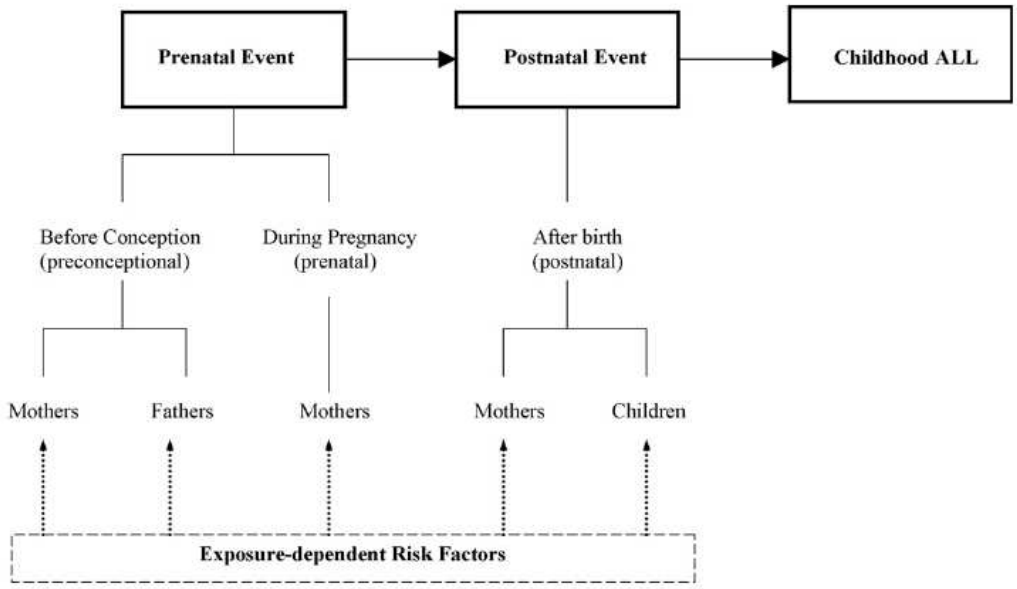

Figure 1.1 Potential relationships among childhood ALL-inducing events, critical exposure windows, and exposure-dependent risk factors (Figure taken from Kim *et al.,* 2006).

The multi-stage model for development of ALL has been supported by many studies. However, the risk factors that cause ALL-inducing events in children, whether occurring prenatally or postnatally, is not fully known yet.

1.2 Risk Factors for the Development of Acute Lymphoblastic Leukemia

As is the case with the other cancer types, it is unlikely that there is a single cause for the development of ALL. The development of ALL is likely to involve an interaction of exposure to environmental factors and inherent genetic susceptibility, where the effects of genetic polymorphisms are modulated by external factors, modifying the child's risk of cancer. There are three factors that are known to influence the disease risk and are likely to be associated with the ALL-inducing events: exposure-independent characteristics, exposure to environmental agents, and genetic factors (Greaves, 2002; Kim *et al.,* 2006).

Besides the above mentioned risk factors, it should also be taken into account that the exposure level and the xenobiotic-metabolizing capacity of children are different from those of adults.

1.2.1 Exposure-Independent Characteristics as Risk Factors

The number of the studies investigating the effect of exposure-independent risk factors is low; however some studies have found increased risk with some factors. A striking risk factor is shown to be the Down syndrome, which increased the risk of incidence ALL 20 to 33-fold (Cnattingius *et al.,* 1995; Murray *et al.,* 2002). Down syndrome is a familial genetic disorder, and is associated with gross genetic alterations, which may also be the initiating events of leukemia.

High birth weight is also shown to be associated with increased risk of childhood ALL in many studies (Ross *et al.,* 1997; Okcu *et al.,* 2002; Paltiel *et al.,* 2004). A statistically significant dose-response relation between birth weight and ALL risk has been reported, with an up to 26%

increase in ALL risk per kg increase in birth weight (Westergaard *et al.,* 1997; Hjalgrim *et al.,* 2004). This increased risk may be explained by an association between birth weight and bone marrow cell number; children with high birth weight have more cells at risk of malignant transformation, thereby increasing ALL risk. It should also be noted that high birth weight also reported to increase the risk of other childhood cancers, such as Wilms' tumor and neuroblastoma (Kim *et al.,* 2006).

Maternal and paternal age was also investigated as a risk factor and older ages for both parents (e.g. more than 40 years) were shown to increase the development of disease (Kaye *et al.,* 1991; Dockerty *et al.,* 2001). Interestingly, ALL incidence is higher in males than in females and the prognosis of the disease is also more severe in males (Paltiel *et al.,* 2004; Pui *et al.,* 2004).

1.2.2 Exposure to Environmental Agents as Risk Factors

The multi-stage model for the development of ALL drives attention to the exposure-dependent risk factors, as mentioned previously. Exposure to environmental factors include preconceptional exposure of both parents, prenatal exposure of mother during pregnancy and of the child through the placental route, and postnatal exposure of breast feeding mother or of the child. Many epidemiological studies have been done in order to determine the environmental risk factors; however there is still little direct evidence linking those factors to the development of ALL. The only well-defined exposure-dependent risk factors for childhood ALL are ionizing radiation and benzene.

1.2.2.1 Ionizing Radiation and Infections

Ionizing radiation is one of the few exposures for which the relationship with childhood leukemia, particularly AML, has been well established (United Nations Scientific Committee on the Effects of Atomic Radiation, 1994; Mahoney *et al.* 2004). The magnitude of the risk depends on the dose of radiation, the duration of exposure, and the age of the individual at the time of exposure. For example, leukemia rates among survivors who were within 1,000 m of the atomic bomb explosions at Hiroshima and Nagasaki, Japan, were 20-fold higher than rates among the general population (Mahoney *et al.* 2004). Preconceptional exposure of fathers (Shu *et al.,* 1994), prenatal exposure of pregnant mothers to diagnostic X-rays (Stewart *et al.,* 1956; Van Steensel-Moll *et al.,* 1985) and postnatal exposure to therapeutic doses (Nishi and Miyake, 1989; Ries *et al.,* 1999; Infante-Rivard, 2003) were shown to be risk factors for ALL. However, these exposures are likely to explain only a small percentage of leukemia in recent years because of the rarity of radiation treatments in children and diagnostic X-rays during pregnancy (Bunin, 2004).

Infection was also considered as a risk factor for ALL development. Some studies suggested that viruses (human T cell leukemia/ lymphoma virus type I, Epstein-Barr virus), and bacteria (*Helicobacter pylori*) may play a part in the development of some leukemia types, however whether they have a major role in childhood ALL is uncertain (Greaves, 2002). Smith et al. (1997) modeled a scenario in which infection-related immune response was associated with prenatal ALL initiation, while Greaves (1988) hypothesized that immune response to exogenous stimuli was associated with postnatal ALL events. The identified infection-related factors appear to influence the leukemic risk by modulating immune responses to infection. Furthermore, the postnatal exposure studies

suggest that while exposure to infection early in life (e.g., in the first year of life) decreases a child's ALL risk, delayed exposure to infection increases the risk (Kim *et al.,* 2006).

1.2.2.2 Chemical Exposure

Apart from ionizing radiation and infection, exposure to chemicals play an important role in the etiology of all cancers, including childhood ALL. Because in the today's modern world, there are lots of routes where individuals are exposed to chemical carcinogens like air pollution, industrialization, automobile exhaust, occupation, tobacco smoke, food additives, drugs, cosmetics and so on. This fact, in part, explains the higher level of cancer incidence in highly-developed /industrialized countries (Bunin, 2004; Ramanakumar, 2007).

Pesticides are among the chemicals that were shown to be associated with risk of childhood ALL. Ma *et al*. (2002) reported that exposure to indoor pesticides during pregnancy increased leukemia risk. Also postnatal exposure of children to indoor pesticide use was shown to be associated with ALL (Steffen *et al.,* 2004).

Hydrocarbons, especially benzene, were also associated with risk of childhood ALL. Hydrocarbons are organic compounds made up primarily of carbon and hydrogen atoms. Hydrocarbons are found in many household and industrial products including paints, paint removers, thinners, and solvents, which are used to dissolve other chemical substances. The most widely recognized hydrocarbon is benzene, a ubiquitous chemical used in the manufacture of paints and plastics and as a constituent in motor fuels and hobby glues. It is also formed during incomplete combustion of fossil fuels (i.e., petroleum products, coal). Other examples of hydrocarbons include gasoline, trichloro-ethylene

(spot remover) and perchloroethylene. These hydrocarbons are also present in air pollution and a study in California suggested that children living in areas with high levels of point source of such pollutants were at an increased risk of developing leukemia (Reynolds *et al.,* 2003). A similar study in Great Britain reported associations between birthplace of children with leukemia and proximity to industrial sites that release volatile organic compounds, dioxins, 1,3-butadiene, and benzo[*a*]pyrene (Knox, 2005). Steffen and co-workers (2004) reported an association between dwellings neighboring a petrol station or a repair garage – benzene emitting sites- during childhood and the risk of childhood leukemia, with a duration trend. A recent occupational study (Glass, 2003) found excess risk for leukemia associated with cumulative benzene exposures and benzene exposure intensities at lower levels (less than 60 ppm-years) than had been previously reported (as high as 220 ppm-years).

Exposure to hazardous chemicals can also be occupational. In a review by Savitz and Chen (1990) on the association between parental occupation and childhood leukemia, exposure to paints and pigments yielded the most consistently positive results, with several studies producing risk factors for ALL and AML of more than 1.5-fold.

Benzene is a known carcinogen and it has a strong positive exposure–response relationship with leukemia, particularly AML. In fact, apart from ionizing radiation, benzene is the only risk factor which was directly shown to be associated with the development of leukemia. Therefore, benzene exposure will be emphasized in more detail in the context of gene-environment relationship in proceeding pages.

Another source of chemical exposure is cigarette, alcohol or illicit drug use, especially of mothers. Although many studies have reported on

this topic, no consistent evidence to confirm links with childhood leukemia has been documented. Sorahan and co-workers (1995, 1997), in their analysis of data from the Oxford Survey of Childhood Cancers, reported a weak association between maternal smoking during pregnancy and childhood ALL, and a significant trend between increasing paternal tobacco consumption and childhood cancer. However, other studies failed to show a relationship between parental smoking and childhood leukemia (Brondum *et al.,* 1999; Pang *et al.,* 2003). On the other hand, maternal marijuana use before and during pregnancy was shown to be associated with childhood AML and ALL (Robison *et al.*, 1989).

Maternal alcohol consumption during pregnancy was shown to be a risk factor for AML (Van Duijn *et al.,* 1994; Shu *et al.*, 1996) and ALL (Shu *et al.*, 1996) however there are also studies which did not find a significant association (Infante-Rivard *et al.,* 2002b).

Many of the chemicals exert their carcinogenic effects after their bioactivation in the body by xenobiotic metabolizing enzymes. Therefore the polymorphisms of the genes of these enzymes also play an important role in the risk of development of childhood ALL.

1.2.3 Genetic Polymorphisms as Risk Factors

It is now apparent that individuals have different degrees of resistance or sensitivity to a toxic or carcinogenic chemical; hence exposure to such chemicals can lead to cancer or another environmental disease in some individuals but not others. Recent advances in genomics area lead to the identification of genetic polymorphisms (differences) among people which influence the phenotype and response to environmental insult. Genes coding for enzymes, receptors or other proteins that interfere with environmental agents and cause alteration of

normal cellular functions, leading ultimately to toxicity or malignancy are called "susceptibility genes" (Nebert and Roe, 2001). The study of the relationship between genetic polymorphisms, cancer susceptibility, toxicity and environmental exposure is a new, exciting and promising area of research. The identification of genetic factors, which might increase the risk of toxicity or cancer, would have important implications for the prevention, early diagnosis and intervention of human disease (Nebert and Roe, 2001).

Development of cancer by environmental exposure requires many steps including generation of DNA damage and mutations that eventually lead to carcinogenesis, as illustrated in Figure 1.2. There are many polymorphic genes that code for enzymes or other proteins which affect the susceptibility of an individual to development of cancer.

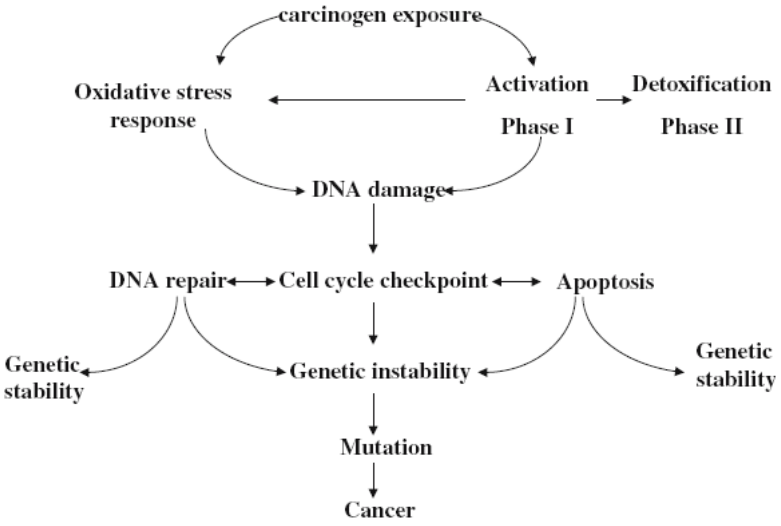

Figure 1.2 Illustration of the process of carcinogenesis and the relationships between distinct pathways (Figure taken from Sinnet *et al.,* 2006).

As can be seen from the upper part of Figure 1.2, the process of DNA damage generation is highly influenced by xenobiotic metabolizing enzymes. Because, most of the chemicals need metabolic activation by phase I xenobiotic metabolizing enzymes in order to make adducts that result in DNA damage. Besides, some phase I enzymes are also have role in generation of reactive oxygen species. On the other hand, especially phase II xenobiotic enzymes are important in the detoxification of these activated metabolites of phase I reactions as well as protection against oxidative stress caused by carcinogen exposure or phase I metabolism. As will be mentioned in more detail later, almost all of the xenobiotic metabolizing enzymes possess genetic polymorphisms which influence the potential of one's DNA damage generation. The lower part of Figure 1.2 illustrates the processes of DNA damage leading to mutations and eventually carcinogenesis. The enzymes and proteins that have roles especially in DNA repair mechanism are also polymorphic, hence are important determinants of one's susceptibility to carcinogenesis.

The polymorphisms in the genes coding for xenobiotic metabolizing enzymes or proteins maintaining DNA stability are important determinants of the individual's susceptibility to cancer. The polymorphisms leading to higher phase I xenobiotic activation results in higher levels of carcinogen activation and oxidative stress, therefore increase the susceptibility; while polymorphisms resulting in higher phase II xenobiotic detoxification leads to more efficient detoxification of activated carcinogens and protection against oxidative stress (however it should be kept in mind that not all Phase II enzyme metabolism results in detoxification). Similarly, polymorphisms lowering the maintenance of DNA stability also lead to higher risk for cancer development. So, exposure to environmental agents in a 'high Phase I, low Phase II' individual might lead to more toxicity than that in a 'low Phase I, high Phase II' individual (Nebert and Roe, 2001). The combined effect of

genetic polymorphisms and chemical exposure on the risk of development of cancer is shown in Figure 1.3.

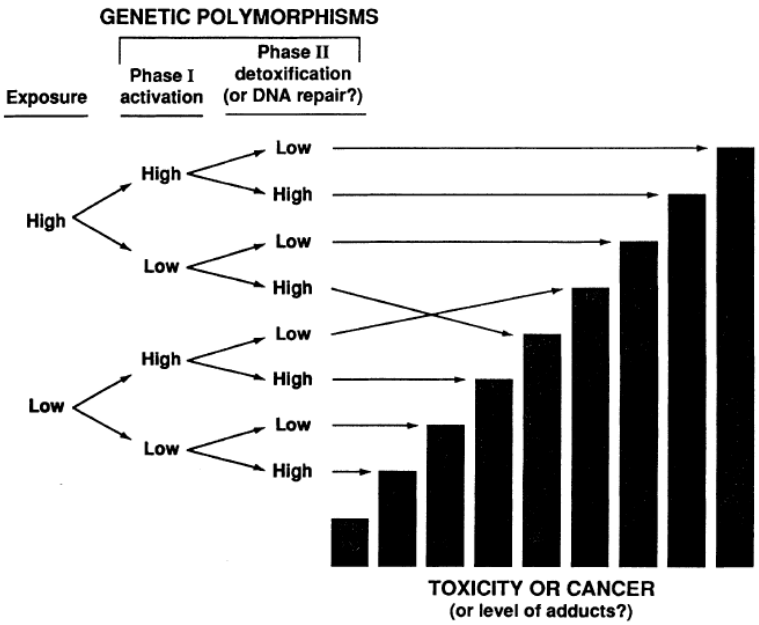

Figure 1.3 The possible combined effects of genetic polymorphisms in susceptibility genes and environmental exposure on development of toxicity or cancer (Figure taken from Nebert and Roe, 2001).

The effect of polymorphisms in susceptibility genes on the development of leukemia including childhood ALL is a topic of interest. The susceptible genes of interest included those coding for xenobiotic metabolizing enzymes and those taking part in maintaining the DNA stability.

1.2.3.1 Genetic Polymorphisms in the Xenobitoic Metabolizing Enzymes

The xenobiotic metabolism is comprised of Phase I and Phase II reactions. The exogenous chemical first encounters with Phase I reactions which convert it to a more soluble form by oxidation, reduction and hydrolysis reactions. The phase II reactions are mainly conjugation reactions that facilitate the elimination of the chemical. Almost all enzymes in phase I and Phase II metabolism possess genetic polymorphisms which may affect the individual's response to exposure.

The genetic polymorphisms in xenobiotic metabolizing enzymes and the risk of development of ALL is the major concern of this study and therefore this topic will be explained in more detail in proceeding sections. However the major enzymes that are thought to be associated with risk of ALL are mentioned below.

The major enzymes of Phase I are cytochrome P450 dependent monooxgenases. Some isozymes of this enzyme superfamily are of particular interest as these isozymes –namely CYP1A1/1A2, CYP1B1, CYP2E1- have roles in the bioactivation of environmental chemicals into carcinogenic forms, hence their polymorphism in the risk of ALL development is of major concern. Another important member in relation to ALL development is NAD(P)H quinone oxidoreductase (NQO1), which detoxifies the carcinogenic metabolites of phase I reactions.

Among the members of Phase II metabolism, polymorphisms of glutathione S-transferases (GSTs) and N-acetyl transferases (NATs) have been of great interest for the risk of development of ALL, as they have roles mainly in detoxification, and sometimes in activation (some

isozymes have dual roles), of carcinogens and protection against oxidative stress.

1.2.3.2 Genetic Polymorphisms in Proteins Responsible for Maintaining DNA Stability

In recent years, it is found that genes coding for proteins that function in the maintenance of DNA stability also bear many polymorphisms which eventually affects individual's susceptibility to accumulate mutations. Enzymes of the DNA repair mechanism are also polymorphic and among them XRCC1 (X-ray repair complementing defective repair in Chinese hamster cells 1) polymorphism was found to be associated with increased risk of childhood ALL (Joseph *et al.,* 2005; Bounegmez Tumer *et al.,* 2008). There are also studies that reported an increased risk of ALL in polymorphisms of MDR1 (membrane transport protein 1) (Jamroziak *et al.,* 2004), cell cycle regulation protein CCND1 (cyclin D1) (Hou *et al.,* 2005) and HLA (human leukocyte antigen) which functions in cell recognition (Taylor *et al.,* 1998, 2002).

In case of childhood ALL, there is another factor that should be taken into account; the patients are children. Infants and children possess differences from adults in exposure levels to toxicants and in disposition capacity.

1.2.4 Differences between Children and Adults with Respect to Exposure and Disposition of Toxicants

There are a number of reasons to suspect that risk assessments based on adults may underpredict the risks of exposure to infants and children. Children have more limited diets than adults and higher intakes per body weight of food, fluids and air. Therefore children receive greater

doses of environmental toxicants on a body weight basis than adults through common exposure pathways, such as inhalation and ingestion. Children have higher oxygen demand compared to adults, because of rapid growth and high levels of physical activity. The mean breathing rate is almost twice as great for children as it is for adults (452 vs. 232 L/kg-day, respectively). Therefore, as children inhale a greater volume of air per unit time and per body weight than adults, they receive higher doses of toxic chemicals present in the air pollution (OEHHA, 2000b).

Children have higher cell proliferation rate, which increases the risk of DNA damage due to misrepair, crosslinks, alterations which may ultimately lead to cancer. During development, a larger percentage of the DNA is transcriptionally active and thus structurally more exposed and vulnerable to damage or alteration by DNA reactive agents (OEHHA, 2001).

Another important difference between children and adults is that children express xenobiotic metabolizing enzymes in different levels than adults, so rates of activation, detoxification and clearance of xenobiotics are also different. Many of the hepatic microsomal enzyme systems responsible for drug are present at birth and their activities increase rapidly to near adult levels early in life (Aranda *et al.,* 1974; Dutton, 1978; Morselli *et al.,* 1980; Morselli, 1989).

Liver phase I enzymes develop rapidly during infancy, with adult levels attained by about six months of age (Aranda et al., 1974; Neims et al., 1976; Dutton, 1978). The total cytochrome P450 content of infant liver microsomes is approximately one third of the total adult content (Treluyer *et al.,* 1991). The ontogeny of phase I xenobiotics metabolizing enzymes is summarized in Table 1.2. Some P450 isoforms like CYP3A7 are present and active in fetal liver, some isoforms including CYP2D6 and

CYP2E1 appear within hours of birth, while others like CYP3A4, CYP2C family and CYP1A2 develop neonatally (Cresteil, 1998). Interestingly for CYP3A family, CYP3A7 is expressed in the fetal tissue and suggested to disappear in the liver after infancy.On the other hand, CYP3A4 expression is transcriptionally activated during the first week after birth and is accompanied by a simultaneous decrease of CYP3A7 expression, in such a way that the overall CYP3A protein content remain nearly constant (Tateishi *et al.,* 1997; Lacroix *et al.,* 1997). Hines (2007) have reported that CYP2E1 was undetectable at the first trimester of fetal life and increase to 10% of adult levels in the third trimester; 25% of the adult levels were detected in neonatals (< 4 weeks) and full activity was seen in infants.

Another enzyme which is important for the risk of ALL is NQO1. Although there is no information on the expression of NQO1 in human fetus, it was found to be very low in rodent liver, rising to adult levels a few weeks after birth (Hommes *et al.,* 1978).

Phase II enzymes are generally low at birth (Milsap and Jusko, 1994). GSTP isoform is present in the fetus and decreases postnatally, while GSTM and GSTA are low at birth but develop during the first three to six months of life (OEHHA, 2001; McCarver and Hines, 2002).

Table 1.2 Postulated developmental expression of some Phase I drug metabolizing enzymes in human liver (taken from Hines and McCarver, 2002).

Isoform	Prenatal Trimester 1st	Prenatal Trimester 2nd	Prenatal Trimester 3rd	Neonate <4 weeks	Infant <12 months	Child <10 years	Adult
CYP1A1	+	+	?	-	-	-	-
CYP1B1	?	±?	?	?	-	-	-
CYP1A2	-	-	-	-	+	+	+
CYP2A	-	-	-	?	+	+	+
CYP2B6	-	-	?	?	?	+	+
CYP2C	-	-	-	+	+	+	+
CYP2D6	-	±	±	+	+	+	+
CYP2E1	?	+?	+?	+	+	+	+
CYP2J1	?	+	?	?	?	+	+
CYP3A7	+	+	+	+	-	-	-
CYP3A4/5	-	-	-	+	+	+	+
FMO1	+	+	+	-	-	-	-
FMO3	±	-	-	±	+	+	+
ADH1	+	+	+	+	-	-	-
ADH2	-	+	+	+	+	+	+
ADH3	-	-	+	+	+	+	+

Smbols for activity or protein: + detectable; - not detectable; ? not determined; ± detectable, but only in a fraction of the samples examined; +? Presence or absence is contoversial.

The multistage model for the development of ALL suggests a postnatal secondary event for development of leukemia. Environmental exposure to chemicals and several genetic polymorphisms have been found to be risk factors for the development of the disease, as mentioned above. It seems that there is gene-environment relationship for the development of the disease. Therefore, rather than single susceptibility genes, investigation of multiple susceptibility genes in the same

metabolic pathway in relation to the exposure would better predict the risk factors for the disease. Benzene is the most evident chemical shown to be associated with leukemia. Benzene also represents a good example for the steps of scientific achievements to prove its carcinogenicity, from individual clinical case reports to epidemiologic studies revealing the importance of gene-environment relationship. The following section gives examples for the importance of gene-environment interaction.

1.2.5 Gene-Environment Relationship

Benzene is the only chemical that is proven to be a risk factor for the development of leukemias. However, there are also other environmental chemicals that are suggested to be associated with the development of leukemia, like carbontetrachloride, pesticides, cigarette smoke, solvents etc. (McKinney *et al*., 1991; Shu *et al*., 1999; Freedman *et al*., 2001; McKinney *et al*., 2003; Menegaux *et al*., 2007).

In 1987, cases of aplastic anemia and acute leukemia due to chronic exposure to benzene were reported. Clinical case reports have been published since then until the first epidemiologic study showing a relationship between benzene exposure and leukemia was published in 1974 by Professor Muzaffer Aksoy, a Turkish hematologist. His epidemiologic study started when he noticed that leukemia cases were higher than expected and observed that a large group of patients were shoemakers or working in leather manufacturing. At that time, benzene was being used as a solvent in leather industry and was a major component of the glue used in shoe making process. Dr. Aksoy and his associates started an epidemiologic study in the shoe making workplaces. They measured the benzene concentration to be 150-220 ppm in these workplaces, and on 28,500 workers, they observed higher incidence of leukemia than general population (13.59 versus 4 per 100,000,

respectively) (Aksoy *et al.,* 1971; Aksoy *et al.,* 1976). This was the first epidemiologic study showing the carcinogenic effect of benzene. His findings caused prohibition and discontinuation of the use of benzene in these workplaces, and a striking decrease in the leukemia cases among workers accompanied (Aksoy *et al.,* 1971; Aksoy *et al.,* 1976). In 1975, Dr. Aksoy was invited to the U.S. Supreme Court of Industry as a scientific expert to provide his scientific findings regarding the health effects of benzene exposure in the workplace. As a result, the legal maximum permissible concentration of benzene was decreased to 1 ppm in the USA (Yaris *et al.,* 2004).

Many studies followed Dr. Aksoy's work and it is now known that benzene exposure is associated with many adverse effects in primarily blood forming organs, like bone marrow damage, changes in circulating blood cells, alterations of the immune system and cancer (OEHHA, 2000a; 2001). Benzene has been implicated as a potential risk factor for the development of childhood leukemia (Smith and Zhang, 1998; U.S. EPA, 1998; Ries *et al.,* 1999; OEHHA, 2001). Therefore the Office of Environmental Health Hazard Assessment (OEHHA) accepted benzene as a toxic air contaminant due to its leukemogenic effects (OEHHA, 2001).

It has been shown by many studies that benzene toxicity results from its metabolism in the body (Snyder and Hedli, 1996). For example, competitive inhibition of metabolism by toluene decreased benzene toxicity. Mice lacking the CYP2E1 gene had decreased metabolism of benzene and, correspondingly, decreased toxicity (Valentine *et al.,* 1996).

The metabolism of benzene is complex, as illustrated in Figure 1.4. It is metabolized primarily in the liver by CYP2E1 forming benzene oxide or its oxepin, latter being cleaved to trans-trans muconaldehyde.

Benzene oxide can be detoxified by GSTs via glutathione conjugation. However, rearrangement of benzene oxide to phenol occurs very rapidly. Phenol can be further oxidized, most likely by CYP2E1, to the polyhydroxylated metabolite, hydroquinone or catechol (Koop *et al.,* 1989; Schlosser *et al.,* 1993; Nedelcheva *et al.,* 1999). These metabolites, as well as phenol, can be detoxified by phase II enzymes such as sulfotransferases and glucuronyltransferases (Seaton *et al.,* 1995), or they can partition into blood and distributed to other tissues, including bone marrow. In bone marrow, these metabolites can be converted non-enzymatically or by myeloperoxidase (MPO) to reactive metabolites 1,4-benzoquinone or 1,2-benzoquinone. These quinone metabolites can be detoxified back to hydroquinone or catechol by NQO1, so kept in a reduced state where they can more readily be conjugated and excreted (Bauer *et al.,* 2003). Benzene toxicity is postulated to be due to its reactive quinone metabolites and trans-trans-muconaldehyde, which can alkylate proteins and DNA (Irons 1985; Soucek *et al.,* 1994).

As can be clearly seen from the figure, many xenobiotic metabolizing enzymes participate in the metabolism of benzene. Among these enzymes, CYP2E1 plays a critical role as it is the key enzyme bioactivating benzene into carcinogenic metabolites. NQO1, which detoxifies the quinone metabolites of benzene in bone marrow, is of crucial importance for the protection against CYP2E1 activated benzene toxicity. GSTs also have an important role as they conjugate benzene oxide, the initial metabolite of CYP2E1. Myeloperoxidase (MPO) is another xenobiotics metabolizing enzyme that converts catechol into carcinogenic metabolite 1,2-benzoquinone. It is a lysosomal enzyme and activates carcinogens from tobacco smoke and organic solvents. It also contributes to oxidative stress by catalyzing the formation of free radicals (Sinnet *et al.,* 2006).

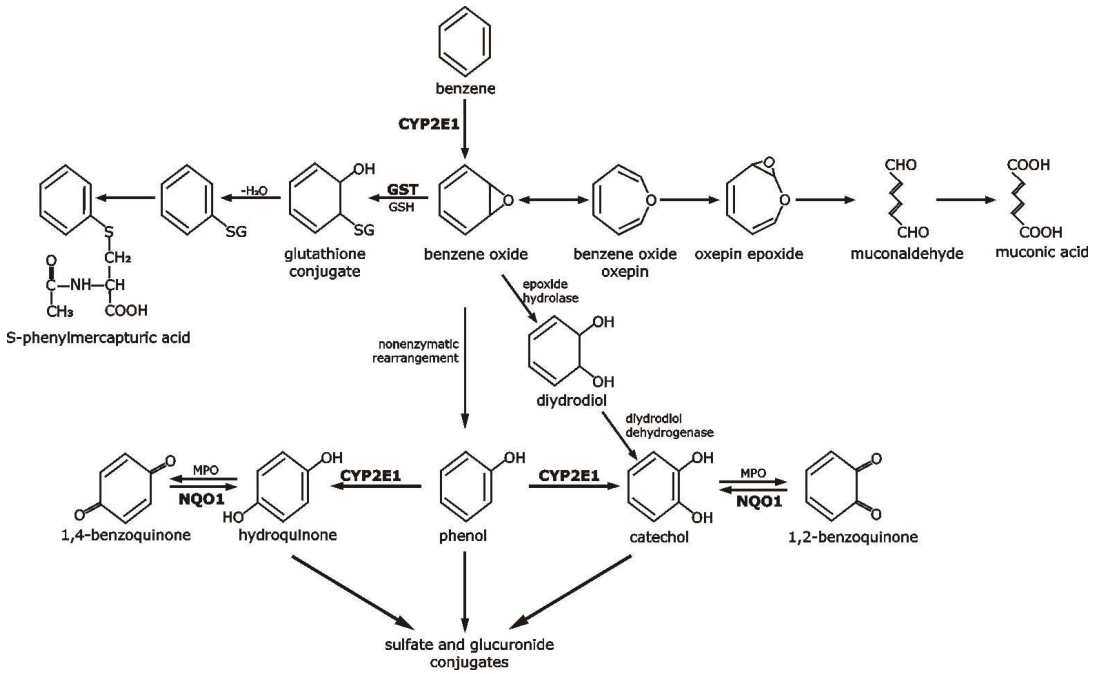

Figure 1.4 Benzene metabolism in liver and bone marrow (Figure adopted from OEHHA, 2001 and Bauer *et al.,* 2003).

The early studies on the benzene metabolism in rabbits with radioactively labeled benzene showed that about 20% of the radioactivity could be recovered as phenol, 5% as hydroquinone, approximately 2% as trans, trans mucoic acid, 1% as catechol and 0.5% as phenyl-mercapturic acid (Parke and Williams, 1954). However, it should be noted that metabolism of benzene is affected from many factors. For example, Phenobarbital and benzene itself were shown to induce the metabolism of benzene. Also cytochrome b5 was shown to stimulate the overall benzene metabolism and increased the relative proportion of hydroquinone formed by CYP2E1 (Snyder and Chatterjee, 1991). The potential pathways of benzene metabolism suggests a central role to benzene oxide, which determines the degree of toxic metabolites formed. Low benzene concentration may lead directly to the formation of phenol, while higher

benzene concentration drive the reaction towards formation of benzene oxide (Snyder and Chatterjee, 1991; Kim *et al.,* 2006).

CYP2E1 is majorly expressed in liver but also found to be expressed in bone marrow (Roberts *et al.,* 1994). Although low levels of CYP2E1 in infants may suggest reduced formation of toxic metabolites, at several months of age, expression of the enzyme reaches to that of adult. On the other hand, detoxifying enzymes NQO1 and GSTs are expressed in many tissues including bone marrow; however it should be noted that GST levels are low in infants and develops in 3-6 months (OEHHA, 2001). There's no information on the NQO1 levels in infants, however it was found to be low in the birth of rodents (Hommes *et al.,* 1978). Low level of protective enzymes NQO1 and GST may increase the risk of toxic metabolite formation, while low levels of CYP2E1 in infants may be protective due to lowered activation of benzene; however there are also other factors like high-inducibility of these enzymes by benzene and other substrates.

As mentioned before, exposure to chemicals can be preconceptional, prenatal (during pregnancy) or postnatal (Figure 1.1). Environmental exposure agents related to childhood ALL risk include inhaled particulate hydrocarbons, timber and wooden furniture, carbontetrachloride, paint and thinners, solvents, pesticides, exposure to artwork, neighbouring repair garages and gas stations, perchloroethylene, trichloroethylene, dioxins, benzo[a]pyrene (McKinney *et al*., 1991; Shu *et al*., 1999; Freedman *et al*., 2001; McKinney *et al*., 2003; Knox, 2005; Menegaux *et al*., 2007; Belson *et al.,* 2007). Many of these exposure agents are source of benzene. But there are also other carcinogens which exposure through pollution or occupation is possible (Bolt *et al.,* 2003). One example is *n*-hexane, which is an industrial solvent, and CYP2E1 was shown to be responsible for the metabolism

(Iba *et al.,* 2000). Acrylonitrile and acrylamide are procarcinogens that are activated by CYP2E1 to carcinogenic epoxide intermediates (Ghanayem *et al*., 1999; Sumner *et al.,* 1999; Nuyan 2008). Other procarcinogens that are activated by CYP2E1 include styrene, chloroform, pyridine, vinyl chloride (Guengerich et al., 1991; Gonzalez and Gelboin, 1994; Guengerich, 1995).

Cigarette smoke is an important agent for risk of childhood ALL as well as other cancer types, as exposure to cigarette smoke either prenatally (exposure of parents) or postnatally is quite common, especially in our country. Cigarette smoke contains many well-established carcinogens, and both active and passive smoking have been implicated in the development of several cancers during adulthood (Boffetta *et al*., 2002). Nearly 70 chemicals in cigarette smoke was reported as carcinogenic (http://info.cancerresearchuk.org/healthyliving/smokeispoison, Cancer Research UK), and some of them, including benzene, 1,3-butadiene, nitrosamines including NDMA are activated by CYP2E1 (Garro *et al.,* 1981; Guengerich, 1995; Arınç *et al.*, 2000a,b; Abdel-Rahman *et al.,* 2000; Bolt *et al.,* 2003). Therefore CYP2E1 pharmacogenetics is important in determining the susceptibility of individuals to cancers, including childhood ALL.

On the other hand, GSTs play important roles in the detoxification of many of these carcinogens or their CYP2E1 metabolites, including acrylamide, acrylonitrile, 1,3-butadiene (Fennell *et al.,* 1991; Thier *et al.,* 1996). They also play roles in the detoxification of many cigarette smoke carcinogens, including benzo(a)pyrene. Therefore, carcinogenicity of many chemicals depend on the activity of CYP2E1 and GSTs, therefore investigation of their genetic polmyorphisms for the risk of childhood ALL is of crucial importance.

Pharmacogenetics is a new research area recently being applied to the epidemiologic studies. Earlier pharmacogenetic studies have focused on single genes in the risk assessment, however as explained above, investigation of multiple genes in the same metabolic pathway may better predict the risk of development of disease. In the case of childhood ALL, polymorphisms of especially *CYP2E1*, *NQO1* and also *GSTs* could have a combined effect on the risk of disease development, as explained before in Figure 1.3. This study focused on the pharmacogenetic factors for the risk of development of ALL, therefore a more detailed explanation of pharmacogenetics and the xenobiotic metabolizing enzymes are given below.

1.3 Pharmacogenetics and Genetic Epidemiology

Exposure to environmental agents is a risk for the development of childhood ALL, and other cancer types, as mentioned before. These chemicals include air pollutants, food additives, industrial chemicals, drugs, herbicides and pesticides, which can be totally called as xenobiotics. Xenobiotic metabolizing enzymes (also called as drug metabolizing enzymes) are responsible for the processing of these xenobiotics to inert derivatives that can be easily eliminated from the body. But they also sometimes mediate the toxicity or carcinogenicity by chemicals mediating metabolic activation of procarcinogens, as in the case of benzene, styrene, nitrosamines, polyaromatic hydrocarbons. Most of the xenobiotic metabolizing enzymes show genetic polymorphisms which create interindividual variability in xenobiotic metabolism and hence in the susceptibility of chemical-related diseases or carcinogenesis.

A genetic polymorphism is defined as a monogenic trait occurring at least in one population that is caused by the presence of more than one allele at the same gene which yields more than one phenotype in the

organism. The frequency of less common allele is usually more than 1%. Polymorphisms generally do not cause any sickness or other problems that would decrease reproductive efficiency. That's why, a polymorphism can be found in a population at such high frequency. In contrast, a genetic defect or mutation that causes a serious disease such as cystic fibrosis are found at extremely low frequencies (less than 1%) in humans because sick people do not efficiently reproduce and transmit the trait (Gonzalez, 1999).

Pharmacogenetics is the study of the linkage between an individual's genotype and that individual's ability to metabolize a foreign compound. In other words, pharmacogenetics deals with the polymorphisms of the xenobiotic metabolizing enzymes and the outcomes of the resultant phenotype, which may include susceptibility towards environmental diseases or response to therapy. Pharmacogenomics has its roots in pharmacogenetics, and is quite broad in scope, uses a genome-wide approach to identify the network of genes that govern an individual's response to therapy or susceptibility (Evans and Johnson, 2001).

The first observation in this field was done by Pythagorous in 510 BC on some people developing hemolytic anemia after consumption of fava bean (Agarwal et al., 2002). In early 1900's, scientists connected drug related disorders with Mendelian genetics. In 1950's, an enzyme variation in glucose 6-phosphate dehydrogenase, and a deficiency of N-acetyltransferase using the drug isoniazid were discovered. Vogel, in Germany, started the use of the term "Pharmacogenetics" in 1957 (Eichelbaum, 1999). The elucidation of the molecular genetic basis for inherited differences in drug metabolism began in the late 1980s, with the initial cloning of a polymorphic human gene encoding the drug metabolizing enzyme debrisoquin hydroxylase (CYP2D6) (Gonzalez et al.,

1988). Pharmacogenomics was born in early 2001 with all other –omics world during the near completion of draft human genome sequence. The technological advancement in genotyping allowed epidemiological studies to be conducted investigating the risk of genetic polymorphisms on disease outcome.

Epidemiology, in most general aspect, can be defined as the study of factors affecting the health and illness of populations, and proves useful in evidence-based medicine for identifying risk factors for disease and determining optimal treatment approaches to clinical practice. Epidemiological studies aim to reveal unbiased relationships between exposures such as alcohol or smoking, biological agents, stress or chemicals to mortality or morbidity.

Genetic epidemiology, on the other hand, studies the role of genetic factors in determining health and disease in families and in populations, and the interplay of such genetic factors with environmental factors. The advances in genotyping after completion of the Human Genome Project lead to investigation of many genetic polymorphisms that influence the risk of developing many common and multigenic diseases, including cancer (Moron, 1982).

Most commonly, genetic epidemiological research rely on the case-control studies. In case-control studies, a group of individuals that are disease positive (the "case" group) is compared with a group of disease negative individuals (the "control" group), with respect to the risk elevating susceptibility genes. The statistic generated to measure association is the “odds ratio (OR)”. In order to calculate the odds ratio, the individuals carrying the risk elevating allele is proportioned to those who do not carry the risk elevating allele for both case and control groups (odds of risk elevating allele for case and control groups), separately. The

proportion of case's odds to control's odds gives the ratio of the risk- the odds ratio- in terms of folds increase. An odds ratio significantly greater than 1 denotes the association of the risk elevating genotype with the disease, while a ratio far less than 1 indicates a protective role of the genotype for the disease. Odds ratio values near to 1 means there is no association between the genotype under question and disease. Odds ratio is an important term for genetic epidemiologic studies showing both the presence or absence of association and the magnitude of it in terms of times–fold of risk (Green *et al.,* 2000).

Pharmacogenetic case-control studies generally involve polymorphisms in single or multiple genes of xenobiotic metabolizing enzymes. Polymorphisms in these genes involve nucleotide or gene insertions and/or deletions, as well as single nucleotide substitutions. The most widely observed polymorphism is nucleotide substitutions, or in other word, single nucleotide polymorphisms (SNPs). SNP is a stable substitution of a single base with a frequency of more than 1% in at least one population (Evans and Johnson, 2001). SNPs are distributed throughout the human genome at an estimated overall frequency of one in every 1900 bp, with 1 SNP per approximately 1080 bases in exons, the frequency being higher in the genes coding for xenobiotic metabolizing enzymes than rest of the genome (Int. SNP Map Work. Group, 2001; Evans and Johnson, 2001).

1.4 Xenobiotic Metabolizing Enzymes

The xenobiotic metabolizing enzymes (or drug metabolizing enzymes) are responsible for protecting the organism by rapidly processing lipophilic chemicals to inert derivatives that can be easily eliminated from the body through urine or bile. Besides detoxification, they also quite often mediate the toxicity of chemicals through metabolic

activation of pro-toxins and pro-carcinogens, so they are also thought to have a role in individual susceptibility to chemical induced diseases and cancer. Liver is the main organ for xenobiotic metabolism and transformation reactions. These occur in two distinct stages; namely phase I and phase II reactions.

Phase I reactions occur by aromatic hydroxylations, aliphatic hydroxylations, oxidative N-dealkylations, oxidative O-dealkylations, S-oxidations, reductions and hydrolysis (Schenkman, 1991). Major phase I enzyme is the superfamily of cytochrome P450-dependent monooxygenases. P450s exist as a large superfamily of proteins and are the principal enzymes involved in oxidation of foreign compounds and the metabolic activation of carcinogens and toxins. Other phase I enzymes include alcohol and aldehyde dehydrogenases (ADH and ALDH, respectively), flavin monooxygenase (FMO), NAD(P)H quinone oxidoreductase (NQO1) and epoxide hydrolase (EH). Often simple functionalization by phase I enzymes could be sufficient to make a drug more soluble, facilitating the elimination through urine or bile. However, many phase I products are not eliminated rapidly and undergo a subsequent reaction with phase II enzymes in which an endogenous substrate combines with the newly incorporated functional group to form a highly polar conjugate.

Phase II enzymes have the role of conjugating either the parent compound or the metabolite from phase I by glucuronidation (by UDP-Glucuronosyltransferases- UGTs), sulfation (by sulfotransferases- STs), acetylation (by N-acetyl transferases- NATs), methylation (for example by thiopurine methyltransferase- TPMT) or glutathione conjugation (by gluthatione transferases- GSTs) to facilitate elimination (Gunaratna, 2000).

As mentioned before, almost all enzymes in xenobiotic metabolism show differences in expression among species and they are highly polymorphic, contributing to interindividual variability in drug response (Gonzalez, 2001). Drug metabolizing enzymes that are known to exhibit genetic polymorphisms with obvious phenotypical consequences are shown in Figure 1.5.

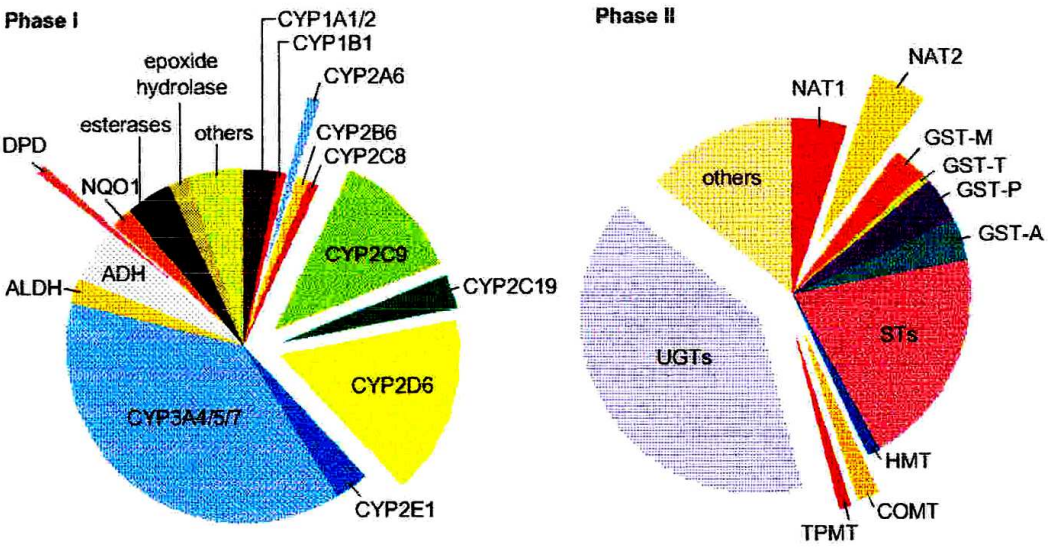

Figure 1.5 Phase I and phase II xenobiotic metabolizing enzymes that exhibit relevant genetic polymorphisms. Enzyme polymorphisms that have already been associated with changes in drug effects are separated from the corresponding pie charts. The percentage of phase I and phase II metabolism of drugs that each enzyme contributes is estimated by the relative size of each section of the corresponding chart (taken from Evans and Relling, 1999).

Many epidemiological studies have been conducted on the pharmacogenetics of these enzymes and the risk of development of childhood ALL. The major concern of this study was the association of

childhood ALL risk and polymorphisms of potential susceptibility genes, therefore some of the drug metabolizing enzymes that are supposed to be associated with the disease are examined thoroughly.

Cytochrome P450s are the most important components of the drug metabolizing system, as most of the drugs or xenobiotics first encounter with these enzymes in the liver, either for detoxification or bioactivation.

1.4.1 Cytochrome P450s

The cytochrome P450s are hemoproteins that play critical roles in the bioactivation and detoxification of a wide variety of xenobiotic substances. Besides, they have also roles in the metabolism and synthesis of endogenous compounds. These enzymes are encoded by a superfamily of genes and in mammalian cells, they are localized predominantly in the smooth endoplasmic reticulum of hepatocytes. The term "cytochrome P450" originates from the observation that the reduced state of the protein has an absorption band with absorption maxima at 450 nm after binding to carbon monoxide (Omura and Sato, 1964).

The cytochrome P450 monooxygenase system functions as a multicomponent electron transport system, which undergoes a cyclic series of reaction. The liver microsomal cytochrome P450 dependent monooxygenase system contains two protein components; cytochrome P450 and cytochrome P450 reductase (Lu and Coon, 1968), and a heat stable factor lipid (Lu *et al.,* 1969); which was later identified as phosphatidylcholine dilauroyl (Lu *et al.,* 1970). Cytochrome P450 catalyzes the monooxygenation reaction, NADPH dependent cytochrome P450 reductase catalyzes the electron transfer from NADPH to cytochrome P450, and lipid facilitates the transfer of electrons from NADPH cytochrome P450 reductase to cytochrome P450 (Lu and Levin,

1974). All three components of cytochrome P450 dependent monooxygenase system (NADPH-cytochrome P450 reductase, cytochrome P450 and lipid) are required to reconstitute the full hydroxylation and oxidation activity (Lu and Coon, 1968; Lu and Levin, 1974; Schenkman and Johnson, 1975; Arınç and Philpot, 1976; Black and Coon, 1986; Adalı and Arınç, 1990; Arınç, 1993; Şen and Arınç, 1998; Arınç and Çelik, 2002; Bozcaarmutlu and Arınç, 2008).

The general reaction catalyzed by cytochrome P450 can be written as follows:

$$NADPH,H^+ + \mathbf{O_2} + RH \longrightarrow NADP^+ + H_2\mathbf{O} + R\mathbf{O}H$$

where R represent the substrate that has a site for oxygenation, such as an alkane, alkene, aromatic ring or heterocyclic substituents. This is a monooxygenation reaction as one of the two oxygens is incorporated into substrate while the other is reduced to water. Depending on the particular reaction and the nature of various unstable intermediates, different reactions can occur. These include oxidative and reductive dehalogenation; N-hydroxylation and N-oxidation; oxidative deamination; S-, N-, and O-dealkylation; and aliphatic and aromatic hydroxylation (Schenkman, 1991; Guengerich, 1993).

Substrates of cytochrome P450s include the majority of drugs and other xenobiotics, together with several types of endogenous compounds (Gonzalez, 1989; Anzenbacher and Anzenbacherova, 2001; Danielson, 2002; Hsu *et al.,* 2007). The endogenous substrates of P450s include saturated and unsaturated fatty acids, eicosanoids, sterols and steroids, bile acids, vitamin D_3 derivatives, retinoids, and uroporphyrinogens. Also, many cytochrome P450 enzymes can metabolize various xenobiotics including drugs, plant- or fungal-derived secondary metabolites

consumed with food, and thousands of environmental pollutants like halogenated hydrocarbons, polycyclic aromatic hydrocarbons, arylamines, ingredients of combustion, industrial complex mixtures, herbicides, and pesticides, resulting in their detoxification (Gonzalez, 1989). Besides, the actions of P450 enzymes can also generate toxic metabolites that contribute to increased risks of cancer, birth defects, and other toxic effects (Nebert and Russell, 2002). Such P450 substrates include polycyclic aromatic hydrocarbons (e.g. benzene), nitrosamines, hydrazines, and arylamines.

P450s have been characterized in many species of organisms, including bacteria, fungi, plants, fish, birds, reptiles, insects and mammalian systems (Lu and Levin, 1974; Philpot et al., 1975; Arınç et al., 1976; Arınç and Philpot, 1976; Coon et al., 1978; Nebert and McKinnon, 1994; Şen and Arınç, 1997, 1998). As of February, 29, 2008, a total of 7232 P450s were identified from various organisms, so that 2565 animal, 2401 plant, 874 bacterial, 210 protist and 1182 fungal P450s were counted. When the pseudogenes were also included, the total number P450s from various organisms reach to 8128 (http://drnelson.utmem.edu/p450stats.Feb2008.htm, P450 Stats).

Presence of so many P450s in various organisms created a need for systemic nomenclature for cytochrome P450s, and it has been devised in 1987 by Nebert and coworkers which is now being used worldwide (Nebert *et al.,* 1987). This systemic nomenclature is based on the structural homology, that is, on amino acid sequence similarities between P450 proteins. According to this system, the cytochrome P450 superfamily is categorized into respective families and subfamilies. P450 proteins (denoted by "CYP") exhibiting more than 40% amino acid sequence similarity are classified within the same family and designated by an Arabic numeral, while proteins exhibiting more than 55% sequence

similarity are grouped into the same subfamily and shown by a capital letter (Nebert and McKinnon, 1994; Nelson et al., 1996). Lastly, the individual isoform is shown by an Arabic numeral following the capital letter. So, for instance, the ethanol-inducible isoform of cytochrome P450 superfamily is designated as CYP2E1, shortly.

1.4.1.1 Human Cytochrome P450s

Although the existence of P450 enzymes in human tissues has been known for many years, Kaschnitz and Coon for the first time, were able to partially separate P450, NADPH-P450 reductase and phospholipid from human liver microsomes in 1975; and demonstrated the need for all three fractions in the reconstitution of catalytic activity (Kaschnitz and Coon, 1975). In the late 1970s efforts in several laboratories led to the purification of human P450s to a high degree of purity. It is now possible to express individual human P450 cDNAs in yeast and mammalian cells, and a number of P450s have been prepared in such a way (Guengerich et al., 1991).

The majority of P450s are expressed in human liver, but they are also expressed in extrahepatic tissues –like lung, kidney, brain, gastrointestinal tract, skin, bone marrow, heart and placenta- on a smaller scale (Raunio *et al.* 1995). A few CYP isoforms participating in the metabolism of foreign compounds are found only in extrahepatic tissues, for example CYP2F is located in lung, CYP4A11/F2 and F3 in kidney, CYP4B1 in lung and placenta, CYP2S1 in lung and gastrointestinal tract, CYP2U1 in brain and thymus, and CYP2W1 in colon tumor tissue (Rylander *et al.,* 2001; Anzenbacher and Anzenbacherova, 2001; Karlgren *et al.,* 2004; Gomez *et al.,* 2007).

The completion of the human genome sequence enabled scientists to determine the human P450 genes. As of January 24, 2007, in humans, 18 P450 families, with a total of 58 P450 genes were determined (http://drnelson.utmem.edu/human.P450.table.html, Human Cytochrome P450s). Figure 1.6 represents the human cytochrome P450 genes identified so far (according to the last update on January, 24, 2007).

Table 1.3 lists the human cytochrome P450 families and their functions. The enzymes in the families 1-3 are mostly active in the metabolism of xenobiotics- thus called xenobiotic metabolizing P450s-, whereas the families 5-51 have important endogenous functions (see Table 1.3). Isoforms in family 4 have also roles in xenobiotic metabolism besides fatty acid hydroxylation. However, some isoforms in xenobiotic metabolizing P450s also metabolize endogenous compounds, such as steroid hormones and arachidonic acid (Gonzalez 1992, Capdevila *et al.* 2000). There is one enzyme in these families, CYP2J2, which has not been shown to metabolize foreign compounds (Wu *et al.* 1996).

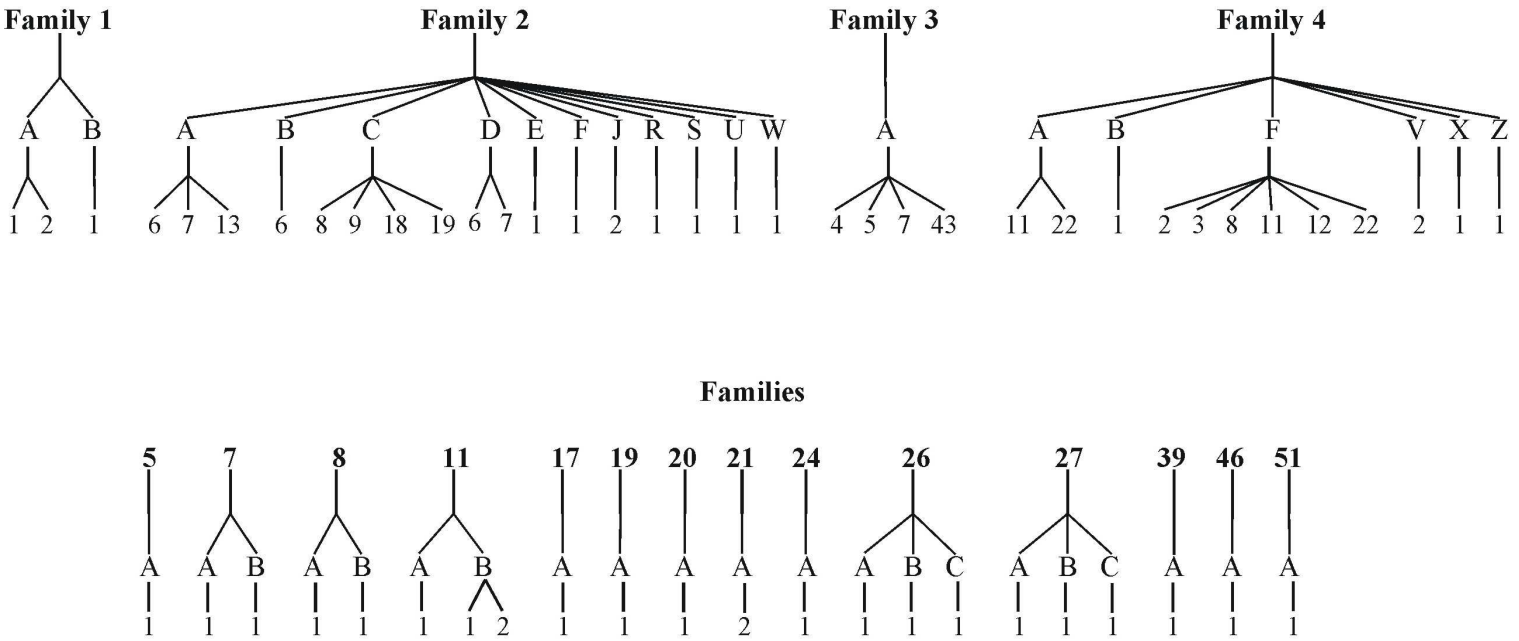

Figure 1.6 Human cytochrome P450 genes (Figure based on Stoilov et al., 2001; and updated from http://drnelson.utmem.edu/human.P450.table.html).

Table 1.3 Human cytochrome P450 families and their main functions (Table adapted from Hukkanen, 2000; Nebert and Russel, 2002).

CYP Family	Main Functions
CYP1	Xenobiotic metabolism
CYP2	Xenobiotic metabolism
	Arachidonic acid metabolism
CYP3	Xenobiotic and steroid metabolism
CYP4	Fatty acid hydroxylation
	Xenobiotic metabolism
CYP5	Thromboxane synthesis
CYP7	Cholesterol 7α-hydroxylation
CYP8	Prostacyclin synthesis
CYP11	Cholesterol side-chain cleavage
	Steroid 11β-hydroxylation
	Aldosterone synthesis
CYP17	Steroid 17α-hydroxylation
CYP19	Androgen aromatization
CYP20	Developmental signalling
CYP21	Steroid 21-hydroxylation
CYP24	Steroid 24-hydroxylation
CYP26	Retinoic acid hydroxylation
CYP27	Steroid 27-hydroxylation
CYP39	24-hydroxycholesterol 7α-hydroxylation
CYP46	Cholesterol 24-hydroxylation
CYP51	Sterol biosynthesis

There are differences between the characteristics of genes encoding xenobiotic metabolism (CYP1-3 and 4), and those of importance for the metabolism of endogenous compounds (CYP5-51). First, the greatest variability in number of members of the subfamilies lies in families 2, 3 and 4, as can be seen in Figure 1.6. In addition, it is evident that the number of polymorphisms is much greater among the *P450* genes coding for drug metabolizing enzymes, so that all *P450* genes belonging to families 1-3 show polymorphisms (Ingelman-Sundberg, 2002). By contrast, so far, only five isoforms in families 5-51 are known to show polymorphisms (http://www.imm.ki.se/CYPalleles, CYP Allele Nomenclature Committee).

Among the xenobiotic metabolizing human P450s, the major isoforms important for drug metabolism are CYP2A6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, whereas CYP1A1, CYP1A2, CYP1B1, and CYP2E1 are the most important isoforms responsible for metabolic activation of procarcinogens (Ingelman-Sundberg, 2002). These xenobiotic-metabolizing P450s comprise approximately 80% of total liver P450 content, and they can be ordered according to their relative content as CYP3A4/3A5 (~35%) > CYP2 (20%, major component being 2C9) > CYP1A2 (~12%) > CYP2E1 (~7%) > CYP2A6 (~4%) > CYP2D6 (~2%) > CYP2B6 = CYP1A1 (~1%) (Tanaka, 2001).

Many genetic epidemiological studies have been conducted on the association of xenobiotic metabolizing P450s and related environmental diseases. One of the isoforms that receives interest in this aspect is the CYP1A1, because of its role in the activation of major tobacco carcinogens like polyaromatic hydrocarbons (PAHs, e.g. benzo[a]pyrene) and aromatic amines. It is an extrahepatic isoform, expressed in lung and epithelial tissues, so many studies focused on the association of CYP1A1 polymorphisms and smoking-related cancers (Bartsch *et al.,* 2000).

CYP1A1 polymorphisms were found to be associated with lung (Coles and Ketterer, 1990; McClellan, 1996), head and neck (Doll, 1998), prostate (Murata *et al.,* 2001) and colorectal (Sivaraman *et al.,* 1994) cancers. Also CYP2C9 and CYP2D6 polymorphisms were shown to be associated with lung cancers (Kawajiri *et al.,* 1995; Bouchardy *et al.* 1996; Nebert *et al.,* 1996).

The carcinogen activating capacity of CYP1A1 made it a susceptibility gene for leukemia cases including childhood ALL. Several studies found an association with **2A* allele (Krajinovic *et al.,* 1999; Joseph *et al.,* 2004), **2B* allele (Infante-Rivard *et al.,* 2000), and *m2* allele (Joseph *et al.,* 2004); however there are also studies that showed no association (Canalle *et al.,* 2004; Pakakasama *et al.,* 2005). There are also studies on *CYP2D6* (Joseph *et al.,* 2004; Aydin-Sayitoglu *et al.,* 2006; Bolufer *et al.,* 2007) and *CYP3A4/3A5* (Pakakasama *et al.,* 2005; Bolufer *et al.,* 2007) polymorphisms and ALL risk, but no significant association was reported.

Among carcinogen activating P450s, CYP2E1 is of crucial importance for the risk of childhood ALL development because of its role in the metabolism of many environmental chemicals including, styrene, vinyl chloride, acrylamide, benzene, chloroform.

1.4.2 Cytochrome P4502E1 (CYP2E1)

CYP2E1 is the ethanol metabolizing isoform of cytochrome P450 superfamily, and its discovery relied on this property. In 1960s, alcohol dehydrogenase was the only enzyme known to metabolize ethanol. However, the morphological observations that, in rats and humans, ethanol feeding resulted in a proliferation of the smooth endoplasmic reticulum (Iseri et al., 1966; Lane and Lieber, 1966), which resembled

the situation observed after P450 induction, raised the idea that ethanol might also be metabolized by a similar process.

Such a cytochrome P450 involving system was then demonstrated in liver microsomes *in vitro* and found to be inducible by chronic ethanol feeding *in vivo* (Lieber and DeCarli, 1970). Ethanol-inducible cytochrome P450 was first purified from rabbits and named as LM3a (Koop *et al.,* 1982; Ingelman-Sundberg and Johansson, 1984) and later in rats, named as P450j (Ryan *et al.,* 1985; Patten *et al.,* 1986) and human (Wrighton *et al.,* 1986, 1987). The cDNA of CYP2E1 was cloned from the rat and human liver in 1986 (Song *et al.,* 1986). According to the new nomenclature system for *CYP* gene superfamily, it was proposed that the ethanol-inducible form be designated as CYP2E1.

1.4.2.1 Features of CYP2E1

CYP2E1 is mainly found in liver, but significant amounts are also found in extrahepatic tissues including lung, kidney, brain, endothelium of large blood vessels, heart, bone marrow and nasopharyngeal tissues (Ding *et al.,* 1986; Ingelman-Sundberg *et al.,* 1993).

A number of different chemicals of diverse structures have been found to be metabolized selectively by CYP2E1. Besides ethanol, CYP2E1 metabolizes some other endogenous compounds but most of its substrates are exogenous, including industrial solvents, procarcinogens and a few pharmaceutical drugs. It is difficult to find a common structure among all these substrates, but it appears that small and hydrophobic compounds in general provide efficient targets for CYP2E1-dependent catalysis (Ingelman-Sundberg *et al.,* 1993). Examples for the substrates, inducers and inhibitors of CYP2E1 are listed in Table 1.4.

Table 1.4 Substrates, inducers and inhibitors of CYP2E1 (combined from Garro *et al.,* 1981; Koop and Casazza, 1985; Peter *et al.,* 1990; Guengerich *et al.,* 1991; Gonzales and Gelboin, 1994; Guengerich, 1995; Melnick and Kohn, 1995; Arınç *et al.,* 2000a,b; Nuyan, 2008)

Substrates		
Endogenous	**Exogenous**	
	Therapeutic drugs/ anesthetic gases	**Solvents and other chemicals**
Ethanol Acetone Acetoacetate Acetol Acetaldeyde Fatty acids (arachidonic acid, lauric acid) Glycerol	Acetaminophen Chlorzoxazone Dapsone Enflurane, sevoflurane, isoflurane Halothane Disulfiram Isoniazid *p*-nitrophenol Phenacetin	Acrylamide Acrylonitrile, methacrylonitrile Alcohols, ethers, alkanes Acetone Benzene (and derivatives) Styrene Chloroform Carbontetrachloride Pyrazole Phenol Pyridine Nitrosamines (e.g. NDMA) Ethyl carbamate, vinyl carbamate Vinyl chloride, vinyl bromide Diethylether Hexane Butadiene Ethylene dibromide Ethylene dichloride Methyl chloride Methylene chloride 1,1,1-trichloropropanol 1,2-dichloropropanol

Inducers	**Inhibitors**
Ethanol Acetone Benzene Acrylamide Isoniazid Isopropanol Pyrazole Pyridine Starvation Diabetes	Diallylsulfide, diallylsulfone Chlormethiazole Diethyldithiocarbamate Isothiocyanates 4-methyl-pyrazole Disulfiram

CYP2E1 is among the most conserved forms in the CYP2 family and the catalytic activities of CYP2E1 across species are quite similar, suggesting that it has a physiological importance. Acetone, a ketone body, is metabolized by CYP2E1 (Koop and Casazza, 1985), therefore it is thought to be involved in the pathway of gluconeogenesis during the fasting state and diabetes (Gonzalez, 1989).

Among the substrates of CYP2E1, the 6-hydroxylation activity on muscle-relaxant chlorzoxazone is being used as a probe substrate to measure CYP2E1 activity in vivo (Peter et al., 1990). Also, N-demethylation of nitrosodimethylamine, aniline 4-hydroxylase and p-nitrophenol hydroxylase activities of CYP2E1 are used to measure the in vitro activity of this enzyme (Lieber, 1997; Arınç et al., 2000a,b; Arslan et al., 2003).

Although the drugs metabolized by CYP2E1 are relatively low in number, it is shown to be the major enzyme responsible for the acetaminophen toxicity, as clearly established in the studies with CYP2E1-null mice protection against acetaminophen toxicity (Gonzalez, 2001). However, as can be seen on the Table 1.4, an important portion of CYP2E1 substrates involves industrial chemicals and procarcinogens/carcinogens. Among them are small molecular weight hydrocarbons, such as benzene and styrene (Guengerich, 1995), butadiene (Melnick and Kohn, 1995), certain chloroalkanes and chloroalkenes, like chloroform, tetrachloromethane, trichloroethane and vinyl chloride (Guengerich *et al.,* 1991; Gonzalez and Gelboin, 1994), and nitrosamines including NDMA (Garro *et al.,* 1981; Arınç *et al.*, 2000a,b). CYP2E1 activates these chemicals into more toxic or carcinogenic forms, therefore receives a great deal of attention in terms of occupational liver-diseases and cancer.

Another important and peculiar feature of CYP2E1 is its capability to reduce molecular oxygen, resulting in the formation of H_2O_2 and $O_2^{\bullet-}$ radicals (Gorsky *et al.,* 1984; Elkstrom and Ingelman-Sundberg, 1989; Persson *et al.,* 1990). It is known that unlike most other P450s, CYP2E1 contains a proportion of the hemoprotein that is present naturally in the high-spin state even in the absence of substrate (Koop *et al.,* 1982; Guengerich and Johnson, 1997), and this feature of CYP2E1 would let it be reduced by an electron provided by NADPH-cytochrome P450 reductase (in the absence of substrate), resulting in the generation of reactive oxygen species (Elkstrom *et al.,* 1986; Cederbaum, 1987; Kukielka and Cederbaum, 1994).

It appears that oxygen radicals generated by CYP2E1 have the capability to initiate membranous lipid peroxidation as verified with studies showing almost complete inhibition of NADPH-dependent lipid peroxidation in microsomes using CYP2E1 antibodies (Elkstrom and Ingelman-Sundberg, 1989). The increased microsomal generation of reactive oxygen derivatives could also contribute to ethanol toxicity through radical-mediated inactivation of metabolic enzymes (Dicker and Cederbaum, 1988), including CYP2E1 itself (Koop and Tierney, 1990). Besides all, reactive oxygen species can cause DNA damage (Halliwell and Gutteridge, 1990; Wiseman and Halliwell, 1996) and can, therefore, represent factors in the overall carcinogenicity of some CYP2E1 substrates and inducers (Parke, 1987, 1994; Terelius *et al.,* 1993; Ioannides *et al.,* 1995).

Most CYP2E1 substrates are shown to be also inducers of the enzyme, like ethanol, isoniazid, acetone, benzene and pyridine. As well, some pathophysiological conditions such as starvation, diabetes, obesity, and high fat feeding are also found to induce CYP2E1 (Koop and Casazza, 1985; Hong *et al.,* 1987, Song *et al.,* 1987; Schenkman *et al.,* 1989; Kim

et al., 1990; Arınç *et al.,* 1991; Yoo *et al.,* 1991; Arınç *et al.,* 2000a,b; Arslan, 2003; Arınç *et al.,* 2005; Arınç *et al.,* 2007). Inhibition of CYP2E1 is observed upon treatment with diallylsulfide, disulfiram, diethlydithiocarbamate, isothiocyanates, and 4-methyl pyrazole (Lieber, 1997; Rendic and Di-Carlo, 1997).

The regulation of CYP2E1 expression is complex, involving transcriptional, posttranscriptional, and posttranslational events with polymorphism playing a role (Song, 1995). Hepatic nuclear factor-1α (HNF1α) (Blumenfeld *et al.,* 1991; Lee *et al.,* 1998), extensive starvation (Albano *et al.,* 1993), and high ethanol concentrations (Badger *et al.,* 1993; Ronis *et al.,* 1993) were shown to induce CYP2E1 transcription. Administration of small organic compounds such as acetone, pyrazole, and low concentrations of ethanol were shown to increase CYP2E1 protein levels either at translational level or by stabilization of the protein in rats (Khani *et al.,* 1987).

CYP2E1 is also shown to be regulated by physiological events. CYP2E1 protein and mRNA levels were shown to be induced in diabetic rats (Song *et al.,* 1986). Fasting also caused an increase in CYP2E1 mRNA and protein (Hong *et al.,* 1987). Recently, studies carried out using rabbits made diabetic experimentally with alloxan treatment have shown that CYP2E1 protein is induced and CYP2E1-associated enzyme activities are stimulated compared to untreated control rabbits (Arslan, 2003; Arslan *et al.,* 2003).

CYP2E1 also shows polymorphisms, some of which are suggested to be associated with several diseases, including cancers.

1.4.2.2 Polymorphisms of *CYP2E1*

The human CYP2E1 gene (accession No. AL161645) is located in 10q24.3-qter region of chromosome 10, and spans 11,413 base pairs with nine exons and a typical TATA box. (Umeno et al., 1988). The gene contains several polymorphisms, some of which seem to effect the expression of the protein. Figure 1.7 shows a schematic representation of the gene and associated polymorphisms.

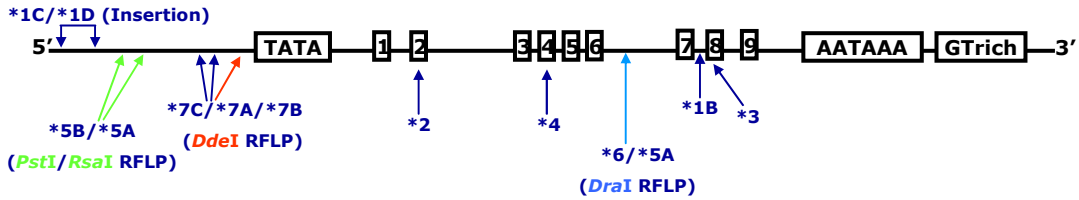

Figure 1.7 Schematic representation of human CYP2E1 gene with associated polymorphisms. The numbered boxes represent the exons; polymorphisms occurring throughout the gene are indicated by dark blue arrows; the polymorphisms investigated in this study are indicated in green, red and light blue arrows. Alleles are designated according to the CYP Allele Nomenclature Committee assignments (http://www.imm.ki.se/CYPalleles).

The most frequently studied genetic polymorphism in *CYP2E1* is the *CYP2E1*5B* variant (rs3813867/rs2031920, *Pst*I/*Rsa*I RFLPs, c2 variant in old designation) located in the 5'-flanking region of the gene with substitutions at positions G-1293C and C-1053T (Watanabe *et al.,* 1990). This variant has been related to high enzyme expression *in vitro* by Hayashi, Watanabe and Kawajiri (Hayashi *et al.*, 1991).

Hayashi *et al.* (1991) showed that reporter gene constructs with **5B*5B* (c2/c2 in old designation) genotype showed 10 times higher reporter gene activity than gene constructs with **1A*1A* (c1/c1 in old designation) genotype, in vitro. The DNA segment in 5'-flanking region of human *CYP2E1* gene that covered both *Pst*I and *Rsa*I RFLP sites was amplified by PCR and fused to a reporter gene (chloramphenicol transferase) with the SV40 promoter. Then the constructs were transfected into the human hepatoma cell line HepG2. They found that the enhancer activity for **5B*5B* (c2/c2) DNA was about 10 times higher than that of **1A*1A* (c1/c1) DNA (for detailed explanation of old and new designations of CYP2E1 polymorphisms, see Ulusoy *et al.,* 2007b). Another polymorphism in intron 6 of gene, the CYP2E1**6* variant (rs6413432, *Dra*I RFLP, D variant in old designation) which is a T to A substitution at position 7632 was also identified (Uematsu *et al.* 1991).

Yang and coworkers have studied the CYP2E1 activity in 14 human lung tissue and its association with *CYP2E1*5B* polymorphism (Yang *et al.,* 2002). In their study, 2-5 grams of lung tissue from normal individuals was separated into mitochondria, microsome, cytosol and DNA fractions. CYP2E1 activity was measured by p-nitrocatechol production from p-nitrophenol using HPLC/EC. They showed that CYP2E1 activity was significantly higher in *CYP2E1*5B* heterozygous (c1c2 genotype) individuals than wild type (c1c1) individuals (Yang *et al.,* 2002).

Styrene is a widely used chemical in the production of plastics, synthetic rubber and polyester resins. Styerene is converted to styrene-7,8-oxide by CYP2E1 (see Table 1.4). IARC has classified styrene-7,8-oxide as a possible human carcinogen (Group 2A) (Vodicka *et al.,* 2001). Association of genetic polymorphisms of *CYP2E1*5B* (c2 variant) and *CYP2E1*6* (C variant) and DNA-strand breaks (measured by Comet assay) in workers occupationaly exposed to styrene was examined

(Vodicka *et al.,* 2001). Study group consisted of 42 hand-lamination workers and 18 unexposed controls. Results showed that the frequency of single strand breaks in DNA was significantly ($p<0.05$) higher in individuals heterozygous for *CYP2E1*5B* (c1/c2 genotype in old designation) and *CYP2E1*6* (C/D genotype in old designation) in comparison to wild type individuals (c1/c1 genotype and D/D genotype). No homozygous mutated individuals (c2/c2 or C/C genotype was found among workers and controls).

Vinyl chloride is a synthetic chemical that is used in the production of polyvinyl chloride (PVC), floor coverings, consumer goods, electrical and transport applications. (IARC, 2008). Its exposure routes were shown to be PVC manufacturing plants, landfill gas and groundwater as a degradation product of chlorinated solvents that were deposited in landfills. Also it was found in PVC products as a residue from production and it was identified in mainstream cigarette smoke (Hoffmann *et al.,* 1976; WHO, 1999; IARC, 2008). Vinyl chloride is absorbed mainly by inhalation and metabolized in liver. First step in the metabolism of vinyl chloride is its oxidation to highly reactive chloroethylene oxide by CYP2E1 (IARC ,2008). It has been shown that this metabolite, chloroethylene oxide, can form DNA adducts (Guengerich, 1992; Müller *et al.,* 1996). Vinyl chloride was as a Group 1 carcinogen by IARC (IARC, 1987). Very recently, association of *CYP2E1*5B* (c2 variant) and DNA damage to peripheral blood lymphocytes (measured by single cell gel electrophoresis assay) was studied in workers occupationaly exposed to vinyl chloride. The study covered 75 PVC polymerization plant workers and unexposed 75 controls. The results showed a significant ($p>0.05$) risk of increased DNA damage for individuals having vinyl chloride exposure and possessing the *CYP2E1*1A*5B* (c1c2) or *CYP2E1*5B*5B* (c2c2) genotypes (Zhu *et al.,* 2008).

These studies associated *CYP2E1*5B* polymorphism with higher CYP2E1 activity, by different measurements. Hayashi *et al.* (1991) showed that higher transcriptional expression was associated with *CYP2E1*5B*5B* (c2c2) variant, in vitro. Yang *et al.* (2002) associated the variant heterozygous genotype (c1c2) with higher CYP2E1 activity in lung. Importantly, study of Vodicka *et al.* (2001) showed that *CYP2E1*5B* and *CYP2E1**6 polymorphisms were associated with increased DNA single strand breaks, which suggests higher CYP2E1 activity in individuals bearing *CYP2E1*5B* (c2 in old designation) and *CYP2E1**6 (C in old designation) variants. Study of Zhu *et al.* (2008) also showed increased DNA single strand breaks, hence higher CYP2E1 activity was associated with *CYP2E1*5B* polymorphism.

The genetic epidemiological studies on *CYP2E1* polymorphisms focused on **5B* and **6* SNPs. The *CYP2E1*6* polymorphism was found to be associated with an increased risk for lung cancer in several studies (Uematsu *et al.,* 1992; Wu *et al.,* 1998; Le Marchand *et al.,* 1998) and for renal carcinoma (Farker *et al.,* 1998). The **5B* polymorphism was found to be associated with adenocarcinoma (El Zein *et al.,* 1997), esophagus cancers (Lin *et al.,* 1998), as well as oral cancer cases (Liu *et al.,* 2001).

Fairbrother and co-workers (1998) have identified *CYP2E1*7B* polymorphism in promoter region, with base substitution at G-71T (rs6413420, *Dde*I RFLP), which is in complete linkage disequilibrium with T-333A SNP (rs2070673). As this polymorphism is located in promoter region, it is suspected to be associated with expression or regulation of the gene.

There are additional polymorphisms on the gene as can be seen from Figure 1.7. An insertion polymorphism in the 5'-flanking region

(*CYP2E1*1C* and **1D*) was found by McCarver and co-workers (1998). Three single nucleotide polymorphisms (*CYP2E1*2, *3* and **4*) in the exons that lead to amino acid changes in the protein were also reported (Hu *et al*., 1997; Fairbrother *et al.,* 1998), but these polymorphisms received not much interest because of their very low frequencies.

Studies on the association of *CYP2E1* polymorphisms on childhood ALL showed inconsistent results. Some research found 2.8 to 3.4 fold increased risk for childhood ALL with the presence of *CYP2E1*5B* allele (Krajinovic *et al.,* 2002b; Aydin-Sayitoglu *et al.,* 2006). Higher odds ratio (4.9 fold increased risk) was observed in the case of postnatal maternal alcohol consumption during nursing period and presence of **5B* allele for the risk of childhood ALL (Infante-Rivard *et al.,* 2002a). However, no association was found in other reports (Bolufer *et al.,* 2007; Canalle *et al.,* 2004). Other variants of *CYP2E1* have not been investigated for this disease yet. So the role of *CYP2E1* polymorphisms in the risk of childhood ALL development still needs to be clarified.

NAD(P)H: quinone oxidoreductase 1 (NQO1) is another important drug metabolizing enzyme that takes role in the metabolism of environmental chemicals.

1.4.3 NAD(P)H: Quinone Oxidoreductase 1

NAD(P)H: quinone oxidoreductase 1 (NQO1) is a ubiquitous flavoprotein and an obligate two-electron reductase that can catalyze, with varying degrees of efficiency, the reduction of quinones, quinone epoxides, aromatic nitro and nitroso compounds, azo dyes and Cr(VI) compounds (Colucci *et al.,* 2008). Quinones are widely distributed in nature, present in all burnt organic materials, including automobile exhaust, cigarette smoke, and urban air particulates and are also found

naturally in many foods. Quinones are highly reactive molecules and readily undergo either one- or two-electron reduction (Jaislaw, 2000). One-electron reduction of quinones and its derivatives by enzymes such as cytochromes P450 (CYP1A1 and CYP1A2), cytochrome P450 reductase, and cytochrome b5 reductase, generate unstable semiquinones that eventually generate reactive oxygen species, leading to toxicity and DNA damage (Jaislaw, 2000). The two-electron reduction and detoxification of quinones without production of reactive oxygen species is catalyzed by NAD(P)H:quinone oxidoreductases. Lars Ernster's laboratory was the first to describe the presence of an oxidoreductase (diaphorase) enzyme activity in the rat liver cytosol that catalyzed reduction of 2,6-dichlorophenolindophenol and partially purified the enzyme in 1958 (Ernster and Navazio, 1958; Ernster *et al.,* 1960). The enzyme NQO1 was initially named DT-diaphorase for its unusual ability to use either NADH (originally designated **D**PNH) or NADPH (**T**PNH). It is related to another cytosolic flavoenzyme, NRH:quinone oxidoreductase 2 (NQO2) that also catalyzes the reductive metabolism of quinones (Colucci *et al.,* 2008). NQO2 is different from NQO1 in cofactor requirement so that it uses dihydronicotinamide riboside (NRH) rather than NAD(P)H as an electron donor (Jaislaw, 2000).

NQO1 is a homodimer with a molecular weight of 60 kDa, and each subunit contains a non-covalently bound molecule of flavin adenine dinucleotide (FAD). The substrate binding site of the enzyme is occupied by both the cofactor (NAD(P)H) and substrate, therefore catalytic activity shows a ping-pong mechanism, where NAD(P)H occupying the binding site transfers its hydride to FAD, resulting $NAD(P)^+$ leaving the site to be replaced by the quinone substrate. Hydride transfer from $FADH_2$ results in reduction of the quinone, and finally the hydroquinone departs to restart the catalytic cycle, as shown in Figure 1.8 (Colucci *et al.,* 2008).

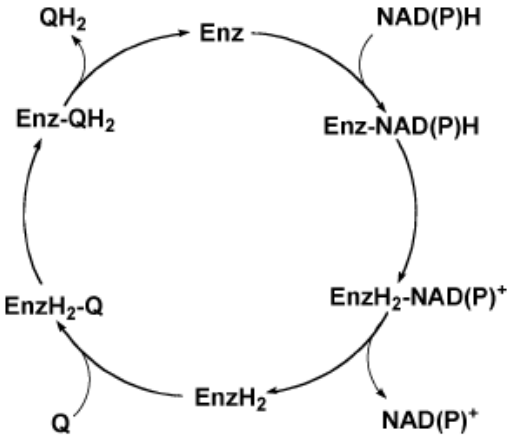

Figure 1.8 Ping-pong reaction mechanism of NQO1 (Figure taken from Colucci *et al.,* 2008).

The enzyme is mainly cytosolic although small mitochondrial and microsomal pools have been identified in rat liver. In mice, rat and humans, NQO1 is mainly localized to epithelial and endothelial tissues. Although the enzyme have been shown to be expressed at high levels in rat and mice livers, only trace amounts of NQO1 could be detected in human liver samples (Ross, 2005; Ross and Siegel, 2004). NOQ1 was not detected in bone marrow hematopoietic cells; however it was induced after high concentrations of benzene metabolite hydroquinone (Bolufer *et al.,* 2006). Also it was shown to be present in endothelial cells lining large blood vessels and sinusoids in the marrow (Ross, 2005).

1.4.3.1 Features of NQO1

NQO1 protects against the deleterious effects of quinones by catalyzing their two-electron reduction, in a single stage, thus bypassing the semiquinone intermediate, as explained above. However, it also

catalyzes the reductive activation of quinolic chemotherapeutic compounds such as mitomycins, anthracyclines, and aziridinyl-benzoquinones. NQO1 is expressed at high levels in many solid tumors including lung, colon, liver, pancreas and breast. This property makes this enzyme an ideal target for development of bio-activable cytotoxic compounds for chemotherapeutic purposes. (Bianchet *et al.,* 2004)

NQO1 also has important roles in the protection against oxidative stress. Ubiquinone (co-enzyme Q) and α-tocopherol-quinone (vitamin E derivative), two important lipid-soluble antioxidants, are substrates for NQO1 in vitro. NQO1 is thought to help maintain these antioxidants in their reduced forms (uniquinol and α-tocopherol-hydroquinone), so plasma membranes are protected against oxidative damage (Beyer *et al.,* 1996, Nioi and Hayes, 2004). It should be also noted that, NQO1 is co-induced with several other genes (like GSTs, UGTs, EH) in response to oxidative stress, so together help protection of the cells against free radical damage, oxidative stress, and neoplasia (Jaislaw, 2000).

Several studies have reported that NQO1 stabilized p53 transcription factor by a redox mechanism (Asher *et al.,* 2002) or by protein-protein interaction (Anwar *et al.,* 2003). Stabilization of p53 by NQO1 in either ways, could help explain the chemoprotective effects of NQO1 in many different systems (Ross, 2005).

1.4.3.2 Polymorphisms of *NQO1*

Human *NQO1* gene is located at chromosome 16, in 16q22.1 region, and associated with two well-characterized polymorphisms –the *NQO1*2* polymorphism located in exon 6 and *NQO1*3* polymorphisms in exon 4. Figure 1.9 shows a schematic representation of *NQO1*2* and *3* variants.

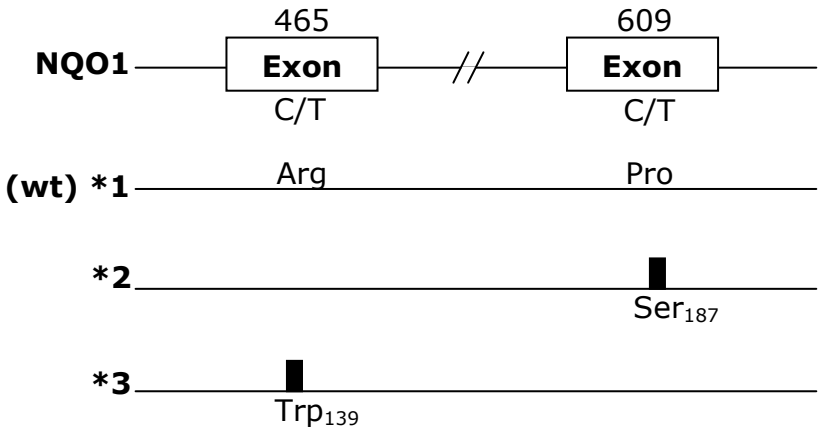

Figure 1.9 Schematic illustration of *NQO1* variants. Two C-to-T transitions at position 609 (Pro to Ser replacement at codon 187) and 465 (Arg to Trp replacement at codon 139) define variants *NQO1*2* and *NQO1*3*, respectively (Figure reproduced from Krajinovic *et al.,* 2002b).

The *NQO1*3* polymorphism is located in exon 4 with a C to T substitution at location 465, which results in a nucleotide substitution of arginine to tryptophan at amino acid 139. The allele frequency of the *NQO1*3* polymorphism is low (<1.0%) and the phenotypic consequences are variable according to substrate (Pan *et al.,* 1995), therefore does not receive much attention in genetic epidemiological studies.

The *NQO1*2* polymorphism is a single nucleotide change from C to T at position 609 of the NQO1 cDNA coding for a proline to serine change at position 187 in the amino acid structure of the protein. It has been shown that, the mutant NQO1*2 protein was degraded rapidly by the ubiquitin proteosomal system (1.5 vs. more than 18 hours in mutant and wild type protein, respectively) Besides, the NQO1*2 protein, unlike the NQO1*1 protein, was found not to associate with the protein chaperones Hsp70 and Hsp40 in a multi-protein complex, which help newly

synthesized proteins fold correctly (Ross D., 2005). Thus, it is proposed that lack of interaction of the NQO1*2 variant protein with chaperons may lead to aberrant folding and accelerated degradation through the ubiquitin proteosomal system. As a result, *NQO1*2* allele results in lack of NQO1 activity, so that individuals with homozygous *NQO1*2/*2* genotype have no activity, and heterozygous individuals with *NQO1*1/*2* genotype have reduced activity.

With the realization of the importance of *NQO1* polymorphisms, research area focused on the consequences of this polymorphism in relation to cancer types. The *NQO1*2* allele was associated with increased risk of urothelial tumors (Schulz *et al.,* 1997), cutaneous basal cell carcinoma (Clairmont *et al.,* 1999), colorectal cancer (Lafuente *et al.,* 2000) and gastric cardiac carcinoma (Zhang *et al.*, 2003).

NQO1 specifically derives attention for the risk of leukemias, because of its detoxification role in benzene metabolism. Several studies have found increased risk of benzene toxicity with *NQO1*2* allele (Rothman *et al.,* 1997; Moran *et al.,* 1999; Wan *et al.,* 2002). Several studies have found an association between *NQO1*2* polymorphism and infant leukemia (OR= 2.5) (Wiemels *et al.,* 1999c), therapy related leukemia and myelodyplastic syndrome (OR = 2.62) (Naoe *et al.,* 2000), adult leukemia (OR = 1.49). (Smith *et al.,* 2001), childhood ALL (OR= 1.7) (Krajinovic *et al.,* 2002b) while some of them found no correlation between *NQO1*2* polymorphism and lymphomas (Soucek *et al.,* 2002), therapy-related acute myeloblastic leukemia (Seedhouse *et al.,* 2002) or acute lymphoblastic leukemias (Blanco *et al.,* 2002; Sirma *et al.,* 2004).

Glutathione S-transferases, the most important of the phase II drug metabolism, are other important polymorphic enzymes for the risk of ALL development.

1.4.4 Glutathione S-Transferases

Glutathione S-transferases (GSTs) are a family of Phase II detoxification enzymes that function to protect cellular macromolecules from attack by reactive electrophiles. Specifically, GSTs catalyze the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous nonpolar compounds that contain an electrophilic carbon, nitrogen, or sulphur atom (Figure 1.10). Their substrates include halogenonitrobenzenes, arene oxides, quinones, and α,*β*-unsaturated carbonyls (Townsend and Tew, 2003; Hayes *et al.,* 2005).

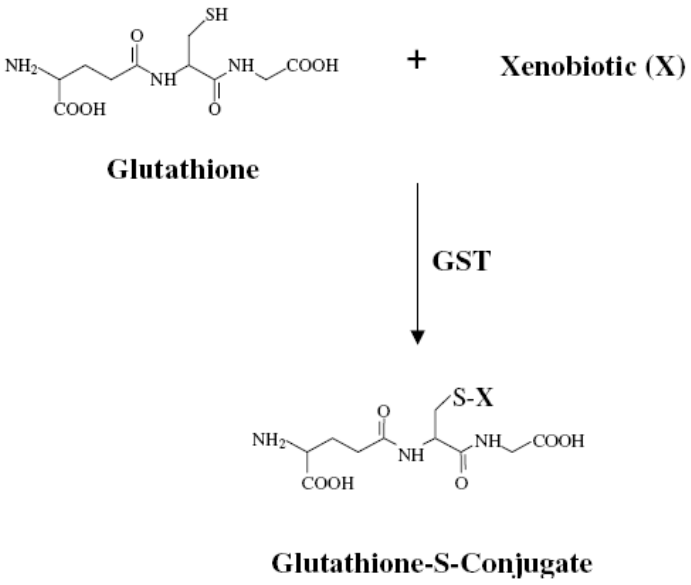

Figure 1.10 Glutathione conjugation to a generic xenobiotic (X) via GST, resulting in the formation of a glutathione-S conjugate (Figure taken from Townsend and Tew, 2003).

There are three major glutathione S-transferase families. Two of these, the cytosolic and mitochondrial GSTs, comprise soluble enzymes that are only distantly related. The third family comprises microsomal GST and is now referred to as membrane-associated proteins in eicosanoid and glutathione (MAPEG) metabolism (Hayes *et al.,* 2005).

Rodent and mammalian cytosolic GST isoenzymes are classified according to their amino acid sequence, and GSTs within a class typically share more than 40% identity in their primary structure, and those between classes share less than 25% identity. Seven classes of cytosolic GSTs are recognized in mammalian species, designated Alpha (GSTA), Mu (GSTM), Pi (GSTP), Sigma (GSTS), Theta (GSTT), Omega (GSTO), and Zeta (GSTZ). The enzymes involved in each class form a separate gene family. Within human genome, at least two genes were found encoding GSTA class, five genes encoding GSTM class, one gene encoding GSTP class, and at least two genes encoding GSTT class (Hayes *et al.,* 2005; Gresner *et al.,* 2007).

Expression of GSTs, although occurring in cells of all tissues and organs, varies considerably. GST expression is regulated by cell-specific environmental, hormonal and genetic agents, and is affected by age, sex, diseases and by various types of endogenous and exogenous chemicals. Highest GST expression was shown in gonads, colon and liver, providing the maximum protection to cells constantly exposed to harmful effects of carcinogenic chemicals (Gresner *et al.,* 2007).

1.4.4.1 Features of GSTs

The substrate specificity of GST isoforms is very low. Therefore, GSTs are among the major mechanisms by which cells are protected

against carcinogenic effects of various endogenous and exogenous compounds, including aflatoxin B1, nitro and nitroso derivatives of hydrocarbons, benzo(a)pyrene, halogenated alkanes and alkenes, as well as the products of oxidative stress (Gresner *et al.,* 2007). Physiologically they function in isomerization of various unsaturated compounds and in the synthesis of prostaglandins and leukotrienes (Hayes *et al.,* 2005). They are also of great pharmacological interest as they provide targets for antiasthmatic and antitumor drug therapies and metabolize cancer chemotherapeutic agents (Hayes *et al.,* 2005).

The activity of GSTs is crucial for plenty of chemical reactions, mostly related with the cell's adaptive response with its activity starting upon chemical exposure. Two isoforms GST-μ and GST-θ are of special interest for their role in carcinogen detoxification and high level of interindividual variation in their expression. The genes coding for *GSTM1* and *GSTT1* are located in chromosomes 1p13.3 and 22q11.2, respectively. Both isoforms are involved in the metabolism of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and are also induced by them. Some examples to these chemicals include benzo-(a)pyrene, styrene-7,8-oxide, and trans-stilbene oxide for GSTM1, and epoxybutanes, ethylene oxide, halomethanes, and methyl bromide for GSTT1 (Rebbeck, 1997).

Both isoforms, as well as other cytosolic GSTs, are subject to significant genetic polymorphisms in human populations, hence leading to high interindividual variability towards protection against carcinogens and oxidative stress (Townsend and Tew, 2003).

1.4.4.2 Polymorphisms of *GSTM1* and *GSTT1*

Both *GSTM1* and *GSTT1* possess null genotypes that result in deletion of the gene, with strikingly high frequencies as high as more than 50% in Caucasians. The null-variant result in the absence of the enzyme activity, hence in the poorer elimination of electrophilic carcinogens. GST deficiencies may therefore result in increased risk of somatic mutation, leading to tumor formation (Booth *et al.,* 1961; Cambes and Stakelum, 1961). If an individual's inherited genotype at a GST locus does not permit the efficient metabolism of compounds involved in carcinogenesis, then that individual may be at increased cancer risk. Therefore many epidemiological studies have been conducted for *GSTM1* and *T1* null on the risk of cancer development. It should also be taken into account that deficiency of GST activity would be more severe if, at the same time, production of reactive metabolites is increased due to increased phase I metabolism by cytochrome P450s.

In general, it has been found that individual *GST* genes alone, do not make a major contribution to susceptibility to cancer, although *GSTM1*null was found to be associated with lung and colorectal cancers (Zhong *et al.,* 1991, 1993), and *GSTT1* null was associated with bladder cancer (Brockmoller *et al.,* 1996).

In the case of leukemia risk and *GS*T null genotypes, contradictory results are present. *GSTM1* null genotype was associated with risk of childhood ALL with odds ratios between 1.8 and 2.6 in some studies (Krainovic *et al.,* 1999, 2002a; Saadat and Saadat, 2000; Alves *et al.,* 2002), while no association was found in others (Davies *et al.,* 2002; Balta *et al.,* 2003; Barnette *et al.,* 2004; Canalle *et al.,* 2004). Similar contradictory results were obtained in the case of *GSTT1* null genotype, no association was shown in childhood ALL (Krainovic *et al.,* 1999; Alves

et al., 2002; Davies *et al.,* 2002; Balta *et al.,* 2003) while one study on adult ALL found 3.1 fold increased risk (Rollinson *et al.,* 2000). *GSTT1* null genotype was also found to be a risk factor for childhood (OR=1.6) and adult (OR=1.7) AML (Davies *et al.,* 2000; D'Alo *et al.,* 2004).

The contradictory results point out the need for investigation of multiple *GST* isoform polymorphisms together with the carcinogenicity increasing polymorphisms of phase I enzymes, like CYP1A1 and CYP2E1. As mentioned previously, genetic epidemiological studies based on the polymorphisms of multiple genes functioning in the same metabolic pathways of chemical exposure would give more reliable results and aid in the clarification of risk assessment for cancers, including childhood ALL.

1.5 Aim of the Study

Childhood acute lymphoblastic leukemia is the most frequent type of cancer seen among children, comprising 30% of all childhood cancers. The improvements in the treatment of ALL increased the overall survival of children with ALL up to 80%; however the exact molecular epidemiology of the disease still remains to be elucidated. Leukemia is an extensively studied type of cancer, and a multistage model for the development of disease has been postulated (Greaves, 1996) which suggests at least two mutations, primary event occurring prenatally and the second occurring postnatally after exposure to risk factors. The model of Greaves' (1996) points out the importance of environmental factors in disease development, at different exposure levels, preconceptional, prenatal and postnatal. Determination of the risk factors at these exposure levels, which eventually lead to leukemigenic mutations, is an important area of research.

The development of ALL is likely to involve an interaction of exposure to environmental chemicals and inherent genetic susceptibility, where the effects of genetic polymorphisms are modulated by external factors, modifying the child's risk of cancer. It is clearly established that the mutagenicity of many chemicals arise or altered after their metabolism in the body. This situation makes it obligatory to consider the enzymes that take role in the metabolic activation or detoxification of the chemicals, in order to determine the risk factors for the disease. It is well known that almost every xenobiotic metabolizing enzyme possess genetic polymorphism which effects the expression or the activity of the enzyme, hence the capacity for the activation or detoxification. Such genetic polymorphisms result in the interindividual variations in susceptibility toward developing cancer, including childhood ALL.

Recent developments in the technology and completion of Human Genome Project lead to determination of polymorphisms in the susceptible genes. Many epidemiological studies have been conducted from then on, aiming to elucidate the role of genetic susceptibility of individuals towards diseases. However, as the genetic epidemiological studies accumulated, several problems accompanied. The initial studies were on single gene or single locus of the genes that possess more than one variant allele, and the results obtained for the risk of cancer development were contradictory. As the metabolism of xenobiotics requires multiple steps involving many enzymes, the overall carcinogenicity of the chemicals depends on combination of metabolic activity rather that single enzyme activities. Therefore, recent genetic epidemiological studies focus on the polymorphisms of enzymes that have major roles in the metabolic pathway of procarcinogens. As mentioned in more detail previously, CYP2E1 is a susceptible enzyme for the development of cancers as it bioactivates many environmental chemicals like nitrosamines, styrene, benzene, vinyl chloride (Table 1.4).

GSTs and NQO1 take role in detoxification of many toxic metabolites of CYP2E1 and other carcinogenic chemicals, as well they have an important role in protecting the cell against oxidative stress. Therefore, this study focused on the effects of *CYP2E1*, *NQO1*, *GSTM1* and *T1* genetic polymorphisms, alone or in combination, as risk factors for the development of childhood ALL. Another possible reason for inconsistent results could be the investigation of single locus instead of multiple loci, or haplotypes. *CYP2E1* possess multiple polymorphisms throughout the gene; however, most of the epidemiological studies focused on a single allele, namely **5B* allele. In consideration that, overall inducibility of the *CYP2E1* gene could be affected by combination of its variant alleles, this study investigated three polymorphic alleles of the gene, **5B*, **6*, and **7B*.

A striking feature of the genetic polymorphisms in xenobiotic metabolizing enzymes is that, the frequency of the polymorphisms show variability in different populations and ethnic groups. So, different populations may show different susceptibilities towards procarcinogens depending on the frequency of the polymorphisms. In this respect, the genetic risk factors for different populations could be of different priority depending on the frequency of the polymorphisms. The current data in the area of cancer etiology and prognosis has been drawn from epidemiological studies conducted in the western world. Nearly one-third of these studies were conducted in either Europe or North America, so the results are representative for only a fraction of the global population. The world does not yet know about the environmental and genetic risk factors of cancer among other countries (Ramanakumar, 2007). Epidemiological studies in these countries could provide valuable new information for the genetic etiology of the cancer. In this respect, genetic epidemiological studies conducted in Turkey are very low in number, and

each study would provide valuable information on the risk assessment regarding our population.

Genetic epidemiological studies also include healthy control subjects; therefore provide information on the polymorphism frequencies in control population. Single epidemiological studies comprise a few hundred subjects at most, while determination of exact population frequencies is thought to require thousands of subjects. The current tendency today is to combine the data from different studies on same populations to obtain high number of subjects that would represent the population. Every study in this respect, add valuable information to the pool of data, which enables to determine the population frequencies of these polymorphisms more precisely.

Regarding all the points above, this genetic epidemiological study was based on a sample of healthy controls and a sample of childhood acute lymphoblastic leukemia patients in Turkey, and the aims of the study included;

- Investigation of three genetic polymorphisms on *CYP2E1* gene, namely *CYP2E1*5B, *6* and **7B* polymorphisms located in 5'-flanking region, intron 6 and promoter region, respectively, on the case and control groups and comparison of the frequencies in these groups to elucidate the possible effect of polymorphism in risk of incidence for childhood ALL. Most epidemiological studies investigated *5B allele only, therefore this study could represent valuable information on the risk of polymorphisms in the multiple loci of *CYP2E1* gene, and **6* and **7B* alleles are investigated as risk factors for childhood ALL for the first time.

- Investigation of *NQO1*2* polymorphisms in control and case groups, and determination of the effect of this polymorphism on the risk of development of childhood ALL.
- Investigation of *GSTM1* and *T1* null in both groups and comparison of their frequencies in order to determine the possible risk association.
- The multi-locus analysis of *CYP2E1* polymorphisms, together with *NQO1* and *GSTM1* and *T1* polymorphisms cover the key points in the metabolism of benzene, hence this study aimed to investigate the effects of combined polymorphisms in the risk of childhood ALL development, which would more efficiently describe the genetic risk factors for the disease.
- Investigation of the interaction between non-genetic risk factors and *CYP2E1, NQO1, GSTM1* and *GSTT1* genetic polmyorphisms in the risk of development of childhood ALL, based on case-only models.
- Investigation of *CYP2E1*5B, *6, *7B, NQO1*2* and *GSTM1* and *T1* polymorphism frequencies in control samples, which would reflect the Turkish population frequencies, and to compare the frequencies of these polymorphisms in Turkish population with those in other populations of different ethnic origins.

In order to achieve these goals, blood samples from healthy control subjects and childhood ALL patients were collected and stored under safe conditions. Genomic DNA was isolated from these blood samples and used for subsequent PCR reactions to determine the genetic polymorphisms. *CYP2E1*5B, *6* and *7B* genotypes, as well as *NQO1*2* genotype were detected by amplification of the corresponding regions of the genes that include the SNPs using polymerase chain reaction (PCR), and subsequently by restriction enzyme digestions. The *GSTM1* and *T1* null were detected simultaneously by a multiplex PCR reaction. Chi-

square analysis was performed on the case and control polymorphism frequencies in order to determine the significance of the differences, as well as to compare different control populations. The risk assessment was based on the odds ratio, with 95% confidence intervals, for both single gene polymorphisms and combination of polymorphisms. Interaction of non-genetic risk factors and genetic polymorphisms in patient population as risk factors was done by determination of case-only odds ratio with 95% confidence intervals. Significance of risk was determined by Chi-square anaylsis.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Subjects and Collection of Blood Samples

Control group comprised of 209 subjects and blood samples were obtained from healthy volunteers with the collaboration of METU Health Center, Biochemistry Laboratory. Written informed consents were taken from each participant, and a copy of the form is given in Appendix A. The consent form also included questions regarding their age, birth place of the volunteer and his/her parents, and any diseases they had had. Subjects having diseases were excluded from the study.

A total of 185 subjects were included in the ALL-patient group. The blood samples for patient group were obtained by the collaboration of Sami Ulus Children's Hospital and Ankara University, Faculty of Medicine, Department of Pediatric Hematology between June 2005 and November 2007. The information on demographic data –like age, birth place of child, mother and father-, clinical diagnosis, treatment protocol, stage of therapy, patient's risk group, familial relationship between mother and father were obtained with a questionnaire. Information on subtype of ALL, age at diagnosis, white blood cell count at diagnosis were obtained from the medical patient's log. Written informed consents were taken

from the parents together with the questionnaire, an example is given in Appendix A.

Questionnaire on the smoking status of mother and father was also obtained for 117 of the patients. Smoking status of mother and father (passive or active smoking), duration of exposure and the number of cigarettes smoked per day for active smokers were obtained. The questionnaire also included information on the smoking status of mother during pregnancy, and postnatal exposure of child to cigarette smoke. Besides, the questionnaire served information on the age of mother, father and child, from which the age of mother and father at the time of conception could be calculated.

4-5 mL of blood samples from control and ALL-patient group subjects were taken in EDTA-containing vacuumed tubes and stored at −20°C till use for DNA isolation.

2.1.2 Enzymes and Chemicals

Agarose (A-9539), acrylamide (A8887), ε-amino caproic acid (ε-ACA, A2504), bovine serum albumin (BSA, A7888), 5-bromo-4-chloro-3-indolyl phosphate (BCIP, B8503), bromophenol blue (B-5525), N-N',dimethyl formamide (D8654), ethidium bromide (E-7637), ethylene diamine tetra acetic acid disodium salt (EDTA; E-5134), glycine (G7126), N-N'-methylene bis acrylamide (BIS, M7256), β-mercaptoethanol (M6250), nitrobluetetrazolium (NBT, N6876), phenazine methosulfate (PMS, P9625), polyxyethylene sorbitan monolaurate (Tween 20, P1376), sodium chloride (NaCl; S-3014), sodium dodecyl sulfate (SDS, L-4390), sodium-potassium tartrate (Rochell salt, S2377), 2-amino-2(hydroxymethyl)-1,3-propandiol (Tris, T-5941) were purchased from Sigma Chemical Company, Saint Louis, Missouri, USA.

Borate (11607), and absolute ethanol (32221) were the products of Riedel de Haën, Seelze. Magnesium chloride (A4425) and potassium chloride (A2939) were purchased from AppliChem, Ottoweg, Darmstadt. Ammonium persulfate (161-0700), and N,N,N,N'-tetramethylene diamine (161-0801) were the products of Bio-Rad Laboratories, Richmond, California, USA. Biocoll Ficoll Separating Solution was purchased from Biochrom AG, Berlin, Germany. Copper (II) sulfate-pentahydrate (02782), glycerol (04093), magnesium chloride (05833), methanol (02500), sodium hydroxide (06462), Sucrose (7653), Triton X-100 (11869), zinc chloride (08515) were the products of Merck & Co., Inc., Whitehouse Station, NJ, USA.

Taq DNA Polymerase –supplied together with $MgCl_2$ and amplification buffer- (#EP0407), dNTP mix (#R0191), Gene Ruler^{TM} 50 bp DNA Ladder (#SM0371) and restriction enzymes *Pst*I (#ER0611), *Rsa*I (#ER1121), *Dra*I (#ER0221), *Hinf*I (#ER0801) –which were supplied with their buffers O^+, $Y^{+/}$TANGO^{TM}, B^+, R, respectively- were purchased from Fermentas, Int., Inc., Ontario, Canada. Restriction enzyme *Dde*I (#R6295) which was supplied together with its buffer G was purchased from GeneMark (Sibenzyme Ltd., Russia).

All chemicals used in this study were of molecular grade and were obtained from commercial sources at the highest grade of purity.

2.1.3 Primers

Primers used throughout the study were selected by literature search and were derived from known sequences of human. The primer pairs were purchased from MWG (MWG Biotech, Ebersberg, Germany) or Metabion (Metabion International AG, Martinsried, Deutschland) and were all purified by HPLC. Primer stocks were brought to 100 pmol/µL

concentration and stored at -20°C. Aliquots of 10 pmol/µL concentration were prepared and used for PCR. The sequences of oligonucleotide primers are given in Table 2.1.

Table 2.1 Sequences of primers used throughout the study

Gene and Allele Name	Forward and Reverse primer sequences	Reference
CYP2E1*5B	5′-CCAGTCGAGTCTACATTGTCA-3′ 5′-TTCATTCTGTCTTCTAACTGG -3′	Hayashi *et al.,* 1991
CYP2E1*6	5′-TCGTCAGTTCCTGAAAGCAGG-3′ 5′-GAGCTCTGATGCAAGTATCGCA-3′	Wu *et al.,* 1998
CYP2E1*7B	5′-GTGGCTGGAGTTCCCCGTTG-3′ 5′-TGCTGCCAGCCCGGGAGGAC-3′	Yang *et al.,* 2001
NQO1*2	5'-CCTCTCTGTGCTTTCTGTATCC-3' 5'-GATGGACTTGCCCAAGTGATG-3'	Eguchi-Ishimae M. *et al.,* 2005
GSTM1	5′-GAACTCCCTGAAAAGCTAAAGC-3′ 5′-GTTGGGCTCAAATATACGGTGG-3′	Abdel-Rahman *et al.,* 1996
GSTT1	5′-TTCCTTACTGGTCCTCACATCTC-3′ 5′-TCACCGGATCATGGCCAGCA-3′	
***CYP1A1* (internal control for GST PCR)**	5′-GAACTGCCACTTCAGCTGTCT-3′ 5′-CAGCTGCATTTGGAAGTGCTC-3′	

2.2 Methods

Preparation of buffers and solutions are described in Appendix B.

2.2.1 Isolation of Genomic DNA from Human Whole Blood Samples

2.2.1.1 Manual Isolation of Genomic DNA

Genomic DNA from human whole blood samples was isolated according to the method of Lahiri and Schnabel (Lahiri and Schnabel, 1993). 500 µL of whole blood, after complete thawing, was treated with an equal volume of (500 µL) low-salt TKME buffer (pH 7.6) and 25 µL of Triton X-100. The cells were lysed by inversions and the suspension was centrifuged at 1000 g for 10 minutes at room temperature using Sigma 1-15 bench top microfuge (Sigma, Postfach 1713-D-37507, Osterode). The pellet was washed three more times with TKME buffer and centrifuged using same conditions. The final pellet was suspended in 0.2 mL of TKME buffer and 20 µL of 10% SDS was added. The suspension was mixed vigorously and incubated at 55°C for 20 minutes in an incubator. Then 75 µL of saturated NaCl (6M) was added to precipitate the proteins, the tube was mixed well and centrifuged at 14000 g for 7 minutes at 4°C. The supernatant, which contained DNA, was precipitated using absolute ethanol, kept in -20°C for 30 min-1 hour to increase the efficiency of precipitation, and DNA pellet was washed once with 70% ethanol. Then, the pellet was air dried, resuspended in 0.2 mL of TE buffer (pH 8.0) and incubated overnight at 37°C in an incubator. The DNA samples were stored at 4°C while they were in active use and kept at -20°C for long-term storage.

2.2.1.2 Genomic DNA Isolation by Nucleospin Blood Kit

Nucleospin Blood Kit (Macherey-Nagel, Germany) was used for isolation of DNA from old blood samples or from the blood samples of ALL patients that have reduced number of white blood cells, which manual

isolation did not give effective PCR results. The basis of isolation depended on lysis of white blood cells in the presence of proteinase K, binding of genomic DNA to the silica membrane of spin-columns, washing off the contaminants and finally elution of pure genomic DNA under low ionic strength conditions in a slightly alkaline elution buffer.

After complete thawing, 200 µL of whole blood was mixed with 25 µL of proteinase-K in a 1.5 mL eppendorf tube, and vortexed immediately. Then 200 µL of lysis buffer "B3" was added, vortexed vigorously for 10-20 seconds and the tube was incubated at 70°C in a heater block for 30 minutes. In every 10 minutes of incubation, tube was vortexed for 10-20 seconds. The lysate turned into a greenish-brownish color at the end of the incubation period. This step was the lysis step of white blood cells in a buffer (B3) containing large amounts of chaotropic ions in the presence of proteinase-K.

At the end of the incubation period, 210 µL of absolute ethanol was added to the mixture in order to create appropriate conditions for binding of DNA to the silica membrane of the spin columns, vortexed and the sample in the tube was transferred to a spin column. The sample in the column was centrifuged at 14000 g for 2 minutes at room temperature using Thermo Microlite RF (refrigerated centrifuge, Waltham, MA, USA) micro centrifuge. The collecting tube with flow-through was discarded. The spin column was placed in a new collecting tube and 500 µL of buffer "BW" was added to the spin column. The sample in the spin column was centrifuged at 14000 g for 2 minutes at room temperature and the flow through was discarded. 600 µL of buffer "B5" was added to the column, centrifuged again as described above, and the flow through was discarded. Then the spin column was centrifuged once more as described above, without adding anything to the column, in order to get rid of the ethanol that was present in buffer "B5" completely. Buffer BW and B5

served to wash the DNA sample and remove the contaminants in the spin column.

In order to elute the DNA in the column, the spin column was placed in a new 1.5 mL eppendorf tube, 100 µL of preheated (to 70°C) buffer "BE" (slightly alkaline elution buffer with low ionic strength) was dispensed directly onto the membrane, the tube was incubated at room temperature for 5 minutes and then centrifuged at 14000 g for 2 minutes at room temperature. The sample eluted from the spin column was loaded onto the same spin column again, incubated for 5 minutes and centrifuged as described above, to increase the yield of DNA.

2.2.2 Spectrophotometric Quantification of Genomic DNA

Concentration of DNA in the sample was determined by measuring the absorbance values at 260 nm and 280 nm in quartz cuvettes using Schimadzu UV-1201 Spectrophotometer (Schimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). As the DNA molecule gave maximum absorption at 260 nm, reading at this wavelength was used to calculate the concentration of nucleic acid in the sample. Based on the knowledge that an optical density of 1.0 corresponded to approximately 50 µg/mL for double-stranded DNA, the concentration of DNA in the sample was calculated according to the formula:

Concentration (µg/mL)= OD_{260nm} x 50 (µg/mL) x Dilution Factor.

The ratio between OD values at 260 nm and 280 nm (OD_{260}/OD_{280} ratio) was used to estimate the purity of the nucleic acid. Pure DNA preparations gave the ratio of 1.8 while the higher or lower values showed either RNA or protein contaminations, respectively.

2.2.3 Qualification of Genomic DNA by Agarose Gel Electrophoresis

Determination of the intactness of DNA samples was performed by agarose gel electrophoresis using Scie-Plas HU13W horizontal gel electrophoresis unit (Warwickshire, England).

Agarose gel was prepared in 0.5% concentration by 0.5x TBE buffer (pH 8.3) using a microwave oven. When the gel solution was cooled enough (approximately 60°C), ethidium bromide was added from a stock solution of 10 mg/mL so as to obtain a final concentration of 0.5 µg/mL and the solution was mixed thoroughly.

The warm agarose solution was poured into the pre-settled mold and any air bubbles –if present-, especially under or between the teeth of the comb were removed by the help of a pipette tip. The gel was allowed to solidify completely for approximately 20-40 minutes at room temperature.

After the agarose gel is solidified, it was mounted in the electrophoresis tank which was filled with 0.5x TBE buffer so that the slots of the gel faced the negative pole-cathode. 5µL of DNA sample was mixed with 1 µL of gel loading dye by the use of a micropipette, and the mixture was slowly added to the wells of the gel. After loading of the DNA samples were completed, the lid of the tank was closed and the electrical leads were attached to the power supply. The power supply was set to a constant voltage so that not more than a voltage of 5V/cm (measured as the distance between the electrodes) was applied (corresponds to a maximum of 150 volts for Scie-Plas HU13W horizontal gel electrophoresis unit.

The gel was run until the bromophenol blue reached to the bottom of the gel, and then examined under UV light and the photograph was taken by Vilber Lourmat Gel Imaging System (Marne La Vallee, Cedex, France) and InfinityCapt (version 12.9) computer software.

2.2.4 Genotyping of Single Nucleotide Polymorphisms

In this study four drug metabolizing genes, *CYP2E1, NQO1*, *GSTM1* and *GSTT1* were genotyped for their single nucleotide polymorphisms. Three SNPs of *CYP2E1,* namely **5B, *6* and **7B*, and *NQO1*2* polymorphisms were identified by PCR amplification of SNP containing regions followed by appropriate restriction enzyme digestions. *GST M1* and *T1* null genotypes were analyzed at the same time by multiplex PCR. The details of these methods were described below. Techne Progene (Cambridge, UK) and Eppendorf Mastercycler (Hamburg, Germany) thermocyclers were used for PCR.

2.2.4.1 Genotyping of *CYP2E1*5B* Polymorphism

2.2.4.1.1 Polymerase Chain Reaction

*CYP2E1*5B* (C-1053T/G-1293C SNPs) genotyping requires amplification of the 5'-flanking region of the gene. The PCR conditions for this polymorphism were optimized before (Ulusoy, 2004) and are presented in Table 2.2.

Table 2.2 Components of PCR mixture for *CYP2E1*5B* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 50 µl reaction mixture
Amplification buffer*	10x	5 µL	1x
$MgCl_2$*	25 mM	3 µL	1.5 mM
dNTP mixture*	10 mM	1 µL	200 µM
Reverse Primer	10 pmol/µL	2 µL	20 pmol
Forward Primer	10 pmol/µL	2 µL	20 pmol
Template DNA	varies	varies	~200 ng
Taq DNA Polymerase*	5 U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

***** see Appendix B for these reagents.

Amplification programme used was as follows:

Denaturation	94°C	1 min	35 cycles
Annealing	55°C	1 min	
Extension	72°C	1 min	
Final extension	72°C	6 min	

PCR products were analyzed on 2% agarose gel as described in section 2.2.3. 10 µL of PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000bp, see Appendix B) was applied to one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.1.2 Restriction Endonuclease Digestion of PCR Products

*CYP2E1*5B* polymorphism is defined with two SNPs (C-1053T/G-1293C SNPs) which are in complete linkage disequilibrium, hence the amplified 5'-flanking region covered both SNP positions. Therefore, the PCR products were digested separately by two restriction endonucleases, *Rsa*I for C-1053T and *Pst*I for G-1293C SNPs. The components of restriction enzyme digestion mixture are given in Table 2.3.

Table 2.3 Components of digestion mixture for C-1053T and G-1293C SNPs of *CYP2E1*5B* polymorphism.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 30 µl reaction mixture
Buffer*,**	10x	3 µL	1x
PCR Product		10 µL	
Restriction enzyme*	10 U/µL	1 µL	10 U
Sterile apyrogen H_2O		16 µL	

***** Buffer Y^+/TANGO™ was the buffer used for *Rsa*I digestion, and Buffer O^+ was the buffer used for *Pst*I digestion.

****** see Appendix B for the components of the buffers.

Both of the *Rsa*I and *Pst*I digestion mixtures were incubated at 37°C for 18 hours for complete digestion and then analyzed on 2.0% agarose gel. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000 bp) was applied to the first well of the gel. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.2 Genotyping of *CYP2E1*6* Polymorphism

2.2.4.2.1 Polymerase Chain Reaction

A region in the intron 6 of *CYP2E1* gene was amplified for determination of *CYP2E1*6* (T7632A SNP) according to the previously optimized PCR conditions (Ulusoy, 2004) as presented in Table 2.4.

Table 2.4 Components of PCR mixture for *CYP2E1*6* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 50 µl reaction mixture
Amplification buffer*	10x	5 µL	1x
$MgCl_2$*	25 mM	3 µL	1.5 mM
dNTP mixture*	10 mM	1 µL	200 µM
Reverse Primer	10 pmol/µL	2 µL	20 pmol
Forward Primer	10 pmol/µL	2 µL	20 pmol
Template DNA	varies	varies	~200 ng
Taq DNA Polymerase*	5 U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

***** see Appendix B for these reagents

Amplification programme used was as follows:

Initial denaturation	94°C	5 min	
Denaturation	94°C	1 min	35 cycles
Annealing	61°C	1 min	
Extension	72°C	1 min	
Final extension	72°C	6 min	

PCR products were analyzed on 2% agarose gel as described in section 2.2.3. 10 µL of PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000bp) was applied to one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.2.2 Restriction Endonuclease Digestion of PCR Products

PCR products were digested with *Dra*I restriction enzyme in a reaction mixture as described in Table 2.5.

Table 2.5 Components of digestion mixture for *CYP2E1*6* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 30 µl reaction mixture
Buffer B*	10x	3 µL	1x
PCR Product		20 µL	
***Dra*I**	10 U/µL	0.6 µL	6 U
Sterile apyrogen H_2O		6.4 µL	

***** see Appendix B for the components of the buffer.

The digestion mixture was incubated at 37°C for 18 hours for complete digestion and then analyzed on 2.0% agarose gel. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000 bp) was applied to the first well of the gel. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.3 Genotyping of *CYP2E1*7B* Polymorphism

2.2.4.3.1 Polymerase Chain Reaction

In order to determine the *CYP2E1*7B* polymorphism (G-71T SNP), a sequence in the promoter region of the gene was amplified. The components for the PCR were given in Table 2.6.

Table 2.6 Components of PCR mixture for *CYP2E1*7B* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 50 µl reaction mixture
Amplification buffer*	10x	5 µL	1x
$MgCl_2$*	25 mM	2 µL	1 mM
dNTP mixture*	10 mM	1 µL	200 µM
Reverse Primer	10 pmol/µL	2 µL	20 pmol
Forward Primer	10 pmol/µL	2 µL	20 pmol
Template DNA	varies	varies	~200 ng
Taq DNA Polymerase*	5 U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

***** see Appendix B for these reagents

Amplification programme used was as follows:

Initial denaturation	94°C	5 min	
Denaturation	94°C	1 min	30 cycles
Annealing	62°C	1.5 min	
Extension	72°C	2 min	
Final extension	72°C	10 min	

PCR products were analyzed on 2% agarose gel as described in section 2.2.3. 10 µL of PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000bp) was applied to one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.3.2 Restriction Endonuclease Digestion of PCR Products

PCR products were digested with *Dde*I restriction enzyme in a reaction mixture as described in Table 2.7.

Table 2.7 Components of digestion mixture for *CYP2E1*7B* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 30 µl reaction mixture
Buffer G*	10x	3 µL	1x
PCR Product		20 µL	
***Dde*I**	20 U/µL	0.2 µL	4 U
Sterile apyrogen H_2O		6.8 µL	

***** see Appendix B for the components of the buffer.

The digestion mixture was incubated at 60°C for 18 hours for complete digestion and then analyzed on 2.0% agarose gel. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000 bp) was applied to the first well of the gel. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.4 Genotyping of *NQO1*2* Polymorphism

2.2.4.4.1 Polymerase Chain Reaction

Determination of *NQO1*2* polymorphism (C609T SNP) requires amplification of a region in exon 6 of the gene. The PCR conditions as given in Table 2.8 were used in this study.

Table 2.8 Components of PCR mixture for *NQO1*2* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 50 µl reaction mixture
Amplification buffer*	10x	5 µL	1x
$MgCl_2$*	25 mM	3 µL	1.5 mM
dNTP mixture*	10 mM	1 µL	200 µM
Reverse Primer	10 pmol/µL	1 µL	10 pmol
Forward Primer	10 pmol/µL	1 µL	10 pmol
Template DNA	varies	varies	~200 ng
Taq DNA Polymerase*	5 U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

***** see Appendix B for these reagents

Amplification programme used was as follows:

Initial denaturation	94°C	5 min	
Denaturation	94°C	1 min	30 cycles
Annealing	61°C	1.5 min	
Extension	72°C	2 min	
Final extension	72°C	10 min	

PCR products were analyzed on 2% agarose gel as described in section 2.2.3. 10 µL of PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000bp) was applied to one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.4.2 Restriction Endonuclease Digestion of PCR Products

PCR products were digested with *Hinf*I restriction enzyme in a reaction mixture as described in Table 2.9.

Table 2.9 Components of digestion mixture for *NQO1*2* SNP.

Constituent	Stock Conc.	Volume to be added	Final Conc. in 30 µl reaction mixture
Buffer R*	10x	3 µL	1x
PCR Product		20 µL	
***Hinf*I**	20 U/µL	0.2 µL	4 U
Sterile apyrogen H_2O		6.8 µL	
***** see Appendix B for the components of the buffer.			

The digestion mixture was incubated at 60°C for 18 hours for complete digestion and then analyzed on 2.0% agarose gel. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000 bp) was applied to the first well of the gel. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.4.5 Genotyping of *GST M1* and *T1* Null by Multiplex Polymerase Chain Reaction

GST M1 and *T1* null genotypes were determined by multiplex PCR where both of these genes and a non-polymorphic region of *CYP1A1* gene were amplified at the same time. The *GST* genotypes were determined according to the presence or absence of the corresponding band and *CYP1A1* region was used as an internal control and amplified in any condition. The PCR components of the multiplex PCR were presented in Table 2.10.

Table 2.10 Components of multiplex PCR mixture for *GSTM1* and *T1.*

Constituent	Stock Conc.	Volume to be added	Final Conc. in 50 µl reaction mixture
Amplification buffer*	10x	5 µL	1x
$MgCl_2$*	25 mM	3 µL	1.5 mM
dNTP mixture*	10 mM	1 µL	200 µM
Reverse Primer for *GSTM1*	10 pmol/µL	3 µL	30 pmol
Forward Primer for *GSTM1*	10 pmol/µL	3 µL	30 pmol
Reverse Primer for *GSTT1*	10 pmol/µL	3 µL	30 pmol
Forward Primer for *GSTT1*	10 pmol/µL	3 µL	30 pmol
Reverse Primer for *CYP1A1*	10 pmol/µL	3 µL	30 pmol
Forward Primer for *CYP1A1*	10 pmol/µL	3 µL	30 pmol
Template DNA	varies	varies	~200 ng
Taq DNA Polymerase*	5 U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

***** see Appendix B for these reagents

Amplification programme used was as follows:

Initial denaturation	94°C	5 min	
Denaturation	94°C	2 min	35 cycles
Annealing	59°C	1 min	
Extension	72°C	2 min	
Final extension	72°C	10 min	

PCR products were analyzed on 2% agarose gel as described in section 2.2.3. 30 µL of PCR product was mixed by 6 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder (50-1000bp) was applied to one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

2.2.5 Isolation of Lymphocytes from Human Whole Blood

Lymphocytes were isolated within four hours of blood collection. Biocoll ficoll separating solution, which was brought to room temperature was added to a sterilized glass tube in the same amount with the whole blood to be added. Then the whole blood was carefully layered onto biocoll. The sample was centrifuged at 400 g for 30 minutes at room temperature by using Sorvall RC-5C Plus high speed centrifuge (mensei), using SS30 rotor with glass tube adapters. The reagents and centrifugation conditions were strictly kept at room temperature as cooler temperatures might cause cell clumping and poor recovery.

After centrifugation was completed, three fractions of whole blood were layered in the tube such that the red blood cell fraction sedimented to the very bottom, the mononuclear cells –including lymphocytes- were layered on top of red blood cells, with a bio-coll layer in between two fractions. Blood plasma was layered on the very top, just above the

lymphocyte layer. The upper plasma layer was aspirated carefully with a disposable sterile Pasteur pipette to within 0.5 cm of the opaque interface containing lymphocytes. The upper plasma layer was discarded, while the opaque lymphocyte interface was transferred to a new tube with a Pasteur pipette.

10 mL of isotonic phosphate buffered saline (PBS) was added on lymphocyte and mixed by gentle aspiration. The sample was centrifuged at 250 g for 10 minutes at room temperature, the supernatant was discarded. The lymphocyte pellet was resuspended with 5.0 mL of isotonic PBS, mixed by gentle aspiration and centrifuged as described above. The supernatant was discarded and the pellet was washed once more with 5.0 mL of PBS as described above. The final pellet was dissolved in 0.5 mL of PBS and stored at -80°C until use.

2.2.6 Sonication of Human Blood Lymphocytes

The lymphocyte samples were sonicated in order to expose the lymphocyte contents into solution prior to protein determination and western blot analyses.

The lymphocyte samples were diluted with lysis buffer (2 mM EDTA pH 7.7, 0.25 mM ε-ACA and 0.1 mM PMSF containing %1.15 KCl solution) in 1:1 ratio and sonicated with thin probe of Cole Parmer Ultrasonic processor (IL, USA) at an amplitude of 40 mHz for 20 seconds continuously (without pulse) 3 to 4 times. The sonicated sample was homogenized by hand several times.

2.2.7 Protein determination of lymphocyte samples

Concentrations of lymphocyte proteins were determined by method of Lowry *et al*. (1951). Bovine serum albumin was used as standard. Rabbit and human lymphocyte samples were diluted in 1:5 ratio. An aliquot of 0.1, 0.25 and 0.5 mL of lymphocytes was mixed with distilled water in order to complete the volume to 0.5 mL in test tubes. After that, they were mixed with 2.5 mL of alkaline copper reagent (ACR) which was prepared freshly by mixing 2% copper sulfate, 2% sodium potassium tartrate and 2% Na_2CO_3 in 0.1 N NaOH in the written order and incubated at room temperature for 10 minutes. Then, 0.25 mL of 1.0 N Folin-Phenol reagent was added to the tubes and incubated at room temperature for 30 minutes. The resulting color intensity was measured at 660 nm. The standard curve of BSA from 20 to 200µg was plotted and used for determination of protein concentration of samples.

2.2.8 Lymphocyte CYP2E1 protein level determination by Western Blotting

Rabbit and human CYP2E1 protein levels were determined as described by Towbin *et al.* (1979) with some modifications. Polyclonal anti-rabbit CYP2E1 (1:1000 dilution) (Oxford Biomedical Research, MI, USA) for rabbit lymphocytes, and Polyclonal anti-human CYP2E1 (1:1000 dilution) (Oxford Biomedical Research, MI, USA) for human lymphocytes were used as primary antibodies. Anti-rabbit IgG (1:5000 dilution) conjugated to alkaline phosphatase was used as secondary antibody.

First, lymphocyte proteins were separated by polyacrylamide gel electrophoresis in the presence of SDS, in a discontinuous buffer system as described by Laemmli (1970) by using 4% stacking gel and 8.5%

separating gel. The separating and stacking gel solutions were prepared just before use as given in Table 2.11 in a given order.

Table 2.11 Components of separating and stacking gel solutions

Constituents	**Separating Gel (8.5%) (0.375 M Tris, pH 8.8)**	**Stacking Gel (4%) (0.125 M Tris, pH 6.8)**
Gel solution (mL)	8.5	1.3
Distilled water (mL)	13.55	6.1
Separating gel buffer (mL)	7.5	-
Stacking Gel buffer (mL)	-	2.5
10% SDS (mL)	0.3	0.1
Ammonium persulfate (mL)	0.15	0.05
Temed (mL)	0.015	0.01
Total volume (mL)	30	10

Vertical slab gel electrophoresis was carried out using Scie-plasV10-CDC vertical electrophoresis unit (Southam, England). Polyacrylamide slab gels were prepared using the gel sandwich. The gel sandwich was prepared between two glass plates leaving 1 mm space between plates and central gel running module was assembled. The module was placed in melted agar and both sides were sealed with agar to prevent a leakage of separating gel and stacking gel solution. First separating gel solution was transferred to the center of gel sandwich until the desired height of the solution in the sandwich was obtained. The top of the gel polymerizing solution was covered with a layer of isobutanol to ensure the formation of smooth gel surface. After polymerization of separating gel, the layer of alcohol was poured off completely. The stacking gel polymerization solution was prepared and poured into the

center of gel sandwich over the separating gel until the sandwich was filled completely. After adding stacking gel, a 1.0 mm Teflon comb with 12 wells was inserted into stacking gel solution. After polymerization, Teflon comb was carefully removed without tearing the wells. Wells were filled with electrode running buffer (25 mM Tris, 192 mM glycine and 0.1% SDS) using syringe with a fine needle to remove any air bubbles and unpolymerized chain particles. Then gel running module was filled with a necessary volume of electrode running buffer. Protein samples were diluted 1:3 (3 part sample and 1 part buffer) with 4× sample dilution buffer containing 0.25 M Tris-HCl, pH 6.8, 8% SDS, 40% glycerol, 20% β-mercaptoethanol, and 0.01% bromophenol blue and were boiled in a boiling water bath for 2 minutes. Each sample was applied to different wells by Hamilton syringe.

After application of the samples, gel running module were placed in the main buffer tank filled with an appreciate amount of electrode running buffer. The electrophoresis unit was connected to the power supply Bio-Rad model 2 (Bio-Rad Laboratories, Richmond, California, USA) and electrophoresis was run at 10mA and 100V in stacking gel and 20mA 200V in separating gel. When electrophoresis was completed, gel was removed for western blot. Gels were equilibrated in transfer buffer (25 mM Tris, 192 mM glycine and 20% methanol) for 15 minutes in order to adjust the final size of gel and remove the buffer salts and SDS which were used in the SDS-Polyacrylamide gel electrophoresis. Nitrocellulose membrane was cut 1 cm larger then the dimension of the gel and two pieces of filter paper (Whatman #1) were cut to a dimension a little bit larger than the membrane. Nitrocellulose membrane, two filter paper and fiber pads of the transfer sandwich were placed in transfer buffer and saturated with this solution. Later, the sandwich was put into the Bio-Rad Trans-Blot Cell and the cell was filled with cold transfer buffer. Voltage and current were set to 90V and 400 mA, respectively. Transfer process

was carried out at cold room (4°C) for 90 minutes. At the end of this period, the membrane having the transferred protein on it, i.e. "blot" was obtained and taken from the cell and placed into a plastic dish in such a way that protein side facing up and washed with TBST (Tris Buffered Saline plus Tween 20: 20mM Tris-HCl, pH 7.4, 0.5 M NaCl, and 0.05 % Tween 20) for 10 minutes in order to remove the salts and buffers of transfer medium. Then the blot was incubated with blocking solution (5% Non-Fat Dry-Milk in TBST) for 40 minutes in order to fill the empty spaces between bound proteins by this way prevent the non-specific binding.

The blot was incubated with primary antibody for 2 hours for rabbit lymphoctes and for 2, 4, and 16 hours for human lymphocytes. Then the blot was washed 3 times with 50 ml TBST for 5 minutes each. The washing steps are necessary to remove excess antibody from the membrane. The blot was then incubated with secondary antibody conjugated to marker enzyme-alkaline phosphatase for 1 hour. The blot was washed three times with TBST for 5 minutes each to remove excess antibody. Since the excess antibody will give reaction with substrate solution nonspecifically the complete removal of the excess antibody between each washing steps are extremely important. Finally, blot was incubated with substrate solution given in Table 2.12 as described by Ey and Ashman (1986) to visualize the specifically bound antibodies. The final images were photographed by using computer based gel imaging instrument (Vilber Lourmat, Marne-la-Vallee Cedex 1, France) by using Infinity-Capt Version 12.9 software.

Table 2.12 Preparation of substrate solution for immunodetection

Solution A: 2.67 mL of 1.5 M Tris-HCl, pH8.8 4.0 mL of 1 M NaCl 0.82 mL of 100 mM $MgCl_2$ 0.04 mL of 100 mM $ZnCl_2$ 0.096 mL of DEA 12.2 mg NBT Distilled water to 40 mL. (pH of the solution was adjusted to 9.55 with saturated Tris buffer before completing the final volume)
Solution B: 2 mg/mL phenazine methosulfate in distilled water
Solution C: 5.44 mg BCIP/0.136 mL N,N-dimethyl formamide
NBT/BCIP substrate solution was prepared by mixing solution A with solution C and 0.286 mL of solution B.

2.2.9 Statistical Analyses

Statistical analyses were conducted using SPSS 16.0 software or by Vassar Stats. The genotype distributions of polymorphisms were compared by χ^2-test (Pearson), Yate's correction factor was used if necessary, or Fisher's exact probability test was applied when the sample number is small so that Pearson's or Yate's χ^2 analysis could not be performed.

Comparison of genotypes in control and patient groups was done by calculating odds ratio (OR). The significance of association was determined by χ^2-test.. For case-only studies, interaction of non-genetic factors and genetic polymorphisms for the risk of development of childhood ALL was analyzed by determining case-only odds ratio (COR) values and the significance was determined by χ^2-test.

CHAPTER 3

RESULTS

3.1 Study Populations

3.1.1 Control Population

Control population comprised of 209 healthy and unrelated Turkish volunteers between the ages of 12 and 65 and mean age of 31.5 ± 12.0. Among the control subjects, 84 were male (mean age: 33.1 ± 12.6; range: 14-65 years) and 125 were female (mean age: 30.4 ± 11.6; range: 12-63 years). The blood samples were collected from Middle East Technical University (METU) Health Center, Biochemistry Laboratory. METU is among the biggest universities of Turkey, located in the capital city, Ankara; therefore it receives many students from different regions of the country. As obtained from the birth place information of volunteer and his/her parents, the sample represented individuals from all seven regions of the country, so the study sample could be said to represent the Turkish population. The distribution of control subjects according to their birth place is given in Figure 3.1.

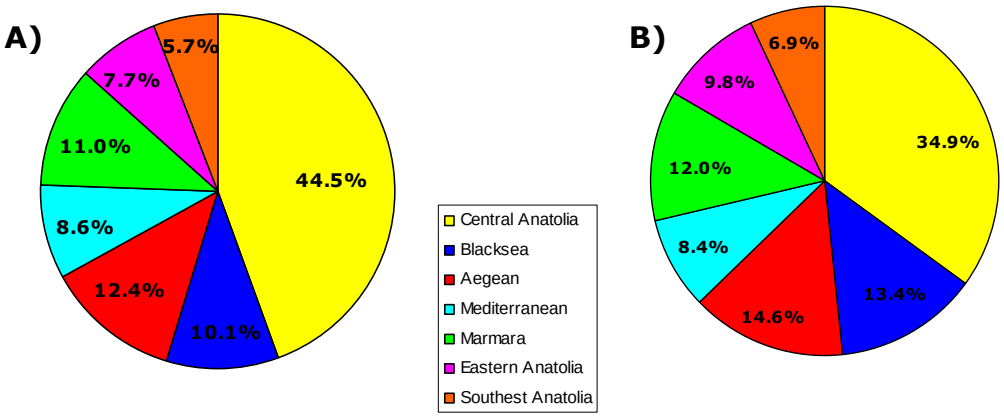

Figure 3.1 Distribution of control subjects to seven regions of Turkey, according to **A)**the birth place of subject; **B)** the birth place of parents of the subjects

3.1.2 Patient Population

The characteristics of the patient population, derived from the informed consent and smoking status questionairres (see Appendix A) are summarized in Table 3.1, and explained below.

There were 185 patients in the study of mean age at diagnosis being 7.0 ± 3.8, age ranging between 0.6 and 16 years. The males counted for 112 (mean age: 7.1 ± 3.9; range: 2-16 years) and females counted for 73 (mean age: 6.8 ± 3.8; range: 0.6-16 years).

Patient sample was also a representative of the Turkish population, as understood from the birth place information of patients and their parents in the questionnaire. Both hospitals -Sami Ulus Children's Hospital and Ankara University, Faculty of Medicine, Department of Pediatric Hematology- received patients from all over the country, both of

them being located in Ankara and serving to many patients all over the country. The distribution of patient subjects according to their birth place is given in Figure 3.2.

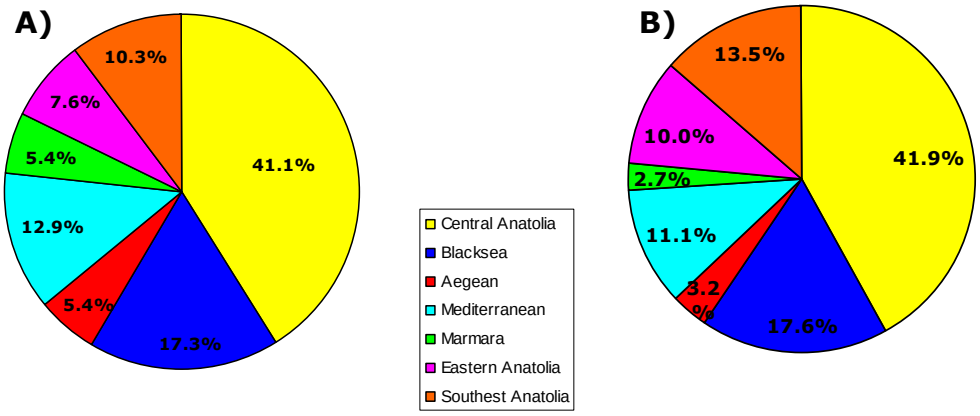

Figure 3.2 Distribution of patient subjects to seven regions of Turkey, according to **A)**the birth place of subject; **B)** the birth place of parents of the subjects

The questionnaires and the patients' medical log presented some information on the patients. These information are given below; however, data was not available for all patients. Because, in some cases, the information was not available (for example, subtype of ALL was not determined for all patients). The questionnaires on the cigarette smoking status of parents were not filled out by all parents.

Table 3.1 Characteristics of the patient population.

Characteristic	Total Patients	Male Patients	Female Patients
N	185	112	73
Age at diagnosis	7.0 ± 3.8 (range: 0.6-16)	7.1 ± 3.9 (range: 2-16)	6.8 ± 3.8 (range: 0.6-16)
Subtype of ALL	154 (31 missing)	93 (19 missing)	61 (12 missing)
B-cell originated	112 (72.7%)	65 (69.9%)	47 (77.0%)
T-cell	32 (20.8%)	20 (21.5%)	12 (19.7%)
B- or T- mixed	10 (6.5%)	8 (8.6%)	2 (3.3%)
Risk Group	183 (2 mising)	112	71 (2 missing)
Low Risk	19 (10.4%)	11 (9.8%)	8 (11.3%)
Standard Risk	72 (39.3%)	41 (36.6%)	31 (43.7%)
High Risk	92 (50.3%)	60 (53.6%)	32 (45.0%)
Familial relationship between parents	175 (10 missing)	108 (4 missing)	67 (6 missing)
Present	49 (28.0%)	31 (28.7%)	18 (26.9%)
Absent	126 (78%)	77 (71.3%)	49 (73.1%)

Information obtained from cigarette smoking questionnairre

Maternal age at conception	**Total Patients** 116(69 missing)	
Mean age	26.5 ± 6.8	
<20 years of age	13 (11.2%)	
20-35 years of age	86 (74.1%)	
>35 years of age	17 (14.7%)	
Paternal age at conception	**Total Patients** N=113 (72 missing)	
Mean age	30.3 ± 8.9	
<40 years of age	98 (86.7%)	
>40 years of age	15 (13.3%)	
Parental Smoking Status	**Maternal**	**Paternal**
N	117 (68 missing)	117 (68 missing)
Non-smoker	31 (26.5%)	22 (18.8%)
Passive smoker	63 (53.8%)	6 (5.1%)
Active smoker	23 (19.7%)	89 (76.1%)
Maternal smoking during pregnancy	**Total Patients** 117 (68 missing)	
Smoked	12 (10.3%)	
Not smoked	105 (89.7%)	
Postnatal exposure of child to cigarette smoke	**Total Patients** 115 (70 missing)	
Exposed	78 (67.8%)	
Not exposed	37 (32.2%)	

The subtype of ALL was available for 154 patients (information on 31 patients was missing). When the subtype of the disease was grouped into three as B-cell originated ALL, T-cell ALL, and B- and T-cell mixed ALL, 112 (72.7%) patients had B-cell originated ALL, 32 (20.8%) patients had T-cell ALL and 10 (6.5%) patients had B- and T-cell mixed ALL. Among 93 males, 65 (69.9%) had B-cell originated ALL, 20 (21.5%) had T-cell ALL, and 8 (8.6%) had B- and T-cell mixed ALL. In 61 females, 47 (77.0%) had B-cell originated ALL, 12 (19.7%) had T-cell ALL, and 2 (3.3%) had B- and T-cell mixed ALL. As can be seen, the T-cell and mixed type ALL were seen in higher frequencies in males compared to females, however the difference was not statistically significant (χ^2=1.93; df=2; p=0.38).

The risk group information (low, standard or high risk), which was assigned at the time of diagnosis to determine the intensity of the therapy, was available for 183 patients (112 male and 71 female; information on 2 patients were missing). In total, 19 (10.4%) patients were assigned to low-risk group, 72 (39.3%) patients were assigned to standard risk group and 92 (50.3%) were assigned to high risk group. Among males 11 (9.8%), 41 (36.6%) and 60 (53.6%) patients were assigned to low, standard and high risk groups, respectively; while among females, 8 (11.3%), 31 (43.7%) and 32 (45.0%) patients were assigned to low, standard and high risk groups, respectively. There was no statistically significant difference between two genders when their distribution into risk groups was considered (χ^2=1.26; df=2; p=0.53).

Age of mother and father at the time of conception was derived from “smoking status questionnaires”. This questionnaire was replied by 117 parents. Among them for 116 mothers the age information was present; accordingly, mothers’ mean age at conception was 26.5 $\pm$ 6.8, 13 of the mothers were younger than 20 years of age while 17 were older

than 35 years of age and 86 were at the ages between 20 and 35 at the time of conception. Age at conception information was available for 113 fathers, and mean age was 30.3 ± 8.9; 98 of the fathers were younger than 40 years of age while 15 were older than 40 at the time of conception.

The "smoking status questionnaire" gathered information on the smoking status of parents, the duration of exposure or smoking, the number of cigarettes smoked per day for regular smokers, smoking status during pregnancy and postnatal exposure of patient (child). In total of 117 parents, 31 mothers and 22 fathers were non-smoker; 63 mothers and 6 fathers reported that they have been passively exposed to cigarette smoke; 23 mothers and 89 fathers were regular smokers. Among 117 mothers, 12 reported that they smoked during pregnancy while 105 did not smoke. Among 115 parents, 78 reported that their child was exposed postnatally to cigarette smoke while 37 reported that the child was not passively exposed to cigarette smoke.

The aim of this study was to determine the genetic risk factors for the development of childhood ALL. However, the non-genetic factors presented above might also effect the association of risk elevating genotypes with childhood cancer. Age of parents at time of conception, and smoking exposure of parents and child could be such non-genetic factors and their combined effects together with susceptible genotypes were also analyzed. These factors were analyzed by case-only studies, as the information was not available for control group. However, the genotyping of single nucleotide polymorphisms were done for all 209 control and 185 patient sample.

3.2 Genotyping of Single Nucleotide Polymorphisms

In this study, four drug metabolizing genes, *CYP2E1, NQO1*, *GSTM1* and *GSTT1* were genotyped for their single nucleotide polymorphisms. PCR-RFLP method was applied in order to determine **5B, *6* and **7B* variants of *CYP2E1,* and *NQO1*2* polymorphism. *GST M1* and *T1* null genotypes were analyzed at the same time by multiplex PCR, using a non-polymorphic region of *CYP1A1* as internal control.

3.2.1 *CYP2E1* Polymorphisms

3.2.1.1 Genotyping for *CYP2E1*5B* Polymorphism

*CYP2E1*5B* polymorphism comprises two single nucleotide substitutions: G-1293C and C-1053T that are in complete linkage disequilibrium with each other. So the amplified PCR product in the 5'-flanking region of *CYP2E1*5B* which was 412 bp long covered both SNPs. Figure 3.3 represents the sequence of amplified region in 5'-flanking region, showing the location of primers, both SNPs and restriction endonuclease recognition sites.

-1293 position of *CYP2E1* gene is occupied by G in wild type alleles, while it is substituted to C in the mutated alleles. In wild types, with G at position –1293, there is no recognition site for *Pst*I in the amplified fragment of 5'-flanking region. (see Figure 3.3 and Figure 3.4; recognition site for *Pst*I is 5'-CTGCA↓G-3'). However, in the mutated allele, that bears C at position –1293, there is a recognition site for *Pst*I (underlined with yellow in Figure 3.3). So, *Pst*I restriction endonuclease can not cut the PCR product in wild types; but an appropriate recognition sequence is present in mutated alleles, therefore *Pst*I cuts the 412 bp PCR product into two fragments of approximately 300bp and 100 bp.

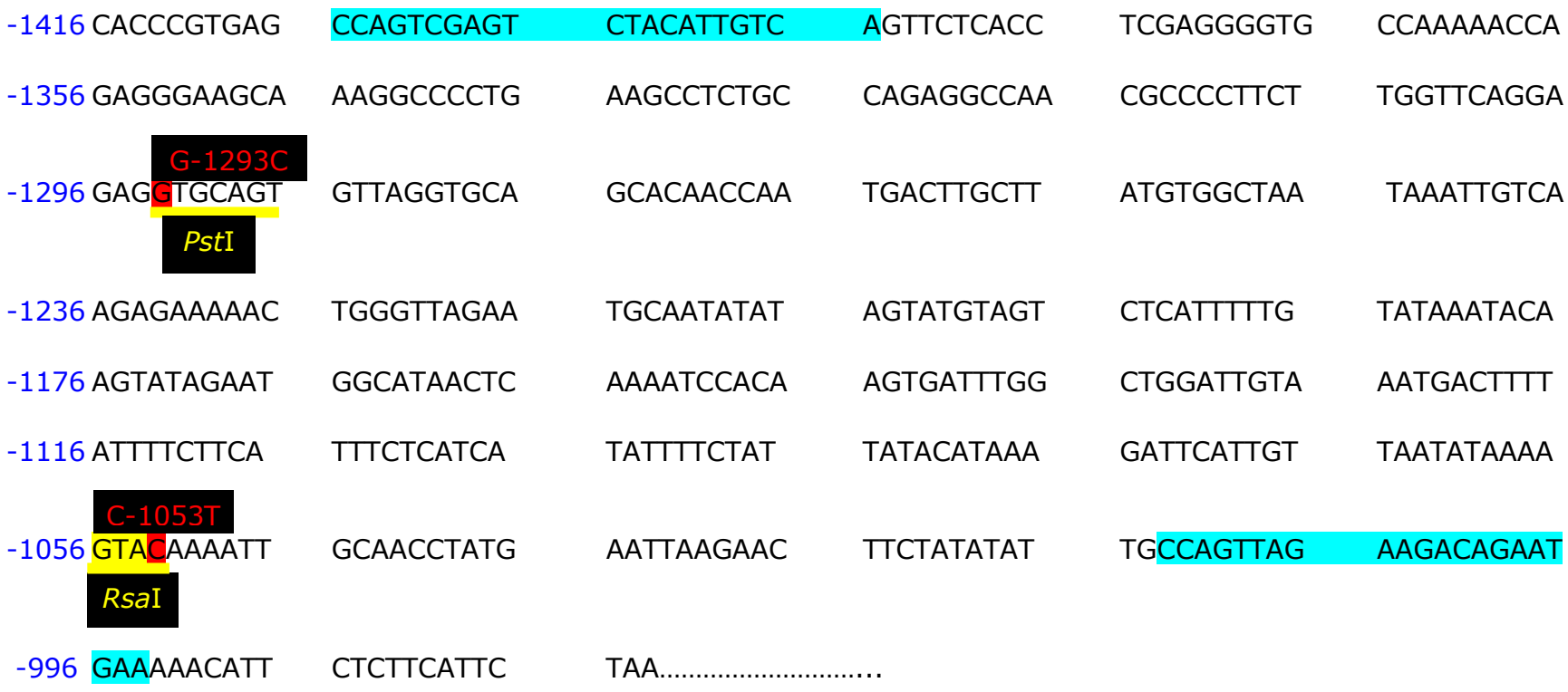

Figure 3.3 Sequence of amplified fragment in 5'-flanking region of *CYP2E1* gene that includes G-1293C/C-1053T single nucleotide polymorphisms. The blue highlighted sequences are forward and reverse primers, red highlighted nucleotides show the location of SNPs, and the yellow underlined and highlighted sequences show the recognition sites for restriction enzymes *Pst*I and *Rsa*I, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

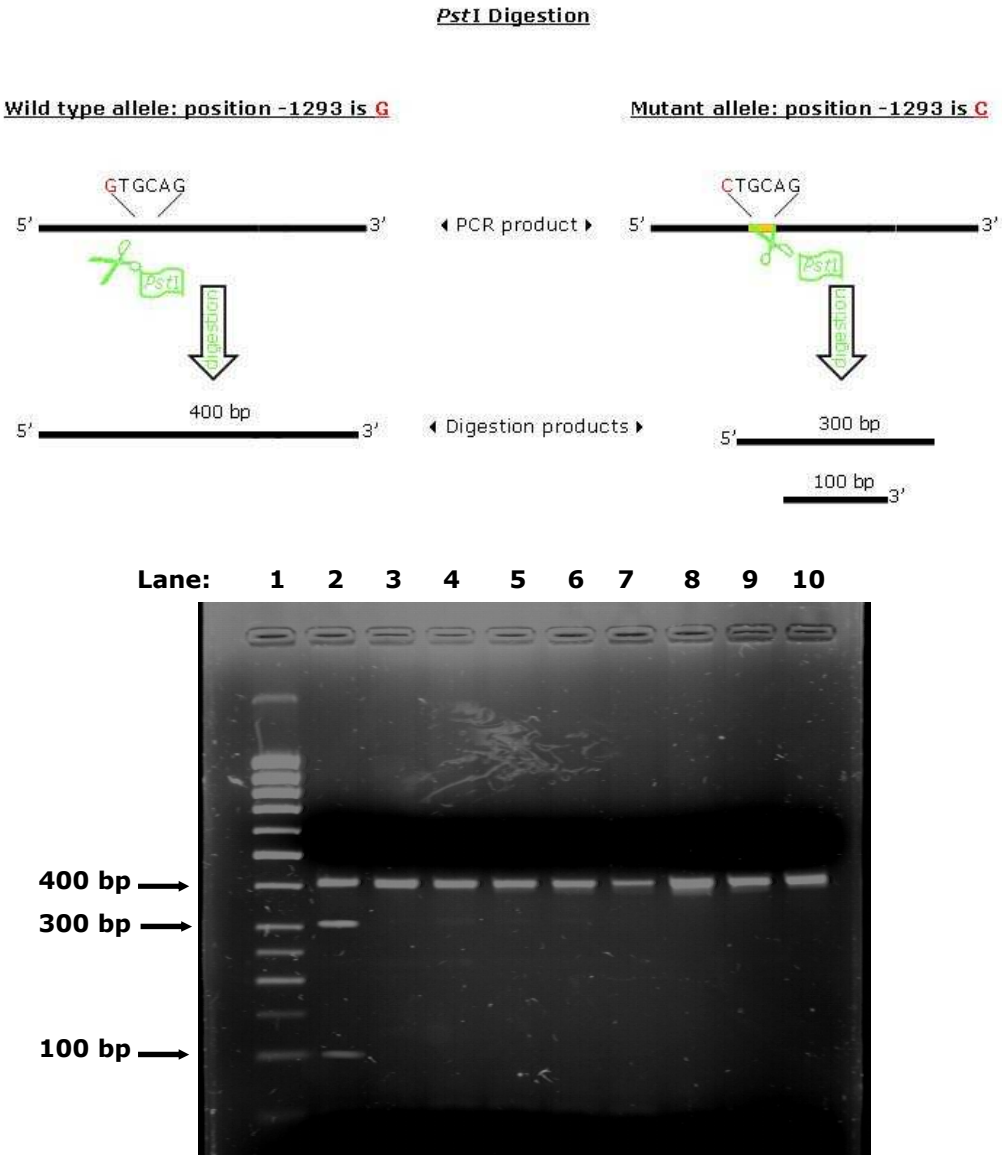

Figure 3.4 Schematic representation (upper part) and agarose gel (2%) photo (lower part) of *Pst*I digestion of the amplified 5'-flanking region of *CYP2E1* gene. In the schematic representation, yellow sign on the genes indicates the presence of a suitable recognition sequence for the enzyme, the scissors are representatives of restriction enzymes. In the gel photo, Lane 1 is DNA ladder (50-1000 bp). Lane 3-9 represents homozygous wild type individuals having a single band of 400 bp (control no's: 67, 68, 69, 70, 71, 72, 73). Lane 2 shows a heterozygous individual having three bands of 400, 300 and 100 bp (control no: 66). Lane 10 is a PCR product which was not subject to digestion.

As a result, when analyzing the *Pst*I digestion results in agarose gel, a homozygous wild type individual would yield a single band of 400 bp, as *Pst*I fails to cut the PCR product; while a homozygous mutated individual would yield two bands of 300 and 100 bp, as the enzyme manages to cut the PCR product. Obviously, heterozygotes were expected to yield three bands with 400, 300 and 100 bp. Throughout this study, a homozygous mutated individual (300 and 100 bp bands) was not encountered. Schematic representation of *Pst*I digestion pattern and an example of the gel photo is depicted in Figure 3.4.

In C-1053T single nucleotide polymorphism, -1053 nucleotide of *CYP2E1* gene is occupied with C in wild type allele, while the same location is T in mutated allele. In wild type allele, with C in position –1053, the PCR product bears a recognition site for restriction endonuclease *Rsa*I (recognition site: 5'-GT↓AC-3'), as can be seen in Figure 3.3 and Figure 3.5. Digestion with *Rsa*I efficiently cuts the PCR product with wild type allele, producing two fragments of approximately 350 bp and 50 bp. However, in the mutated allele, -1053 position is occupied with T, so there is no suitable sequence that *Rsa*I can recognize and cut the PCR product. In this case, digestion of PCR products with *Rsa*I yields one fragment of 412 bp, the undigested PCR product itself.

If the individual is homozygous wild type, the presence of recognition site for *Rsa*I would yield two bands of 350 bp and 50 bp, while in a homozygous mutated individual, a single 412 bp fragment would be the result. In heterozygotes, as expected, 412 bp, 350 bp and 50 bp bands would be observed. Throughout the study, a homozygous mutated individual was not encountered. The scheme of digestion pattern of *Rsa*I in wild type and mutated alleles, and a representative agarose gel photo is given in Figure 3.5. The photo comprised the same individuals as in the photo (in Figure 3.4) showing *Pst*I digestion.

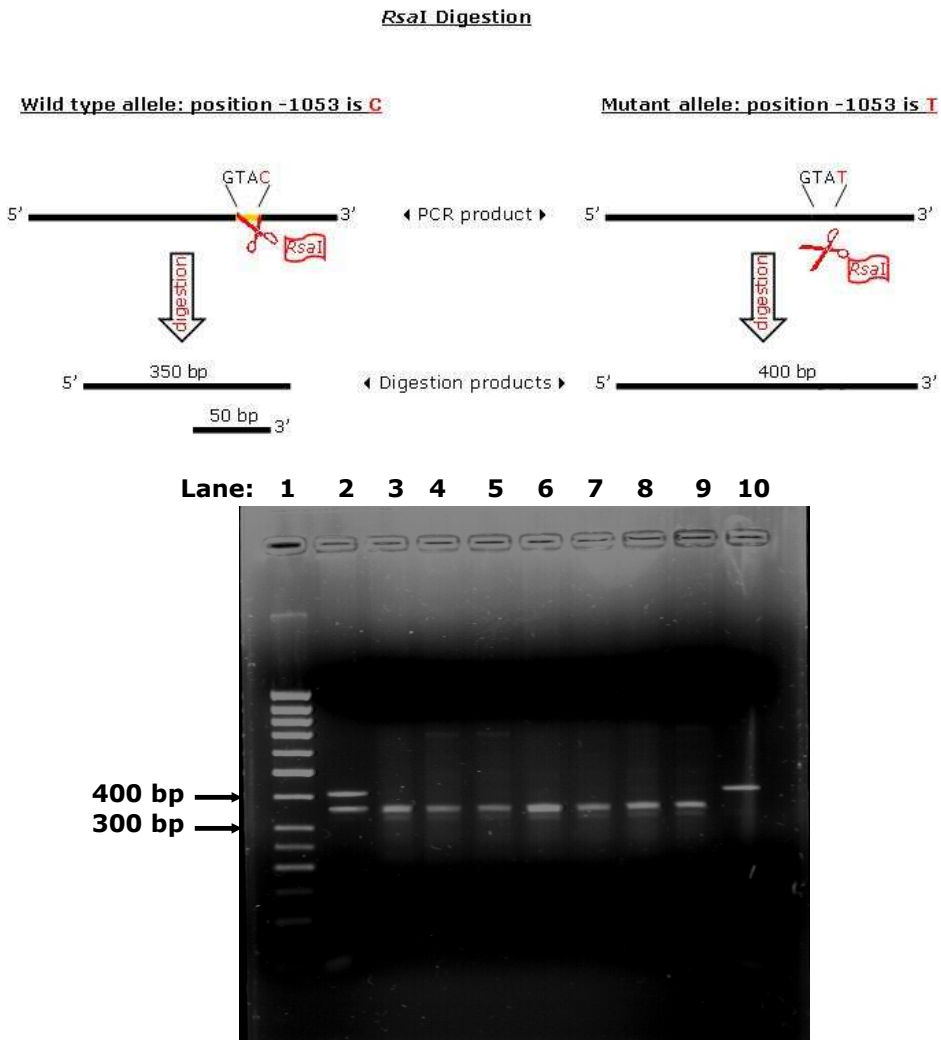

Figure 3.5 Schematic representation (upper part) and agarose gel (2%) photo (lower part) of *Rsa*I digestion of the amplified 5'-flanking region of *CYP2E1* gene. In the schematic representation, yellow sign on the genes indicates the presence of a suitable recognition sequence for the enzyme, the scissors are representatives of restriction enzymes. In the gel photo, Lane 1 is DNA ladder (50-1000 bp). Lane 3-9 represents homozygous wild type individuals having two bands of 350bp and 50 bp (control no's: 67, 68, 69, 70, 71, 72, 73). Lane 2 is a heterozygous individual (control no: 66), as both 400 bp and 350 bp bands are observed. Lane 10 is the PCR product, which was not subjected to digestion. All lanes (except 10) also contain a 50 bp band which is not observable in the photo.

The two single nucleotide polymorphisms, G-1293C and C-1053T are located in the 5'-flanking region of the *CYP2E1* gene with close proximity to each other. It is well established that these two polymorphisms are in complete linkage disequilibrium (Watanabe *et al.,* 1990; Hayashi *et al.,* 1991). Complete linkage disequilibrium indicates that the two SNPs are associated and always inherited together, for example, if –1053 is occupied with C, -1293 is always occupied with G, but never C. So, in wild types, position -1293 is occupied by G and -1053 with C and designated as **1A* according to CYP allele nomenclature committee (http://www.imm.ki.se/CYPalleles). Similarly, mutated allele, with C at position -1293 and T at position -1053, are designated as **5B*. It should also be noted that, the old designation for wild type allele was c1 and for mutated allele was c2.

The genotype distribution and allele frequencies of *CYP2E1*5B* polymorphism are given in Table 3.2. Comparison of the genotype distribution in control and patient samples are also expressed in terms of odds ratio (OR) with 95% confidence intervals. Among 209 healthy controls, 201 (96.2%) had **1A*1A* genotype and 8 (3.8%) had **1A*5B* genotype. No homozygous mutated (**5B*5B*) individual was detected in control sample. The wild type allele (**1A*) frequency was 98.1% and the mutated allele (**5B*) frequency was 1.9% for the control group. In patient group of 185 children with ALL, 173 (93.5%) were homozygous wild type (**1A*1A*) and 12 (6.5%) were heterozygote (**1A*5B*). As in controls, no homozygous mutated (**5B*5B*) individual was present in patient group. The allele frequencies were 96.8% for wild type (**1A*) and 3.2% for mutated (**5B*) allele in patient sample.

Table 3.2 Genotype distribution and allele frequencies of *CYP2E1*5B* SNP in control and patient samples.

*CYP2E1*5B* SNP *Pst*I / *Rsa*I RFLP		Control		Patient		OR (95% CI)	*p*
		N (209)	%	N (185)	%		
Genotype	**1A*1A* (c1c1)	201	96.2	173	93.5	1 (ref)	
	**1A*5B* (c1c2)	8	3.8	12	6.5	1.7 (0.7-4.4)	0.23
	**5B*5B* (c2c2)	0	0	0	0	-	-
Allele	**1A* (c1)	410	98.1	358	96.8	1 (ref)	
	**5B* (c2)	8	1.9	12	3.2	1.7 (0.7-4.2)	0.24

The heterozygosity was higher in patients when compared with controls (3.8% and 6.5% in control and patient groups, respectively), so that the odds ratio was 1.7; however, this difference was not statistically significant (95% CI:0.7-4.4, p=0.23). The **5B* allele also was not significantly associated with risk of childhood ALL (OR=1.7, 95%CI: 0.7-4.2, p=0.24).

3.2.1.2 Genotyping for *CYP2E1*6* Polymorphism

The 1000 bp amplified region in the intron 6 of *CYP2E1* gene included a single nucleotide polymorphism at nucleotide position of 7632. This position is occupied by T in wild type alleles and A in mutants, respectively. Figure 3.6 represents the partial nucleotide sequence of the amplified region, with primers, the SNP and the recognition sites of *Dra*I. The amplified fragment contained two recognition sites as shown in Figure 3.6, one of which included the SNP in its sequence.

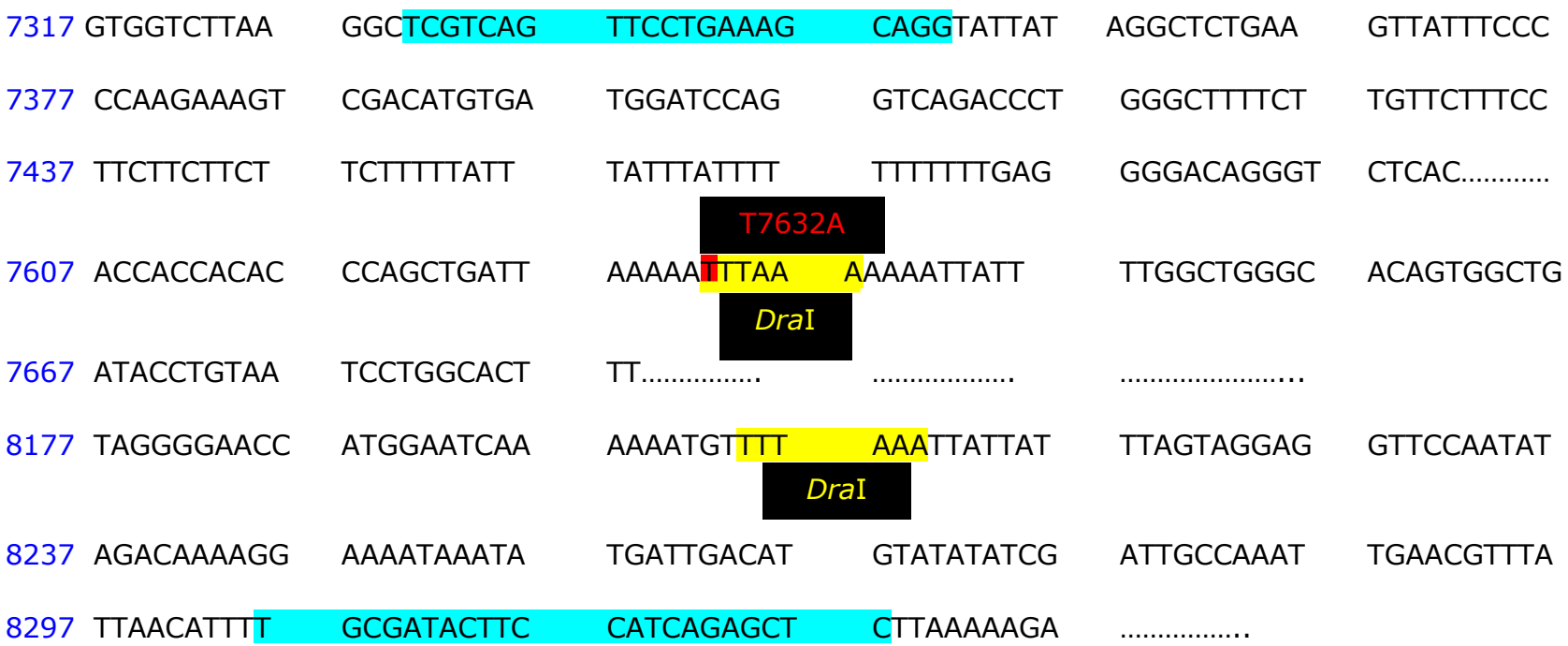

Figure 3.6 Sequence of amplified fragment in intron 6 of *CYP2E1* gene that includes T7632A single nucleotide polymorphism. The amplified region contains two recoginition sites for *Dra*I restriction enzyme, and T7632A SNP is located in one of them. The blue highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the yellow highlighted sequences show the recognition sites for restriction enzyme *Dra*I, as indicated. The dots indicates that there are many nucleotides there, which are not presented for convenience (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov)

The wild type alleles that contain a T at position 7632, also bear a recognition site around that nucleotide, enabling *Dra*I restriction endonuclease (recognition sequence: 5'-TTT↓AAA-3') to cut the PCR product from that position. But in the mutant alleles with A in position 7632, there is not a recognition site around the SNP, preventing *Dra*I to cut the fragment (Figure 3.6 and Figure 3.7). As the PCR product contains an additional recognition sequence for the restriction enzyme, the 1000 bp PCR product is cut into two; yielding 900 bp and 100 bp fragments upon *Dra*I digestion. In the wild type alleles, the 900 bp fragment is further cut into 600 bp and 300 bp fragments, as a second recognition site is present. In mutated allele, however, the corresponding recognition site at position 7632 is absent, and further digestion does not occur, resulting two fragments of 900 and 100 bp. The scheme of digestion pattern of *Dra*I in wild type and mutated alleles, and a representative agarose gel photo is given in Figure 3.7.

In *CYP2E1*6* polymorphism, the mutated allele is designated as **6* and wild type allele as **1A*. The old designation for mutated and wild type alleles are C and D, respectively. The genotype distribution and allele frequencies of control (N=209) and patient (N=185) groups are presented in Table 3.3. The distribution of homozygous wild type (**1A*1A*), heterozygote (**1A*6*) and homozygous mutated (**6*6*) individuals were 176 (84.2%), 32 (15.3%) and 1 (0.5%), respectively in control group. The wild type (**1A*) allele frequency was 91.9% and the mutated (**6*) allele frequency was 8.1 in control group. There were 153 (87.7%) homozygous wild type (**1A*1A*) and 32 (17.3%) heterozygotes (**1A*6*) in patients group, but there was no homozygous mutated (**6*6*) individual. Correspondingly, the wild type (**1A*) allele frequency was 91.4% and mutated (**6*) allele frequency was 8.6% in patient group.

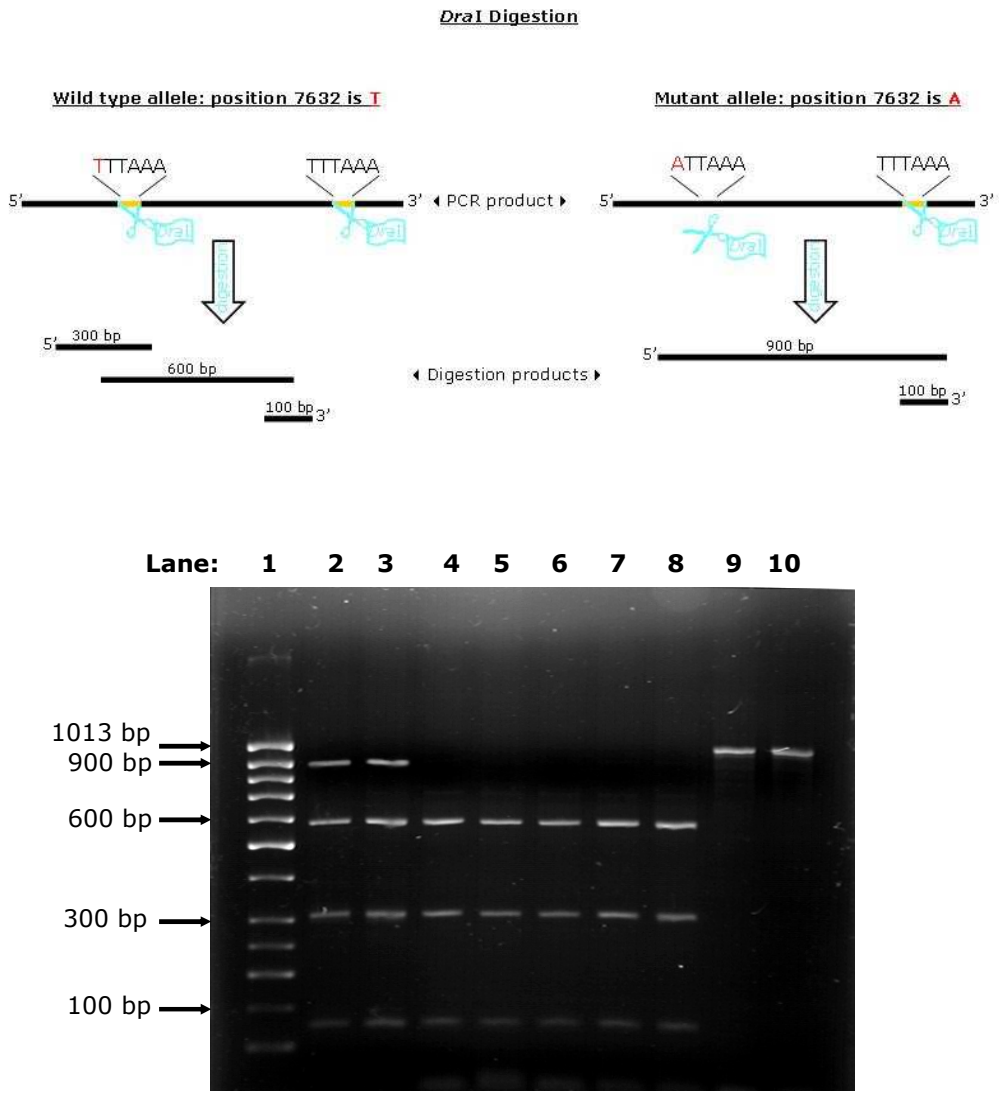

Figure 3.7 Schematic representation (upper part) and agarose gel (2%) photo (lower part) of *Dra*I digestion of intron 6 of *CYP2E1* gene. In the schematic representation, yellow signs on the genes indicate the presence of a suitable recognition sequence for the enzyme, the scissors are representatives of restriction enzymes. In the gel photo, Lane 1 is DNA ladder (50-1000 bp).In lane 2 and 3, four bands of 900, 600, 300, and 100 bp indicates heterozygotes (control no's: 13, 14). Lanes 4-8 with 600, 300 and 100 bp bands belong to homozygous wild type individuals (control no's: 15, 16, 17, 18, 19). Lanes 9 and 10, with single 1000 bp bands, are PCR products that were not subject to *Dra*I digestion.

Table 3.3 Genotype distribution and allele frequencies of *CYP2E1*6* SNP in control and patient samples.

*CYP2E1*6* SNP *Dra*I RFLP		Control		Patient		OR (95% CI)	*p*
		N (209)	%	N (185)	%		
Genotype	**1A*1A* (DD)	176	84.2	153	87.7	1 (ref)	
	**1A*6* (DC)	32	15.3	32	17.3	1.2 (0.7-2.0)	0.26
	**6*6* (CC)	1	0.5	0	0	-	-
Allele	**1A* (D)	384	91.9	338	91.4	1 (ref)	
	**6* (C)	34	8.1	32	8.6	1.1 (0.6-1.8)	0.79

The distribution of *CYP2E1*6* genotypes were not significantly different in control and patient groups. The **6*6* genotype was not observed in patient group, hence a comparison was not possible, but the **1A*6* genotype did not show significant association with the risk of ALL (OR=1.2, 95% CI: 0.7-2.0, p=0.26). The mutated allele (**6*) also could not be considered as a risk factor (OR=1.1, 95%CI: 0.6-1.8, p=0.79).

3.2.1.3 Genotyping for *CYP2E1*7B* Polymorphism

*CYP2E1*7B* polymorphism is a base substitution from G to T at position -71 near the TATA box and the PCR region involving the SNP was 360 bp long. The sequence of the PCR product, showing the primers, the single nucleotide polymorphism and the recognition site of the restriction enzyme *Dde*I is presented in Figure 3.8.

Figure 3.8 Sequence of amplified fragment covering the G-71T single nucleotide polymorphism of *CYP2E1*. The blue highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the yellow highlighted sequences show the recognition sites for restriction enzyme *Dde*I, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

In the wild type alleles, with G at position -71, there is a suitable recognition site for the restriction enzyme *Dde*I (recognition sequence: 5'-C↓TNAG-3') so that the enzyme cuts the PCR fragment into two pieces of approximately 310 and 50 bp long. In the mutated allele, however, the nucleotide at position -71 is occupied with T, which leads to absence of a suitable recognition site for the restriction enzyme (Figure 3.8 and 3.9). The case of mutated allele results in a single band of 360 bp long, which is the undigested PCR product.

Schematic representation of *Dde*I digestion pattern in wild type and mutated individuals, and a representative agarose gel photo is given in Figure 3.9. Homozygous wild type individuals yielded two bands of approximately 310 bp and 50 bp long. On the other hand, homozygous mutated individuals yield uncut PCR fragment of 360 bp long. The heterozygotes result in three bands of 360 bp, 310 bp and 50 bp long as can be observed in the gel photo in Figure 3.9.

The designation of *CYP2E1*7B* polymorphism is **1A* (previously named as G) for wild type allele and **7B* (previously named as T) for mutated allele. The genotype distribution and allele frequencies in control and patient groups, and their comparison, are given in Table 3.4. In control group of 209 individuals, 182 (87.1%) were homozygous wild type (**1A*1A*), 26 (12.4%) were heterozygote (**1A*7B*), and 1 (0.5%) was homozygous mutated (**7B*7B*). The mutated (**7B*) allele frequency was 6.7% while the wild type (**1A*) allele frequency was 93.3%. Both heterozygosity and homozygous mutated genotypes were more common in patient group, such that the distribution of **1A*1A*, **1A*7B*, and **7B*7B* genotypes were 153 (82.7%), 29 (15.7%) and 3 (1.6%), respectively, in patient group. Accordingly, mutated (**7B*) allele frequency, 9.5%, was higher than that of control group. The wild type allele (**1A*) frequency was 90.5% in patient group.

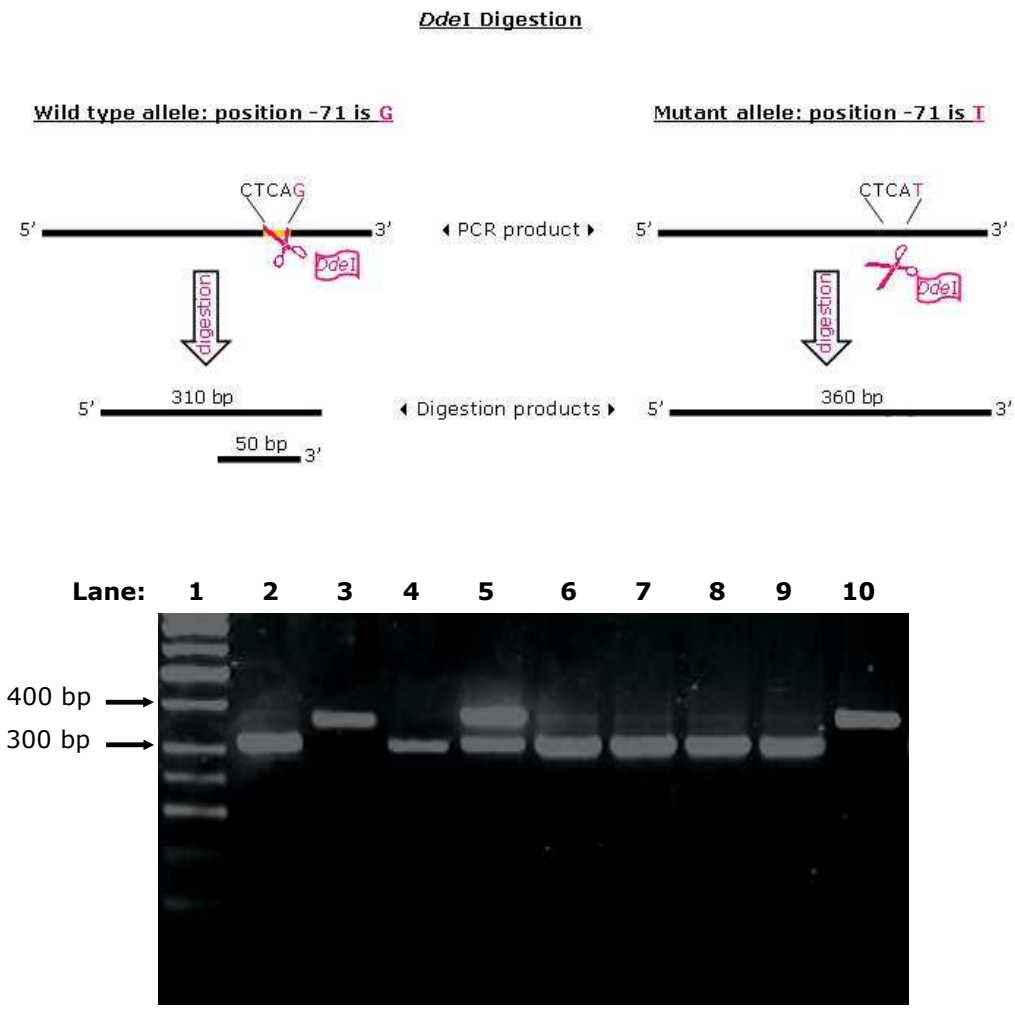

Figure 3.9 Schematic representation (upper part) and agarose gel (2%) photo (lower part) of *Dde*I digestion of promoter region of *CYP2E1* gene. In the schematic representation, yellow sign on the genes indicates the presence of a suitable recognition sequence for the enzyme, the scissors are representatives of restriction enzymes. In the gel photo, Lane 1 is DNA ladder (50-1000 bp). Lanes 2, 4, 6-9 are homozygous wild type individuals with a band of 310 bp (patient no's: 153, 155, 157, 158, 159, 167). Lane 3 is a homozygous mutated individual with 360 bp band (patient no: 154). Lane 5 is a heterozygous individual with both 360 and 310 bp bands (sample no: 156). Note that the 50 bp bands are not seen in the photo. Lane 10 represents a PCR product of 360 bp which was not subjected to *Dde*I digestion.

Table 3.4 Genotype distribution and allele frequencies of *CYP2E1*7B* SNP in control and patient samples.

*CYP2E1*7B* SNP *Dde*I RFLP		Control		Patient		OR (95% CI)	*p*
		N (209)	%	N (185)	%		
Genotype	**1A*1A* (GG)	182	87.1	153	82.7	1 (ref)	
	**1A*7B* (GT)	26	12.4	29	15.7	1.3 (0.7-2.3)	0.94
	**7B*7B* (TT)	1	0.5	3	1.6	3.6 (0.4-34.7)	0.25
Allele	**1A* (G)	390	93.3	335	90.5	1 (ref)	
	**7B* (T)	28	6.7	35	9.5	1.5 (0.9-2.4)	0.15

When *CYP2E1*7B* genotypes were investigated as risk factors for the development of childhood ALL, homozygous mutated (**7B*7B*) genotype resulted in 3.6 fold increased risk, however this was not statistically significant (95% CI: 0.4-34.7, p=0.25). Heterozygosity (**1A*7B*) was not a risk factor also (OR=1.3, 95% CI: 0.7-2.3, p= 0.94). Similarly, when the allele frequencies were considered, mutated allele (**7B*) did not show a significant association with the risk of childhood ALL (OR=1.5, 95%CI: 0.9-2.4, p=0.15).

3.2.2 Genotyping for *NQO1*2* Polymorphism

*NQO1*2* single nucleotide polymorphism is a C to T base substitution at position 609 of NQO1 cDNA. The amplified PCR product in exon 6 bears two recognition sites for the restriction enzyme *Hinf*I (recognition sequence: 5'-G↓ANTC-3'), one of them is present only if the nucleotide at position 609 is occupied by T (mutated allele), the other is

present in any condition. Figure 3.10 presents the sequence of the approximately 250 bp long PCR product in exon 6 of *NQO1*, highlighting the sequence of primers, location of SNP and the recognition sequence of the restriction enzyme *Hinf*I.

Upon digestion with the restriction enzyme *Hinf*I, the PCR product is cut into two bands of 210 bp and 40 bp long, independently from the SNP (Figure 3.10). Wild type alleles possess the nucleotide C at position 609, and a suitable recognition sequence is not present for *Hinf*I restriction enzyme in this case. So wild type alleles result in 210 bp and 40 bp bands. On the other hand, in mutated individuals, the nucleotide at position 609 is T, which creates an additional recognition site for *Hinf*I, hence upon digestion with the restriction enzyme, the 210 bp band is further cut into 150 bp and 60 bp bands.

Schematic representation of *Hinf*I digestion pattern in wild type and mutated individuals, and a representative agarose gel photo is given in Figure 3.11. Homozygous wild type individuals yielded two bands of approximately 210 bp and 40 bp long, which is the result of SNP-independent recognition site. On the other hand, in homozygous mutated individuals, three bands of 150 bp, 60 bp and 40 bp are obtained. The heterozygotes result in four bands of 210 bp, 150 bp, 60 bp and 40 bp (Figure 3.11).

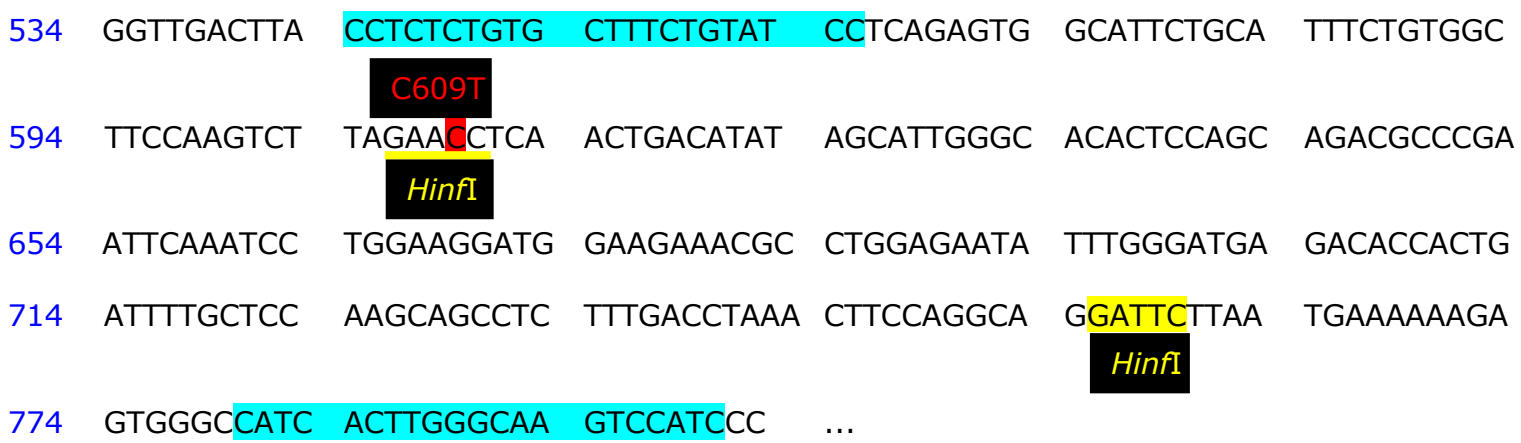

Figure 3.10 Sequence of amplified fragment covering the C609T single nucleotide polymorphism of *NQO1*. The blue highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the yellow highlighted sequences show the recognition sites for restriction enzyme *Hinf*I, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

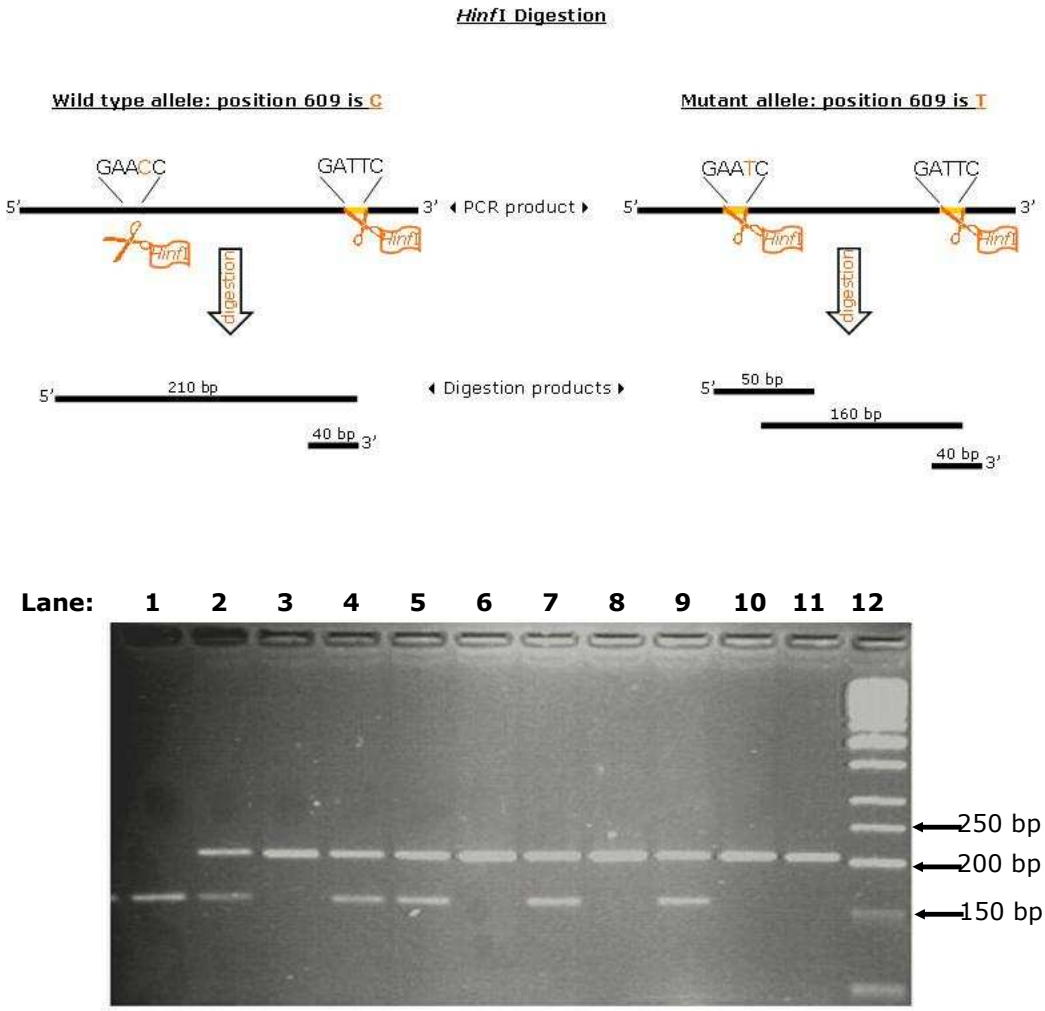

Figure 3.11 Schematic representation (upper part) and agarose gel (2%) photo (lower part) of *Hinf*I digestion of exon 6 of *NQO1* gene. In the schematic representation, yellow signs on the genes indicate the presence of a suitable recognition sequence for the enzyme, the scissors are representatives of restriction enzymes. In the gel photo, Lane 1 and 14 are DNA ladder (50-1000 bp). Lane 2 is PCR product which was not subjected to digestion. Lane 3 is a homozygous mutated individual with a band of 160 bp (patient no: 1). Lanes 4, 6, 7, 9, 11 are heterozygotes with 210bp and 160 bp bands (patient no's: 2, 4, 5, 7, 9). Lanes 5, 8, 10, 12, 13 are homozygous wild type individuals with 210 bp band (patient no's: 3, 6, 8, 10,11). Bands of 50 and 40 bp long are not seen in the photo.

Wild type and mutated alleles are designated as **1* (C in old designation) and **2* (T in old designation) for *NQO1*2* polymorphism. The genotype distribution and allele frequencies of *NQO1*2* polymorphism are presented in Table 3.5 for control and patient groups. Among 209 control subjects, 122 (58.4%) had homozygous wild type (**1*1*) genotype, 75 (35.9%) had heterozygous (**1**2) genotype, and 12 (5.7%) had homozygous mutated genotype. The mutated (**2*) allele frequency was 26.7% and the wild type (**1*) allele frequency was 76.3% in control group. In patient group of 185 subjects, 104 (56.2%) had homozygous wild type (**1*1*) genotype, 68 (36.8%) had heterozygous (**1*2*) genotype, and 13 (7.0%) had homozygous mutated (**2*2*) genotype. The mutated (*2) allele frequency was 25.4% and wild type (**1*) allele frequency was 74.6% in patient group. As can be seen from Table 3.5, the distribution of *NQO1*2* genotypes was similar both in control and patient groups.

Table 3.5 Genotype distribution and allele frequencies of *NQO1*2* SNP in control and patient samples.

*NQO1*2* SNP *Hinf*I RFLP		Control		Patient		OR (95% CI)	*p*
		N (209)	%	N (185)	%		
Genotype	**1*1* (CC)	122	58.4	104	56.2	1 (ref)	
	**1*2* (CT)	75	35.9	68	36.8	1.1 (0.7-1.6)	0.78
	**2*2* (TT)	12	5.7	13	7.0	1.3 (0.6-2.9)	0.32
Allele	**1* (C)	319	76.3	276	74.6	1 (ref)	
	**2* (T)	99	26.7	94	25.4	1.1 (0.8-1.5)	0.58

When the risk of heterozygosity (**1*2*) or homozygous mutated (**2**2) genotypes were investigated, it was observed that neither of the genotypes was associated with the risk of development of childhood ALL (for **1*2* genotype, OR=1.1, 95% CI: 0.7-1.6, *p*=0.78; for **2*2* genotype, OR=1.3, 95% CI: 0.6-2.9, *p*=0.32). Considering the mutated allele frequencies in control and patient groups, mutated allele **2* was not a risk factor for childhood ALL (OR=1.1, 95% CI: 0.8-1.5, *p*=0.58).

3.2.3 Genotyping for *GSTM1* and *GSTT1* Null

GST M1 and *T1* genes possess null polymorphisms which is resultant of deletion of the genes, so that the enzyme is not expressed. Null polymorphisms of both genes are detected by multiplex PCR, where amplified PCR products are selected from the regions coding for the gene. Figure 3.12 presents the sequences amplified for *GST M1* and *T1* from chromosomes 1 and 22, respectively, where the genes are located. Figure also shows the sequence of amplified region of *CYP1A1* gene in chromosome 15. This region is not polymorphic and it is amplified in any case. It is used as internal control, to assure that the absence of the band is due to deletion of the gene, not from unsuccessful PCR. The PCR medium contained primer pairs for all three genes (*GSTM1, GSTT1* and *CYP1A1*) and all three regions were amplified at the same time.

GSTT1

22706890	GAACAAGGCC	TTCCTTACTG	GTCCTCACAT	CTCCTTAGCT	GACCTCGTAG	CCATCACGGA
22706830	GCTGATGCAT	GTGAGTGCTG	TGGGCAGGTG	AACCCACTAG	GCAGGGGGCC	CTGGCTAGTT
22706770	GCTGAAGTCC	TGCTTATGCT	GCCACACCGG	GCTATGGCAC	TGTGCTTAAG	TGTGTGTGCA
22706710	AACACCTCCT	GGAGATCTGT	GGTCCCCAAA	TCAGATGCTG	CCCATCCCTG	CCCTCACAAC
22706650	CATCCATCCC	CAGTCTGTAC	CCTTTTCCCC	ACAGCCCGTG	GGTGCTGGCT	GCCAAGTCT T
22706590	CGAAGGCCGA	CCCAAGCTGG	CCACATGGCG	GCAGCGCGTG	GAGGCAGCAG	TGGGGGAGGA
22706530	CCTCTTCCAG	GAGGCCCATG	AGGTCATTCT	GAAGGCCAAG	GACTTCCCAC	CTGCAGACCC
22706470	CACCATAAAG	CAGAAGCTGA	TGCCCTGGGT	GCTGGCCATG	ATCCGGTGAG	CTGGG...

CYP1A1

45803500	GGAGCTCCAC	TCACTTGACA	CTTCTGAGCC	CTGAACTGCC	ACTTCAGCTG	TCTCCCTCTG
45803440	GTTACAGGAA	GCTATGGGTC	AACCCATCTG	AGTTCCTACC	TGAACGGTTT	CTCACCCCTG
45803380	ATGGTGCTAT	CGACAAGGTG	TTAAGTGAG	AGGTGATTAT	CTTTGGCATG	GGCAAGCGGA
45803320	AGTGTATCGG	TGAGACCATT	GCCCGCTGGG	AGGTCTTTCT	CTTCCTGGCT	ATCCTGCTGC
45803260	AACGGGTGGA	ATTCAGCGTG	CCACTGGGCG	TGAAGGTGGA	CATGACCCCC	ATCTATGGGC
45803200	TAACCATGAA	GCATGCCTGC	TGTGAGCACT	TCCAAATGCA	GCTGCGCT..	

GSTM1

110034433	GTACTTGGAG	GAACTCCCTG	AAAAGCTAAA	GCTCTACTCA	GAGTTTCTGG	GGAAGCGGCC
110034493	ATGGTTTGCA	GGAAACAAGG	TAAAGGAGGA	GTGATATGGG	GAATGAGATC	TGTTTTGCTT
110034553	CACGTGTTAT	GGAGGTTCCA	GCCCACATAT	TCTTGGCCTT	CTGCAGATCA	CTTTTGTAGA
110034613	TTTTCTCGTC	TATGATGTCC	TTGACCTCCA	CCGTATATTT	GAGCCCAAGT	...

Figure 3.12 Sequence of amplified fragments of *GSTT1, CYP1A1* and *GSTM1* genes, in order. The blue highlighted sequences are forward and reverse primers (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

Both for GSTM1 and T1, if the gene is deleted, the PCR product is not produced, on the other hand, if the gene is present on the chromosome, the amplification reaction yields a product. In heterozygotes, one allele possessing the gene results in amplification of PCR product. So presence of the band denotes that the individual is either homozygous wild type or heterozygous, expressing the enzyme in either case. Absence of the band shows the individual is homozygous mutated, and does not express the enzyme. Figure 3.13 presents an example for the agarose gel electrophoresis result of multiplex PCR. The multiplex PCR results in a band of 219 bp for *GSTM1,* 459 bp band for *GSTT1,* and 314 bp band for *CYP1A1*, the internal control.

As mentioned above, the genotyping of *GSTM1* and *T1* gave results as "null", indicating the gene is not expressed (homozygous mutated) or "present", indicating the gene is expressed (either homozygous wild type or heterozygote). The frequencies of null and present genotypes for *GSTM1* and *GSTT1* in control and patient groups are presented in Table 3.6.

The null genotype was strikingly high for *GSTM1* both in control and patient groups. In 85 (40.7%) of control subjects the enzyme was present, while in 124 (59.3%) of them, the enzyme was not expressed (null genotype). In patients, 89 (48.1%) individuals expressed the enzyme while 96 (51.9%) did not (null genotype). The risk of *GSTM1* null genotype for ALL was 0.7, which was not statistically significant (95% CI: 0.5-1.1, p= 0.14).

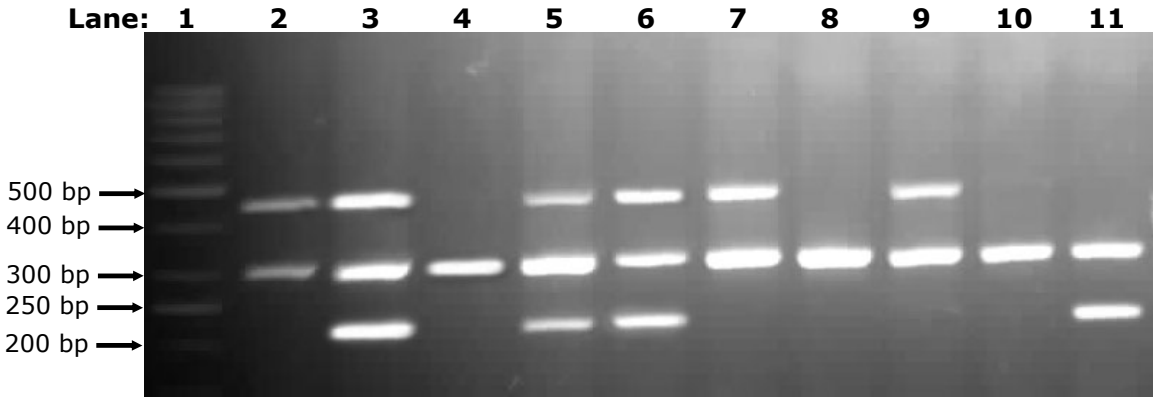

Figure 3.13 Agarose gel (2%) photograph of multiplex *GSTM1* and *GSTT1* PCR. Band of 314 bp is *CYP1A1*, the internal control. Presence of 459 bp denotes expression of GSTT1, presence of 219 bp band denotes expression of GSTM1. Accordingly, Lanes 2, 7 and 9 show individuals that express only GSTT1 (control no's: 115, 124 and 142). Lanes 3, 5 and 6 show individuals expressing both GSTM1 and GSTT1 (control no's: 116, 118 and 121). Lanes 4, 8 and 10 show individuals who do express neither GSTM1 nor GSTT1 (control no's: 117, 141, and 145). Lane 11 shows an individual expressing only GSTM1 (control no: 148).

Table 3.6 Genotype frequencies of *GSTM1* and *GSTT1* null in control and patient samples.

GST		Control		Patient		OR (95% CI)	*p*
		N (209)	%	N (185)	%		
M1	present	85	40.7	89	48.1	1 (ref)	
	null	124	59.3	96	51.9	0.7 (0.5-1.1)	0.14
T1	present	165	78.9	125	67.6	1 (ref)	
	null	44	21.1	60	32.4	1.8 (1.1-2.8)	0.01*

* statistically significant association.

When *GSTT1* was considered, in control group of 209 subjects, the gene was present in 165 (78.9%) individuals, and absent in 44 (21.1%) individuals. In patient group of 185 subjects, the gene was present in 125 (67.6%) subjects and absent in 60 (32.4%) subjects. When the null genotype of *GSTT1* was investigated as a risk factor for childhood ALL, it was observed that the null *GSTT1* null genotype increased the risk of ALL 1.8-fold, which was statistically significant (95% CI: 1.1-2.8, p=0.01).

3.3 Genetic Risk Factors for The Development of Childhood ALL: Case-Control Analyses.

This study involved four susceptible genes, *CYP2E1* –three polymorphisms throughout the gene, *NQO1*, *GSTM1* and *GSTT1*, hence in total six polymorphisms as possible genetic risk factors for the development of childhood ALL. In previous section, risk of each genotype for each polymorphism was analyzed when the genotype frequencies were presented, and among six polymorphisms, only *GSTT1* null genotype was significantly associated with ALL (OR=1.8, 95% CI: 1.1-2.8, p=0.01).

A common problem in genetic epidemiologic studies is the low frequency of possible risk elevating genotypes in the population, as in the case of *CYP2E1* and *NQO1* polymorphisms. One approach to overcome this problem is to group the genotypes such that the possible risk elevating alleles are combined in a single group. For all *CYP2E1* polymorphisms (**5B*, **6* and **7B*), presence of the mutated allele is a possible risk factor, as either of these mutations are suspected to increase the inducibility or expression of the enzyme. So in the following analyses, the heterozygous and homozygous mutated genotypes were combined under "risk" group, and analyzed against homozygous wild type genotype, which was placed in "no-risk" group.

For *NQO1*2* polymorphism, presence of mutated allele results in aberrant folding and rapid degradation of enzyme so that in homozygous mutated individuals there is no NQO1 activity, while in heterozygotes there is low level of activity. Therefore, in the case of *NQO1*2* polymorphism, homozygous mutated (**2*2*) and heterozygous (**1*2*) genotypes, which result in deficient or low activity, respectively, were combined and grouped under "risk", while homozygous wild type (**1*1*) genotype, was placed under "no risk" group.

GSTM1 and *GSTT1* null genotypes already present a combined genotype, such that null genotype (the homozygous mutated genotype) was placed under "risk" group, and presence of gene (homozygous wild types or heterozygotes) were placed under "no risk" group.

Grouping the genotypes for all polymorphisms under "risk" and "no risk" titles aimed to ease the reading of the tables by avoiding excessive use of genotype (or combination of genotypes) designations separately for each polymorphism.

3.3.1 Single Polymorphisms as Genetic Risk Factors

Table 3.7 presents the analyses for single nucleotide polymorphisms as grouped into "risk" or "no risk" genotypes. The odds ratio was calculated by the formula:
$OR = (Patient_{risk}/Patient_{no\ risk}) / (Control_{risk}/Control_{no\ risk})$.

For example, for *CYP2E1*5B* polymorphism, OR was calculated as (the first row in Table 3.7):
OR= (12/173) / (8/201) = 1.7.

Table 3.7 Analyses of single nucleotide polymorphisms as risk factors for the development of childhood ALL.

SNP	Control (N=209)		Patient (N=185)		OR (95% CI)	*p*
	no risk	risk	no risk	risk		
*CYP2E1*5B*	201	8	173	12	1.7 (0.7-4.4)	0.23
*CYP2E1*6*	176	33	153	32	1.1 (0.7-1.9)	0.69
*CYP2E1*7B*	182	27	153	32	1.4 (0.8-2.5)	0.22
*NQO1*2*	122	89	104	81	1.1 (0.7-1.6)	0.75
GSTM1	85	124	89	96	0.7 (0.5-1.1)	0.14
GSTT1	**165**	**44**	**125**	**60**	**1.8 (1.1-2.8)**	**0.01***

* statistically significant association.

Accordingly, none of the polymorphisms were risk factors alone, except *GSTT1*, which increased the risk of development of childhood ALL significantly, 1.8 fold (95% CI: 1.1-2.8, p=0.01).

3.3.2 Combination of Polymorphisms as Genetic Risk Factors

As mentioned in introduction part, single polymorphisms may not be effective in determining the risk associated with the disease. Rather, current research focused on the association of combined genotypes, or haplotypes that function in a metabolic mechanism. This study focused on the xenobiotic metabolizing enzymes that have key roles in the metabolism of benzene, a chemical which was proven to be associated with development of leukemia. In this section, combinations of six polymorphisms were analyzed for the risk of childhood ALL.

Table 3.8 presents the analyses of combinations of two polymorphisms as risk factors for the development of childhood ALL. The subjects that carried possible risk elevating genotypes for both polymorphisms under investigation at the same time were grouped under "risk". The "no risk" group consisted of individuals who were homozygous wild type for both genotypes under question. For example, in the analysis of *CYP2E1*5B* and *CYP2E1*6* combination, the individuals grouped under "risk" carried both **5B* and **6* alleles (either as homozygous mutated or heterozygotes), and individuals who were homozygous wild type for both CYP2E1**5B* and **6* alleles were grouped under "no risk".

When combinations of two SNPs on the same individual was considered as risk factor, combination of *CYP2E1*5B* and **6* polymorphisms was found to increase the risk of ALL development 2.7 fold (95% CI: 0.9-7.9, p= 0.04). Co-presence of *CYP2E1*6* and *GSTT1* polymorphisms increased the risk of ALL 4.2 fold, which was statistically significant (95% CI: 1.3-13.2, p=0.02). Co-presence of *CYP2E1*7B* and *GSTT1* polymorphisms increased the risk 4.1 fold (95% CI: 1.1-15.7, p=0.03), which was statistically significant. It should also be pointed out that copresence of *CYP2E1*5B* and *NQO1*2* polymorphisms increased the risk 4.1 fold, although with borderline significance (95% CI: 0.8-20.2, p= 0.06).

Interestingly, 12 patients who were heterozygous (**1A*5B)* for CYP2E1**5B* polymorphism were also heterozygous (**1A*6)* for *CYP2E1*6* polymorphism. So in patient group, all individuals carrying a **5B* allele also carried a **6* allele, which was not the case in the control group. This fact suggested a combined effect for **5B* and **6* polymorphisms, and that combination was found to increase the risk 2.7 fold (see Table 3.8).

Table 3.8 Analyses of the combinations of two single nucleotide polymorphisms as risk factors for the development of childhood ALL.

Combination of 2 SNPs[a]	Control (N=209)		Patient (N=185)		OR (95% CI)	*p*
	no risk	risk	no risk	risk		
***5B-*6**	**173**	**5**	**153**	**12**	**2.7 (0.9-7.9)**	**0.04***
**5B-*7B*	174	0	142	1	-	-
***5B-NQ**	**116**	**2**	**99**	**7**	**4.1 (0.8-20.2)**	**0.06**
**5B-M1*	83	6	85	8	1.3 (0.4-3.9)	0.63
**5B-T1*	159	2	119	6	4.0 (0.8-20.2)	0.07
**6-*7B*	149	0	125	4	-	-
**6-NQ*	102	13	90	18	1.6 (0.7-3.4)	0.24
**6-M1*	72	20	75	18	0.9 (0.4-1.8)	0.68
***6-T1**	**136**	**4**	**106**	**13**	**4.2 (1.3-13.2)**	**0.02***
**7B-NQ*	109	14	85	13	1.2 (0.5-2.7)	0.67
**7B-M1*	70	12	75	18	1.4 (0.6-3.1)	0.40
***7B-T1**	**141**	**3**	**102**	**9**	**4.1 (1.1-15.7)**	**0.03***
NQ-M1	52	54	54	46	0.8 (0.5-1.4)	0.48
NQ-T1	100	22	70	26	1.7 (0.9-3.2)	0.11
M1-T1	70	29	69	40	1.4 (0.8-2.5)	0.26

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

* statistically significant association.

Combination of three genotypes, that is, the risk analysis for the co-presence of three polymorphisms on the same individual was analyzed as presented in Table 3.9. The co-presence of *CYP2E1*5B, CYP2E1*6* and *GSTT1* polymorphisms in same individual considerably increased the risk for ALL to 7.6 fold, but with borderline significance level (95% CI: 0.9-64.0, p=0.04). Other combination of three SNPs were not associated with the risk of development of childhood ALL, as can be seen from Table 3.9.

Table 3.9 Analyses of combinations of three single nucleotide polymorphisms as risk factors for the development of childhood ALL.

Combination of 3 SNPs[a]	Control (N=209)		Patient (N=185)		OR (95% CI)	*p*
	no risk	risk	no risk	risk		
**5B-*6-*7B*	146	0	125	1	-	-
**5B-*6-NQ*	99	2	90	7	3.9 (0.8-19.0)	0.07
**5B-*6-M1*	71	4	75	8	1.9 (0.5-6.6)	0.24
****5B-*6-T1***	**134**	**1**	**106**	**6**	**7.6 (0.9-64.0)**	**0.04***
**5B-*7B-NQ*	103	0	80	1	-	-
**5B-*7B-M1*	68	0	71	1	-	-
**5B-*7B-T1*	135	0	97	0	-	-
**5B-NQ-M1*	50	2	54	3	1.4 (0.2-8.7)	0.54
**5B-NQ-T1*	95	1	69	2	2.7 (0.2-31.0)	0.39
**5B-M1-T1*	69	1	66	5	5.2 (0.6-45.9)	0.10
**6-*7B-NQ*	89	0	73	2	-	-
**6-*7B-M1*	57	0	63	2	-	-
**6-*7B-T1*	112	0	85	2	-	-
**6-NQ-M1*	44	8	48	10	1.1 (0.4-3.2)	0.79
**6-NQ-T1*	82	2	63	6	3.9 (0.8-20.0)	0.08
**6-M1-T1*	58	3	60	8	2.6 (0.7-10.2)	0.14
**7B-NQ-M1*	44	7	46	7	1.0 (0.3-3.0)	0.92
**7B-NQ-T1*	87	3	56	4	2.1 (0.4-9.6)	0.29
**7B-M1-T1*	56	2	58	6	2.9 (0.6-15.0)	0.17
NQ-M1-T1	46	13	43	17	1.4 (0.6-3.2)	0.42

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

* statistically significant association.

Risk analysis for the combination of four genotypes on the same individual is presented in Table 3.10. Risk analysis for the combination of five and six genotypes is presented in Table 3.11. However, the number of "risk" genotypes was very low in such combinations so that it was not possible to make an analysis, or the association was not significant.

Table 3.10 Analyses of combinations of four single nucleotide polymorphisms as risk factors for the development of childhood ALL.

Combination of 4 SNPs[a]	Control (N=209)		Patient (N=185)		OR (95% CI)	*p*
	no risk	risk	no risk	risk		
**5B-*6-*7B-NQ*	86	0	73	1	-	-
**5B-*6-*7B-M1*	56	0	63	1	-	-
**5B-*6-*7B-T1*	110	0	85	0	-	-
**5B-*6-NQ-M1*	43	2	48	3	1.3 (0.2-8.4)	0.56
**5B-*6-NQ-T1*	80	1	63	2	2.5 (0.2-28.6)	0.42
**5B-*6-M1-T1*	58	1	60	5	4.8 (0.5-43.6)	0.13
**5B-*7B-NQ-M1*	42	0	46	1	-	-
**5B-*7B-NQ-T1*	82	0	55	0	-	-
**5B-*7B-M1-T1*	55	0	55	0	-	-
**5B-NQ-M1-T1*	45	1	43	1	1.0 (0.1-17.3)	0.74
**6-*7B-NQ-M1*	36	0	41	1	-	-
**6-*7B-NQ-T1*	69	0	50	1	-	-
**6-*7B-M1-T1*	44	0	50	1	-	-
**6-NQ- M1-T1*	38	1	38	2	2.0 (0.2-23.0)	0.51
**7B-NQ- M1-T1*	38	2	36	2	1.1 (0.1-7.9)	0.67

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

Table 3.11 Analyses of combinations of five and six single nucleotide polymorphisms as risk factors for the development of childhood ALL.

Combination of 5 SNPs[a]	Control (N=209)		Patient (N=185)		OR (95% CI)	*p*
	no risk	risk	no risk	risk		
**5B-*6-*7B-NQ-M1*	35	0	41	1	-	-
**5B-*6-*7B-NQ-T1*	67	0	50	0	-	-
**5B-*6-*7B-M1-T1*	44	0	50	0	-	-
**5B-*6-NQ-M1-T1*	38	1	38	1	1.1 (0.1-16.6)	0.75
**5B-*7B-NQ-M1-T1*	37	0	36	0	-	-
**6-*7B-NQ-M1-T1*	30	0	32	0	-	-
Combination of 6 SNPs[a]						
**5B-*6-*7B-NQ-M1-T1*	30	0	32	0	-	-

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

3.4 Effect of Non-genetic Factors on The Risk of Genetic Factors for The Development of Childhood ALL: Case-Only Analyses

The majority of the genetic epidemiologic studies are based on case-control populations; however, increasingly, a case-series design has been promoted as an approach that can be used to evaluate gene-environment interaction in disease etiology (Khoury *et al.,* 1996; Goodman and Flanders, 2007). In this design, investigators use case subjects only to assess the magnitude of the association between the exposure of interest and the susceptibility genotype. The basic setup for analysis is a new 2 X 2 table, from which Case-only Odds Ratio (COR) can be calculated. A model for calculating COR is presented in Table 3.12.

Table 3.12 Model for gene-environment interaction analysis in the context of a case-only study (Khoury *et al.,* 1996).

Environmental Factor	Susceptibility Genotype	
	-	+
-	a	b
+	c	d

Case-only Odds Ratio (COR) can be calculated as:
COR= (d/c) / (b/a)

This approach was suggested as a simple tool to screen for gene-environment interaction in disease etiology. It was reported as appropriate to be used in the context of crude analysis of a 2 X 2 table or in the context of logistic models when other covariates need to be

adjusted for (Khoury *et al.,* 1996). COR values calculated from only case population were shown to be similar to OR values calculated for the case-control population, where the case population was the same for both analyses (Khoury *et al.,* 1996).

However, there are some issues that must be considered in case-only studies. First of all, independence between the environmental factor and genotype must be assumed in order to apply this method. For example, alcohol dependence and alcohol dehydrogenase polymorphisms may be dependent on each other (alcohol demand of individuals may be different because of the polymorphisms of alcohol dehydrogenase), hence such a case-only study would not be appropriate in that specific circumstance. Still this assumption is valid for many gene-environment studies. However, as independence between different non-genetic factors (like smoking, nutrition, occupational exposure) can not be assumed, case-only analyses can not be applied to examine the interaction between different non-genetic factors. It should also be noted that, the case-only approach does not allow the investigators to evaluate the independent effects of the exposure alone or the genotype alone, merely their interaction (Khoury *et al.,* 1996; Goodman and Flanders, 2007).

In this study, there are non-genetic factors which could be examined for their interaction with the genetic factors in the risk of development of childhood ALL, like parental age at conception or smoking exposure of parents or child. As information on these factors was not present for control population, case-only approache was applied to examine the interaction. This section presents the case-only analyses for interaction of non-genetic factors and genetic polymorphisms for the risk of development of ALL. Throughout the analyses, the genotypes for all polymorphisms were grouped under "risk" and "no risk" titles, as explained before in section 3.3.

3.4.1 Interaction of Age of Parents at Conception with the Genetic Polymorphisms in Risk of Childhood ALL

Several studies have reported that young (<20) and old (>35) maternal ages at the time of conception was a risk factor for the development of childhood ALL. Also paternal ages older than 40 years was shown to be risk factor (Kaye *et al.,* 1991; Dockerty *et al.,* 2001).

Age at conception information was available for 116 mothers and the ages were grouped into three as younger than 20 years (Age <20) which included 13 mothers, between 20 and 35 years (20-35) with 86 mothers, and older than 35 years (Age >35) with 17 mothers. Age group of "20-35" was taken as the reference group and COR values were calculated for "Age <20" and "Age >35" groups, and the results are presented in Table 3.13.

The maternal age older than 35 years and *CYP2E1*5B* polymorphism together were significantly associated with the risk of childhood ALL (COR=9.0, 95% CI: 1.4-58.8, p= 0.03). The risk for maternal age younger than 20 years and *CYP2E1*5B* was considerably high, 7.6 fold, however was not statistically significant (95% CI: 1.0-59.8, p= 0.08).

Table 3.13 Analysis of interaction of single genetic polymorphisms and maternal age at conception as risk factors for development of childhood ALL.

SNP	Age 20-35 (N=86) no risk	risk	Age <20 (N=13) no risk	risk	Age>35 (N=17) no risk	risk	COR for Age<20 (95% CI)	COR for Age>35 (95% CI)
CYP2E1*5B	84	2	11	2	14	3	7.6 (1.0-59.9)	**9.0 (1.4-58.8)***
*CYP2E1*6*	74	12	11	2	13	4	1.1 (0.2-5.7)	1.9 (0.5-6.8)
*CYP2E1*7B*	72	14	10	3	14	3	1.5 (0.4-6.3)	1.1 (0.3-4.3)
*NQO1*2*	51	35	8	5	7	10	0.9 (0.3-3.0)	2.1 (0.7-6.0)
GSTM1	42	44	5	8	6	11	1.5 (0.5-5.0)	1.8 (0.6-5.2)
GSTT1	61	25	8	5	15	2	1.5 (0.5-5.1)	1.8 (0.6-5.2)

* statistically significant association, $p<0.05$.

The case-only odds ratio for maternal age and combination of two genotypes were also done (data not shown). Maternal age older than 35 years, and co-presence of *CYP2E1*5B* and **6* polymorphisms together showed a significantly 8.5 fold increased risk for childhood ALL (95% CI: 1.3-56.2, p= 0.04). The COR value for maternal age younger than 20 years and co-presence of *CYP2E1*5B* and **6* was 6.7, but was not statistically significant (95% CI: 0.9-52.8, p= 0.10). Interestingly, co-presence of *CYP2E1*5B* and *NQO1*2* polymorphisms and maternal age older than 35 years showed a significantly increased risk for the development of childhood ALL, 16.7-fold (95% CI: 1.3-212.5, p= 0.046).

Information on the paternal age at conception was available for 113 fathers. Among them, only 4 fathers were younger than 20 years of age, which was a very low number to make analyses, for that reason "<20 years of age" grouping was not done. When the paternal age older than 35 years was considered, there was no significant association with neither of the polymorphisms for the risk of childhood ALL (data not shown). The analysis of interaction of the paternal age at conception older than 40 years and genetic polymorphisms for the risk of childhood ALL is presented in Table 3.14. Among 113 fathers considered, 98 of them was younger than 40 years of age (comprised "<40 years" group) while 15 were older than 40 years of age (comprised ">40 years" group).

Paternal age older than 40 years and *CYP2E1*5B* polymorphism was significantly associated with the risk of childhood ALL (COR=5.9, 95% CI: 1.2-29.5, p=0.04). Interaction of paternal age older than 40 years and *CYP2E1*6* also increased the risk of childhood ALL 3.3 fold, but with borderline significance (95% CI: 1.0-11.1, p=0.05).

Table 3.14 Analysis of interaction of single genetic polymorphisms and paternal age at conception as risk factors for development of childhood ALL.

SNP	Age <40 (N=98)		Age >40 (N=15)		COR (95% CI)	*p*
	no risk	risk	no risk	risk		
CYP2E1*5B	**94**	**4**	**12**	**3**	**5.9 (1.2-29.5)**	**0.04***
CYP2E1*6	**85**	**13**	**10**	**5**	**3.3 (1.0-11.1)**	**0.05***
*CYP2E1*7B*	82	16	11	4	1.9 (0.5-6.6)	0.16
*NQO1*2*	57	41	7	8	1.6 (0.5-4.7)	0.15
GSTM1	46	52	7	8	1.0 (0.3-3.0)	0.22
GSTT1	70	28	11	4	0.9 (0.3-3.1)	0.24

* statistically significant association

The co-presence of two polymorphisms were analyzed for their interaction with older (>40 years) paternal age in the risk of ALL (data not shown). Combination of *CYP2E1*5B* and **6* polymorphisms and >40 years of age resulted in significant association (COR=6.4, 95% CI: 1.2-32.7, *p*=0.04). Co-presence of *CYP2E1*6* and *NQO1*2* polymorphisms and >40 years of paternal age also increased the risk of childhood ALL significantly (COR=5.6, 95% CI: 1.2-25.5, *p*=0.04). It should also be pointed out that co-presence of *CYP2E1*5B* and *NQO1*2* and >40 years of paternal age also showed an increased risk of 9.2-fold, however it was not statistically significant (95% CI: 1.1-77.4, *p*= 0.07). Because of the significant association of co-presence of *CYP2E1*5B* and **6*, and also *CYP2E1*6* and *NQO1*2*, the triple combination of these polymorphisms, that is co-presence of *CYP2E1*5B, *6* and *NQO1*2* for the interaction with >40 years of paternal age was examined in the risk of development

of ALL and it was observed that the risk increased to 8.3 fold, however it was not statistically significant (95% CI: 1.0-70.5, p= 0.08).

3.4.2 Interaction of Cigarette Smoking Status of Parents with the Genetic Polymorphisms in Risk of Childhood ALL

Cigarette smoking questionnaires were filled up by 117 parents. The questionnaire gave information on the smoking status of parents, smoking status of mother during pregnancy and postnatal exposure of the children.

Smoking status of the parents was grouped as "non smoker" "passive smoker" or "active smoker". Although information was also available for the duration of passive smoking, for duration and number of cigarettes for active smoking, and for duration of postnatal exposure of child; further categorization under passive or active smoking with respect to dose of smoking/exposure was not done because it would disperse the number of "risk" polymorphisms among too many categories which would prevent healthy analysis.

Among 117 mothers participated, 31 of them were non smokers, 63 of them were passive smokers and 23 of them were active smokers. Case-only odds ratio analysis was done for passive and active smokers against non-smokers for all genetic polymorphisms, but no significant association was observed (data not shown). In other words, neither passive nor active smoking of mother, together with genetic polymorphisms, was a risk factor for childhood ALL. Another exposure model for investigation of smoking status was done by grouping the mothers as "not exposed" which comprised of non smokers (N=31), and "exposed" which involved the mothers who were either passive or active smokers (N=86). The result of the analysis of exposure of mother and

genetic polymorphisms as risk factors for the development of childhood ALL is presented in Table 3.15.

Table 3.15 Analysis of interaction of single genetic polymorphisms and maternal smoking exposure as risk factors for development of childhood ALL.

SNP	not exposed (N=31)		exposed (N=86)		COR (95% CI)	*p*
	no risk	risk	no risk	risk		
*CYP2E1*5B*	29	2	81	5	0.9 (0.2-4.9)	0.33
*CYP2E1*6*	24	7	75	11	0.5 (0.2-1.4)	0.10
*CYP2E1*7B*	26	5	71	15	1.1 (0.4-3.3)	0.86
NQO1*2	**22**	**9**	**44**	**42**	**2.3 (1.0-5.6)**	**0.05***
GSTM1	**19**	**12**	**34**	**53**	**2.5 (1.1-5.7)**	**0.03***
GSTT1	24	7	60	26	1.5 (0.6-3.9)	0.42

* statistically significant association

The COR analyses showed that exposure of mother either passively or by active smoking, together with *GSTM1* null, increased the risk of childhood ALL significantly 2.5 fold (95%CI: 1.1-5.7, *p*=0.03). Exposure of mother and *NQO1*2* polymorphism also showed an association for the risk of ALL, but with borderline significance (COR=2.3, 95% CI: 1.0-5.6, *p*=0.05). When combination of two polymorphisms and exposure of mother was investigated for the risk of childhood ALL, it was observed that co-presence of *NQO1*2* and *GSTM1* null and exposure of mother showed a significantly increased association with risk of childhood ALL as 4.4 fold (95% CI: 1.3-14.4, *p*= 0.01). Also, co-presence of *GSTM1* and *GSTT1* null showed a risk of 3.4-fold, however it was not statistically significant (95% CI: 0.9-13.6, *p*= 0.05).

The analysis of exposure of fathers either passively or as active smoker with genetic polymorphisms for the risk of childhood ALL is presented in Table 3.16. Among 117 fathers, 22 were non smokers, 6 were passive smokers and 89 were active smokers. The analysis for passive or active smoking of father compared to non smokers did not show a significant risk factor for childhood ALL with neither of the polymorphisms. Grouping passive and active smokers under "exposed" title, that is the exposure model, is presented in Table 3.16, but as can be seen from the table, no significant association was present for neither of the genetic polymorphisms. However, when the analysis was done with the combination of two genetic polymorphisms, it was observed that co-presence of *NQO1*2* and *GSTM1* null and paternal exposure to cigarette smoke (either passively or as active smoker) was associated with the risk of childhood ALL significantly (COR=4.1, 95% CI: 1.0-16.7, p=0.04).

Table 3.16 Analysis of interaction of single genetic polymorphisms and paternal smoking exposure as risk factors for development of childhood ALL.

SNP	not exposed (N=22)		exposed (N=95)		COR (95% CI)	p
	no risk	risk	no risk	risk		
*CYP2E1*5B*	21	1	89	6	1.4 (0.2-12.4)	0.40
*CYP2E1*6*	17	5	82	13	0.5 (0.2-1.7)	0.14
*CYP2E1*7B*	19	3	78	17	1.4 (0.4-5.2)	0.23
*NQO1*2*	16	6	50	45	2.4 (0.9-6.7)	0.09
GSTM1	13	9	40	55	2.0 (0.8-5.1)	0.14
GSTT1	18	4	66	29	2.0 (0.6-6.4)	0.11

Another analysis regarding the smoking status was the case of mothers that smoked during pregnancy. Among 117 mothers, 12 of them had smoked during pregnancy, while 105 did not. The analysis of association of maternal smoking status during pregnancy, which was grouped as "smoked" or "not smoked", with genetic polymorphisms as risk factor for development of childhood ALL is presented in Table 3.17. No significant association was observed for neither of the genotypes and maternal smoking during pregnancy for the risk of development of childhood ALL. When the analysis of maternal smoking during pregnancy and co-presence of two polymorphisms were done, again no significant association was found for the risk of development of childhood ALL (data not shown).

Table 3.17 Analysis of interaction of single genetic polymorphisms and maternal smoking during pregnancy as risk factors for development of childhood ALL.

SNP	not smoked (N=105)		smoked (N=12)		COR (95% CI)	*p*
	no risk	risk	no risk	risk		
*CYP2E1*5B*	99	6	11	1	1.5 (0.2-13.6)	0.39
*CYP2E1*6*	89	16	10	2	1.1 (0.2-5.6)	0.31
*CYP2E1*7B*	88	17	9	3	1.7 (0.4-7.0)	0.21
*NQO1*2*	59	46	7	5	0.9 (0.3-3.1)	0.89
GSTM1	47	58	6	6	0.8 (0.2-2.7)	0.73
GSTT1	73	32	11	1	0.2 (0.03-1.7)	0.08

The last analysis for cigarette smoking was the interaction of the postnatal exposure of child to cigarette smoke with genetic polymorphisms for the risk of development of childhood ALL. Postnatal exposure information was present for 115 children, among which 37 of them were not exposed and 78 were exposed to cigarette smoke postnatally. The results of the analysis are presented in Table 3.18.

Table 3.18 Analysis of interaction of single genetic polymorphisms and postnatal exposure of child to cigarette smoke as risk factors for development of childhood ALL.

SNP	not exposed (N=37)		exposed (N=78)		COR (95% CI)	*p*
	no risk	risk	no risk	risk		
*CYP2E1*5B*	33	4	75	3	0.3 (0.1-1.6)	0.12
*CYP2E1*6*	29	8	68	10	0.5 (0.2-1.5)	0.22
*CYP2E1*7B*	29	8	67	11	0.6 (0.2-1.6)	0.31
*NQO1*2*	24	13	41	37	1.7 (0.7-3.7)	0.21
GSTM1	**23**	**14**	**30**	**48**	**2.6 (1.2-5.9)**	**0.02***
GSTT1	28	9	55	23	1.3 (0.5-3.2)	0.56

* statistically significant association

Among six polymorphisms examined, none but *GSTM1* null and postnatal exposure to cigarette smoke was significantly associated with the risk of childhood ALL (COR= 2.6, 95% CI: 1.2-5.9, p=0.02). In the analysis of combination of two polymorphisms, co-presence of *NQO1**2 and *GSTM1* null and postnatal exposure showed an increased risk of 3.6-fold, which was statistically significant (95% CI: 1.2-10.7, p= 0.02).

3.5 CYP2E1 Protein Levels in Lmyphocytes

In order to optimize the conditions for CYP2E1 level in human lymphocytes, first western blot analysis was done using rabbit lymphoctes. Then CYP2E1 protein was determined in two human lymphocyte samples.

The western blot result on rabbit lymphoctes are shown in Figure 3.14. The protein content of rabbit liver microsome was 35.6 mg/mL while protein content of rabbit lymphocte was much lower, 0.92 mg/mL. Accordingly, the amount of lymphocyte protein loaded to the gel was lower than liver microsome protein. As can be seen in Figure 3.14, CYP2E protein was shown in rabbit lymphocytes, and the molecular weight was similar to that of liver protein.

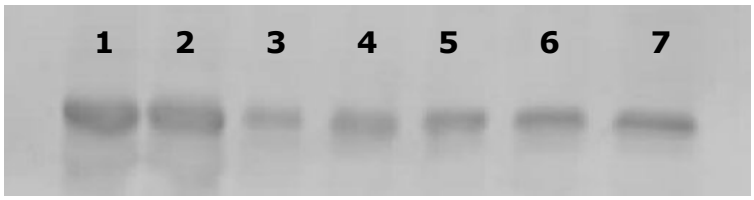

Figure 3.14 CYP2E protein in rabbit lymphoctes. Lanes 1 and 2 contain 10 mg and 15 mg of rabbit liver microsome, respectively. Lanes 3-7 contain rabbit lymphocte samples containing 5µg, 7.5µg, 7.5µg, 10µg ve 15µg protein, in order.

The western blot result for human lymphoctes are given in Figure 3.15. Considering the low protein content of lymphocytes and low amounts of blood that can be taken from human subjects, different

incubation times of 2, 4 and 16 hour was tested for primary antibody. The protein content of rabbit liver microsome was 35.6 mg/mL, while protein contents of human lymphoctes were much lower (0.81 mg/mL for sampleH1, 1.09 mg/mL for sample H2). Both human subjects were healthy individuals with homozygous wild type genotypes for *CYP2E1*5B, *6* and **7B* polymorphisms.

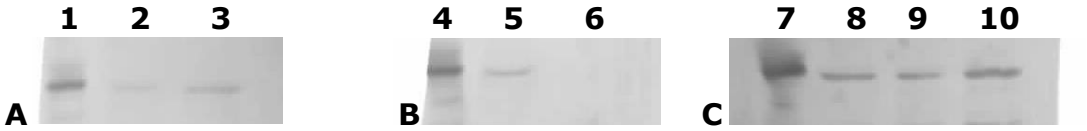

Figure 3.15 CYP2E1 protein in human lymphocytes. **A)** 2 hour incubation in primary antibody. Lane 1, rabbit liver microsome (15 mg); Lane 2, human lymphocte (15 µg, Sample H1), Lane 3, human lymphocyte (25 µg, sample H2). **B)** 4 hour incubation in primary antibody. Lane 4, rabbit liver microsome (15 mg); Lane 5, human lymphocte (15 µg, Sample H1), Lane 6, human lymphocyte (25 µg, sample H2). **C)** 16 hour incubation in primary antibody. Lane 7, rabbit liver microsome (15 mg); Lane 8, human lymphocte (15 µg, Sample H1), Lanes 9 and 10, human lymphocyte (15 µg and 25 µg respectivley, sample H2).

As can be seen from Figure 3.15, CYP2E1 protein was detected in human lymphocytes, and 16 hour incubation in primary antibody yielded better results. The molecular weight of human lymphocyte CYP2E1 protein was nearly the same as rabbit liver microsomal CYP2E protein.

After showing CYP2E1 protein in human lymphocytes, blood samples have been collected from patients with known genotypes between May-September 2008. In average 4 to 6 mL of blood samples could be collected from each patient, the lymphoctye protein contents and genotypes of patients are given in Table 3.19. As can be seen from

the table, the protein content of lymphocytes were too low to make reliable western blot analysis (protein contents were between 0.02-0.36 mg/mL, and 0.92 mg/mL of protein was obtained only from one sample). For that reason, western analysis was not done with lymphocytes of these patients.

Table 3.19 Lymphocyte protein contents and genotypes of childhood ALL patients whose blood was recollected for western blot analysis.

Patient No	Lymphocyte Protein Content (mg/mL)	Genotype		
		*CYP2E1*5B*	*CYP2E1*6*	*CYP2E1*7B*
5	0.044	**1A*1A*	****1A*6***	****1A*7B***
7	0.16	**1A*1A*	**1A*1A*	**1A*1A*
13	0.073	****1A*5B***	****1A*6***	**1A*1A*
18	0.019	**1A*1A*	**1A*1A*	****1A*7B***
22	0.10	****1A*5B***	****1A*6***	**1A*1A*
49	0.15	**1A*1A*	**1A*1A*	**1A*1A*
51	0.92	**1A*1A*	**1A*1A*	**1A*1A*
54	0.055	**1A*1A*	**1A*1A*	****1A*7B***
57	0.21	**1A*1A*	**1A*1A*	**1A*1A*
62	0.027	**1A*1A*	**1A*1A*	**1A*1A*
64	0.27	**1A*1A*	**1A*1A*	**1A*1A*
65	0.046	**1A*1A*	****1A*6***	**1A*1A*
71	0.18	**1A*1A*	**1A*1A*	**1A*1A*
87	0.054	**1A*1A*	**1A*1A*	**1A*1A*
91	Not determined	**1A*1A*	**1A*1A*	**1A*1A*
92	0.059	**1A*1A*	**1A*1A*	**1A*1A*
94	0.36	**1A*1A*	**1A*1A*	****1A*7B***
111	0.15	**1A*1A*	**1A*1A*	**1A*1A*

CHAPTER 4

DISCUSSION

Leukemia is a common type of cancer among children, acute lymphoblastic leukemia being the most frequent (30% of all childhood malignancies) type observed. The multistage development of childhood ALL, as hypothesized by Greaves (1996) suggested an interaction of exposure to environmental factors and inherent genetic susceptibility. Besides, children are thought to be at greater risk to toxic environmental substances from the onset of the prenatal period because of their greater relative exposure, immature metabolism, and higher levels of cell division and growth, as explained in introduction. In this context, understanding the interaction between various predisposing genes and environmental factors in the pathogenesis of childhood ALL is of considerable importance.

Many environmental factors have been investigated as risk factors for the development of leukemia, and among them, benzene was clearly established to be a risk factor (Aksoy *et al.,* 1971; Aksoy *et al.,* 1976; Smith and Zhang, 1998; U.S. EPA, 1998; Ries *et al.,* 1999; OEHHA, 2001). Other environmental chemicals that was associated with risk of leukemia include carbontetrachloride, paint and thinners, solvents, pesticides, neighbouring repair garages and gas stations, perchloroethylene, trichloroethylene, dioxins, benzo[a]pyrene, and cigarette smoke (McKinney *et al*., 1991; Shu *et al*., 1999; Freedman *et*

al., 2001; McKinney *et al*., 2003; Knox, 2005; Menegaux *et al*., 2006; Belson *et al.,* 2007). The overall toxicity of most of these chemicals depend on their metabolism in the body. Therefore this study included the genetic polymorphisms of xenobiotics metabolizing enzymes that are important either in the bioactivation or detoxification of environmental chemicals, namely CYP2E1, NQO1, GSTM1 and GSTT1.

It should be pointed out that the age at diagnosis distribution in the patient sample of this study showed the typical distribution for ALL incidence. The age at diagnosis distribution in the sample of childhood ALL patients in Turkish population of this study and the annual rate in US population is given in Figure 4.1. It is apparent from both graphs that the peak incidence is occurring at the ages of 2-5 and decreasing at higher and lower ages. The distinctive shape of the age-incidence curve for ALL - peak incidence occurring at the ages of 2-5 and then decrease in the incidence- also supported the model of Greaves (1996) which assumed that childhood ALL results from two events required for full malignant transformation with the first of these events occurring *in utero*.

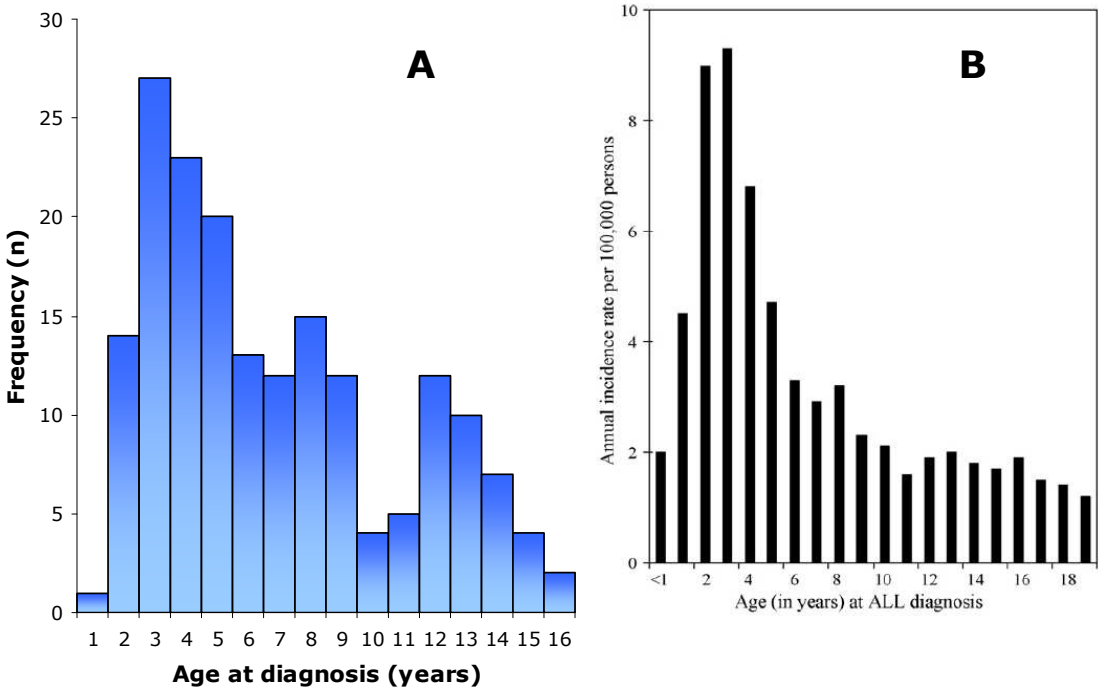

Figure 4.1 Age at diagnosis distribution of children with ALL. **A)** The age distribution in a sample of 185 childhood ALL patients in Turkish population, which was the patient sample of this study. **B)** Graph was taken from Kim *et al.,* (2006) and represents the age specific incidence rates of childhood ALL in U.S, between the years 1993-2002.

The data of this study served to make analyses in three ways; first was the genotype distributions of polymorphisms in healthy control population, which let to determine the genotype frequencies of Turkish population and compare with other ethnicities; second was the case-control analyses that was used to determine the genetic risk factors for childhood ALL, alone or in combinations; lastly case-only analyses were done to investigate the interaction of environmental factors with genetic polymorphisms in the risk of development of childhood ALL. Discussion of those analyses will follow the same order.

Genotype Distributions of *CYP2E1*, *NQO1*, *GSTM1* and *GSTT1* Polymorphisms in Turkish Population and Other Ethnicities

As genetic epidemiological studies also include healthy control subjects, they provide information for the polymorphism frequencies of populations. It is evident from many studies that the frequencies of genetic polymorphisms show variability in different ethnicities, therefore determination of polymorphism frequencies in different populations is of crucial importance.

In general, genetic epidemiological studies comprise a few hundred subjects at most, while determination of exact population frequencies is thought to require thousands of subjects. A solution to determine the population frequencies more precisely is to combine the data from different studies on same populations to obtain high number of subjects that would represent the population or ethnicities. An example is the study of Garte *et al.* (2001), where the data for genotype and allele frequencies of eight metabolic genes from a total of 73 separate studies were gathered together covering 16,000 control subjects to determine the genotype distributions in major races. In this respect, every study adds valuable information to the pool of data, which enables to determine the population frequencies of these polymorphisms more precisely.

It has also been observed that different groups of investigators reported different frequencies for the same gene in the same population. For example, *GSTM1* null frequency was reported to be 18.0% by Pinarbasi *et al.* (2003) in Turkish population, while Aydin-Sayitoglu *et al.* (2006) and Balta *et al.* (2003) reported higher frequencies of 55.0% and 54.6%, respectively, for Turkish population. There were also different frequencies reported for *GSTM1* and *GSTT1* null in Japanese population by different groups. *GSTM1* null frequency was determined around 51%

in Japanese by Naoe *et al.* (2000) and Harada *et al.* (2001), while Sunaga *et al.* (2002) reported a lower frequency of 36.8% for Japanese population. *GSTT1* null frequency in Japanese was shown to be 35.3% and 38.8% by Garte *et al.* (2001) and Sunaga *et al.* (2002), but another group reported a higher *GSTT1* null frequency of 54.0% for Japanese (Naoe *et al.,* 2000). These examples show clearly that it is of crucial importance to verify the genotype frequencies even in the same population.

Control sample of this study comprised of 209 healthy individuals. The blood samples were collected from METU Health Center, Biochemistry Laboratory. METU is among the biggest universities in Turkey, located in the capital city; therefore it receives many students from different regions of the country. As obtained from the birth place information of volunteer and his/her parents, the study sample represented individuals from all seven regions of the country, so the study sample represented the Turkish population (see Figure 3.1).

Genotype Distributions of CYP2E1 Polymorphisms in Turkish Population and Other Ethnicities

Among *CYP2E1* genetic polymorphisms, **5B* is the most commonly studied one; hence data on the genotype distribution of *CYP2E1*5B* polymorphism were the most available, while studies on **6* and **7B* polymorphisms were relatively less common. The genotype distributions of *CYP2E1*5B, *6* and **7B* polymorphisms in Turkish population, using the control population of this study was published previously (the publication included 206 subjects while this study included 209 subjects) (Ulusoy *et al.,* 2007b). Table 4.1 represents the comparison of genotype distributions of *CYP2E1*5B* polymorphism in various populations.

Table 4.1 Population differences in the observed genotypes for *CYP2E1*5B* polymorphism. *P*- values express the genotype frequency comparisons between the respective populations and Turkish population of this study.

Population	N	*CYP2E1*5B* genotype freq.(%) *1A*1A* (c1c1)	*1A*5B* (c1c2)	*5B*5B* (c2c2)	*p*	Reference
Turkish (This study)	**209**	**92.6**	**3.8**	**0.0**		
British Caucosoids	155	96.8	3.2	0.0	NS	Yang *et al.,* 2001
Canadian	302	97.0	3.0	0.0	NS	Krajinovic *et al.,* 2002b
French	172	91.6	4.7	0.0	NS	Bouchardy *et al.,* 2000
German	373	94.3	5.7	0.0	NS	Brockmöller *et al.,* 1996
German	297	94.9	4.4	0.7	NS	Neuhaus *et al.,* 2004
Italian	114	91.0	9.0	0.0	NS	Ingelman-Sundberg *et al.,* 1993
Spanish	390	96.1	5.9	0.0	NS	Bolufer *et al.,* 2007
Turkish	140	95.7	4.3	0.0	NS	Aydin-Sayitoglu *et al.,* 2006
Turkish	153	96.1	3.9	0.0	NS	Omer *et al.,* 2001
Indian	227	98.0	2.0	0.0	NS	Sikdar *et al.,* 2003
Brazilian*	221	89.1	10.4	0.5	0.02	Canalle *et al*., 2004
Brazilian*	191	90.0	9.0	1.0	0.035	Nishimoto *et al.,* 2000
Chilean*	148	71.0	27.0	2.0	<0.0001	Quinones *et al.,* 2001
Mexican-American*	92	70.6	28.3	1.1	<0.0001	Wu *et al.,* 1997
African-American*	114	86.8	12.3	0.9	0.0059	Wu *et al.,* 1997
Chinese*	122	51.6	43.5	4.9	<0.0001	Persson *et al.,* 1999
Chinese*	181	53.6	41.4	5.0	<0.0001	Wang *et al.,* 2003
Japanese*	196	61.2	34.7	4.1	<0.0001	Ogawa *et al.,* 2003
Japanese*	612	63.9	32.0	4.1	<0.0001	Oyama *et al.*, 1997
Taiwanese*	231	58.0	35.1	6.9	<0.0001	Wang *et al.,* 1999

NS indicates that there is no significant difference in genotype frequencies between respective populations and Turkish population of this study.

* Statistically significant difference between respective populations and Turkish population of this study, as determined by χ^2-test, with Yate's correction where necessary.

As can be seen from Table 4.1, there's no significant difference between Turkish population and other white populations like British, Canadian, French, German, Italian and Spanish. The genotype distribution was also similar to Indian population, which is Dravidian in ethnic origin. In those populations, the prevalence of **5B* variant genotypes were very low, such that the variant **5B* allele frequency was between 1.1-4.4% in the above mentioned populations. The variant **5B* allele frequency was found to be 1.9% for Turkish population, in this study and similar to what was observed in this study, it was reported to be 2.0 and 2.1% in Turkish population by other studies (Omer *et al.,* 2000; Aydin-Sayitoglu *et al.,* 2006).

The genotype distribution of Turkish population was significantly different from Latin-American populations like Brazilians, Chileans and Mexican-Americans (Table 4.1). The variant **5B* allele frequency was higher in these populations, 5.5-5.7% in Brazilians, 15.5% in Chileans and 15.2% in Mexican-Americans. The population of Latin America is a composite of ethnic groups and races, Amerindians (aboriginal population of Latin America), African, Asian and European ancestries dominating in different countries. Brazilians presents a mixed population of white, mulatto (mixed European- African ancestry) and black ethnicities, while in Chile, whites and white-Amerindians dominate. Mexican population is predominantly mestizo (mixed European-Amerindian) and Amerindian in origin (https://www.cia.gov/library/publications/the-world-factbook/fields/2075.html, The World Factbook). It could be hypothesized that African ancestry in Brazilians and Amerindian ancestry in Chilean and Mexican-American populations might be reflected in the higher *CYP2E1*5B* allele frequency in those populations compared to other Caucasian populations.

*CYP2E1*5B* genotype distribution of Turkish population was also significantly different from African-Americans, the variant **5B* allele frequency being higher in African-Americans (7.0%). The similarity of genotype distributions and variant **5B* allele frequencies of African-Americans and Brazilians should also be pointed out (see Table 4.1 for genotype distributions; allele frequencies: 7.0% and 5.5-5.7%, respectively), considering the partial African ancestry in Brazil.

There is major difference between Asian and Caucasian populations in frequency of xenobiotics metabolizing enzymes (Garte *et al.,* 2001). Accordingly, *CYP2E1*5B* genotype distribution in Turkish population was found to be significantly different from Asian populations like Chinese, Japanese and Taiwanese. The **5B* variant allele frequency was considerably high in Asian populations, ranging between 20.1-26.6% in above mentioned Asian populations, while it was 1.9% in Turkish population.

Table 4.2 presents the genotype distributions of *CYP2E1*6* polymorphism in various populations and comparison with Turkish population of this study.

As was the case for *CYP2E1*5B* polymorphism, *CYP2E1*6* genotype distribution in Turkish population was similar to other Caucasian populations like British, Finnish, French, German and Italians. In this study, **6* variant allele frequency was found to be 8.1% for Turkish population, and a very similar frequency -8.2%, was also reported by Omer *et al.* (2001). The **6* variant allele frequency was between 6.4-10.7% in above mentioned populations.

Table 4.2 Population differences in the observed genotypes for *CYP2E1*6* polymorphism. *P-* values express the genotype frequency comparisons between the respective populations and Turkish population of this study.

Population	N	*CYP2E1*6* genotype freq.(%) *1A*1A* (DD)	*1A*6* (DC)	*6*6* (CC)	*p*	Reference
Turkish (This study)	**209**	**84.2**	**15.3**	**0.5**		
British Caucosoids	155	83.2	16.1	0.7	NS	Yang *et al.,* 2001
Finnish	121	79.0	20.0	1.0	NS	Hirvonen *et al.,* 1993
French	172	87.8	11.6	0.6	NS	Bouchardy *et al.,* 2000
German	373	87.3	12.4	0.3	NS	Brockmöller *et al.,* 1996
German	236	83.1	16.5	0.4	NS	Neuhaus *et al.,* 2004
Italian	114	83.0	17.0	0.0	NS	Ingelman-Sundberg *et al.,* 1993
Turkish	153	84.3	15.0	0.7	NS	Omer *et al.,* 2001
Indian*	227	64.8	32.1	3.1	<0.0001	Sikdar *et al.,* 2003
Chilean*	129	63.6	31.0	5.4	<0.0001	Quinones *et al.,* 2001
Mexican-American*	104	72.1	24.0	3.9	0.01	Konishi *et al.,* 2003
Chinese*	122	48.4	46.7	4.9	<0.0001	Persson *et al.,* 1999
Japanese*	76	56.6	28.9	14.5	<0.0001	Uematsu *et al.,* 1994
Taiwanese*	231	53.7	37.7	8.6	<0.0001	Wang *et al.,* 1999

NS indicates that there is no significant difference in genotype frequencies between respective populations and Turkish population of this study.

* Statistically significant difference between respective populations and Turkish population of this study, as determined by χ^2-test, with Yate's correction where necessary.

The *CYP2E1*6* genotype distribution was again different from Chilean and Mexican-Americans. The frequency of this variant allele was higher in these Latin American populations (20.9% in Chileans and 15.9% in Mexican-Americans). Prevalence of **6* variant allele was considerably higher in Indian population (19.2%), and the genotype distribution was significantly different from Turkish population. *CYP2E1*5B* genotype distribution, on the other hand, was not different between Turkish and Indian populations (see Table 4.1).

As expected, *CYP2E1*6* genotype distribution in Turkish population was significantly different from Asian populations like Chinese, Japanese and Taiwanese, the variant allele frequency being much more higher in those populations, ranging between 27.5-28.9%.

The last variant for *CYP2E1* was **7B* variant, and genotype distributions of this variant in different populations and comparison with Turkish population is presented in Table 4.3. *CYP2E1*7B* is a relatively recently discovered polymorphism compared to other variants, hence studies on this polymorphism were low in number. Information was available for only some Caucasian populations, like British, German and Northern European where no significant difference was present between them and Turkish population. The **7B* variant allele frequency ranged between 4.1-7.1% in above mentioned populations, and it was found to be 6.7% for Turkish population in this study.

Table 4.3 Population differences in the observed genotypes for *CYP2E1*7B* polymorphism. *P*- values express the genotype frequency comparisons between the respective populations and Turkish population of this study.

		*CYP2E1*7B* genotype freq.(%)				
Population	N	**1A*1A* (GG)	**1A*7B* (GT)	**7B*7B* (TT)	*p*	Reference
Turkish (This study)	**209**	**87.1**	**12.4**	**0.5**		
British Caucosoids	155	90.3	9.0	0.7	NS	Yang *et al.,* 2001
German	56	85.7	14.3	0.0	NS	Thier *et al.,* 2002
German	299	92.6	7.4	0.0	NS	Neuhaus *et al.,* 2004
Northern European	115	89.6	10.4	0.0	NS	Fairbrother *et al.,* 1998
Swedish	37	91.9	8.1	0.0	NS	Ernstgard *et al.,* 2004

NS indicates that there is no significant difference in genotype frequencies between respective populations and Turkish population of this study.

It should be pointed out that, previously no information was available for the genotype distribution of *CYP2E1*7B* polymorphism in Turkish population, so *CYP2E1*7B* genotype frequencies in Turkish population was determined for the first time by the control population of this study.

In summary, it could be said that *CYP2E1* polymorphisms in Turkish population showed similar genotype distributions with other white populations, but differed significantly from Asian and Latin American populations.

*Genotype Distributions of NQO1*2 Polymorphism in Turkish Population and Other Ethnicities*

The genotype distribution of *NQO1*2* polymorphism in various populations and comparison between different populations with Turkish population of this study is presented in Table 4.4. Information on *NQO1*2* polymorphism was available for several white and Asian populations, but information for African populations was not available.

The variant *NQO1*2* frequency was determined to be 26.7% for Turkish population in this study. Previously, Sirma *et al.* (2004) also reported a similar frequency (24.8%) for Turkish population. The genotype distribution of *NQO1*2* in Turkish population was not significantly different from other white populations like British, French, Spanish and US populations, as well as Hawaiians (who are Polynesian in origin), although the variant *NQO1*2* allele frequency was higher, but not significantly, in Turkish population when compared (the variant allele frequency was between 17.0-22.1% for above mentioned populations).

Table 4.4 Population differences in the observed genotypes for *NQO1*2* polymorphism. *P-* values express the genotype frequency comparisons between the respective populations and Turkish population of this study.

Population	N	*NQO1*2* genotype freq.(%) *1*1* (CC)	*1*2* (CT)	*2*2* (TT)	*p*	Reference
Turkish (This study)	**209**	**58.4**	**35.9**	**5.7**		
British (UK)	100	67.0	32.0	1.0	NS	Wiemels *et al.,* 1999c
French	210	64.8	31.4	3.8	NS	Longuemaux *et al.,* 1999
Hawaii Caucasian	171	61.4	36.3	2.3	NS	Chen *et al.,* 1999
Spanish	447	59.9	35.8	4.3	NS	Bolufer *et al.,* 2007
Turkish	286	53.8	42.7	3.5	NS	Sirma *et al.,* 2004
US white	258	67.5	29.0	3.5	NS	Kiffmeyer *et al.,* 2004
US	1123	65.3	31.0	3.7	NS	Xu *et al.,* 2001
Hawaiian	102	58.8	38.2	2.9	NS	Chen *et al.,* 1999
Chinese*	84	31.0	48.8	20.0	<0.0001	Yin *et al.,* 2001
Hmong*	198	12.0	54.0	34.0	<0.0001	Kiffmeyer *et al.,* 2004
Hawaii Japanese*	167	38.3	46.7	15.0	<0.0001	Chen *et al.,* 1999
Japanese*	150	34.0	55.3	10.6	<0.0001	Naoe *et al.,* 2000
Japanese*	152	34.2	50.7	15.1	<0.0001	Sunaga *et al.,* 2002
Japanese*	197	44.7	42.6	12.7	0.006	Eguchi-Ishimae *et al.,* 2005

NS indicates that there is no significant difference in genotype frequencies between respective populations and Turkish population of this study.

* Statistically significant difference between respective populations and Turkish population of this study, as determined by χ^2-test, with Yate's correction where necessary.

The *NQO1*2* variant allele frequency was much higher in Asian populations like Chinese and Japanese, ranging between 34.0-44.6%. The genotype distributions of these Asian populations were significantly different from Turkish population. Interesting information was available for Hmong population from the study of Kiffmeyer *et al.* (2004). As can be seen from Table 4.4, the frequency of *NQO1*2* variant carrying genotypes was strikingly high, and the homozygous wild type (**1*1*) genotype frequency was accordingly very low (12%) in Hmong

population. The genotype frequency of this population was also significantly different from Turkish population, the variant *NQO1*2* allele frequency being as high as 61.1%. As Kiffmeyer *et al*. (2004) reported, Hmongs are an isolated ethnic group that settled in the mountainous regions of what today are Vietnam, Cambodia, and Laos. At the conclusion of the Vietnam War, many Hmong were relocated from refugee camps in Thailand to the United States and settled in New Jersey, Texas, Wisconsin, California, and Minnesota, and the study was conducted on Hmongs in Minnesota.

In summary *NQO1*2* genotype distribution of Turkish population was similar to other white populations, and significantly different from Asian populations.

Genotype Distributions of GSTM1 and *GSTT1 Null in Turkish Population and Other Ethnicities*

GSTM1 and *GSTT1* null have been studied widely throughout the world, hence information on many populations were available. As mentioned before, *Garte* et al. (2001) have combined many studies from the International Project on Genetic Susceptibility to Environmental Carcinogens (GSEC) database, and determined the polymorphism frequencies from many populations with higher number of samples. *GSTM1* and *GSTT1* null were among the selected polymorphisms, hence information on these polymorphism frequencies of various populations from the combined studies were also available. Besides individual studies, the frequency data obtained by Garte *et al.* (2001) were also presented in the following Tables 4.5 and 4.6, by referring the author.

Table 4.5 presents the *GSTM1 null* frequencies in various populations and comparison of frequencies between these populations and Turkish population of this study. Noticeably, there are inconsistencies for the allele frequencies of some populations from different study groups, like Turkish and Japanese populations (Table 4.5). As mentioned before, this situation points out the importance of conducting multiple studies by different groups, even for the same population.

Unlike other xenobiotic metabolizing enzyme polymorphisms examined, *GSTM1* null showed significance variations among white populations also. The *GSTM1* null genotype frequency was reported to be 59.3% for Turkish population in this study. Aydin-Sayitoglu *et al.* (2006) Balta *et al*. (2003) and Ada *et al.* (2004) also reported similar null allele frequencies (55.0%, 54.6% and 51.9%, respectively) for Turkish population but another study (Pinarbasi *et al.,* 2003) had reported a much lower null allele frequency of 18.0%, which was significantly different from other studies on Turkish population.

The *GSTM1* null genotype frequency in Turkish population of this study showed no difference with some of the white populations including British, Canadian, Danish, French, German, Portuguese, Slovakian, Slovenian, Spanish, Swedish and US; also no difference was observed with Saudi Arabians. The null allele frequency ranged between 51.2-58.3% in the above mentioned populations. However, there was significant difference between Turkish and some other white populations like Finnish, Norwegian, Dutch and Italian populations, which had lower *GSTM1* null frequencies (46.9%-50.6%). Other populations that show significantly low frequency of *GSTM1* null when compared to Turkish population are Indian (24.6%), Iranian (32.0%), and Brazilian (45.7%) populations, as can be seen from Table 4.5.

Table 4.5 Population differences in the observed genotypes for *GSTM1 null*. *P*-values express the genotype frequency comparisons between the respective populations and Turkish population of this study.

Population	N	*GSTM1* freq. (%) present	null	*p*	Reference
Turkish (This study)	**209**	**40.7**	**59.3**		
British (UK)	1122	42.2	57.8	NS	Garte *et al.,* 2001
Canadian	304	48.7	51.3	NS	Garte *et al.,* 2001
Canadian	304	48.7	51.3	NS	Krajinovic *et al.,* 1999
Danish	537	46.4	53.6	NS	Garte *et al.,* 2001
Dutch*	419	49.6	50.4	0.034	Garte *et al.,* 2001
Finnish*	482	53.1	46.9	0.0027	Garte *et al.,* 2001
French	1184	46.6	53.4	NS	Garte *et al.,* 2001
German	734	48.4	51.6	NS	Garte *et al.,* 2001
Italian	810	50.6	49.4	0.01*	Garte *et al.,* 2001
Norwegian*	423	49.4	50.6	0.038	Garte *et al.,* 2001
Portuguese	501	41.7	58.3	NS	Garte *et al.,* 2001
Slovakian	332	48.8	51.2	NS	Garte *et al.,* 2001
Slovenian	102	48.0	52.0	NS	Garte *et al.,* 2001
Spanish	451	48.6	51.4	NS	Bolufer *et al.,* 2007
Swedish	544	44.1	55.9	NS	Garte *et al.,* 2001
Turkish*	206	82.0	18.0	<0.0001	Pinarbasi *et al.,* 2003
Turkish	140	45.0	55.0	NS	Aydin-Sayitoglu *et al.,* 2006
Turkish	185	45.5	54.6	NS	Balta *et al.,* 2003
US	1751	45.7	54.3	NS	Garte *et al.,* 2001
US white	267	46.5	53.5	NS	Kiffmeyer *et al.,* 2004
US white	213	46.5	53.5	NS	Chen *et al.,* 1997
US white	532	46.0	54.0	NS	Davies *et al.,* 2002
Brazilian*	221	54.3	45.7	0.0047	Canalle *et al.,* 2004
Indian*	118	75.4	24.6	<0.0001	Joseph *et al.* 2004
Iranian*	75	68.0	32.0	<0.0001	Saadat and Saadat, 2000
Saudi Arabian	895	43.7	56.3	NS	Garte *et al.,* 2001

table continued on the next page

Table 4.5 continued

Population	N	*GSTM1* freq. (%) present	null	*p*	Reference
Turkish (This study)	**209**	**40.7**	**59.3**		
US black*	203	72.4	27.6	<0.0001	Chen *et al.,* 1997
US black*	201	68.0	32.0	<0.0001	Davies *et al.,* 2002
Hmong*	200	18.5	81.5	<0.0001	Kiffmeyer *et al.,* 2004
Japanese	150	48.7	51.3	NS	Naoe *et al.,* 2000
Japanese	100	49.0	51.0	NS	Harada *et al.,* 2001
Japanese*	152	63.2	36.8	<0.0001	Sunaga *et al.,* 2002
Japanese*	639	52.4	47.6	0.0032	Garte *et al.,* 2001
Korean	165	47.9	52.1	NS	Garte *et al.,* 2001
Singaporean	244	43.8	56.2	NS	Garte *et al.,* 2001
Thai	320	40.3	59.7	NS	Pakakasama *et al.,* 2005

NS indicates that there is no significant difference in genotype frequencies between respective populations and Turkish population of this study.

* Statistically significant difference between respective populations and Turkish population of this study, as determined by χ^2-test, with Yate's correction where necessary.

When Turkish population of this study and African populations were compared, a significant difference was observed in *GSTM1* null frequency, being lower in African-American populations (27.6 and 32.0% in US blacks, 59.3% in Turkish population).

Interestingly, there was no significant difference in *GSTM1* null frequencies in Turkish population and some Asian populations like Korean, Singaporean, Thai and Japanese, allele frequency ranging between 51.0-59.7% in those Asian populations. However, two studies conducted on Japanese population yielded significantly lower null allele frequencies (47.6 and 36.8%) compared to Turkish population (Garte e*t al.,* 2001; Sunaga *et al.,* 2002). Hmong population, with strikingly high

GSTM1 null frequency of 81.5%, was also significantly differed from Turkish population of this study.

In summary, there occurs a wide heterogeneity in *GSTM1* null frequency in different ethnicities, and in different populations of same ethnic origin. *GSTM1* null frequency was higher in Turkish population when the populations that possess significant difference was considered, except the Hmong population with higher null allele frequency than Turkish population.

The *GSTT1* null frequencies in different populations and comparison with the Turkish population of this study is presented in Table 4.6. In the case of *GSTT1* null, less heterogeneity is observed among different populations, compared to *GSTM1* null. However still, some inconsistent results were present, for example in Spanish and Japanese populations (Table 4.6). The *GSTT1* null was reported to be 21.1% for Turkish population in this study, and similar frequencies as 22.7%, 20.7% and 17.3% were also reported for Turkish population in previous studies (Balta *et al.,* 2003; Ada *et al.,* 2004; Aydin-Sayitoglu *et al.,* 2006).

In general, *GSTT1* null frequency of Turkish population and other white populations, like British, Canadian, Dutch, French, German, Italian, Portuguese, Slovakian, Slovenian, Spanish and US were similar. But it should be noted that two studies on Spanish population gave different frequencies for null allele, such that one study with 20.5% null allele frequency showed similarity to Turkish population (Garte *et al.,* 2001), while another with 13.4% null allele frequency differed significantly (Bolufer *et al.,* 2007). Interestingly, Nordic populations -Danish, Finnish and Swedish, had significantly lower *GSTT1* null frequencies (13%) compared to Turkish population of this study (21.1%).

Table 4.6 Population differences in the observed genotypes for *GSTT1* null. *P*-values express the genotype frequency comparisons between the respective populations and Turkish population of this study.

Population	N	*GSTT1* freq. (%) present	null	*p*	Reference
Turkish (This study)	**209**	**78.9**	**21.1**		
British (UK)	422	79.5	20.5	NS	Garte *et al.,* 2001
Canadian	274	82.2	17.2	NS	Garte *et al.,* 2001
Canadian	274	82.8	17.2	NS	Krajinovic *et al.,* 1999
Danish*	358	87.1	12.9	0.0099	Garte *et al.,* 2001
Dutch	419	77.1	22.9	NS	Garte *et al.,* 2001
Finnish*	385	87.0	13.0	0.01	Garte *et al.,* 2001
French	512	83.2	16.8	NS	Garte *et al.,* 2001
German	487	80.5	19.5	NS	Garte *et al.,* 2001
Italian	553	83.7	16.3	NS	Garte *et al.,* 2001
Portuguese	102	74.5	25.5	NS	Alves *et al.,* 2002
Slovakian	322	82.0	18.0	NS	Garte *et al.,* 2001
Slovenian	102	74.5	25.5	NS	Garte *et al.,* 2001
Spanish	312	79.5	20.5	NS	Garte *et al.,* 2001
Spanish*	455	86.6	13.4	0.012	Bolufer *et al.,* 2007
Swedish*	423	87.0	13.0	0.0088	Garte *et al.,* 2001
Turkish	140	79.3	20.7	NS	Aydin-Sayitoglu *et al.,* 2006
Turkish	185	77.3	22.7	NS	Balta *et al.,* 2003
US	286	72.4	27.6	NS	Garte *et al.,* 2001
US white	213	85.0	15.0	NS	Chen *et al.,* 1997
US white	270	82.5	17.5	NS	Kiffmeyer *et al.,* 2004
US white	532	84.0	16.0	NS	Davies *et al.,* 2002
Brazilian	221	80.5	19.5	NS	Canalle *et al.,* 2004
Indian*	118	91.5	8.5	0.0033	Joseph *et al.,* 2004

table continued on the next page

Table 4.6 continued

Population	N	*GSTT1* freq. (%) present	null	*p*	Reference
Turkish (This study)	**209**	**78.9**	**21.1**		
US black	203	75.9	24.1	NS	Chen *et al.,* 1997
US black	201	72.0	28.0	NS	Davies *et al.,* 2002
Hmong*	200	39.0	61.0	<0.0001	Kiffmeyer *et al.,* 2004
Japanese*	167	64.7	35.3	0.002	Garte *et al.,* 2001
Japanese*	150	46.0	54.0	<0.0001	Naoe *et al.,* 2000
Japanese*	152	61.2	38.8	0.0002	Sunaga *et al.,* 2002
Korean*	165	48.5	51.5	<0.0001	Garte *et al.,* 2001
Singaporean*	243	48.1	51.9	<0.0001	Garte *et al.,* 2001
Thai*	320	61.9	38.1	<0.0001	Pakakasama *et al.,* 2005

NS indicates that there is no significant difference in genotype frequencies between respective populations and Turkish population of this study.
* Statistically significant difference between respective populations and Turkish population of this study, as determined by χ^2-test, with Yate's correction where necessary.

Brazilian population –the Latin American population, which was shown to be different for other polymorphisms investigated, was not significantly different from Turkish population with respect to *GSTT1* null. Indians had significantly low *GSTT1* null frequency (8.5%) compared to Turkish population. The African originated population frequencies for *GSTT1* null (24.1 and 28.0%, Table 4.6) were similar to that of Turkish population. Interestingly, the *GSTM1* null was significantly different in black populations compared to Turkish population (Table 4.5).

Asian populations –Hmong, Japanese, Korean, Singaporean and Thai, all possessed significantly higher frequencies for *GSTT1* null, ranging between 35.3-61.0%, compared to Turkish and other white populations. It should be noted that, among three studies presented in

Table 4.6 for Japanese population, there is a heterogeneity in null allele frequencies so that Naoe *et al.* (2000) reported the frequency as 54.0%, while other two studies reported much lower frequencies (35.3% and 38.8%) for Japanese population (Garte *et al.,* 2001; Sunaga *et al.,* 2002).

As a summary, *GSTT1* null frequency in Turkish population showed similarity with most white populations and black populations, while differed significantly from Nordic populations (null allele frequency being higher in Turkish population), and Asian populations (null allele frequency being higher in Asian populations).

The analyses on control sample of this study revealed information on the genotype distribution of *CYP2E1*5B, *6,*7B, NQO1*2, GSTM1* null and GSTT1 null in Turkish population, *CYP2E1*7B* being the first time. As discussed above, major differences were observed among Asian and African ethnicities, besides, the comparisons showed that Latin-American and Nordic populations also derive attention for their different genotype distributions. Although some information was available for Indian, Iranian and Arabic populations, still it is not possible to make generalizations about comparison with these ethnicities, because of low number of studies in these populations.

Genetic Polymorphisms of *CYP2E1, NQO1, GSTM1* and *GSTT1,* Alone or in Combination, as Risk Factors for the Development of Childhood Acute Lymphoblastic Leukemia: Case-Control Analyses

As examined above in detail, genetic polymorphism frequencies in xenobiotics enzymes show variability among different populations and ethnicities, which mean the genetic susceptibility of different populations towards procarcinogens could be different. Currently, much of the

information on genetic epidemiologic studies were generated in either Europe or North America, hence information on other parts of the world still needs to be elucidated (Ramanakumar, 2007). In this respect, even the number of such studies had increased in recent years for Turkey, still more research is needed that would provide valuable information on the risk assessment regarding our population.

Genetic epidemiological studies on case-control analyses can be designed in two ways, one is to match the age and gender of the subjects in case and control groups, so that effect of age and gender can be examined as parameters besides the genetic risk factors, as several groups have studied for leukemia (Infante-Rivard *et al.,* 2002a; Balta *et al.,* 2003; Joseph *et al.,* 2004). However, as finding control subjects that match for gender and age with case subjects is practically is not easy, especially for the children, and many studies use databanks to gather control population or use control samples of previous studies; many research depended on case-control analyses that do not strict control subjects to case subjects in terms of age and gender, and that is the second way of case-control study design. Greater number of studies were available for genetic epidemiological studies of leukemia, that had unmatched case-control groups (Krajinovic *et al.,* 1999; Alves *et al;* 2002; Davies *et al.,* 2002; Pakakasama *et al.,* 2005; Canalle *et al.,* 2004; Aydin-Sayitoglu *et al.,* 2006; Bolufer *et al.,* 2007).

In this study, an unmatched case-control design was used, mainly because of the difficulty to find healthy children, especially of ages smaller than five, which made up the larger proportion of the patient group (see Figure 4.1 for age distribution of patient group). Rather, a control sample which was previously used to determine the *CYP2E1* polymorphism frequencies in Turkish population (Ulusoy, 2004; Ulusoy *et al.,* 2007b), was used as the control group of this study. Because the

study was designed on unmatched controls and cases, analysis for the risk of age and gender together with genetic polymorphisms were not done.

This study focused on four xenobiotic metabolizing genes, *CYP2E1, NQO1, GSTM1* and *GSTT1*, which are important in the metabolism of many environmental chemicals, for the risk of development of childhood ALL. Case-control analyses were done on 209 healthy subjects and 185 patients with childhood ALL, and the effect of these four genes, and a total of six polymorphisms, were investigated as risk factors either alone, or in combinations. It should also be noted that the risk analyses for *CYP2E1*5B, *6* and **7B* polymorphisms for development of childhood ALL was published before using a part of this study subjects (207 control and 168 patient subjects) (Ulusoy *et al.,* 2007a).

As described in detail under results section, the genotypes for each polymorphism was grouped as "risk" or "no risk", and odds ratio was calculated for each single polymorphism, and combinations (co-presence) of polymorphisms. The results were presented in Tables 3.6 through 3.10, but for the ease of discussion, the situations that were found to be important for risk analysis are summarized below, in Table 4.7.

As can be seen, when single polymorphisms were examined, only *GSTT1* null was shown to be a risk factor for childhood ALL (OR= 1.8, 95% CI: 1.1-2.8, p=0.01). None of the *CYP2E1* polymorphisms were associated with childhood ALL, when examined alone. However, *CYP2E1* polymorphisms turned out to be important as risk factors when multi-locus analyses were examined.

Table 4.7 Analyses of single nucleotide polymorphisms, alone or in combination, as risk factors for the development of childhood ALL. The table was derived from Tables 3.6 to 3.10.

SNPs[a]	Control (N=209)		Patient (N=185)		OR (95% CI)	*p*
	no risk	risk	no risk	risk		
Single SNP						
*CYP2E1*5B*	201	8	173	12	1.7 (0.7-4.4)	0.23
*CYP2E1*6*	176	33	153	32	1.1 (0.7-1.9)	0.69
*CYP2E1*7B*	182	27	153	32	1.4 (0.8-2.5)	0.22
GSTT1	**145**	**44**	**125**	**60**	**1.8 (1.1-2.8)**	**0.01***
Two SNPs						
****5B-*6***	**173**	**5**	**153**	**12**	**2.7 (0.9-7.9)**	**0.04***
**5B-T1*	159	2	119	6	4.0 (0.8-20.2)	0.07
****6-T1***	**136**	**4**	**106**	**13**	**4.2 (1.3-13.2)**	**0.02***
****7B-T1***	**141**	**3**	**102**	**9**	**4.1 (1.1-15.7)**	**0.03***
Three SNPs						
****5B-*6-T1***	**134**	**1**	**106**	**6**	**7.6 (0.9-64.0)**	**0.04***

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

* statistically significant association.

As mentioned previously, all patients that carried a variant *CYP2E1*5B* allele also carried the **6* variant, which was not the case in control group. In multi-locus analysis, it was observed that co-presence of *CYP2E1*5B* and **6* alleles increased the risk of individuals 2.7 fold, (95% CI: 0.9-7.9, p=0.04). This pointed out a combined effect for *CYP2E1*5B* and **6* polymorphisms. Supporting the importance of *CYP2E1* polymorphisms for childhood ALL risk, co-presence of *GSTT1* null and any

of *CYP2E1* polymorphisms increased the risk of childhood ALL 4.0 fold or more. Although co-presence of *GSTT1* and *CYP2E1*5B* was not significant, co-presence of *GSTT1* and *CYP2E1*6* or *GSTT1* and *CYP2E1*7B* both increased the risk significantly to 4.2 and 4.1 fold, respectively. Moreover, co-presence of *GSTT1* null, *CYP2E1*5B* and **6* alleles together increased the risk of development of childhood ALL strikingly, 7.6 fold (95% CI: 0.9-64.0, p= 0.04). It should also be noted that copresence of *CYP2E1*5B* and *NQO1*2* polymorphisms were associated with the risk of childhood ALL with borderline significance (OR=4.1, 95%CI: 0.8-20.2, p=0.06).

As mentioned in more detail previously, CYP2E1 activates many procarcinogens like styrene, benzene, vinyl chloride, acrylamide, pyridine, nitrosamines etc. into carcinogenic forms (Garro *et al.,* 1981; Guengerich *et al.,* 1991; Gonzalez and Gelboin, 1994; Arınç *et al.*, 2000a,b; Bolt *et al.,* 2003; Nuyan, 2008). Also CYP2E1 has a unique capability to reduce molecular oxygen, resulting in the generation of reactive oxygen species, which eventually could cause DNA damage and carcinogenesis (Elkstrom *et al.,* 1986; Cederbaum, 1987; Parke, 1987, 1994; Terelius *et al.,* 1993; Kukielka and Cederbaum, 1994; Ioannides *et al.,* 1995). Therefore, CYP2E1 is a dangerous enzyme for development carcinogenesis, and its expression at higher levels due to polymorphisms could increase the risk of development of cancer. On the other hand, GSTT1 has important roles in detoxification of many carcinogens involving epoxybutanes, ethylene oxide, halomethanes, and methyl bromide for GSTT1 (Rebbeck, 1997), and in protection against oxidative stress. So *GSTT1*null, which leads to lack of enzyme expression, would increase the individuals risk for cancer development.

In the literature, there were contradictory results for the association of genetic polymorphisms on the risk of development of childhood ALL. While some studies on *CYP2E1*5B* polymorphism found no association with childhood ALL (Canalle *et al.,* 2004; Bolufer *et al.,* 2007), some others found significant association, one of them being conducted in Turkish population (Krajinovic *et al.,* 2002b; Aydin-Sayitoglu *et al.,* 2006). Also, case only studies have showed significant association of *CYP2E1*5B* polymorphism with risk of childhood ALL in the case of prenatal exposure to trihalomethanes and maternal alcohol consumption (Infante-Rivard *et al.,* 2002a, b).

It should be noted that studies regarding *CYP2E1* polymorphisms and childhood ALL have focused on **5B* variant only. However, this study found an interaction between **5B* and **6* alleles in patient groups, and association with risk of childhood ALL was observed only for the case of co-presence of **5B* and **6* alleles (OR=2.7, 95% CI: 0.9-7.9, p=0.04), while individual *CYP2E1* polymorphisms studied showed no significant association (Table 4.7). The polymorphisms on the *CYP2E1* gene are likely to increase the expression or inducibility of the enzyme rather than changing the protein structure. Therefore co-presence of multiple SNPs on *CYP2E1* gene would be more effective in altering the expression level of the enzyme than presence of single polymorphisms. This study presented the first work in the literature that showed a haplotype (**5B* and **6*) of *CYP2E1* was associated with the risk of childhood ALL, and *CYP2E1*6* and **7B* polymorphisms possessed a risk together with *GSTT1* null.

Regarding the studies on *GSTT1* null and childhood ALL risk, Bolufer *et al.* (2007) reported a 2.15 fold increased risk for childhood ALL, and Infante-Rivard *et al.* (2002a) reported a significant risk of 9.1 fold for post natal exposure to high levels of trihalomethanes. In this

study, *GSTT1* null was found to increase the childhood ALL risk 1.8 fold (95% CI: 1.1-2.8, p=0.01, Table 4.7).

When *NQO1*2* polymorphism is considered for the risk of childhood ALL, similar to the results of this study, Sirma *et al.* (2004) also did not find an association between *NQO1*2* polymorphism and risk of childhood ALL in Turkish population. Another study conducted in Japanese also resulted in no association (Eguchi-Ishimae *et al.,* 2005), while other studies found increased risk of 1.7 to 4.2 fold (Krajinovic *et al.,* 2002b; Lanciotti *et al.,* 2005; Bolufer *et al.,* 2007). But it should be noted that in this study, copresence of *NQO1*2* and *CYP2E1*5B* polymorphisms was associated with 4.1 fold increased risk of childhood ALL, with borderline significance.

Although there were genetic epidemiological studies on the risk of childhood ALL in Turkish population (Balta *et al.,* 2003; Sirma *et al.,* 2004; Aydin-Sayitoglu *et al.,* 2006), none of them investigated the risk of co-presence of genetic polymorphisms. In this respect, this study presented the first effort in Turkish population, on the association of combination of genes for the risk of childhood ALL, and showed that co-presence of *GSTT1* null and *CYP2E1* polymorphisms had a combined effect on the risk of childhood ALL, increasing the risk from 1.8 fold (risk of *GSTT1* null alone) to approximately 4 fold (co presence of *GSTT1* and *CYP2E1*6* or *GSTT1* and *CYP2E1*7B*), and even much higher to 7.6 fold (*GSTT1* and *CYP2E1*5B* and **6*) (Table 4.7). It should also be noted that, in this respect, this study is the first one not only in Turkish population, but also in the global scientific literature in the sense that it showed interaction of *GSTT1* and *CYP2E1* polymorphisms and *CYP2E1**5B and *CYP2E1*6* alleles for the risk of childhood ALL.

This study also aimed to investigate the CYP2E1 protein levels in lymphocytes of individuals with different *CYP2E1* genotypes. For that reason, western blot analysis was done on human lymphocytes and it was shown that lymphocytes express CYP2E1 protein. The molecular weight of the protein was similar to rabbit liver microsomal CYP2E, which was around 51 kDa (Arslan, 2003). Similar results were obtainen by other studies on human lymphocyte CYP2E1 protein expression (Hannon-Fletcher *et al.,* 1997; Raucy *et al.,* 1997). Blood samples were collected from patients with known *CYP2E1* genotypes, however the amount of protein in lymphocyte samples was not enough to make reliable western blot analysis. Because low amount of blood samples were collected as the chemotherapy also prevented angiogenesis in the patients. Also, the white blood cell numbers were generally reduced in the patients, due to the disease or chemotherapy.

Interaction of Non-genetic Factors and Genetic Polymorphisms on the Risk of Development of Childhood ALL: Case-Only Analyses

Risk factors for childhood ALL does not include only genetic polymorphisms, non-genetic risk factors have also been defined for the disease, as explained previously in introduction part. Hence, interaction of these non-genetic risk factors with genetic polymorphisms would also provide valuable information in terms of disease etiology. The case-only analyses of this study searched for these interactions. Studies investigating such interactions were low in number, and not present for Turkish population.

A non-genetic risk factor for the development of childhood ALL is parental age at conception. It has been shown that risk of childhood ALL was significantly higher among children of older mothers and fathers, and significant trends with increasing mothers' ($P < 0.001$) and fathers' ($P =$

0.002) ages were found, especially for parents older than 35 years of age in the study of Dockerty *et al.* (2001), and 2.14 fold increased risk was observed for mothers older than 35 years of age and 1.62 fold for older fathers (Kaye *et al.,* 1991). However, interaction of genetic polymorphisms and parental age was not investigated to the knowledge of literature so far. This study investigated the risk of development of childhood ALL in terms of parental age and genetic polymorphism. The results of importance are summarized in Table 4.8.

At the first glance, it seemed that *CYP2E1*5B, *6* and *NQO1*2* polymorphisms and older parental ages were together risk elevating factors for childhood ALL. For the case of maternal age younger than 20 years, *CYP2E1*5B*, and co-presence of **5B and *6* alleles increased the risk 7.6 and 6.7 folds, respectively; however the results were not statistically significant. However for maternal age older than 35 years and presence of *CYP2E1*5B* allele, the risk associated with childhood ALL was 9.0 fold, which was statistically significant (95% CI: 1.4-58.8, p=0.03). Again for maternal age older than 35 years and co-presence of *CYP2E1*5B* and **6* alleles, the risk was 8.5 fold and significant (95%CI: 1.3-56.2, p=0.04), however it should be noted that co-presence of *CYP2E1*6* allele did not show a combined effect, meaning its presence did not increase the risk associated with *CYP2E1*5B* alone. However co-presence of *CYP2E1*5B* and *NQO1*2* in children with mothers older than 35 years of age at conception, showed a combined effect and increased the risk strikingly to 16.7 fold (95% CI: 1.3-212.5, p=0.046) (Table 4.8).

Table 4.8 Risk of genetic polymorphisms in interaction with parental age for development of childhood ALL. This table was based mainly on data of Tables 3.13 and 3.14.

Parental age	Polymorphism	COR (95% CI) *p*
Maternal age <20	*CYP2E1*5B*	7.6 (1.0-59.9) 0.08
	**5B-*6*	6.7 (0.9-52.8) 0.10
Maternal age >35	***CYP2E1*5B***	**9.0 (1.4-58.8) 0.03***
	****5B-*6***	**8.5 (1.3-56.2) 0.04***
	****5B-NQ***	**16.7 (1.3-212.5) 0.046***
Paternal age >40	***CYP2E1*5B***	**5.9 (1.2-29.5) 0.04***
	CYP2E1*6	**3.3 (1.0-11.1) 0.05***
	****5B-*6***	**6.4 (1.2-22.7) 0.04***
	****6-NQ***	**5.6 (1.2-25.5) 0.04***
	**5B-NQ*	9.2 (1.1-77.4) 0.07
	**5B-*6-NQ*	8.3 (1.0-70.5) 0.08

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

* statistically significant association.

In the case of paternal age older than 40 years, presence of *CYP2E1*5B* alone*,* presence of *CYP2E1*6* alone*,* or co-presence of both alleles showed a significant risk for development of childhood ALL as 5.9, 3.3 and 6.4 fold, respectively, indicating a combined effect for *CYP2E1*5B* and **6* polymorphisms (Table 4.8). Also for the case of paternal age older than 40 years, co-presence of *CYP2E1*6* and *NQO1*2* polymorphisms showed an association with disease development, showing 5.6 fold increased risk (95%CI: 1.2-25.5, p=0.04). Co-presence of *CYP2E1*5B* and *NQO1*2*, or co-presence of *CYP2E1*5B, *6* and

*NQO1*2* also showed increased risks of 9.2 and 8.3 folds, respectively, however; these associations were not statistically significant.

This study, for the first time, showed that *CYP2E1*5B, *6* and *NQO1*2* polymorphisms, and older parental ages at conception were associated with the development of childhood ALL. Older parental ages as risk factors could be explained by accumulating mutations, especially in the germ cells of parents with increasing age, which could increase the possibility of the prenatal first hit mutation described in Greaves' (1996) model for the development of ALL. Both *CYP2E1* and *NQO1* were susceptibility genes for the disease development, and *CYP2E1*5B* and **6* polymorphisms together was already found as a risk factor, interestingly *NQO1*2* polymorphism did not show an association when analyzed regardless of the parents age. One explanation could be that if the older parental ages result in a more damaged genome of the child, which could be more prone to environmental factors or accumulating more mutations, presence of *CYP2E1* and *NQO1* polymorphisms would increase the potential of already damaged genome to accumulate second hit of postnatal mutations described in Greaves' (1996) model. Another hypothesis would rely on the parental genotypes, as the child inherit his/her genome from parents, then either one or both parents should also be polymorphic for these genes, which would alter the susceptibility of the parents to accumulate mutations in their germ cells, especially through exposure to environmental factors, and the risk would increase more as the parents are aged older. However the latter hypothesis would have been more effective if the parental genotypes were also examined. Storage of blood spots of newborns in databanks, as done in Europe, would have given the opportunity to retrospectively investigate the chromosomal damage of the children with older parents, which in turn would help to explain these findings more accurately.

The other case only analyses were done on the smoking exposure of mother, father or postnatally, child. Table 4.9 represents the summary of the results obtained. Apparently seen from the table, the genes that were associated with childhood ALL development together with smoking exposure were *NQO1*2* and *GSTM1*.

Table 4.9 Risk of genetic polymorphisms in interaction with exposure to cigarette smoke for development of childhood ALL. This table was based mainly on data of Tables 3.15 to 3.18.

Exposure to cigarette smoke	Polymorphism	COR (95% CI) *p*
Maternal	***NQO1*2***	**2.3 (1.0-5.6) 0.05***
	GSTM1	**2.5 (1.1-5.7) 0.03***
	NQ-M1	**4.4 (1.3-14.4) 0.01***
	M1-T1	3.4 (0.9-13.6) 0.05
Paternal	*NQO1*2*	2.4 (0.9-6.7) 0.09
	GSTM1	2.0 (0.8-5.1) 0.14
	NQ-M1	**4.1 (1.0-16.7) 0.04***
Child, postnatal	*NQO1*2*	1.7 (0.7-3.7) 0.21
	GSTM1	**2.6 (1.2-5.9) 0.02***
	NQ-M1	**3.6 (1.2-10.7) 0.02***

[a] The polymorphism names were given short designations for convenience as: *CYP2E1*5B* as **5B; CYP2E1*6* as **6; CYP2E1*7B* as **7B; NQO1*2* as *NQ; GSTM1* as *M1; GSTT1* as *T1.*

* statistically significant association.

Together with maternal exposure, both *NQO1*2* and *GSTM1* null showed increased risks of 2.3 and 2.5 folds, respectively (Table 4.9). Co-presence of these alleles and maternal exposure showed a combined effect and increased the risk to 4.4 fold, which was also statistically significant (95%CI: 1.3-14.4, p=0.01). It should also be noted than co-presence of *GSTM1* and *GSTT1* null also had a risk of 3.4 fold, with a borderline significance. Maternal cigarette smoking during pregnancy and genetic polymorphisms was not found to be associated with risk of childhood ALL; low number of smokers during pregnancy (n=12) could have prevented to identify an association.

For paternal smoking exposure, neither *NQO1*2* nor *GSTM1* showed a significant risk for disease development, however co-presence of both polymorphisms in children whose father was exposed to cigarette smoke, associated with a significant risk of 4.1 fold (95% CI: 1.0-16.7, p=0.04).

On the other hand, when postnatal exposure of child was considered, *GSTM1* null was associated significantly with risk of childhood ALL, 2.6 fold (95%CI: 1.2-5.9, p=0.02). Besides, co-presence of *NQO1*2* and *GSTM1* null and exposure to smoke showed a combined effect and increased risk of development 3.6 fold, which was significant (95%CI: 1.2-10.7, p=0.02).

Neither *NQO1*2* nor *GSTM1* null showed an association with the risk of childhood ALL development when investigated regardless of the smoking exposure, but they definitely seemed to present a risk factor when smoking exposure was taken into account. Both enzymes have roles in the metabolism of tobacco smoke related chemicals and are also induced by them (Rebbeck, 1997; Hayes *et al.,* 2005; Gresner *et al.,* 2007). Also NQO1 and GSTs, together with several other genes, are co-

induced in response to oxidative stress to protect the cell against free radical damage (Jaislaw, 2000).

Relying on these findings, the explanation for the association of *NQO1*2* and *GSTM1* null and cigarette smoke exposure with risk of leukemia could be that these enzymes exert their effect especially upon induction by cigarette smoke related chemicals or oxidative stress, however in the polymorphic individuals, the enzymes are either not expressed at all or degraded quite rapidly. Hence polymorphic individuals are supposed to suffer more from exposure, in this case from exposure to cigarette smoke, overall result most probably being higher genotoxic damage, either prenatally to parents including germ cells or postnattally to child.

In the literature it is unclear whether maternal or paternal cigarette smoking was a risk factor for developing childhood leukemia. Many studies could not find an association between parental smoking and childhood leukemia (Brondum *et al.,* 1999; Schuz *et al.,* 1999; Alexander *et al.,* 2001; Okcu *et al.,* 2002; Pang *et al.,* 2003; Chang *et al.,* 2006; MacArthur *et al.,* 2008). Some studies found an association with childhood ALL and paternal smoking (Sorahan *et al.,* 2001; Rudant *et al.,* 2008) and maternal smoking (Menegaux *et al.,* 2007). In the study of Lee *et al.* (2009), significant association was found between paternal smoking and childhood ALL, and the risk was increased with *CYP1A1* polymorphisms. Another study by Clavel *et al.* (2005) found a significant association for *CYP1A1*2A* and *GSTM1* null and maternal smoking during pregnancy.

The uncertainty of the findings for parental smoking status could be overcome by involving the risk elevating genetic polymorphisms in such studies. This study, in that respect, provided valuable information to

the literature pointing out the importance of *NQO1*2* and *GSTM1* null polymorphisms and parental or postnatal exposure to cigarette smoke. It should also be noted that, the interaction was found when the analyses were done on the exposed and not exposed groups, which the active and passive smokers were combined in the "exposed" group. This modeling would reflect the importance of any kind of exposure to chemicals found in the cigarette smoke, either actively or passively.

In summary, this study provided information on the polymorphism frequencies in Turkish population, to the pool of data which would aid in determining more accurate frequencies for the Turkish population. Also the case-control analyses revealed that *GSTT1* null alone, combination of *CYP2E1*5B* and **6* polymorphisms, and combination of *GSTT1* null and *CYP2E1* polymorphisms were risk factors for the development of childhood ALL. Parental age at conception, *CYP2E1* and *NQO1* polymorphisms were together associated with the development of the disease and *NQO1*2* and *GSTM1* null and parental or postnatal exposure to cigarette smoke was significantly associated with the risk of development of ALL. Many of these findings are new for the Turkish population and also for the literature to date.

CHAPTER 5

CONCLUSION

ALL is the most common type of childhood malignancy in the world as well as in our country. Although many studies have been conducted on the molecular etiology of the disease, still the exact mechanism remains to be elucidated. Therefore genetic epidemiological studies have been conducted by many groups in many countries. Each study surely provides valuable information, as the genetic polymorphisms in xenobiotic metabolizing enzymes show variability in different ethnicities, and the outcome and risk of the polymorphisms are highly prone to haplotypes and exposure to environmental factors. Although some studies have been conducted on the genetic risk factors for childhood ALL in Turkey, they remained to investigate the effects of single polymorphisms as risk factors rather than combinations.

This study focused on the genetic polymorphisms of the enzymes that function in the metabolism of many environmental chemicals, with the hypothesis that the development of childhood ALL could be associated with these polymorphisms either alone or in combination, which together would affect the amount of carcinogen produced. In this respect, three polymorphisms of *CYP2E1 (*5B, *6* and *7B*), *NQO1*2, GSTM1 null* and *GSTT1* null were selected for the study. The genotyping for all these polymorphisms were performed on 209 healthy controls and 185 patients with childhood ALL.

The genotyping for control sample of this study also served to determine the population frequencies of these polymorphisms, and to make comparison with other population frequencies. *CYP2E1*5B* genotype distribution was found to be similar to other Caucasian populations while differed significantly from African-Americans and Asian populations. *CYP2E1*5B* genotype distribution was also found to be different from Latin-American populations which could be explained in part by their African and Amerindian ancestral origins.

*CYP2E1*6* genotype distribution showed similarity to other Caucasian populations and different from Asian and Latin-American populations, as was the case for *CYP2E1*5B. CYP2E1*6* polymorphism showed significant difference between Turkish and Indian populations, variant allele frequency being higher in Indian population.

*CYP2E1*7B* genotype distribution was determined for the first time in Turkish population by the control population of this study. The number of studies on *CYP2E1*7B* was low in literature, and information was available only for some Caucasian populations, which no significant difference was observed when compared to Turkish population.

*NQO1*2* allele frequency in Turkish population (26.7%) was slightly higher than other Caucasian populations (17.0-22.1%), however there were no significant difference between them. The genotype distribution was significantly different from Asian populations, frequency being much higher in latter ones.

Many studies were available for *GSTM1* and *GSTT1* null for various populations, and more heterogeneity was observed even in the populations of same race. The prevalence of *GSTM1* null was quite high in Turkish (59.3%) and other Caucasian populations. *GSTM1* null frequency

in Turkish population showed similarity to most Caucasian populations, however differed significantly from Finnish, Norwegian, Dutch, and Italian populations, as well as from Brazilian, Indians and Iranians, the null frequency being higher in Turkish population. Also *GSTM1* null frequency was found to be significantly lower in African-American populations. Interestingly, *GSTM1* null frequency of Turkish population showed similarity with some Asian populations like Korean, Singaporean, Thai and Japanese, but differed from Hmong population which showed an strikingly high null allele frequency. Also it should be noted that inconsistent results were present for Japanese population.

GSTT1 null frequency in Turkish population was found to be 21.2%, and similar to other Caucasian populations as well as African-Americans. When compared to Asian populations, it was observed that the null frequency was higher in those populations than Turkish population.

As a summary for the population comparisons, major differences were observed among Asian and African ethnicities, besides, the comparisons showed that Latin-American populations also derive attention for their different genotype distributions. Although some information was available for Indian, Iranian and Arabic populations, still it is not possible to make generalizations about comparison with these populations, because of low number of studies in these populations.

In the case control analyses, the risk of *CYP2E1*5B, *6,*7B, NQO1*2, GSTM1* and *GSTT1* null were investigated alone or in combination, as risk factors for the development of childhood ALL. When investigated alone, only *GSTT1* null was found to be associated with the development of disease (OR= 1.8, p=0.01). However, when combinations of polymorphisms were investigated, *CYP2E1*5B* and **6*

combination showed an increased risk of 2.7 fold (p= 0.04). Also co-presence of *CYP2E1*5B-GSTT1, CYP2E1*6-GSTT1,* and *CYP2E1*7B-GSTT1* polymorphisms showed a combined effect and increased the risk to approximately 4.0 fold, risk of the latter two combinations being statistically significant. The risk increased even more to 7.6 fold, when *CYP2E1*5B,*6* and *GSTT1* null were considered together (p=0.04).

To date, studies on *CYP2E1* polymorphisms focused on **5B* alone, so this study presented to be the first one investigating **6* and **7B* polymorphisms for the risk of childhood ALL, hence the first one showing association of these alleles with the risk of childhood ALL.

Association of parental ages and exposure to cigarette smoke was investigated on the data from patient sample only. Older parental ages were reported to be associated with the risk of childhood ALL previously. This study, for the first time to the literature knowledge, investigated the parental ages together with genetic polymorphisms and found that *CYP2E1*5B, *6* and *NQO1*2* polymorphisms and parental age were significantly associated with the risk of development of childhood ALL. The risk was strikingly high in the case of maternal age older than 35 years, being 9.0 fold for *CYP2E1*5B* (p=0.03), 8.5 fold for co-presence of *CYP2E1*5B* and **6* (p=0.04), and 16.7 fold for *CYP2E1*5B* and *NQO1*2* (p=0.046). The genetic polymorphisms and parental age older than 40 years also revealed significant results of 5.9 fold for *CYP2E1*5B* (p=0.04), 3.3 fold for *CYP2E1*6* (p=0.05), 6.4 fold for co-presence of *CYP2E1*5B* and **6* (p=0.04), and 5.6 fold for *CYP2E1*6* and *NQO1*2* (p=0.04). The polymorphisms and older parental ages being risk factors for the development of childhood ALL could be explained by the accumulating mutations in germ cells of parents and further increase of risk postnatally by the associated polymorphisms.

Interaction of exposure to cigarette smoke and genetic polymorphisms for the risk of development of childhood ALL was examined. Exposure of mother and *NQO1*2* alone or *GSTM1* null alone showed significant associations of 2.3 and 2.5 folds, respectively ($p<0.05$), and co-presence of *NQO1*2* and *GSTM1* null showed a combined effect increasing the risk significantly to 4.4 fold ($p=0.01$). For paternal exposure, only co-presence of *NQO1*2* and *GSTM1* null was associated with increased risk of 4.1 fold ($p=0.04$). When the postnatal exposure of the child was considered, *GSTM1* null alone showed an association of 2.6 fold increased risk ($p=0.02$) and co-presence of *NQO1*2* and *GSTM1* null again showed a combination effect increasing the risk to 3.6 fold ($p=0.02$).

Although there were many studies on parental smoking and risk of development of childhood ALL, inconsistent results had been established so far when only smoking was examined without considering genetic polymorphisms. In this respect, this study was first to show an association of *NQO1*2* and *GSTM1* null and parental or postnatal exposure to cigarette smoke as significant risk factors for the disease.

Collecting the blood samples from patients, especially when the patient is a child, was a hard effort and reaching to sufficiently high number of subjects required a long time. To make such samples available to all researchers, and to share the scientific information; consortiums and blood, cell or DNA databanks have been established in many countries like Genetic Epidemiology of Chronic Lymphocytic Leukemia Consortium (http://mayoresearch.mayo.edu/mayo/research/slager_lab/gec.cfm) in USA, European Leukemia Net (http://www.leukemia-net.org/content/ network_services/registries/project_info) and European Leukemia Trial Registry (http://www.leukemia-net.org/content/ leukemias/trial_registry/trial_ registry) in Europe and The Korean

Leukemia Cell and Gene Bank (http://www.klcgb.or.kr/eng/about/1_2.htm) in Korea. Such efforts would improve the scope and the strength of genetic epidemiological studies. Number of genetic epidemiological studies had increased considerably in our country in recent years. Efforts to establish such consortiums would be beneficial for the scientific community and in long term, for public health.

REFERENCES

Abdel-Rahman SZ, Salama SA, Au WW, Hamada FA (2000) Role of polymorphic CYP2E1 and CYP2D6 genes in NNK-induced chromosome aberrations in cultured human lymphocytes. *Pharmacogenetics.* 10(3):239-49.

Ada AO, Süzen SH, Iscan M (2004) Polymorphisms of cytochrome P450 1A1, gluthatione S-transferases M1 and T1 in a Turkish population. Toxicology Letters. 151, 311-315.

Adalı O and Arınç E (1990) Electrophoretic, spectral, catalytic and immunochemical properties of highly purified cytochrome P450 from sheep lung. *International Journal of Biochemistry* 22: 1433-1444.

Agarwal S., Chaturvedi A., Roy N. (2002) Pharmacogenomics: The concepts behind personalized medicine.CRIPS 3:3

Aksoy M, Dincol K, Akgun T, Erdem S, Dincol G (1971) The hematologic effects of chronic benzene poisoning in 217 workers. *Brit. J. Ind. Med.* 28:296.

Aksoy M, Erdem S, Dincol G (1976) Types of leukemia in chronic benzene poisoning: A study in 34 patients. *Acta Hematol* 55:65-72.

Albano E, Clot P, Tabone M, Arico S, and Ingelman-Sundberg M (1993) Oxidative damage and human alcoholic liver disease. Experimental and clinical evidence. **In** *Oxy radicals* (Edited by: Poli G, Albano E, and Dianzani M) pp: 310-322. Birkenhauser, Basel, Switzerland.

Alexander FE, Patheal SL, Biondi A, Brandalise S, Cabrera ME, Chan LC, Chen Z, Cimino G, Cordoba JC, Gu LJ, Hussein H, Ishii E, Kamel AM, Labra S, Magalhães IQ, Mizutani S, Petridou E, de Oliveira MP, Yuen P, Wiemels JL, Greaves MF (2001) Transplacental chemical exposure and risk of infant leukemia with MLL gene fusion. *Cancer Research* 61: 2542–2546.

Alves S, Amorim A, Ferreira F, Norton L, Prata MJ (2002) The GSTM1 and GSTT1 genetic polymorphisms and susceptibility to acute lymphoblastic leukemia in children from north Portugal. *Leukemia* 16: 1565–1567.

Anwar A, Dehn D, Siegel D, Kepa JK, Tang LJ, Pietenpol JA, Ross D (2003) Interaction of human NAD(P)H:quinone oxidoreductase 1 (NQO1) with the tumor suppressor protein p53 in cells and cell-free systems, *Journal of Biological Chemistry* 278: 10368–10373.

Anzenbacher P and Anzenbacherova E (2001) Cytochromes P450 and metabolism of xenobiotics. *CMLS.* 58: 737-747.

Aranda JV, MacLeod SM, Renton KW, Eade NR (1974) Hepatic microsomal drug metabolism and electron transport in newborn infants. *The Journal of Pediatrics* 85:534-542.

Arınç E (1993) Extrahepatic Microsomal Forms: Lung Microsomal Cytochrome P450 Isozymes. In: Schenkman JB, Greim H (Eds.), Handbook of Experimental Pharmacology, vol. 105. pp. 373-386. Springer, Heidelberg.

Arınç E and Philpot RM (1976) Preparation and properties of partially purified pulmonary cytochrome P-450 from rabbits. *Journal of Biological Chemistry* 251: 3213-3220.

Arınç E, Adalı O, Gençler-Özkan AM (2000a) Induction of N-nitrosodimethylamine metabolism in liver and lung by in vivo pyridine treatments of rabbits. *Archives of Toxicology* 74: 329-334.

Arınç E, Adalı O, Gençler-Özkan AM (2000b) Stimualtion of aniline, *p*-nitrophenol and N-nitrosodimethylamine metabolism in kidney by pyridine treatments of rabbits. *Archives of Toxicology* 74: 527-532.

Arınç E, Adalı O, İşcan M, Giray T (1991) Stimulatory effects of benzene on rabbit liver and kidney microsomal cytochrome P-450 dependent drug metabolizing enzymes. *Archives of Toxicology* 65: 186-190.

Arınç E, Arslan Ş, Adalı O (2005) Differential effects of diabetes on CYP2E1 and CYP2B4 preoteins and associated drug metabolizing enzyme activities in rabbitliver. *Archives of Toxicology* 79: 427-433.

Arınç E, Arslan Ş, Bozcaarmutlu A, Adalı O (2007) Effects of Diabetes on rabbit kidney and lung CYP2E1 and CYP2B4 expression and drug metabolism and potentiation of carcinogenic activity of N-nitrosodimethyl amine in kidney and lung. *Food Chemical Toxicology* 45: 107-118.

Arınç E, Philpot RM, Fotus JR (1976) Partial purification of hepatic microsomal cytochrome P450 and NADPH-cytochrome c reductase from little skate, *Raja erinacea*, and reconstitution of mixed function oxidase activity. *Fedn. Proc.* 36: 666.

Arinç E and Celik H (2002) Biochemical characteristics of purified beef liver NADPH-cytochrome P450 reductase. *Journal of Biochemistry and Molecular Toxicology*. 16(6):286-297.

Arslan Ş (2003) Cytochrome P450 dependent drug metabolism in diabetic rabbits. *MS. Thesis.* Middle East Technical University, Ankara.

Arslan Ş, Adalı O, Arınç E (2003) Diabetes stimulates the formation of bioactive compounds by inducing p-nitrophenol hydroxylase activity in rabbit liver. *Turkish Journal of Biochemistry* 28(3): 177-178.

Asher G, Lotem J, Kama R, Sachs L, Shaul Y (2002) NQO1 stabilizes p53 through a distinct pathway. *PNAS USA* 99: 3099–3104.

Aydin-Sayitoglu M., Hatirnaz O., Erensoy N., Ozbek U. (2006) Role of CYP2D6, CYP1A1, CYP2E1, GSTT1, and GSTM1 Genes in the Susceptibility to Acute Leukemias. *American Journal of Hematology* 81:162–170

Badger TM, Huang J, Ronis M, Lumpkin CK (1993) Induction of cytochrome P450 2E1 during chronic ethanol exposure accurs via transcription of the CYP2E1 gene when blood alcohol concentrations are high. *Biochemical and Biophysical Research Communications* 190: 780-785.

Balta G, Yuksek N, Ozyurek E, Ertem U, Hicsonmez G, Altay C, Gurgey A (2003) Characterization of MTHFR, GSTM1, GSTT1, GSTP1, and CYP1A1 Genotypes in Childhood Acute Lymphoblastic Leukemia. American Journal of Hematology. 71:154-160.

Barnette P, Scholl R, Blandford M, Ballard L, Tsodikov A, Magee J, Williams S, Robertson M, Ali-Osman F, Lemons R, Keller C (2004) High-throughput detection of glutathione s-transferase polymorphic alleles in a pediatric cancer population. *Cancer Epidemiology Biomarkers and Prevention* 13: 304–313.

Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K (2000) Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of Tobacco-related cancers. *Cancer Epidemiology, Biomarkers and Prevention* 9(1):3-28

Bauer AK, Faiola B, Abernethy DJ, Marchan R, Pluta LJ, Wong VA, Roberts K, Jaiswal AK, Gonzalez FJ, Butterworth BE, Borghoff S, Parkinson H, Everitt J, Recio L (2003) Genetic Susceptibility to Benzene-induced Toxicity: Role of NADPH: Quinone Oxidoreductase-1. *Cancer Research* 63: 929-935.

Belson M, Kingsley B, Holmes A (2007) Risk Factors for Acute Leukemia in Children: A Review. *Environmental Health Perspectives* 115(1):138-145.

Beyer RE, Segura-Aguilar J, Di Bernardo S, Cavazzoni M, Fato R, Fiorentini D, Galli M, Setti L, Landi G, Lenaz G (1996) The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane systems. *PNAS USA* 93: 2528–2532.

Bianchet MA, Faig M, Amzel LM (2004) Structure and Mechanism of NAD(P)H: Quinone Acceptor Oxidoreductases (NQO) **in:** Methods in Enzymology Vol 382-Quinones and Quinone Enzymes, Part B (Ed: Sies H and Paska L) Elsevier Publishing.

Black SD and Coon MJ (1986) Comparative structures of P-450 cytochromes. **In** C*ytochrome P-450, structure, mechanism and biochemistry* (Edited by: Ortiz de Montellano), pp: 161-216, Plenum Press, New York.

Blanco JG, Edick MJ, Hancock ML, Winick NJ, Dervieux T, Amylon MD, Bash RO, Behm FG, Camitta BM, Pui CH, Raimondi SC, Relling MV (2002) Genetic polymorphisms in CYP3A5, CYP3A4 and NQO1 in children who developed therapy-related myeloid malignancies. *Pharmacogenetics* 12: 605.

Blumenfeld M, Maury M, Chouard T, Yaniv M, Condamine H (1991) Hepatic nuclear factor 1 (HNF1) shows a wider distribution than products of its own target genes in mouse. *Development* 113: 589-599.

Bolt H.M. et al (2003) The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. *Int Arch Occup Environ Health* 76:174 –185

Bolufer P, Barragan E, Collado M, Cervera J, L´opez J-A, Sanz MA (2006) Influence of genetic polymorphisms on the risk of developing leukemia and on disease progression. *Leukemia Research* 30: 1471–1491.

Bolufer P, Collado M, Barragán E, Cervera J, Calasanz M-J, Colomer D, Roman-Gómez J, Sanz MA (2007) The potential effect of gender in combination with common genetic polymorphisms of drug-metabolizing enzymes on the risk of developing acute leukemia. *Haematologica* 92:308-314.

Booth J, Boyland E, Sims P (1961) An enzyme from rat liver catalyzing conjugations with glutathiane. *Biochemical Journal 79: 516-524*.

Bouchardy C, Benhamou S, Dayer P (1996) The effect of tobacco on lung cancer risk depends on CYP2D6 activity. *Cancer Research* 56(2): 251–253.

Bouchardy C, Hirvonen A, Coutelle C, Ward PJ, Dayer P, Benhamou S (2000) Role of alcohol dehydrogenase 3 and cytochrome P-4502E1 genotypes in susceptibility to cancers of the upper aerodigestive tract. *International Journal of Cancer* 87: 734-740.

Boyunegmez Tumer T, Ulusoy G, Adali O, Sahin G, Gozdasoglu S, Arinc E (2007) The low frequency of defective TPMT alleles in Turkish population: A study on pediatric patients with acute lymphoblastic leukemia. *American Journal of Hematology* 82:906–910.

Boyunegmez Tumer T, Yilmaz D, Tanrikut C, Ulusoy G, Arinc E (2008) DNA Repair Gene XRCC1 Arg399gln Polymorphisms as a Risk Factor for Childhood Acute Lymphoblastic Leukemia. *EMBO Young Scientist Forum*. February 20-22, Istanbul, TURKEY.

Bozcaarmutlu A and Arinç E (2008) Purification of CYP2B-like protein from feral leaping mullet (Liza saliens) liver microsomes and its biocatalytic, molecular, and immunological characterization. *Journal of Biochemistry and Molecular Toxicology* 22(4):284-298.

Brockmöller J, Cascorbi I, Kerb R, Roots I (1996) Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1, microsomal, and cytochrome P450 enzymes as modulators of bladder cancer risk. *Cancer Research* 56(17): 3915-3925.

Brondum J, Shu XO, Steinbuch M, Severson RK, Potter JD, Robison LL (1999) Parental cigarette smoking and the risk of acute leukemia in children. *Cancer* 85,: 1380–1388

Bunin GR (2004) Nongenetic causes of childhood cancers: evidence from international variation, time trends, and risk factor studies. *Toxicology and Applied Pharmacology* 199: 91-103.

Cambes B and Stakelum GSA (1961) Liver enzyme that conjugates sulfobromaphtalien sodium with glutathione. *Journal of Clinical Investigations* 40: 981-988.

Canalle R, Burim RV, Tone LG, Takahashi CS (2004) Genetic Polymorphisms and Susceptibility to Childhood Acute Lymphoblastic Leukemia. *Environmental and Molecular Mutagenesis* 43:100–109.

Cancer Research UK. http://info.cancerresearchuk.org/healthyliving/smokeispoison (accessed on February 20, 2009)

Capdevila JH, Falck JR, Harris RC (2000) Cytochrome P450 and arachidonic acid bioactivation: molecular and functional properties of the arachidonate monooxygenase. *Journal of Lipid Research* 41: 163-181.

Caporaso N and Goldstein A (1995) Cancer genes—single and susceptibility—exposing the difference. *Pharmacogenetics* 5: 59–63.

Cederbaum AI (1987) Microsomal generation of hydroxyl radicals: Its role in microsomal ethanol oxidizing system (MEOS) activity and requirements for iron. *Annals of the New York Academy of Sciences* 492: 35-49.

Chan KW (2002) Acute Lymphoblastic Leukemia. *Current Problems in Pediatrics* 32: 40-49.

Chang JS, Selvin S, Metayer C, Crouse V, Golembesky A, Buffler PA (2006) Parental smoking and the risk of childhood leukemia. *American Journal of Epidemiology* 163: 1091–1100.

Chen CL, Liu Q, Pui CH, Rivera GK, Sandlund JT, Ribeiro R, Evans WE, Relling MV (1997) Higher frequency of glutathione S-transferase deletions in black children with acute lymphoblastic leukemia. *Blood* 89(5):1701-7

Chen H, Lum A, Seifried A, Wilkens LR, Le Marchand L (1999) Association of the NAD(P)H:quinone oxidoreductase 609C-->T polymorphism with a decreased lung cancer risk. *Cancer Research* 59(13):3045-3048.

Clairmont A, Sies H, Ramachandran S, Lear JT, Smith AG, Bowers B, Jones PW, Fryer AA, Strange RC (1999) Association of NAD(P)H:quinone oxidoreductase (NQO1) null with numbers of basal cell carcinomas: use of a multivariate model to rank the relative importance of this polymorphism and those at other relevant loci. *Carcinogenesis* 20:235–1240.

Clavel J, Bellec S, Rebouissou S, Ménégaux F, Feunteun J, Bonaïti-Pellié C, Baruchel A, Kebaili K, Lambilliotte A, Leverger G, Sommelet D, Lescoeur B, Beaune P, Hémon D, Loriot MA (2005) Childhood leukaemia, polymorphisms of metabolism enzyme genes, and interactions with maternal tobacco, coffee and alcohol consumption during pregnancy. *European Journal of Cancer Prevention* 14(6):531-540.

Cnattingius S, Zack MM, Ekbom A, Gunnarskog J, Kreuger A, Linet M, Adami HO (1995) Prenatal and neonatal risk factors for childhood lymphatic leukemia, *Journal of the National Cancer Institute* 87: 908–914.

Coles B and Ketterer B (1990) The role of glutathione transferase in chemical carcinogenesis. CRC *Critical Reviews in Biochemistry and Molecular Biology* 25: 47–70.

Colucci MA, Moody CJ, Couch GD (2008) Natural and synthetic quinones and their reduction by the quinone reductase enzyme NQO1: from synthetic organic chemistry to compounds withanticancer potential. *Organic and Biomolecular Chemistry* 6: 637–656.

Coon MJ, Van der Hoeven TP, Dahl SB, Haugen DA (1978) Two forms of liver microsomal cytochrome P-450 –P-450 LM2 and P-450 LM4 (rabbit liver). **In** *Methods in Enzymology* (Edited by: Colowick SP and Kaplan NO) vol:52, pp:109-117. Academic Press, New York.

Cresteil T (1998) Onset of xenobiotic metabolism in children: toxicological implications. *Food Additives and Contaminants* 15 (Suppl.):45-51.

CYP Allele Nomenclature Committee. Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee, http://www.imm.ki.se/CYPalleles. (last updated on 09.10.2008, accessed on September, 28, 2008).

D'Alò F, Voso MT, Guidi F, Massini G, Scardocci A, Sica S, Pagano L, Hohaus S, Leone G (2004) Polymorphisms of CYP1A1 and glutathione S-transferase and susceptibility to adult acute myeloid leukemia. *Haematologica* 89: 664–670.

Danielson PB (2002) The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Current Drug Metabolizm.* 3(6):561-597

Davies SM, Bhatia S, Ross JA, Kiffmeyer WR, Gaynon PS, Radloff GA, Robison LL, Perentesis JP (2002) Glutathione S-transferase genotypes, genetic susceptibility, and outcome of therapy in childhood acute lymphoblastic leukemia. *Blood* 100: 67-71

Davies SM, Robison LL, Buckley JD, Radloff GA, Ross JA, Perentesis JP (2000) Glutathione s-transferase polymorphisms in childhood with myeloid leukaemia: a children's cancer group study. *Cancer Epidemiology Biomarkers and Prevention* 9: 563–566.

Dicker A and Cederbaum AI (1988) Increased oxygen radical dependent inactivation of metabolic enzymes by liver microsomes after chronic ethanol consumption. *FASEB Journal* 2: 2901-2906.

Ding X, Koop DR, Crumb BL, Coon MJ (1986) Immunochemical identification of cytochrome P-450 isozyme 3a ($P450_{ALC}$) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Molecular Pharmacology* 30: 370-378.

Dockerty JD, Draper G, Vincent T, Rowan SD, Bunch KJ (2001) Case-control study of parental age, parity and socioeconomic level in relation to childhood cancers. *International Journal of Epidemiology* 30: 1428–1437.

Doll R (1998) Uncovering the risks of smoking: historical perspective. *Stat. Meth. Med. Res.* 7: 87–117.

Dutton GJ (1978) Developmental aspects of drug conjugation, with special reference to glucuronidation. *Annual Review of Pharmacology and Toxicology* 18:17-35.

Eguchi-Ishimae M, Eguchi M, Ishii E, Knight D, Sadakane Y, Isoyama K, Yabe H, Mizutani S, Greaves M (2005) The Association of a Distinctive Allele of NAD(P)H: Quinone Oxidoreductase with Pediatric Acute Lymphoblastic Leukemias with MLL Fusion Genes in Japan. *Haematologia/The Hematology Journal* 90(11): 1511-1515.

Eichelbaum M (1999) Clinical aspects of polymorphic drug metabolism in humans. **In** *Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes* (Edited by: Arınç E, Schenkman JB, Hodgson E) NATO ASI Series A: Life Sciences Volume:303, pp 111-126, Kluwer Academic / Plenum Publishers, New York.

Elkstrom G and Ingelman-Sundberg M (1989) Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIEI). *Biochemical Pharmacology* 38: 1313-1319.

Elkstrom G, Cronholm T, Ingelman-Sundberg M (1986) Hydroxyl radical production and ethanol oxidation by liver microsomes from ethanol-treated rats. *Biochemical Journal* 233: 755-761.

El-Zein R, Zwischenbergher JB, Wood TG, Abdel Rahman SZ, Brekelbaum C, Au WW (1997) Combined genetic polymorphism and risk for development of lung cancer. *Mutation Research* 381: 189-200.

Ernster L, Ljunggren M, Danielson L (1960) Purification and some properties of a highly dicumarol-sensitive liver diaphorase. *Biochemical and Biophysical Research Communications* 2:88–92.

Ernster L, Navazio F (1958) Soluble diaphorases in animal tissues. *Acta Chemica Scandinavica* 12:595.

Ernstgard L, Warholm M, Johanson G. (2004) Robustness of chlorzoxazone as an *in vivo* measure of cytochrome P450 2E1 activity. *British Journal of Clinical Pharmacology* 58:2, 190–200

European Leukemia Net (ELN). European Leukemia Information Center (ELIC), http://www.leukemia-net.org/content/network_services/registries/project_info. (last updated: 02.14.2006, accessed on January 28, 2009).

European Leukemia Trial Registry (ELTR), European Leukemia Information Center, http://www.leukemia-net.org/content/leukemias/trialregistry/trial_registry. (last updated: 21.09.2006, accessed on January 28, 2009).

Evans WE and Relling MV (1999) Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science.* 286: 487-491.

Evans WE, and Johnson JA (2001) Pharmacogenomics: The inherited basis for interindividual differences in drug response. *Annual Review of Genomics and Human Genetics* 2: 9-39.

Ey PL and Ashman LK (1986) The use of alkaline phosphatase conjugated anti-immunoglobilin with immunoblots for determining the specificity monoclonal antibodies to protein mixtures. *Methods in Enzymology*. 121:497-509.

Fairbrother A, Grove J, de Waziers I, Steimel DT, Day CD, Crespi CL, Daly AK (1998) Detection and characterization of novel polymorphisms in the CYP2E1 gene. *Pharmacogenetics* 8: 543-552.

Farker K, Lehmann MH, Oelschlagel B, Haerting J, Hoffmann A, Janitzky V, Schubert J (1998) Impact of *CYP2E1* genotype in renal cell and urothelial cancer patients. *Experimental Toxicology and Pathology* 50: 425-431.

Fennell TR, Kedderis GL, Sumner CJ (1991) Urinary metabolites of 1,2,3-(^{13}C)-acrylonitrile in rats and mice detected by ^{13}C nuclear magnetic resonance spectroscopy. *Chem Res Toxicol* 4:678–687

Ford AM, Ridge SA, Cabrera ME, Mahmoud H, Steel CM, Chan LC, Greaves M (1993) In utero rearrangements in the trithorax-related oncogene in infant leukaemias. *Nature* 363:358-360.

Freedman DM, Stewart P, Kleinerman RA, Wacholder S, Hatch EE, Tarone RE, Robison LL, Linet MS (2001) Household Solvent Exposures and Childhood Acute Lymphoblastic Leukemia. *Am J Public Health.* 91:564–567.

Gale KB, Ford AM, Repp R, Borkhardt A, Keller C, Eden OB, Greaves MF (1997) Back-tracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proceedings of the National Academy of Sciences of the USA* 94:13950-13954.

Garro AJ, Seitz HK, Lieber CS (1981) Enhancement of dimethylnitrosamine metabolism and activation to a mutagen following chronic ethanol consumption. *Cancer Research* 41: 120-124.

Garte S, Gaspari L, Alexandrie A-K, Ambrosone C, Autrup H, Autrup JI, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, Brockmöller J, Cascorbi I, Clapper ML, Coutelle C, Daly A, Dell'Omo M, Dolzana V, Dresler CM, Fryer A, Haugen A, Hein DW, Hildesheim A, Hirvonen A, Hsich L-L, Ingelman-Sundberg M, Kalina I, Kang D, Kihara M, Kiyohara C, Kremers P, Lazarus P, LeMarchand L, Lechner MC, Lieshout EMM, London S, Manni JJ, Maugard CM, Morita S, Nazar-Stewart V, Noda K, Oda Y, Parl FF, Pastorelli R, Persson I, Peters WHM, Rannug A, Rebbeck T, Risch A, Roelandt L, Romkes M, Ryberg D, Salagovic J, Schoket B, Seidegard J, Shileds PG, Sim E, Sinhet D, Strange RC, Stücker I, Sugimura H, To-Figueras J, Vineis P, Yu MC, Taioli E (2001) Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiology, Biomarkers and Prevention* 10: 1239-1248.

GATA Tıbbi Onkoloji Bilim Dalı Homepage. Türkiye ve Dünyada Kanser Epidemiyolojisi. http://www.gata.edu.tr/dahilibilimler/onkoloji/kanser_epidemiyolojisi.htm. (Latest Update: 04.10.2007, accessed on September 28, 2008).

Genetic Epidemiology of Chronic Lymphocytic Leukemia Consortium (GEC), Mayo Foundation for Medical Education and Research , http:// mayoresearch.mayo.edu/mayo/research/slager_lab/gec.cfm. (accessed on January 28, 2009).

Ghanayem BI, Sanders M, Chanas B, Burka LT, Gonzalez FJ (1999) Role of cytochrome P-450 2E1 in methacylonitrile metabolism and disposition. *J Pharmacol Exp Ther* 289:1054–1059

Glass DC (2003) Leukemia risk associated with low-level benzene exposure. *Epidemiology* 14:569–577.

Gomez A, Karlgren M, Edler D, Bernal ML, Mkrtchian S, Ingelman-Sundberg M (2007) Expression of CYP2W1 in colon tumors: regulation by gene methylation. *Pharmacogenomics.* 8(10):1315-1325.

Gonzalez FJ (1989) The molecular biology of cytochrome P450s. *Pharmacological Reviews.* 40(4): 243-288.

Gonzalez FJ (1992) Human cytochromes P450: problems and prospects. *Trends in Pharmacological Sciences* 13: 346-352.

Gonzalez FJ (1999) Pharmacogenetics: Polymorphisms in xenobiotic metabolism. **In** *Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes* (Edited by: Arınç E, Schenkman JB, Hodgson E) NATO ASI Series A: Life Sciences Volume: 303, pp 91-110, Kluwer Academic / Plenum Publishers, New York.

Gonzalez FJ (2001) The use of gene knockout mice to unravel the mechanisms of toxicity and chemical carcinogenesis. *Toxicology Letters.* 120: 199-208.

Gonzalez FJ and Gelboin HV (1994) Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metabolism Reviwes* 26: 165-183.

Gonzalez FJ, Skodat RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP, Meyer UA (1988) Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* 331: 442-446.

Goodman M, Flanders WD (2007) Study Design Options in Evaluating Gene-Environment Interactions: Practical Considerations for a Planned Case-Control Study of Pediatric Leukemia. *Pediatric Blood and Cancer* 48:375–379

Gorsky LD, Koop DR, Coon MJ (1984) On the stochiometry of the oxidase and monooxygenase reactions catalyzed by liver microsomal cytochrome P-450. *Journal of Biological Chemistry* 259: 6812-6817.

Greaves M (2002) Childhood leukaemia. *BMJ* 324:283–287

Greaves M (2003) Pre-natal origins of childhood leukemia. *Reviews in Clinical and Experimental Hematology* 7: 233–245.

Greaves MF (1988) Speculations on the cause of childhood acute lymphoblastic leukemia. *Leukemia* 2: 120–125.

Greaves MF (1996) Infant leukaemia biology, aetiology and treatment. *Leukemia* 10: 372–377.

Greaves MF, Maia AT, Wiemels JL, Ford AM (2003) Leukemia in twins: lessons in natural history. *Blood* 102: 2321–2333.

Green MD, Freedman DM, Gordis L (2000) Reference Guide on Epidemiology **in**: Reference Manual on Scientific Evidence, 2nd Edition. Federal Judicial Center Publication. Available at: www.fjc.gov/public/pdf.nsf/lookup/sciman06.pdf/$file/sciman06.pdf

Gresner P, Gromadzinska J, Wasowicz W (2007) Polymorphism of selected enzymes involved in detoxification and biotransformation in relation to lung cancer. *Lung Cancer* 57: 1—25.

Guengerich FP (1992) Roles of the vinyl chloride oxidation products 1-chlorooxirane and 2-chloroacetaldehyde in the in vitro formation of etheno adducts of nucleic acid bases. *Chem.Res. Toxicol.* 5: 2–5.

Guengerich FP (1993) Metabolic reactions: Types of reactions of cytochrome P450 enzymes. **In** *Handbook of Experimental Pharmacology.* (Edited by: Schenkman JB and Greim H), Volume: 105: pp:89-103. Springer-Verlag Press, Heidelberg.

Guengerich FP (1995) Human cytochrome P450 enzymes. **In** *Cytochrome P450* (Edited by: Ortiz de Montellano), pp:473-535. Plenum Publishers, New York.

Guengerich FP and Johnsson WW (1997) Kinetics of ferric cytochrome P450 reduction by NADPH-cytochrome P450 reductase: rapid reduction in the absence of substrate and variations among cytochrome P450 systems. *Biochemistry.* 36: 14741-14750.

Guengerich FP, Kim D-H, Iwasaki M (1991) Role of human cytochrome P450 IIEI in the oxidation of many low molecular weight cancer suspects. *Chemical Research in Toxicology* 4: 168-179.

Gunaratna C (2000) Drug metabolism and pharmacokinetics in drug discovery: A primer for bioanalytical chemists, Part I. *Current Separations.* 19(1): 17-23.

Halliwell B and Gutteridge JMC (1990) Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology* 186: 1-85.

Hannon-Fletcher MPA, O'Kane MJ, Moles KW, Barnett YA, Barnett CR (2001) Lymphocyte cytochrome P450-CYP2E1 expression in human IDDM subjects. *Food and Chemical Toxicology* 39: 125-132.

Harada S, Fujii C, Hayashi A, Ohkoshi N (2001) An association between idiopathic Parkinson's disease and polymorphisms of phase II detoxification enzymes: glutathione S-transferase M1 and quinone oxidoreductase 1 and 2. *Biochem Biophys Res Commun.* 288(4):887-892.

Hayashi H, Watanabe J, Kawajiri K (1991) Genetic polymorphism in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *Journal of Biochemistry* 110:559-565.

Hayes JD, Flanagan JU, Jowsey IR (2005) Glutathione Transferases. *Annual Reviews of Pharmacology and Toxicology* 45:51–88.

Hines RN (2007) Ontogeny of Human Hepatic Cytochromes P450. *Journal of Biochemistry and Molecular Toxicology* 21(4): 169-175.

Hines RN and McCarver DG (2002) The Ontogeny of Human Drug-Metabolizing Enzymes: Phase I Oxidative Enzymes. *The Journal Of Pharmacology And Experımental Therapeutıcs* 300(2):355–360

Hirvonen A, Husgafvel-Pursiainen K, Anttila S, Karjalainen A, Vainio H (1993) The human CYP2E1 gene and lung cancer: DraI and RsaI restriction fragment length polymorphisms in a Finnish study population. *Carcinogenesis* 14(1):85-88.

Hjalgrim LL, Rostgaard K, Hjalgrim H, Westergaard T, Thomassen H, Forestier E, Gustafsson G, Kristinsson J, Melbye M, Schmiegelow K (2004) Birth weight and risk for childhood leukemia in Denmark, Sweden, Norway, and Iceland, *Journal of the National Cancer Institute* 96: 1549–1556.

Hoffmann D, Patrianakos C, Brunnemann KD, Gori GB (1976) Chromatographic determination of vinyl chloride in tobacco smoke. *Anal Chem* 48: 47–50.

Hommes FA, Everts RS, Havinga H (1978) The development of DT-diaphorase in rat liver and its induction by benzo(a)pyrene. *Biology of the Neonate* 4(5-6):248-252.

Hong J-Y, Pan J, Gonzales FJ, Gelboin HV, Yang CS (1987) The induction of a specific form of cytochrome P-450 (P-450j) by fasting. *Biochemical and Biophysical Research Communications* 142: 1077-1083.

Hou X, Wang S, Zhou Y, Xu Z, Zou Y, Zhu X, Han M, Pang T, Han ZC (2005) Cyclin D1 gene polymorphism and susceptibility to childhood acute lymphoblastic leukemia in a Chinese population *International Journal of Hematol* 82: 206–209.

Hsu MH, Savas U, Griffin KJ, Johnson EF (2007) Human cytochrome p450 family 4 enzymes: function, genetic variation and regulation. *Drug Metabolism Reviews.* 39(2-3):515-538.

Hu Y, Oscarson M, Johansson I, Yue QY, Dahl ML, Tabone M, Arinco S, Albano E, Ingelman-Sundberg M (1997) Genetic polymorphisms of human CYP2E1: characterization of two variant alleles. *Molecular Pharmacology* 51: 370-376.

Hukkanen J (2000) Xenobiotic metabolizing cytochrome P450 enzymes in human lung, *Acta Univ. Oul. D 621,* Oulu University Press, Finland.

Human Cytochrome P450s. Dr. Nelson's Cytochrome P450 Homepage, http://drnelson. utmem.edu/human.P450.table.html. (last updated on 17.05.2007, accessed on September, 28, 2008).

IARC, 2008. International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 97. available at: http://monographs.iarc.fr/ENG/Monographs/vol97/index.php.

Iba MM, Fung J, Gonzalez FJ (2000) Functional Cyp2e1 is required for substantial in vivo formation of 2,5-hexanedione from n-hexane in the mouse. *Arch Toxicol* 74:582–586

Infante-Rivard C (2003) Diagnostic X rays, DNA repair genes and childhood acute lymphoblastic leukemia *Health Physics* 85: 60–64.

Infante-Rivard C, Amre D, Sinnett D (2002a) GSTT1 and CYP2E1 polymorphisms and trihalomethanes in drinking water: effect on childhood leukemia. *Environmental Health Perspectives* 110(6): 591–593.

Infante-Rivard C, Krajinovic M, Labuda D, Sinnet D (2002b) Childhood Acute Lymphoblastic Leukemia Associated with Parental Alcohol Consumption and Polymorphisms of Carcinogen-Metabolizing Genes. *Epidemiology* 13(3): 277-281

Infante-Rivard C, Krajinovic M, Labuda D, Sinnett D (2000) Parental smoking, CYP1A1 genetic polymorphisms and childhood leukemia (Quebec, Canada). *Cancer Causes Control* 11(6): 547–553.

Ingelman-Sundberg M (2002) Polymorphism of cytochrome P450 and xenobiotic toxicity. *Toxicology* 181-182: 447-452.

Ingelman-Sundberg M and Johansson I (1984) Mechanisms of hydroxyl radical formation and ethanol oxidation by ethanol-inducible and other forms of rabbit liver microsomal cytochromes P-450. *Journal of Biological Chemistry* 259: 6447-6458.

Ingelman-Sundberg M, Johansson I, Yin H, Terelius Y, Eliason E, Clot P, Albano E (1993) Ethanol-inducible cytochrome P4502E1: Genetic polymorphism, regulation, and possible role in the etiology of alcohol-induced liver disease. *Alcohol* 10: 447-452.

Int. SNP Map Work. Group (2001) A map of the human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 409: 928-933.

Ioannides C, Lewis DFV and Parke DV (1995) Mechanisms of chemical carcinogenesis and molecular parametric analyis in the safety evaluation of chemicals. **In** *Drugs, Diet and Disease* (Edited by: Ioannides C and Lewis DFV). vol:1, pp:147-160. Ellis Horwood Publishers, Chichester.

Irons RD (1985) Quinones as toxic metabolites of benzene. *Journal of Toxicology and Environmental Health* 16: 673-678.

Iseri OA, Gottlieb LS, Lieber CS (1966) The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *American Journal of Pathology* 48: 535-555.

Jaislaw AK (2000) Regulation of Genes Encoding NAD(P)H: Quinone Oxidoreductases. *Free Radical Biology and Medicine* 29(3/4):254-262.

Jamroziak K, Mlynarski W, Balcerczak E, Mistygacz M, Trelinska J, Mirowski M, Bodalski J, Robak T (2004) Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *European Journal of Haematology* 72: 314–321.

Joseph T, Kusumakumary P, Chacko P, Abraham A, Pillai MR (2004) Genetic Polymorphism of CYP1A1, CYP2D6, GSTM1 and GSTT1 and Susceptibility to Acute Lymphoblastic Leukaemia in Indian Children. *Pediatric Blood Cancer* 43:560–567

Joseph T, Kusumakumary P, Chacko P, Abraham A, Pillai MR (2005) DNA repair gene XRCC1 polymorphisms in childhood acute lymphoblastic leukemia. *Cancer Lett*ers 217: 17–24.

Karlgren M, Backlund M, Johansson I, Oscarson M, Ingelman-Sundberg M (2004) Characterization and tissue distribution of a novel human cytochrome P450-CYP2U1. *Biochem Biophys Res Commun.* 315(3):679-685.

Kaschnitz RM and Coon MJ (1975) Drug and fatty acid hydroxylation by solubilized human liver microsomal cytochrome P-450 phospholipid requirement. *Biochemical Pharmacology* 24: 295-297.

Kawajiri K, Watanabe J, Eguchi H, Nakachi K, Kiyohara C, Hayashi S (1995) Polymorphisms of human *Ah receptor* gene are not involved in lung cancer. *Pharmacogenetics* 5: 151–158.

Kaye SA, Robison LL, Smithson WA, Gunderson P, King FL, Neglia JP (1991) Maternal reproductive history and birth characteristics in childhood acute lymphoblastic leukemia. *Cancer* 68: 1351–1355.

Khani CS, Zaphiropoulos PG, Fujita VS, Porter TD, Koop DR, Coon MJ (1987) cDNA and derived amino acid sequence of ethanol-inducible rabbit liver cytochrome P-450 isozyme 3a (P-450$_{ALC}$). *PNAS USA* 84: 638-642.

Khoury MJ and Flanders WD (1996) Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: Case-control studies with no controls! *American Journal of Epidemiology* 144(3):207–213.

Kiffmeyer WR, Langer E, Davies SM, Envall J, Robison LL, Ross JA (2004) Genetic polymorphisms in the Hmong population: implications for cancer etiology and survival. *Cancer* 100(2):411-417.

Kim AS, Eastmond DA, Preston RJ (2006) Childhood acute lymphocytic leukemia and perspectives on risk assessment of early-life stage exposures. *Mutation Research* 613: 138–160.

Kim S, Lan Q, Waidyanatha S, Chanock S, Johnson BA, Vermeulen R, Smith MT, Zhang L, Li G, Shen M, Yin S, Rothman N, Rappaport SM (2007) Genetic polymorphisms and benzene metabolism in humans exposed to a wide range of air concentrations. *Pharmacogeneics and Genomics* 17(10):789-801.

Kim SG, Shehin SE, States JC, Novak RF (1990) Evidence for increased translational efficiency in the induction of P450IIEI by solvents: Analysis of P450IIEI mRNA polyribosomal distribution. *Biochemical and Biophysical Research Communications* 172: 767-774.

Klug WS and Cummings MR (1997) Concepts of Genetics (Fifth Edition), Prentice-Hall, Inc USA. ISBN: 0-13-531062-8

Knox EG (2005) Childhood cancers and atmospheric carcinogens. *Journal of Epidemiology and Community Health* 59:101–105.

Konishi T, Calvillo M, Leng A-S, Feng J, Lee T, Lee H, Smith JL, Sial SH, Berman N, French S, Eysselein V, Lin K-M, Wan Y-JY (2003) The ADH3*2 and CYP2E1 c2 alleles increase the risk of alcoholism in Mexican American men. *Exp. Mol. Pathol.* 74:183–189.

Koop DR and Casazza JP (1985) Identification of ethanol-inducible P-450 isozyme 3a as the acetone and acetol monooxygenase of rabbit microsomes. *J Journal of Biological Chemistry* 260: 13607-13612.

Koop DR and Tierney DJ (1990) Multiple mechanisms in the regulation of ethanol inducible cytochrome P-450IIEI. *Bioessays* 9: 429-435.

Koop DR, Laethem CL, Schnier GG (1989) Identification of ethanolinducible P450 isozyme 3a (P450I1E1) as a benzene and phenol hydroxylase. *Toxicology and Applied Pharmacology* 98:278-288.

Koop DR, Morgan ET, Tarr GE, Coon MJ (1982) Purification and characterization of a unique isozyme of cytochrome P-450 from liver microsomes of ethanol treated rabbits. *Journal of Biological Chemistry* 257: 8472-8480.

Krajinovic M, Labuda D, Mathonnet G, Labuda M, Moghrabi A, Champagne J, Sinnett D (2002a) Polymorphisms in Genes Encoding Drugs and Xenobiotic Metabolizing Enzymes, DNA Repair Enzymes, and Response to Treatment of Childhood Acute Lymphoblastic Leukemia. Clinical Cancer Research 8:802-810

Krajinovic M, Labuda D, Richer C, Karimi S, Sinnett D (1999) Susceptibility to Childhood Acute Lymphoblastic Leukemia: Influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 Genetic Polymorphisms. *Blood* 93: 1496-1501

Krajinovic M., Sinnet H., Richer C., Labuda D., Sinnet D. (2002b) Role of *NQO1, MPO* and *CYP2E1* Genetic Polymorphisms in The Susceptibility to Childhood Acute Lymphoblastic Leukemia. *International Journal of Cancer* 97:230-236.

Kukielka E and Cederbaum AI (1994) DNA strand cleavage as a sensitive assay for the production of hydroxyl radicals by microsomes: role of cytochrome P4502E1 in the increased activity after ethanol treatment. *Biochemical Journal* 302: 773-779.

Kutluk T, Yeşilipek A (2008) Türk Pediatrik Onkoloji Grubu (TPOG)/ Türk Pediatrik Hematoloji Derneği (TPHD), Pediatrik Tümör Kayıtları.

Lacroix D, Sonnier M, Moncion A, Cheron G, Cresteil T (1997) Expression of CYP3A in the human liver-evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *European Journal of Biochemistry.* 247(2):625-634.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-684.

Lafuente MJ, Casterad X, Trias M, Ascaso C, Molina R, Ballesta A, Zheng S, Wiencke JK, Lafuente A (2000) NAD(P)H:quinone oxidoreductase-dependent risk for colorectal cancer and its association with the presence of K*ras* mutations in tumors. *Carcinogenesis* 21: 1813–1819.

Lahiri DK, Schnabel B (1993) DNA Isolation by a rapid method from human blood samples: Effects of $MgCl_2$, EDTA, storage time and tempertaure on DNA yield and quality. *Biochemical Genetics* 31(7/9): 321-328.

Lanciotti M, Dufour C, Corral L, Di Michele P, Pigullo S, De Rossi G, Basso G, Leszl A, Luciani M, Lo Nigro L, Micalizzi C, Valsecchi MG, Biondi A, Haupt R (2005) Genetic polymorphism of NAD(P)H:quinone oxidoreductase is associated with an increased risk of infant acute lymphoblastic leukemia without MLL gene rearrangements. *Leukemia* 19(2):214-6.

Lane BP and Lieber CS (1966) Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. *American Journal of Pathology* 49: 593-603.

Le Marchand L, Sivaraman L, Pierce L, Seifried A, Lum A, Wilkens LR, Lau AF (1998) Associations of *CYP1A1, GSTM1,* and *CYP2E1* polymorphisms with lung cancer suggests cell type specificities to tobacco carcinogens. *Cancer Research* 58: 4858-4863.

Lee KM, Ward MH, Han S, Ahn HS, Kang HJ, Choi HS, Shin HY, Koo HH, Seo JJ, Choi JE, Ahn YO, Kang D (2009) Paternal smoking, genetic polymorphisms in CYP1A1 and childhood leukemia risk. *Leukemia Research* 33(2):250-258.

Lee YH, Sauer B, Gonzales FJ (1998) Laron dwarfism and non-insulin dependent diabetes mellitus in the Hnf-1alpha knockout mice. *Molecular and Cellular Biology* 18: 3059-3068.

Lieber CS (1997) Cytochrome P-4502E1: Its physiological and pathological role. *Physiological Reviews* 77(2): 517-543.

Lieber CS and DeCarli LM (1970) Hepatic microsomal ethanol oxidizing system. In vivo characteristic and adaptive properties in vivo. *Journal of Biological Chemistry* 245: 2505-2512.

Lightfoot TJ and Roman E (2004) Causes of childhood leukaemia and lymphoma. *Toxicology and Applied Pharmacology* 199: 104–117.

Lin DX, Tang YM, Peng Q, Lu SX, Ambrosone CB, Kadlubar FF (1998) Susceptibility to esophageal cancer and genetic polymorphisms in glutathione S-transferases T1, P1 and M1 and cytochrome P450 2E1. *Cancer Epidemiology Biomarkers and Prevention* 7: 1013-1018.

Liu S, Park JY, Schantz SP, Stern JC, Lazarus P (2001) Elucidation of CYP2E1 5' regulatory *Rsa*I/*Pst*I allelic variants and their role in risk for oral cancer. *Oral Oncology* 37: 437-445.

Longuemaux S, Deloménie C, Gallou C, Méjean A, Vincent-Viry M, Bouvier R, Droz D, Krishnamoorthy R, Galteau MM, Junien C, Béroud C, Dupret JM. (1999) Candidate genetic modifiers of individual susceptibility to renal cell carcinoma: a study of polymorphic human xenobiotic-metabolizing enzymes. *Cancer Research* 59(12):2903-2908.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin Phenol reagent. *Journal of Biological Chemistry* 193: 265-275.

Lu AY and Coon MJ (1968) Role of hemoprotein P450 in fatty acid w-hydroxylation in a soluble enzyme system from liver microsomes. *The Journal of Biological Chemistry* 243: 1331-1332.

Lu AY and Levin W (1974) The resolution and reconstitution of the liver microsomal hydroxylation system. *Biochimica et Biophysica Acta* 344: 205-240.

Lu AY, Strobel HW, Coon MJ (1969) Hydroxylation of benzphetamine and other drugs by a solubilized form of cytochrome P-450 from liver microsomes: lipid requirement for drug demethylation. *Biochem Biophys Res Commun.* 36(4):545-51.

Lu AY, Strobel HW, Coon MJ (1970) Properties of a solubilized form of the cytochrome P-450-containing mixed-function oxidase of liver microsomes. *Molecular Pharmacology* 6(3):213-20.

Ma X, Buffler PA, Gunier RB, Dahl G, Smith MT, Reinier K, Reynolds P (2002) Critical windows of exposure to household pesticides and risk of childhood leukemia. *Environmental Health Perspectives* 110: 955– 960.

MacArthur AC, McBride ML, Spinelli JJ, Tamaro S, Gallagher RP, Theriault G (2008) Risk of childhood leukemia associated with parental smoking and alcohol consumption prior to conception and during pregnancy: the cross-Canada childhood leukemia study. *Cancer Causes Control* 19: 283–295.

Mahoney MC, Moysich KB, McCarthy PL Jr, McDonald RC, Stepanenko VF, Day RW, Michalek AM (2004) The Chernobyl childhood leukemia study: background & lessons learned. *Environmental Health* 3(1):12.

McCarver DG, Byun R, Hines RN, Hichme M, Wegenek W (1998) A genetic polymorphism in the regulatory sequences of human CYP2E1: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. *Toxicology and Applied Pharmacology* 152: 276-281.

McCarver DG, Hines RN (2002) The Ontogeny of Human Drug-Metabolizing Enzymes: Phase II Conjugation Enzymes and Regulatory Mechanisms. *The Journal Of Pharmacology And Experımental Therapeutıcs* 300(2):361–366

McClellan RO (1996) Tobacco-specific *N*-nitrosamines. Recent Adv. *Critical Reviews of Toxicology* 26*:* 119–253.

McKinney PA, Alexander FE, Cartwright RA, Parker L (1991) Parental occupations of children with leukaemia in west Cumbria, north Humberside, and Gateshead. *BMJ* 302:681-687.

McKinney PA, Fear NT, Stockton D (2003) Parental occupation at periconception: findings from the United Kingdom Childhood Cancer Study. *Occup Environ Med* 60:901–909.

McLeod HL, Coulthard S, Thomas AE, Pritchard SC, King DJ, Richards SM, Eden OB, Hall AG, Gibson BE (1999) Analysis of thiopurine methyltransferase variant alleles in childhood acute lymphoblastic leukaemia. *British Journal of Haematology* 105(3):696–700.

MedWatch. The FDA Safety Information and Adverse Event Reporting Program, U.S. Food and Drug Administration, http://www.fda.gov/medwatch/SAFETY/2003/03DEC_PI /Tabloid_PI.pdf. (last updated on 30.01.2009).

Melnick RL and Kohn MC (1995) Mechanistic data indicate that 1,3-butadiene is a human carcinogen. *Carcinogenesis* 16: 157-163.

Menegaux F, Ripert M, Hémon D, Clavel J (2007) Maternal alcohol and coffee drinking, parental smoking and childhood leukaemia: a French population-based case-control study. *Paediatric and Perinatal Epidemiology* 21(4):293-299.

Milsap RL, Jusko WJ (1994) Pharmacokinetics in the infant. *Environmental Health Perspectives* 102(Suppl 11):107-110.

Moran JL, Siegel D, Ross D (1999) A potential mechanism underlying the increased susceptibility of individuals with a polymorphism in NAD(P)H:quinone oxidoreductase 1 (NQO1) to benzene toxicity. *PNAS USA* 96: 8150–8155.

Moron NE (1982) Outline of Genetic Epidemiology. August 1982. S. Karger Publishing.

Morselli PL (1989) Clinical pharmacology of the perinatal period and early infancy. *Clinical Pharmacokinetics* 17(Suppl.1):13-28.

Morselli PL, Franco-Morselli R, Bossi L (1980) Clinical pharmacokinetics in newborns and infants. Age-related differences and therapeutic implications. *Clinical Pharmacokinetics* 5:485-527.

Murata M, Watanabe M, Yamanaka M, Kubota Y, Ito H, Nagao M, Katoh T, Kamataki T, Kawamura J, Yatani R, Shiraishi T (2001) Genetic polymorphisms in cytochrome P450 (CYP)1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. *Cancer Letters* 165: 171-177

Murray L, McCarron P, Bailie K, Middleton R, Davey Smith G, Dempsey S, McCarthy A, Gavin A (2002) Association of early life factors and acute lymphoblastic leukaemia in childhood: historical cohort study, *British Journal of Cancer* 86: 356–361.

Müller M, Belas F, Ueno H, Guengerich FP (1996) Development of a mass spectrometric assay for 5,6,7,9-tetrahydro-7-hydroxy-9-oximidazo[1,2-alpha] purine in DNA modified by 2-chloro-oxirane. *Adv. exp. Med. Biol.* 387: 31–36.

Naoe T, Takeyama K, Yokozawa T, Kiyoi H, Seto M, Uike N, Ino T, Utsunomiya A, Maruta A, Jin-nai I, Kamada N, Kubota Y, Nakamura H, Shimazaki C, Horiike S, Kodera Y, Saito H, Ueda R, Wiemels J, Ohno R (2000) Analysis of genetic polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4 in 469 Japanese patients with therapy-related leukemia/ myelodysplastic syndrome and de novo acute myeloid leukemia. *Clinical Cancer Research* 6(10):4091-4095.

National Cancer Institute (NCI) Leukemia Homepage, US National Institutes of Health. http://www.cancer.gov/cancertopics/types/leukemia. (accessed on September 28, 2008).

Nebert DW and McKinnon RA (1994) Cytochrome P450: Evolution and functional diversity. *Progress in Liver Diseases* 12: 63-97.

Nebert DW and Roe AL (2001) Ethnic and genetic differences in metabolism genes and risk of toxicity and cancer. *The Science of the Total Environ-ment* 274: 93-102.

Nebert DW and Russell DW (2002) Clinical importance of the cytochromes P450. *Lancet.* 360: 1155-1162.

Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzales FJ, Guengerih FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Philips IR, Sato R, Waterman MR (1987) The P450 gene superfamily: Recommended nomenclature. *DNA.* 6, 1-13.

Nebert DW, McKinnon RA, Puga A (1996) Human drug-metabolizing enzyme polymorphisms: effects on risk of toxicity and cancer. *DNA and Cell Biology* 15: 273–280, 1996.

Nedelcheva V, Gut I, Soucek P, Tichavska B, Tynkova L, Mraz J, Guengerich F P, Ingelman-Sundberg M (1999) Metabolism of benzene in human liver microsomes: individual variations in relationto CYP2E1 expression. *Archives of Toxicology* 73: 33-40.

Neims AH, Warner M, Loughnan PM, Aranda JV (1976) Developmental aspects of the hepatic cytrochrome P450 monooxygenase system. *Annual Review of Pharmacology and Toxicology* 16:427-445.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus LC, Nebert DW (1996) P450 superfamily: Update on new sequences, gene mapping, accession numbers, and nomenclature. *Pharmacogenetics.* 6: 1-42.

Neuhaus T, Ko Y-D, Lorenzen K, Fronhoffs S, Harth V, Bröde P, Vetter H, Bolt HM, Pesch B, Brüning T (2004) Association of cytochrome P450 2E1 Polymorphisms and head and neck squamous cell cancer. *Toxicology Letters* 151: 273-282.

Nioi P and Hayes JD (2004) Contribution of NADP(H):Quinone Oxidoreductase 1 to Protection Against Carcinogenesis, and Regulation of its Gene by The Nrf2 Basic-region Leuicine Zipper and The Arylhydrocarbon Receptor Basic Helix-loop-helix Transcription Factors. *Mutation Research* 555: 149-171.

Nishi M, Miyake H (1989) A case-control study of non-T cell acute lymphoblastic leukaemia of children in Hokkaido, Japan. *Journal of Epidemiology and Community Health* 43: 352–355.

Nishimoto IN, Hanaoka T, Sugimura H, Nagura K, Ihara M, Li X-J, Arai T, Hamada GS, Kowalski LB, Tsugane S (2000) Cytochrome P450 2E1 polymorphism in gastric cancer in Brazil: cas-control studies of Japanese Brazilians and non-Japanese Brazilians. *Cancer Epidemiology, Biomarkers and Prevention* 9: 675-680.

Nuyan M (2008) In vivo interaction of carcinogenic acrylamide with cytochrome p450 isozymes and phase II enzymes in rabbit liver, kidney and lung. *MSc. Thesis.* Middle East Technical University, Ankara, TURKEY.

OEHHA, Office of Environmental Health Hazard Assessment (2000a). Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Office of Environmental Health Hazard Assessment, Cal/EPA April 2000. Available at: www.oehha.ca.gov.

OEHHA, Office of Environmental Health Hazard Assessment (2000b) Air Toxics Hot Spots Program Risk Assessment Guidelines Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis. Office of Environmental Health Hazard Assessment, Cal/EPA. September 2000. Available at www.oehha.ca.gov.

OEHHA, Office of Environmental Health Hazard Assessment (2001) Prioritization of Toxic Air Contaminants Under the Children's Environmental Health Protection Act: Final Report. October 2001. Available at: http://www.oehha.org/air/toxic_contaminants/SB25finalreport.html#download

Ogawa K. Suno M, Shimizu K, Yoshida M, Awaya T, Matsubara K, Shiono H (2003) Genotyping of cytochrome P450 isoform genes is useful for identiication of cadaver. *Legal Medicine* 5(3):132-138

Okcu MF, Goodman KJ, Carozza SE, Weiss NS, Burau KD, Bleyer WA, Cooper SP (2002) Birth weight, ethnicity, and occurrence of cancer in children: a population-based, incident case–control study in the State of Texas, USA. *Cancer Causes Control* 13: 595–602.

Omer B, Ozbek U, Akkose A, Kilic G (2001) Genetic polymorphism of cytochrome P450 2E1 in the Turkish population. *Cell Biochemistry and Function* 19: 273-275.

Omura T and Sato R (1964) The carbon-monoxide binding pigment of liver microsomes I. Evidence for its hemoprotein nature. *The Journal of Biological Chemistry* 239: 2370-2378.

Oyama T, Kawamato T, Mizoue T, Sugio K, Kodama Y, Mitsudomi T, Yasumoto K (1997) Cytochrome P4502E1 polymorphism as a risk factor for lung cancer: In relation to p53 gene mutation. *Anticancer Research* 17: 583-588.

P450 Stats. Dr. Nelson's Cytochrome P450 Homepage. http://drnelson.utmem.edu/p450stats.Feb2008.htm, (last updated on 27.02.2008, accesses on September, 28, 2008).

Pakakasama S, Mukda E, Sasanakul W, Kadegasem P, Udomsubpayakul U, Thithapandha A, Hongeng S (2005) Polymorphisms of Drug-Metabolizing Enzymes and Risk of Childhood Acute Lymphoblastic Leukemia. *American Journal of Hematology* 79:202-205.

Paltiel O, Harlap S, Deutsch L, Knaanie A, Massalha S, Tiram E, Barchana M, Friedlander Y (2004) Birth weight and other risk factors for acute leukemia in the Jerusalem Perinatal Study cohort. *Cancer Epidemiology Biomarkers and Prevention* 13: 1057–1064.

Pan SS, Forrest GL, Akman SA, Hu L-T (1995) NAD(P)H:quinone oxidoreductase expression and mitomycin C resistance developed by human colon cancer HCT 116 cells. *Cancer Research* 55: 330–335.

Pang D, McNally R, Birch JM, on behalf of the UK Childhood Cancer Study Investigators (2003) Parental smoking and childhood cancer: results from the United Kingdom Childhood Cancer Study. *British Journal of Cancer* 88: 373– 381.

Parke D and William RT (1954) Detoxication XLIV. Metabolism of benzene containing ^{14}C benzene. *Biochem. J.* 54:231.

Parke DV (1987) Activation mechanisms to chemical toxicity. *Archives of Toxicology* 60: 5-15.

Parke DV (1994) The cytochromes P450 and and mechanisms of chemical carcinogenesis. *Environmental Health Perspectives* 102: 852-853.

Patten CJ, Ning SM, Lu AYH, Yang CS (1986) Acetone-inducible cytochrome P-450: Purification, catalytic activity, and interaction with cytochrome b_5. *Archives of Biochemistry and Biophysics* 251: 629-638.

Persson I, Johansson I, Lou YC, Yue QY, Duan LS, Bertilsson L, Ingelman-Sundberg M (1999) Genetic polymorphism of xenobiotic metabolizing enzymes among Chinese kung cancer patients. *International Journal of Cancer* 81: 325-329.

Persson JO, Terelius Y, Ingelman-Sundberg M (1990) Cytochrome P-450 dependent formation of reactive oxygen radicals: isozyme-specific inhibition of P-450-mediated reduction oxygen and carbon tetrahloride. *Xenobiotica*. 20: 887-900.

Peter R, Böker R, Beaune PH, Iwasaki M, Guengerich FP, Yang CS (1990) Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIEI. *Chemical Research in Toxicology* 3: 566-573.

Philpot RM, Arınç E, Fotus JR (1975) Reconstitution of the rabbit pulmonary microsomal mixed-function oxidize system from solubilized components. *Drug Metabolism and Disposition* 3: 118-126.

Pinarbasi H, Silig Y, Cetinkaya O, Seyfikli Z, Pinarbasi E (2003) Strong association between the GSTM1-null genotype and lung cancer in a Turkish population. *Cancer Genetics and Cytogenetics* 146(2):125-129.

Pui C-H, Schrappe M, Ribeiro RC, Niemeyer CM (2004) Childhood and Adolescent Lymphoid and Myeloid Leukemia. *Hematology, American Society of Hematology Education Program* 118-145.

Quinones L, Lucas D, Godoy J, Caceres D, Berthou F, Varela N, Lee K, Acevedo C, Martinez L, Aguilera AM, Gil L (2001) CYP1A1, CYP2E1 and GSTM1 genetic polymorphisms. The effect of single and combined genotypes on lung cancer susceptibility in Chilean people. *Cancer Letters* 174: 35-44.

Ramanakumar AV (2007) Need for epidemiological evidence from the developing world to know the cancer-related risk factors. *Journal of Cancer Research and Therapeutics* 3:29-33

Raucy JL, Schultz ED, Wester MR, Arora S, Johnston DE, Omdahl JL, Carpenter SP (1997) Human lymphocyte Cytochrome P450 2E1, a putative marker for alcohol-mediated changes in hepatic chlorzoxazone activity. *Drug Metabolism and Disposition* 25 (12): 1429-1435.

Raunio H, Pasanen M, Mäenpää J, Hakkola J, Pelkonen O (1995) Expression of extrahepatic cytochrome P450 in humans. **In** *Advances in Drug Metabolism in Man* (Edited by: Pacifici GM and Fracchia GN) pp: 234-287. European Commission, Office for Official Publications of the European Communities, Luxembourg.

Rebbeck TR (1997) Molecular Epidemiology of the Human Glutathione S-Transferase Genotypes *GSTM1* and *GS7T1* in Cancer Susceptibility. *Cancer Epidemiology Biomarkers and Prevention* 6: 733-743.

Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, Pui CH, Evans WE (1999) Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *Journal of the National Cancer Institute* 91:2001–2008.

Rendic S and Di-Carlo FJ (1997) Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers and inhibitors. *Drug Metabolism Reviews* 29: 413-580.

Reynolds P, Behren JV, Gunier RB, Goldberg DE, Hertz A, Smith DF (2003) Childhood cancer incidence rates and hazardous air pollutants in California: an exploratory analysis. *Environmental Health Perspectives* 111: 663–668.

Ries LAG, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, Bunin GR (Eds.) (1999) Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975–1995. National Cancer Institute, Bethesda, MD. Available at: http://seer.cancergov/publications/ childhood/leukemia.pdf

Roberts BJ, Shoaf SE, Jeong KS, Song BJ (1994) Induction of CYP2E1 in liver, kidney, brain and intestine during chronic ethanol administration and withdrawal: evidence that CYP2E1 possesses a rapid phase half-life of 6 hours or less. *Biochemical and Biophysical Research Communications* 205: 1064–1071.

Robison LL, Buckley JD, Daigle AE, Wells R, Benjamin D, Arthur DC, Hammond GD (1989) Maternal drug use and risk of childhood nonlymphoblastic leukemia among offspring: an epidemiologic investigation implicating marijuana (a report from the Children's Cancer Study Group). *Cancer* 63: 1904–1911.

Rollinson S, Roddam P, Kane E, Roman E, Cartwright R, Jack A, Morgan GJ (2000) Polymorphic variation within the glutathione S-transferase genes and risk of adult acuteleukaemia. *Carcinogenesis* 21: 43–47.

Ronis MJ, Huang J, Crouch J, Mercado C, Irby D, Valentine CR, Lumpkin CK, Ingelman-Sundberg M, Badger TM (1993) Cytochrome P450 CYP2E1 induction during chronic alcohol exposure occurs by a two step mechanism associated with blood alcohol concentration in rats. *Journal of Pharmacology and Experimental Therapeutics* 246: 944-950.

Ross D (2005) Functions and distribution of NQO1 in human bone marrow: Potential clues to benzene toxicity. *Chemico-Biological Interactions* 153–154: 137–146.

Ross D, and Siegel D (2004) NAD(P)H: Quinone Oxidoreductase 1 (NQO1, DT-Diaphorase), Functions and Pharmacogenetics. **in:** Methods in Enzymology Vol 382-Quinones and Quinone Enzymes, Part B (Ed: Sies H. And Paska L.) Elsevier Publishing.

Ross JA, Potter JD, Shu XO, Reaman GH, Lampkin B, Robison LL (1997) Evaluating the relationships among maternal reproductive history, birth characteristics, and infant leukemia: a report from the Children's Cancer Group. *Annals of Epidemiology* 7:172–179.

Rothman N, Smith MT, Hayes RB, Traver RD, Hoener B, Campleman S, Li GL, Dosemeci M, Linet M, Zhang L, Xi L, Wacholder S, Lu W, Meyer KB, Titenko-Holland N, Stewart JT, Yin S, Ross D (1997) Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609C-T mutation and rapid fractional excretion of chlorzoxazone. *Cancer Research* 57: 2839– 2842.

Rudant J, Menegaux F, Leverger G, Baruchel A, Lambilliotte A, Bertrand Y, Patte C, Pacquement H, Vérité C, Robert A, Michel G, Margueritte G, Gandemer V, Hémon D, Clavel J (2008) Childhood hematopoietic malignancies and parental use of tobacco and alcohol: the ESCALE study (SFCE). *Cancer Causes and Control* 19(10):1277-1290.

Ryan DE, Ramanthan L, Iida S, Thomas PE, Haniu M, Shively JE, Lieber CS, Levin W (1985) Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. *Journal of Biological Chemistry* 260: 6385-6393.

Rylander T, Neve EPA, Ingelman-Sundberg M, Oscarson M (2001) Identification and tissue distribution of the novel human cytochrome P450 2S1 (CYP2S1). *Biochemical and Biophysical Research Communications* 281: 529-535.

Saadat I and Saadat M (2000) The glutathione S-transferase mu polymorphism and susceptibility to acute lymphocytic leukemia *Cancer Letters* 158: 43-45

Savitz DA and Chen J (1990) Parental occupation and childhood cancer: review of epidemiologic studies. *Environmental Health Perspectives* 88:325–337.

Schenkman JB (1991) Cytochrome P450 dependent monooxygenase: An overview. **In** *Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compounds.* (Edited by: Arınç E, Schenkman JB, Hodgson E) pp: 1-13. Kluwer Academic / Plenum Publishers, New York

Schenkman JB and Johnsson I (1975) Interaction between microsomal electron transfer pathways. *Advanced in Experimental Medicineand Biology* 58: 387-404.

Schenkman JB, Thummel KE, Favreu LV (1989) Physiological and patho-physiological alterations in rat hepatic cytochrome P450. *Drug Metabolism Reviews* 20: 557-584.

Schlosser PM, Bond JA, Medinsky MA (1993) Benzene and phenol metabolism by mouse and rat liver microsomes. *Carcinogenesis* 14:2477-2486.

Schulz WA, Krummeck A, Rosinger I, Eickelmann P, Neuhas C, Ebert T, Schmitz-Drager B, Sies H (1997) Increased frequency of a null allele for NAD(P)H:quinone oxidoreductase in patients with urological malignancies, *Pharmacogenetics* 7: 235–239.

Schuz J, Kaatsch P, Kaletsch U, Meinert R, Michaelis J (1999) Association of childhood cancer with factors related to pregnancy and birth. *International Journal of Epidemiology* 28: 631–639.

Seaton MJ, Schlosser PM, Bond JA, Medinsky MA (1995) In vitro conjugation of benzene metabolites by human liver: potential influence of interindividual variability on benzene toxicity. *Carcinogenesis* 16:1519-1527.

Seedhouse C, Bainton R, Lewis M, Harding A, Russell N, Das-Gupta E (2002) The genotype distribution of the XRCC1 gene indicates a role for base excision repair in the development of therapy-related acute myeloblastic leukemia. *Blood* 100: 3761.

Shu XO, Reaman GH, Lampkin B, Sather HN, Pendergrass TW, Robison LL (1994) Association of paternal diagnostic X-ray exposure with risk of infant leukemia. Investigators of the Childrens Cancer Group, *Cancer Epidemiology Biomarkers and Prevention* 3: 645–653.

Shu XO, Ross JA, Pendergrass TW, Reaman GH, Lampkin B, Robison LL (1996) Parental alcohol consumption, cigarette smoking, and risk of infant leukemia: a Children's Cancer Group study. *Journal of National Cancer Institute* 88(1):24–31.

Shu XO, Stewart P, Wen W-Q, Han D, Potter JD, Buckley JD, Heineman E, Robison LL (1999) Parental Occupational Exposure to Hydrocarbons and Risk of Acute Lymphocytic Leukemia in Offspring. *Cancer Epidemiology, Biomarkers and Prevention* 8:783-791.

Sikdar N, Mahmud ASk, Paul RR, Roy B (2003) Polymorphism in CYP1A1 and CYP2E1 genes and susceptibility to leukoplakia in Indian tobacco users. *Cancer Letters* 195(1): 33-42.

Sinnett D, Labuda D, Krajinovic M (2006) Challenges identifying genetic determinants of pediatric cancers – the childhood leukemia experience. *Familial Cancer* 5:35–47.

Sirma S, Agaoglu L, Yildiz I, Cayli D, Horgusluoglu E, Anak S, Yuksel L, Unuvar A, Celkan T, Apak H, Karakas Z, Devecioglu O, Ozbek U (2004) NAD(P)H:Quinone Oxidoreductase 1 Null Genotype Is not Associated With Pediatric De Novo Acute Leukemia. *Pediatr Blood Cancer* 43:568–570

Sivaraman L, Leatham MP, Yee J, Wilkens LR, Lau AF, Le Marchand L (1994) CYP1A1 genetic polymorphisms and in situ colorectal cancer. *Cancer Research* 54:3692–3695.

Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P, Gelber R, Heerema N, Korn EL, Link M, Murphy S, Pui CH, Pullen J, Reamon G, Sallan SE, Sather H, Shuster J, Simon R, Trigg M, Tubergen D, Uckun F, Ungerleider R (1996) Uniform approach to risk classification and treatment for children with acute lymphoblastic leukemia. *Journal of Clinical Oncology* 14:18-24.

Smith MA, Chen T, Simon R (1997) Age-specific incidence of acute lymphoblastic leukemia in U.S. children: in utero initiation model. *Journal of National Cancer Institute* 89: 1542–1544.

Smith MT and Zhang L (1998) Biomarkers of leukemia risk: benzene as a model. *Environmental Health Perspectives* 106(Suppl 4):937-946.

Smith MT, McHalea CM, Wiemelsb JL, Zhanga L, Wienckeb JK, Zhengb S, Gunna L, Skibolaa CF, Maa X, Bufflera PA (2005) Molecular biomarkers for the study of childhood leukemia. *Toxicology and Applied Pharmacology* 206: 237–245.

Smith MT, Wang Y, Kane E, Rollinson S, Wiemels JL, Roman E, Roddam P, Cartwright R, Morgan G (2001) Low NAD(P)H:quinone oxidoreductase 1 activity is associated with increased risk of acute leukemia in adults. *Blood* 97:1422

Snyder R and Chatterjee SP (1991) Benzene Metabolism **in:** Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compounds. Edited by Arinc E, Schenkman JB and Hodgson E. Plenum Publishers, New York.

Snyder R, Hedli CC (1996) An overview of benzene metabolism. *Environmental Health Perspectives* 104(Suppl. 6):1165-1171.

Song BJ (1995) Gene structure and multiple regulations of the ethanol-inducible cytochrome P4502E1 (CYP2E1) subfamily. **In** *Drug and Alcohol Abuse Reviews*. (Edited by: Watson RR) Vol: 6, pp: 177-192. Humana Press, Totowa, NJ.

Song BJ, Gelboin HV, Park S-S, Yang CS, Gonzales FJ (1986) Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450: Transcriptional and posttranscriptional regulation of the rat enzyme. *Journal of Biological Chemistry* 261: 16689-16697.

Song BJ, Matsunaga T, Hardwick JP, Park SS, Veech RL, Yang CS, Gelboin HV, Gonzales FJ (1987) Stabilization of cytochrome P450j messenger ribonucleic acid in the rat. *Molecular Endocrinology* 1: 542-547.

Sorahan T, Lancashire RJ, Hulte'n MA, Peck I, Stewart AM (1997) Childhood cancer and parental use of tobacco: deaths from 1953 to 1955. *British Journal of Cancer* 75: 134– 138.

Sorahan T, Lancashire RJ, Prior P, Peck I, Stewart AM, (1995) Childhood cancer and parental use of alcohol and tobacco. *Annals of Epidemiology* 5: 354–359.

Sorahan T, McKinney PA, Mann JR, Lancashire RJ, Stiller CA, Birch JM, Dodd HE, Cartwright RA (2001) Childhood cancer and parental use of tobacco: findings from the inter-regional epidemiological study of childhood cancer (IRESCC). *British Journal of Cancer* 84: 141–146.

Soucek P, Filipcova B, Gut I (1994) Cytochrome P450 destruction and radical scavenging by benzene and its metabolites. Evidence for the key role of quinones. *Biochemical Pharmacology* 47: 2233-2242.

Soucek P, Sarmanová J, Kristensen VN, Apltauerová M, Gut I (2002) Genetic polymorphisms of biotransformation enzymes in patients with Hodgkin's and non-Hodgkin's lymphomas. *International Archives of Occupational and Environmental Health* 75(Suppl.1): 86-92.

Spire-Vayron de la Moureyre C, Debuysere H, Mastain B, Vinner E, Marez D, Lo Guidice JM, Chevalier D, Brique S, Motte K, Colombel JF, Turck D, Noel C, Flipo RM, Pol A, Lhermitte M, Lafitte JJ, Libersa C, Broly F (1998) Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene (TPMT) in a European population. *British Journal of Pharmacology* 125:879–887.

Steffen C, Auclerc MF, Auvrignon A, Baruchel A, Kebaili K, Lambilliotte A, Leverger G, Sommelet D, Vilmer E, Hemon D, Clavel J (2004) Acute childhood leukaemia and environmental exposure to potential sources of benzene and other hydrocarbons; a case-control study. *Occupational and Environmental Medicine* 61: 773–778.

Stewart A, Webb J, Giles D, Hewitt D, (1956) Malignant disease in childhood and diagnostic irradiation in utero. *Lancet* 2: 447– 448.

Stoilov I, Jansson I, Sarfarazi M, Schenkman JB (2001) Roles of cytochrome P450 in development. *Drug Metabolism and Drug Interactions* 18(1): 33-55.

Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol* 12:1110–1116

Sunaga N, Kohno T, Yanagitani N, Sugimura H, Kunitoh H, Tamura T, Takei Y, Tsuchiya S, Saito R, Yokota J (2002) Contribution of the NQO1 and GSTT1 polymorphisms to lung adenocarcinoma susceptibility. *Cancer Epidemiol Biomarkers Prev.* 11(8):730-738.

Şen A and Arınç E (1997) Separation of three P450 isozymes from liver microsomes of gilthead seabream treated with β-naphtoflavone and partial purification. *Biochemistry and Molecular Biology International* 41: 134-141.

Şen A and Arınç E (1998) Preparation of highly purified cytochrome P4501A1 from leaping mullet (*Liza saliens*) liver microsomes and biocatalytic, molecular and immunochemical properties. *Comparative Biochemistry and Physiology* 121C: 249-265.

Şen A, and Arinc E (1998) Purification and characterization of cytochrome P450 reductase from liver microsomes of feral leaping mullet (Liza saliens). *Journal of Biochemistry and Molecular Toxicology* 12(2):103-113.

Tanaka E (2001) Polymorphism of drug metabolizing enzymes in humans. *Sepsis*. 4: 247-254.

Tateishi T, Nakura H, Asoh M, Watanabe M, Tanaka M, Kumai T, Takashima S, Imaoka S, Funae Y, Yabusaki Y, Kamataki T, Kobayashi S (1997) A comparison of hepatic cytochrome P450 protein expression between infancy and postinfancy. *Life Sciences* 61(26):2567-2574.

Taylor GM, Dearden S, Payne N, Ayres M, Gokhale MA, Birch JM, Blair V, Stevens RF, Will AM, Eden OB (1998) Evidence that an HLA-DQA1-DQB1 haplotype influences susceptibility to childhood common acute lymphoblastic leukaemia in boys provides further support for an infection-related aetiology. *British Journal of Cancer* 78: 561–565.

Taylor GM, Dearden S, Ravetto P, Ayres M, Watson P, Hussain A, Greaves M, Alexander F, Eden OB (2002) Genetic susceptibility to childhood common acute lymphoblastic leukaemia is associated with polymorphic peptide-binding pocket profiles in HLA-DPB1*0201. *Human Molecular Genetics* 11: 1585–1597.

Terelius Y, Lindros KO, Albano E, Ingelman-Sundberg M (1993) Isozyme specificity of cytochrome P450-mediated hepatotoxicity. **In** *Frontiers of biotransformation.* (Edited by: Rein H and Ruckpaul K). vol: 8, pp:186-232. Akademie Verlag, Berlin.

The Korean Leukemia Cell and Gene Bank (KLCGB), Molecular Genetic Research Lab. The Catholic University of Korea. http://www.klcgb.or.kr/eng/about/1_2.html. (accessed on January 28, 2009).

The Leukemia and Lymphoma Society (LLS). Leukemia Facts and Statistics. http://www.leukemia-lymphoma.org/all_page.adp?Item_id=9346. (last update on 07.18.2008, accessed on September 28, 2008).

The World Factbook, Central Intelligence Agency Central Intelligence Agency, https://www.cia.gov/library/publications/the-world-factbook/fields/2075.html. (last update: 22.01.2009, accessed on 29.01.2009).

Thier R, Lewalter J, Selinski S, Bolt HM. (2002) Possible impact of human CYP2E1 polymorphisms on the metabolism of acrylonitrile. *Toxicol Letters* 128: 249–255.

Thier R, Pemble SE, Kramer H, Taylor JB, Guengerich FP, Ketterer B (1996) Human glutathione S-transferase T1-1 enhances mutagenicity of 1,2-dibromoethane, dibromomethane and 1,2,3,4-diepoxybutane in Salmonella typhimurium. *Carcinogenesis* 17:163–166

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of National Academy of Science.* 76(9):4350-4354.

Townsend DW and Tew KD (2003) The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene* 22: 7369–7375.

Treluyer JM, Jacqz-Aigrain E, Alvarez F, Cresteil T (1991). Expression of CYP2D6 in developing liver. *European Journal of Biochemistry* 202:583-588.

U.S. EPA (1998) Carcinogenic effects of benzene: an update. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C., EPA/600/P-97/001F.

Uematsu F, Ikawa S, Kikuchi H, Sagami I, Kanamaru R, Abe T, Satoh K, Motomiya M, Watanabe M (1994) Restriction fragment length polymorphism of the human CYP2E1 (cytochrome P450IIE1) gene and susceptibility to lung cancer: possible relevance to low smoking exposure. *Pharmacogenetics* 4(2):58-63.

Uematsu F, Kikuchi H, Motomiya Abe T, Ishioka C, Kanamaru R, Sagami I, Watanabe M (1992) Human cytochrome *P450IIEI* gene: *Dra*I polymorphism and susceptibility to cancer. *Tohoku Journal of Experimental Medicine* 168: 113-117.

Uematsu F, Kikuchi H, Motomiya Abe T, Sagami I, Ohmachi T, Wakui A (1991) Association between restriction fragment length polymorphism of the human cytochrome P450 2E1 gene and susceptibility to lung cancer. *Japanese Journal of Cancer Research* 82: 254-256.

Ulusoy G (2004) Genetic Polymorphisms of Alcohol Inducible CYP2E1 in Turkish Population. *MS. Thesis.* Middle East Technical University, Ankara.

Ulusoy G, Adali O, Boyunegmez Tumer T, Sahin G, Gozdasoglu S, Arinç E (2007a) Significance of genetic polymorphisms at multiple loci of *CYP2E1* in the risk of development of childhood acute lymphoblastic leukemia. *Oncology* 72(1-2): 125-131.

Ulusoy G, Arinç E, Adali O (2007b) Genotype and allele frequencies of polymorphic *CYP2E1* in the Turkish population. *Archives of Toxicology* 81:711-718.

Umeno M, McBridge OW, Yang CS, Gelboin HV, Gonzales FJ (1988) Human ethanol-inducible P450IIEI: complete gene sequence, promoter characterization, chromosome mapping and cDNA-directed expression. *Biochemistry* 27: 9006-9013.

United Nations Scientific Committee on the Effects of Atomic Radiation (1994) Sources and Effects of Ionizing Radiation. New York:United Nations.

Valentine JL, Lee SST, Seaton MJ, Asgharian B, Farris G, Corton JC (1996) Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. *Toxicology and Applied Pharmacology* 141(1):205-213.

Van Duijn CM, Van Steensel-Moll HA, Coebergh JW, Van Zanen GE (1994) Risk factors for childhood acute non-lymphocytic leukemia: an association with maternal alcohol consumption during pregnancy? *Cancer Epidemiology Biomarkers and Prevention* 3:457–460.

Van Steensel-Moll HA, Valkenburg HA, Vandenbroucke JP, van Zanen GE (1985) Are maternal fertility problems related to childhood leukaemia? *International Journal of Epidemiology* 14: 555–559.

Vodicka P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, Vodickova L, Koskinen M, Zwart FA, Natajaran AT, Hemminki K (2001) Association between genetic polymorphisms and biomarkers in styrene-exposed workers. *Mutation Research* 482: 89-103.

Vodicka P,Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, Vodickova L, Koskinen M, Zwart FA, Natarajan AT, Hemminkif K (2001) Association between genetic polymorphisms and biomarkers in styrene-exposed workers. Mutation Research 482: 89–103

Wan J, Shi J, Hui L, Wu D, Jin X, Zhao N, Huang W, Xia Z, Hu G (2002) Association of genetic polymorphisms in CYP2E1, MPO, NQO1, GSTM1, and GSTT1 genes with benzene poisoning. *Environmental Health Perspectives* 110 (12): 1213-1218.

Wang J, Deng Y, Li L, Kuriki K, Ding J, Pan X, Zhuge X, Jiang J, Luo C, Lin P, Tokudome S (2003) Association of GSTM1, CYP1A1 and CYP2E1 genetic polymorphisms with susceptibility to lung adenocarcinoma: A case-control study in Chinese population. *Cancer Science* 94(5):448-452

Wang S-L, Lee H, Chen K-W, Tsai K-J, Chen C-Y, Lin P (1999) Cytochrome P4502E1 genetic polymorphisms and lung cancer in a Taiwanese population. *Lung Cancer* 26: 27-34.

Watanabe J, Hayashi S, Nakachi K, Imai K, Suda Y, Sekine T, Kawajiri K (1990) PstI and RsaI RFLPs in complete linkage disequilibrium at the CYP2E gene. *Nucleic Acids Res.* 18(23): 7194.

Westergaard T, Andersen PK, Pedersen JB, Olsen JH, Frisch M, Sorensen HT, Wohlfahrt J, Melbye M (1997) Birth characteristics, sibling patterns, and acute leukemia risk in childhood: a population-based cohort study, *Journal of the National Cancer Institute* 89: 939–947.

WHO (1999) *Vinyl Chloride* (Environmental Health Criteria 215), Geneva, World Health Organization.

Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, Saha V, Biondi A, Greaves MF (1999a) Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet* 354:1499-1503.

Wiemels JL, Ford AM, Van Wering ER, Postma A, Greaves M (1999b) Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion in utero. *Blood* 94:1057-1062.

Wiemels JL, Pagnamenta A, Taylor GM, Eden OB, Alexander FE, Greaves MF (1999c) A lack of a functional NAD(P)H:quinone oxidoreductase allele is selectively associated with pediatric leukemias that have MLL fusions. United Kingdom Childhood Cancer Study Investigators. *Cancer Research* 59: 4095-4099.

Wiseman H and Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemical Journal* 313: 17-29.

Wrighton SA, Thomas PE, Molowa DT, Haniu M, Shively JE, Maines SL, Watkins PB, Parker G, Mendez-Picon G, Levin W, Guzelian PS (1986) Characterization of ethanol-inducible human liver N-nitrosodimethylamine demethylase. *Biochemistry* 25: 6731-6735.

Wrighton SA, Thomas PE, Ryan DE, Lewin W (1987) Purification and characterization of ethanol-inducible human hepatic cytochrome P-450j. *Archives of Biochemistry and Biophysics* 258: 292-297.

Wu S, Moomaw CR, Tomer KB, Falck JR, Zeldin DC (1996) Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *Journal of Biological Chemistry* 271: 3460-3468.

Wu X, Amos CI, Kemp BL, Shi H, Jiang H, Wan Y, Spitz MR (1998) Cytochrome P450 2E1 *Dra*I polymorphism in lung cancer in minority populations. *Cancer Epidemiology Biomarkers and Prevention* 7: 13-18.

Wu X, Shi H, Jiang H, Kemp B, Hong WK, Delclos GL, Spitz MR (1997) Associations between cytochrome P4502E1 genotype, mutagen sensitivity, cigarette smoking and susceptibility to lung cancer. *Carcinogenesis* 18(5): 967-73.

Xu LL, Wain JC, Miller DP, Thurston SW, Su L, Lynch TJ, Christiani DC (2001) The NAD(P)H:quinone oxidoreductase 1 gene polymorphism and lung cancer: differential susceptibility based on smoking behavior. *Cancer Epidemiology Biomarkers and Prevention* 10(4):303-309.

Yang BM, O'Reilly DA, Demaine AG, Kingsnorth AN (2001) Study of polymorphisms in the CYP2E1 gene in patients with alcoholic pancreatitis. *Alcohol* 23: 91-97.

Yang M, Coles BF, Delongchamp R, Lang NP, Kadlubar FF (2002) Effects of the ADH3, CYP2E1 and GSTP1 genetic polymorphisms on their expressions in Caucasian lung tissue. *Lung Cancer* 38:15-21

Yaris F, Dikici M, Akbulut T, Yaris E, Sabuncu H (2004) Story of benzene and leukemia: Epidemiologic approach of Muzaffer Aksoy. *Journal of Occupational Health* 46: 24-247.

Yin L, Pu Y, Liu TY, Tung YH, Chen KW, Lin P (2001) Genetic polymorphisms of NAD(P)H quinone oxidoreductase, CYP1A1 and microsomal epoxide hydrolase and lung cancer risk in Nanjing, China. *Lung Cancer* 33(2-3):133-141.

Yoo JSH, Ning SM, Pantuck CB, Pantuck EJ, Yang CS (1991) Regulation of hepatic microsomal cytochrome P4502E1 level by dietary lipids and carbohydrates in rats. *Journal of Nutrition* 121: 959-965.

Zhang J-H, Li Y, Wang R, Geddert H, Guo W, Wen D-G, Chen Z-F, Wei L-Z, Kuang G, He H, Zhang L-W, Wu ML, Wang S-J (2003) *NQO1* C609T polymorphism associated with esophageal cancer and gastric cardiac carcinoma in North China. *World Journal Gastroenterology* 9: 1390–1393.

Zhong S, Howie AF, Ketterer B, Taylor J, Hayes JD, Beckett GJ, Wathen CG, Wolf CR, Spurr NK (1991) Glutathione S-transferase μ locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. *Carcinogenesis (Lond.)* 12: 1533-1537.

Zhong S, Wyllie AH, Barnes D, Wolf CR, Spurr NK (1993) Relationship between the *GSTM1* genetic polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis (Lond.)* 14: 1821-1824.

Zhu SM, Xia ZL, Wang AH, Ren XF, Jiao J, Zhao NQ, Qian J, Jin L, Christiani DC (2008) Polymorphisms and haplotypes of DNA repair and xenobiotic metabolism genes and risk of DNA damage in Chinese vinyl chloride monomer (VCM)-exposed workers. *Toxicology Letters.* 178(2):88-94.

APPENDIX A

INFORMED CONSENTS AND QUESTIONAIRRES

A.1 Written Informed Consent Form For Control Group

ONAY BELGESİ No:38

Benden alınan 3-5 mL kan örneğinin, O.D.T.Ü. Biyoloji Bölümü'nde Araştırma Görevlisi Gülen Ulusoy'un "Türkiye popülasyonunda insan CYP2E1 enziminin polimorfizmi" konulu Yüksek Lisans Tez çalışması için kullanılacağını biliyorum ve onay veriyorum.

Kan verenin Adı, Soyadı:

İmza

ANKET

1. Kadın | Erkek

2. Yaş:.

3. Doğum yeri ve yılı:

4. Annenizin doğum yeri:
Babanızın doğum yeri:

5. Sağlığınızla ilgili gönüllü olarak vermek istediğiniz bilgiler (geçirdiğiniz hastalıklar, örneğin diyabet, kanser vb.).

A.2 Written Informed Consent Form For All-Patient Group

ONAY BELGESİ

Hastanın tedaviye cevabını gösterecek olan ve O.D.T.Ü Biyoloji Bölümünde yütütülecek 'Türkiye'de Akut Lösemi Hastası Çocuklarda İlaç Metabolizmasında Rol Oynayan Enzimlerin Genetik Polimorfizmlerinin Araştırılması' için, çocuğumdan alınan 2 – 3 mL kan örneğinin kullanılacağını biliyor ve onay veriyorum.

Anne veya Babanın adı soyadı: Tarih:
İmza:

HASTA BİLGİLERİ

1. Hastanın adı soyadı:
2. Hastanın takip edildiği hastane ve protokol numarası:
3. Kız Erkek
4. Yaş:
5. Doğum yeri ve yılı:
6. Eş akrabalığı var mı?:
7. Anne doğum yeri: Baba doğum yeri:
8. Hastanın klinik tanısı:
9. Tedavinin aşaması:
10. Hastanın risk grubu:
11. Tedavi şekli (eğer sabit bir tedavi protokolü varsa belirtilmeli, böyle bir protokol yoksa kullanılan ilaçların adı ve dozajı belirtilmelidir):
12. Hasta son 3 aylık dönemde kan transfüzyonu aldı mı? (evet ise en son kan transfüzyonun yapıldığı tarihi belirtiniz):
13. Çocuğun daha önce geçirmiş olduğu ya da halen var olan (lösemi dışındaki) önemli hastalıklar varsa belirtiniz:
14. Ailede başka kanser hastası var mı? Varsa kanserin çeşidini ve bu kişinin hasta ile akrabalık derecesini belirtiniz:
15. Tam kan sayımı sonuçları:
    Not: Sonuçlar bilgisayar çıktısı halinde ise onay belgesinin arkasına zımbalanmalıdır.
16. Karaciğer fonkisyon testleri sonuçları:

A.3 Cigatte Smoking Status Questionnaire

MALİGNNİTE VE SİGARA İLİŞKİSİ FORMU

ADI SOYADI:

YAŞI:

MALİGNNİTE TİPİ:

EVRESİ:

REMİSYON EVET HAYIR

ANNE YAŞI:

SİGARA İÇME: AKTİF PASİF( )SÜRESİ:

BAŞLAMA YAŞI:

KAÇ YILDIR İÇİYOR:

HAMİLELİKTE İÇME: EVET HAYIR

MİKTARI: TANE/GÜN

BABA YAŞI:

SİGARA İÇME: AKTİF PASİF

BAŞLAMA YAŞI:

KAÇ YILDIR İÇİYOR:

MİKTARI: TANE/GÜN

POSTNATAL SİGARAYA MARUZİYET: EVET HAYIR

EVETSE SÜRESİ(AY OLARAK):

AKRABALIK DÜZEYİ:

AYLIK EKONOMİK NET GELİR(YTL):

APPENDIX B

BUFFERS AND SOLUTIONS

All the glassware used for DNA isolation, genotyping and lymphocyte isolation procedures were sterilized by autoclaving and sterilized distilled water was used for the preparation of solutions.

Tris-HCl, pH 8.0 (100 mM);

12.1 g Tris was weighed and dissolved in 700 mL of dH_2O. pH was adjusted to 8.0 with concentrated HCl and volume was completed to 1 L. Solution was autoclaved for sterilization and stored at 4°C.

EDTA, pH 8.0 (500 mM);

186.1 g $Na_2EDTA.2H_2O$ was weighed and dissolved in 700 mL dH_2O. Dissolution of EDTA was achieved by adjusting the pH to 8.0 with NaOH. Volume was completed to 1 L. Solution was autoclaved for sterilization and stored at 4°C.

TKME (Tris-KCl-$MgCl_2$-EDTA) Buffer, pH 7.6;

10mM Tris-HCl (pH 7.6), 10 mM KCl, 4 mM $MgCl_2$, 2 mM EDTA.
Solution was autoclaved for sterilization and stored at 4°C.

Saturated NaCl (6M)

3.5064 g NaCl was weighed and dissolved in 10 mL of sterilized dH_2O.
Solution was autoclaved for sterilization and stored at 4°C.

TE (Tris-EDTA) Buffer, pH 8.0;

10 mM Tris-HCl (pH 8.0), 1mM EDTA (pH 8.0).

Solution was autoclaved for sterilization and stored at 4°C.

TBE (Tris-Borate-EDTA) Buffer, pH 8.3;

5x stock solution: 54 g Trizma-base and 27.5 g boric acid were weighed and dissolved in appropriate amount of water. 20 mL of 500 mM EDTA (pH 8.0) was added. pH was set to 8.3. Volume was completed to 1 L. Solution was autoclaved for sterilization and stored at room temperature.

0.5x solution: The stock solution was diluted 10 times with sterilized dH_2O prior to use to achieve 45 mM Tris-borate, 1 mM EDTA.

Ethidium Bromide (10 mg/mL);

0.1 g ethidium bromide was dissolved in 10 mL dH_2O. Solution was stirred on magnetic stirrer for several hours to ensure that dye had completely dissolved. As this solution is light sensitive, the bottle was covered with aluminum foil and stored at room temperature.

Gel loading dye

0.25% bromophenol blue, 40% sucrose in sterilized dH_2O.

Solution is stored at 4°C.

PCR Amplification Buffer (10x) (Fermentas);

100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 0.8% Nonidet P40.

This buffer and 25 mM $MgCl_2$ solution were supplied together with Taq DNA Polymerase. The solutions were stored at -20°C.

dNTP Mixture (Fermentas);

10 mM of each dATP, dCTP, dGTP and dTTP in aqueous solution. The solution was stored at -20°C.

Buffer O^+ (digestion buffer of *Pst*I) (Fermentas);

50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/mL BSA.

This buffer was supplied together with the restriction enzyme *Pst*I. The restriction enzyme and buffer were stored at -20°C.

Buffer Y^+/TANGO™ (digestion buffer of *Rsa*I) (Fermentas);

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

This buffer was supplied together with the restriction enzyme *Rsa*I. The restriction enzyme and buffer were stored at -20°C.

Buffer B^+ (digestion buffer of *Dra*I) (Fermentas);

10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mg/mL BSA.

This buffer was supplied together with the restriction enzyme *Dra*I. The restriction enzyme and buffer were stored at -20°C.

Buffer R (digestion buffer of *Hinf*I) (Fermentas);

10 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA.

This buffer was supplied together with the restriction enzyme *Hinf*I. The restriction enzyme and buffer were stored at -20°C.

Buffer G (digestion buffer of *Dde*I) (GeneMark);

10 mM Tris-HCL (pH 7.6), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT.

This buffer was supplied together with the restriction enzyme *Ddef*I. The restriction enzyme and buffer were stored at -20°C.

Gene Ruler 50 bp DNA Ladder (0.5 mg DNA/mL) (Fermentas);

This commercial DNA ladder was prepared from a specially designed plasmid pEJ3 DNA, containing pUC, λ phage and yeast genome sequences. The ladder was dissolved in storage buffer (10 mM Tris-HCl (pH 7.6), 1 mM EDTA).

6x Loading dye solution: 0.09% bromophenol blue, 0.09% xylene cyanol FF, 60% glycerol, 60 mM EDTA.

The ladder was prepared by mixing DNA ladder: 6x loading dye solution: dH_2O in 1:1:4 ratio, mixed well and applied to the gel.

The DNA ladder contained the following discrete fragments (in base pairs):

1031 900 800 700 600 500 400 300 250 200 150 100 50

APPENDIX C

GENOTYPE LIST FOR THE STUDY POPULATIONS

Table C.1 Genotype List For Control Samples

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
1	♂	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
2	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
3	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
4	♀	40	*1A*1A	*1A*6	*1A*1A	*1*2	+	null
5	♀	39	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
6	♀	21	*1A*1A	*1A*6	*1A*1A	*1*1	null	null
7	♀	40	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
8	♂	58	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
9	♂	22	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
10	♂	20	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
11	♀	54	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
15	♀	37	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
16	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
17	♂	19	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
18	♀	40	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
19	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
20	♀	40	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
21	♂	22	*1A*1A	*1A*6	*1A*1A	*1*2	+	+
22	♂	20	*1A*5B	*6*6	*1A*1A	*1*2	null	+
23	♂	20	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+

Table C.1 Continued.

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
24	♂	50	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
25	♂	28	*1A*1A	*1A*1A	*1A*7B	*1*1	+	null
26	♀	19	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
27	♀	29	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
28	♀	26	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
29	♀	20	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
30	♂	47	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
31	♂	49	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
32	♀	20	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
33	♂	43	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
34	♀	20	*1A*1A	*1A*1A	*1A*1A	*2*2	null	null
35	♂	62	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
36	♀	30	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
37	♀	22	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
39	♀	19	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
40	♀	16	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
41	♀	21	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
42	♀	40	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
43	♂	39	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
44	♀	19	*1A*1A	*1A*1A	*1A*7B	*2*2	+	+
45	♀	48	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
46	♀	46	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
47	♂	43	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
48	♀	19	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
49	♂	34	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
50	♂	55	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
51	♀	25	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
52	♂	48	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
53	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
54	♀	57	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
55	♀	20	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+

Table C.1 Continued.

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
56	♀	55	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
57	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
58	♀	23	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
59	♀	22	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
60	♀	40	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
61	♂	18	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
62	♀	45	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
63	♀	38	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
64	♀	21	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
65	♂	42	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
66	♀	19	*1A*5B	*1A*1A	*1A*1A	*1*1	null	+
67	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
68	♂	38	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
69	♀	23	*1A*1A	*1A*1A	*1A*7B	*2*2	+	+
70	♀	22	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
71	♀	26	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
72	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
73	♂	22	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
74	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
75	♂	31	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
76	♀	46	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
77	♂	22	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
78	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
79	♂	42	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
80	♂	53	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
81	♀	40	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
82	♀	19	*1A*1A	*1A*1A	*1A*1A	*2*2	null	null
83	♀	38	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
84	♂	41	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
85	♂	38	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
86	♀	39	*1A*1A	*1A*6	*1A*1A	*1*2	null	+

Table C.1 Continued.

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
87	♀	23	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
88	♀	42	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
89	♀	26	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
90	♂	36	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
91	♀	22	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
92	♀	57	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
93	♀	28	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
94	♂	27	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
95	♂	47	*1A*5B	*1A*6	*1A*1A	*1*1	null	+
96	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
97	♀	25	*1A*1A	*1A*1A	*1A*7B	*1*2	null	null
98	♂	50	*1A*1A	*1A*6	*1A*1A	*1*2	+	+
99	♀	47	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
100	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
101	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
102	♂	35	*1A*1A	*1A*6	*1A*1A	*1*1	null	null
103	♀	17	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
104	♀	24	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
105	♀	42	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
106	♂	19	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
107	♀	22	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
108	♂	23	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
109	♂	27	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
110	♂	48	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
111	♂	43	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
112	♂	24	*1A*5B	*1A*1A	*1A*1A	*1*1	+	null
113	♂	24	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
114	♀	23	*1A*5B	*1A*1A	*1A*1A	*1*1	null	+
115	♀	14	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
116	♀	24	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
117	♀	63	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null

Table C.1 Continued.

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
118	♀	62	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
119	♂	24	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
120	♀	27	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
121	♀	25	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
122	♀	22	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
123	♂	65	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
124	♀	12	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
125	♂	34	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
126	♂	26	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
127	♀	50	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
128	♂	49	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
129	♀	50	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
130	♀	44	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
131	♀	30	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
132	♀	40	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
133	♂	48	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
134	♂	57	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
135	♀	43	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
136	♀	57	*1A*1A	*1A*6	*1A*1A	*2*2	null	+
137	♀	49	*1A*1A	*1A*1A	*1A*1A	*2*2	+	+
138	♀	28	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
139	♂	21	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
140	♀	53	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
141	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
142	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
143	♂	14	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
144	♀	43	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
145	♂	44	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
146	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
147	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
148	♂	41	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null

Table C.1 Continued.

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
149	♀	33	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
150	♀	25	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
151	♂	38	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
152	♀	20	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
153	♀	21	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
154	♀	19	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
155	♀	52	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
156	♂	27	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
157	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
158	♀	25	*1A*1A	*1A*1A	*7B*7B	*1*1	null	+
159	♀	39	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
160	♂	21	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
161	♀	31	*1A*1A	*1A*6	*1A*1A	*1*2	+	+
162	♂	25	*1A*1A	*1A*1A	*1A*1A	*2*2	+	+
163	♀	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
164	♂	40	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
165	♀	25	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
166	♂	30	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
167	♀	32	*1A*1A	*1A*1A	*1A*7B	*1*2	+	null
168	♀	25	*1A*1A	*1A*1A	*1A*1A	*2*2	+	+
169	♀	24	*1A*5B	*1A*6	*1A*1A	*1*1	null	+
170	♀	33	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
171	♀	32	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
172	♀	34	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
173	♀	36	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
174	♀	30	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
175	♀	27	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
176	♀	23	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
177	♂	24	*1A*5B	*1A*6	*1A*1A	*1*1	+	+
178	♂	32	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
179	♂	27	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+

Table C.1 Continued.

Sample No	Gender	Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
180	♀	31	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
181	♂	45	*1A*5B	*1A*6	*1A*1A	*1*2	null	null
182	♀	25	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
183	♀	26	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
184	♀	21	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
185	♀	25	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
186	♂	56	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
187	♀	19	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
188	♀	19	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
189	♀	20	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
190	♂	22	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
191	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
192	♀	20	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
193	♂	20	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
194	♀	21	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
195	♀	17	*1A*1A	*1A*1A	*1A*7B	*2*2	+	+
196	♂	23	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
197	♀	38	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
198	♂	20	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
199	♂	20	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
200	♀	26	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
201	♂	50	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
202	♂	24	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
203	♀	19	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
204	♀	25	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
205	♀	40	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
206	♂	21	*1A*1A	*1A*1A	*1A*7B	*1*2	null	null
207	♂	30	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
208	♂	31	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
209	♂	47	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
210	♂	22	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null

Table C.2 Genotype List for Patient Samples

Sample No	Gender	Diagn. Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
1	♀	15	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
2	♀	14	*1A*5B	*1A*6	*1A*1A	*1*2	+	+
3	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
4	♀	14	*1A*5B	*1A*6	*1A*1A	*1*2	+	+
5	♂	4,5	*1A*1A	*1A*6	*1A*7B	*1*2	+	null
6	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
7	♀	2,5	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
8	♂	11	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
9	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
10	♂	3	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
11	♂	7	*1A*1A	*1A*1A	*1A*7B	*1*1	null	null
12	♂	9	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
13	♀	9	*1A*5B	*1A*6	*1A*1A	*1*2	null	+
14	♂	12	*1A*1A	*1A*6	*1A*1A	*2*2	+	null
15	♀	5	*1A*5B	*1A*6	*1A*1A	*1*1	null	+
16	♀	5	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
17	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
18	♂	8	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
21	♀	3,5	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
22	♂	2,5	*1A*5B	*1A*6	*1A*1A	*1*2	+	+
23	♂	5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
24	♂	2,5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
27	♂	5	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
28	♀	3,5	*1A*1A	*1A*6	*1A*1A	*2*2	null	+
29	♂	8	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
30	♀	12	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
31	♂	4	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
32	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
34	♂	7	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
35	♀	10	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
37	♀	10	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+

Table C.2 Continued.

Sample No	Gender	Diagn. Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
41	♂	13	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
43	♂	9	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
44	♀	6	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
45	♂	3,5	*1A*5B	*1A*6	*1A*1A	*1*1	null	null
48	♂	2,5	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
49	♀	3	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
50	♂	4,5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
51	♀	5	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
52	♂	4	*1A*5B	*1A*6	*1A*1A	*1*2	+	null
53	♂	2,5	*1A*1A	*1A*6	*1A*1A	*1*1	null	null
54	♂	7	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
55	♂	4	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
56	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
57	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
58	♀	3	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
59	♂	7	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
60	♂	3,5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
61	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
62	♂	4	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
63	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
64	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
65	♀	13	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
66	♂	3,5	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
67	♀	5	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
68	♂	14	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
69	♂	7	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
70	♂	5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
71	♂	4	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
72	♂	3	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
73	♀	3,5	*1A*5B	*1A*6	*1A*1A	*1*1	null	null
74	♂	3,5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null

Table C.2 Continued.

Sample No	Gender	Diagn. Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
76	♀	11	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
77	♀	13	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
78	♀	12	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
79	♀	5	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
80	♀	9	*1A*1A	*1A*1A	*1A*7B	*1*1	null	null
81	♂	9	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
82	♂	15	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
83	♂	11	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
84	♀	3,5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
85	♀	8	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
86	♀	5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
87	♀	9	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
88	♂	14	*1A*1A	*1A*1A	*1A*1A	*2*2	+	+
89	♂	13	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
90	♂	10	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
91	♀	3,5	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
92	♀	11	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
93	♂	7	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
94	♂	8	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
95	♀	6	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
96	♀	10	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
97	♂	4	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
98	♀	8	*1A*1A	*1A*6	*1A*1A	*2*2	null	+
99	♂	2,5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
100	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
101	♀	15	*1A*1A	*1A*6	*1A*1A	*1*1	+	null
102	♀	3	*1A*1A	*1A*6	*1A*1A	*1*2	+	+
103	♂	5	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
104	♂	3	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
105	♂	9	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
107	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null

Table C.2 Continued.

Sample No	Gender	Diagn. Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
108	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
109	♂	3	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
110	♂	3	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
111	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
113	♂	7	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
114	♂	4	*1A*1A	*1A*1A	*1A*7B	*1*2	+	+
115	♂	4	*1A*1A	*1A*6	*1A*7B	*1*1	null	null
116	♂	5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
117	♂	4	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
118	♀	3	*1A*1A	*1A*1A	*1A*7B	*1*1	+	null
119	♂	13	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
123	♂	13	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
124	♀	3	*1A*1A	*1A*1A	*1A*1A	*2*2	+	null
125	♂	2	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
127	♂	3	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
128	♂	12	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
129	♂	3,5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
130	♀	7	*1A*5B	*1A*6	*1A*1A	*1*2	null	null
131	♀	12	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
132	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
133	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
134	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
135	♂	14	*1A*1A	*1A*1A	*1A*1A	*2*2	+	+
136	♂	8	*1A*5B	*1A*6	*1A*1A	*1*1	null	null
137	♂	9	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
138	♂	5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
139	♂	13	*1A*1A	*1A*6	*1A*7B	*1*1	+	+
140	♀	2	*1A*1A	*1A*1A	*1A*7B	*1*2	+	null
141	♂	5	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
142	♂	2	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
143	♀	7	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+

Table C.2 Continued.

Sample No	Gender	Diagn. Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
144	♂	2,5	*1A*1A	*1A*6	*1A*1A	*1*1	null	+
145	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
146	♂	9	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
147	♀	10	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
149	♂	8	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
150	♀	11	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
151	♂	14	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
153	♀	6	*1A*1A	*1A*1A	*1A*1A	*2*2	null	null
154	♀	4	*1A*1A	*1A*1A	*7B*7B	*1*1	null	+
155	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*2	null	+
156	♀	4,5	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
157	♀	5	*1A*1A	*1A*1A	*1A*1A	*2*2	+	+
158	♂	3.5	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
159	♂	2,5	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
160	♂	12	*1A*1A	*1A*1A	*1A*7B	*1*1	null	null
161	♀	13	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
162	♂	3	*1A*1A	*1A*1A	*7B*7B	*1*2	null	+
163	♂	15	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
164	♂	4	*1A*1A	*1A*1A	*1A*7B	*1*2	null	+
165	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
166	♂	12	*1A*1A	*1A*1A	*7B*7B	*1*1	null	+
167	♀	9	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
169	♂	5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
172	♀	8	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
173	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
174	♀	5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
176	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
177	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
178	♀	7	*1A*1A	*1A*6	*1A*1A	*1*2	null	+
179	♂	3	*1A*1A	*1A*1A	*1A*7B	*1*2	null	null
180	♀	4,5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null

Table C.2 Continued.

Sample No	Gender	Diagn. Age	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	NQO1*2	GSTM1	GSTT1
181	♂	4	*1A*1A	*1A*1A	*1A*1A	*2*2	null	+
182	♂	5,5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
184	♀	9	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
185	♀	15	*1A*5B	*1A*6	*1A*7B	*1*2	null	+
186	♂	3,5	*1A*5B	*1A*6	*1A*1A	*1*1	null	null
187	♂	6	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
188	♀	1,5	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
190	♂	14	*1A*1A	*1A*6	*1A*1A	*1*2	null	null
191	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
192	♂	13	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
193	♀	7	*1A*1A	*1A*1A	*1A*7B	*1*2	null	null
194	♀	2,5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
195	♂	4	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
196	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
197	♂	3	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
198	♀	0,7	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
199	♂	16	*1A*1A	*1A*1A	*1A*7B	*1*1	+	+
200	♀	3	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+
201	♂	7	*1A*1A	*1A*1A	*1A*1A	*1*1	+	null
202	♂	6	*1A*1A	*1A*1A	*1A*7B	*1*1	null	+
203	♀	2,5	*1A*1A	*1A*6	*1A*1A	*1*1	+	+
204	♂	6,5	*1A*1A	*1A*6	*1A*1A	*2*2	+	null
205	♀	4	*1A*1A	*1A*1A	*1A*1A	*1*2	+	+
206	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
207	♀	8	*1A*1A	*1A*1A	*1A*1A	*1*1	null	null
208	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*1	+	+
209	♂	2	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
210	♂	12	*1A*1A	*1A*1A	*1A*1A	*1*2	+	null
211	♀	8	*1A*1A	*1A*1A	*1A*1A	*1*2	null	null
212	♀	5	*1A*1A	*1A*1A	*1A*1A	*1*1	null	+

VITAE

PERSONAL INFORMATION

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Education

2001- 2004 M.Sc. in Joint Graduate Program in Biochemistry, Middle East Technical University, Ankara, TURKEY.

1997 - 2001 B.S. in Biology (C.GPA: 3,17 / 4)
Middle East Technical University, Department of Biology
Ankara, Turkey

1993 - 1997 Private Yuce High School , Ankara, TURKEY

WORKING EXPERIENCES

October 2001- October 2008: Research Assistant in Middle East Technical University, Faculty of Arts and Sciences, Department of Biology

Spring 2000: Study on undergraduate research project at the Middle East Technical University Department of Biology: Cytochrome P450 dependent microsomal mixed function oxidases. Particularly, determination of the stability of fish cytochrome b5 reductase at different conditions.

July-August 2000, Summer Practice: Study on DNA damage by radiation using capillary electrophoresis at Hacettepe University, Faculty of Medicine, Biochemistry Department, Ankara - TURKEY

Teaching experience:
- Bio251, Cell Biology Laboratory for undergraduate
- Bio310, Biochemistry Laboratory for undergraduate

PUBLICATIONS

Master of Science Thesis

Ulusoy G. (January 2004) Genetic Polymorphisms of Alcohol-Inducible CYP2E1 in Turkish Population. MSc Thesis. 123 pages, Middle East Technical University, Ankara. Advisor: Prof. Dr. Orhan Adalı, Covisor: Prof. Dr. Emel Arınç.

Publications (Indexed in Science Citation Index)

1. Ulusoy G., Adali O., Boyunegmez Tumer T., Sahin G., Gozdasoglu S., Arinc E. (2007) Significance of Genetic Polymorphisms at Multiple Loci of CYP2E1 in The Risk of Development of Childhood Acute Lymphoblastic Leukemia. Oncology. 72(1-2):125-31.

2. Boyunegmez Tumer T., **Ulusoy G.,** Adali O., Sahin G., Gozdasoglu S., Arinc E. (2007) The Low frequency of defective TPMT alleles in Turkish population: A study on Pediatric Patients with Acute Lymphoblastic Leukemia. Am J Hematol. 82(10):906-10.

3. Ulusoy G., Arinc E., Adali O. (2007) Genotype and allele frequencies of polymorphic CYP2E1 in the Turkish population. Arch. Toxicol. 81(10):711-8**.**

Citations

Publication: Ulusoy G, Adali O, Boyunegmez Tumer T, Sahin G, Gozdasoglu S, Arinc E (2007) Significance of Genetic Polymorphisms at Multiple Loci of CYP2E1 in The Risk of Development of Childhood Acute Lymphoblastic Leukemia. *Oncology.* 72(1-2):125-31.
Cited by: 1) Matsunaga N *et al.,* (2008) *Hepatology* 48 (1):240-251.

Publication: Ulusoy G, Arinc E, Adali O (2007) Genotype and allele frequencies of polymorphic CYP2E1 in the Turkish population. *Archives of Toxicology* 81(10):711-8**.**
Cited by: **1)** Guo Y-M et al., (2007) *World Chinese Journal of Digestology* 15 (16): 1825-1829.
2) Hengstler JG and Bolt HM (2008) *Archives of Toxicology* 82 (10):665-666.
3) Bolt HM and Hengstler JG (2008) *Archives of Toxicology* 82 (7): 413-414.
4) Ulusoy G. *et al.,* (2007) *Oncology.* 72(1-2):125-31.

Publication: Ulusoy G., Arinc E., Adali O. (2005) CYP2E1 genotyping in Turkish population: Determination of *CYP2E1*5B*,**6,* and **7B* alleles. Drug Metabolism Reviews. 37(Suppl. 2): 349.

Cited by: 1) Ulusoy G *et al.,* (2007) *Oncology.* 72(1-2):125-131.

2) Tumer TB *et al.,* (2007) *American Journal of Hematology*. 82 (10): 906-910.

Publication: Ulusoy G, Boyuneğmez T, Bozcaarmutlu A, Arınç E (2002) Kinetic and biochemical evidence suggest that at least two isoforms of P450 participates in N-demethylation of cocaine in rabbit. Abstract Book, XVII National Biochemistry Congress, Ankara, Turkey, June 24-27, pp. 533-534.

Cited by: 1) Arinç E, Bozcaarmutlu A (2003) *Journal of Biochemical and Molecular Toxicology* 17(3): 169-176.

PRESENTATIONS

Presentations in International Congresses

1. **Ulusoy G.,** Boyunegmez Tumer T., Adali O., Sahin G., Arinc E., (2008) Significance of CYP2E1 and NQO1 Polymorphisms in the risk development of childhood acute lymphoblastic leukemia. Drug Metabolism Reviews- Abstracts from the 10 th European ISSX Regional Meeting, May 22-28, Vienna, AUSTRIA Vol: 40, Supplement 1, pp: 134-135.

2. Boyunegmez Tumer T., **Ulusoy G.,** Adali O., Sahin G., Arinc E., (2008) The Role Of SULT1A1 and EPHX1 Polymorphisms as Risk Modifiers In Childhood Acute Lymphoblastic Leukemia in Turkish Population: A Case-Control Study. Drug Metabolism Reviews- Abstracts from the 10 th European ISSX Regional Meeting, May 22-28, Vienna, AUSTRIA Vol:40, Supplement 1, pp: 141-142.

3. Boyunegmez Tumer T., Yilmaz D., Tanrikut C., **Ulusoy G.,** Arinc E., (2008) DNA Repair Gene XRCC1 Arg399gln Polymorphisms as a Risk Factor for Childhood Acute Lymphoblastic Leukemia. EMBO Young Scientist Forum. February 20-22, İstanbul, TURKEY.

4. **Ulusoy G.,** Boyunegmez Tumer T., Adali O., Sahin G., Cevik E., Arinc E. (2007) Possible risk factors for childhood acute lymphoblastic leukemia: CYP2E1 and NQO1 polymoprhisms. ESF-UB Conference in Biomedicine Pharmacogenetics and Pharmacogenomics, June 15-20, Sant Feliu de Guixols, SPAIN. Book of Abstract pp: 27.

5. Boyunegmez Tumer T., **Ulusoy G.,** Adali O., Sahin G., Gozdasoglu S., Arinc E. (2007) The Low Frequency Of Major TPMT Variants in Turkish Populatıon: A Study Based On Pediatric Patients With Acute Lymphoblastic Leukemia And Healthy Subjects. ESF-UB Conference in Biomedicine Pharmacogenetics and Pharmacogenomics, June 15-20, Sant Feliu de Guixols, SPAIN. Book of Abstract pp: 6

6. Boyunegmez Tumer T., **Ulusoy G.,** Adali O., Sahin G., Gozdasoglu S., Arinc E. (2006) Genetic polymorphism of Thiopurine Methyltransferase Enzyme in Children with Acute Lymphoblastic Leukemia in Turkish population: A Preliminary Study on *TPMT *3A* and *TPMT *3C* allele. Drug Metabolism Reviews- Abstracts from the 14th North American ISSX Meeting, October 22-26, Rio Grande, PuertoRico, USA. Vol:38, Supplement 2, pp: 205.

7. Ulusoy G., Arinc E., Adali O. (2006) Carcinogen activating cytochrome P4502E1 variants in Turkish Population. The FEBS Journal- Abstracts from 31th FEBS Congress, June 24-29, Istanbul, TURKEY. Vol :273, Supplement 1, pp: 259.

8. Ulusoy G., Adali O., Boyunegmez Tumer T., Sahin G., Gozdasoglu S., Arinc E. (2006) *CYP2E1*5B* and **6* Polymorphisms are Possible Risk Factors in the Development of Childhood Acute Lymphoblastic Leukemia. Drug Metabolism Reviews- Abstracts from the 9th European ISSX/ 20th JSSX Meeting, June 4-7, Manchester, UK. Vol:38, Supplement 1, pp:21.

9. Ulusoy G., Arinc E., Adali O. (2005) CYP2E1 genotyping in Turkish population: Determination of *CYP2E1*5B*,**6,* and **7B* alleles. Drug Metabolism Reviews- Abstracts from the 13th NA ISSX/ 20th JSXX Meeting, October 23-27, Maui, Hawaii. Vol:37, Supplement 2, pp 349.

10. Ulusoy, G., Arınç, E., and Adalı O. (2004) Genetic Polymorphisms of Alcohol Inducible CYP2E1 in Turkish Population. 19th Eurepean Workshop on Drug Metabolism. October 03-08, Antalya, TURKEY.

11. Ulusoy, G., Arınç, E., and Adalı O. (2003) Polymorphisms of Human Drug Metabolizing Enzymes: Study Of DraI Genetic Polymorphism Of CYP2E1. 13th Balkan Biocehmical Biophsical Days and Meeting on Metabolic Disorders. October 12-15, Kuşadasi, TURKEY

Presentations in National Congresses

1. Ulusoy G., Boyuneğmez T., Bozcaarmutlu A., and Arınç E., (2002), Kinetic and biochemical evidence suggest that at least two isoforms of P450 participates in N-demethylation of cocaine in rabbit. 17th Biochemistry Congress, June 24-27, Ankara, TURKEY. Book of Abstract pp:534.

CONGRESSES ATTENDED

1. May 22-28, 2008. 10 th European ISSX Regional Meeting, Vienna, AUSTRIA. **Poster presentation**

2. June 15-20, 2007. ESF-UB Conference in Biomedicine, Pharmacogenetics and Pharmacogenomics, Sant Feliu de Guixols, SPAIN. **Poster Presentation.**

3. June 24-29, 2006. 31st FEBS Congress, İstanbul, TURKEY. **Poster Presentation.**

4. June 4-7, 2006. 9th European ISSX/ 20th JSSX Meeting, Manchester, UK. **Oral Presentation.**

5. October 23-27, 2005. 13th North American ISSX/ 20th JSSX Meeting, Maui, Hawaii, USA. **Poster Presentation.**

6. October 03-08 2004, 19th Eurepean Workshop on Drug Metabolism, Kemer-Antalya, TURKEY. **Poster Presentation.**

7. October 12-15 2003, 13th Balkan Biochemical Biophysical Days and Meeting on Metabolic Disorders. Kusadasi, TURKEY **Poster presentation.**

8. June 24-27 2001, 17th National Biochemistry Congress, METU, Ankara, TURKEY. **Poster presentation.**

COURSES ATTENDED

1. February 20-22, 2008. EMBO Young Scientist Forum. İstanbul, TURKEY.

2. June 22-24, 2006. FEBS Forum for Young Scientists, İstanbul, TURKEY.

3. September 21-27, 2003 FEBS Advanced Course on Recombinant DNA Technology and Protein Expression, Bucharest, ROMANIA.

SOCIETY MEMBERSHIP

Turkish Biochemical Society (member of FEBS)
International Society for the Study of Xenobiotics (ISSX)