ASSOCIATION OF THE CYP2E1, FMO3, NQO1, GST AND NOS3 GENETIC POLYMORPHISMS WITH ISCHEMIC STROKE RISK IN TURKISH POPULATION

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ABSTRACT

ASSOCIATION OF THE CYP2E1, FMO3, NQO1, GST AND NOS3 GENETIC POLYMORPHISMS WITH ISCHEMIC STROKE RISK IN TURKISH POPULATION

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Stroke, a major cause of death and disability, is described as interruption or severe reduction of blood flow in cerebral arteries. Oxidative stress plays an important role in the pathogenesis of atherosclerosis and carotid atherosclerosis is a risk factor for stroke. Combination of multiple environmental and genetic risk factors is thought to increase susceptibility to the development of this disease. Therefore, investigation of the polymorphisms of drug metabolizing enzymes is of crucial importance to determine the molecular etiology of the disease. The main objective of this study was to investigate the possible association between polymorphisms of enzymes causing oxidative stress (CYP2E1, FMO3 and NOS3) and enzymes protecting against oxidative stress (GST and NQO1), and the pathogenesis of atherosclerosis and ischemic stroke risk.

The study population consisted of 245 unrelated ischemic stroke patients and 145 healthy control subjects. There was no statistically difference between the patient and control groups in terms of age and gender. Hypertension, diabetes, smoking and obesity were found to be at least 2 times more common in stroke patients than controls. While total-cholesterol, triglyceride and LDL-cholesterol level

were higher in stroke patients, HDL-cholesterol level was lower in stroke patients when compared to controls.

In the case-control analyses for the risk of ischemic stroke, CYP2E1*5B mutant allele, *5B was found to be associated with the development of disease (Odds Ratio; OR=7.876, 95%CI=1.025-60.525, *P*=0.019). In addition, significant difference was observed between stroke patients and controls with respect to CYP2E1*5B genotype distribution (OR=0.869, 95%CI=1.044-62.339, *P*=0.017). On the other hand, in the NQO1*2 polymorphism, together with NQO1 heterozygote (*1*2), NQO1 homozygote mutant (*2*2) genotype was found protective against ischemic stroke (OR=0.627, 95%CI=0.414-0.950, *P*=0.027). The risk of hypertensive individuals having stroke was highest in the FMO3 472GA group (OR=6.110, *P*=0.000). In diabetics, GSTP1 313AG genotype was found to be the highest risk factor for stroke (OR=3.808 *P*=0.001). On the other hand, NQO1 *1*2 heterozygote genotype was associated with 5 times increased risk for stroke in smokers (OR=5.000, *P*=0.000). In addition GSTM1 present genotype constituted 8 times increased stroke risk in obese individuals (OR=8.068, *P*=0.001). Logistic regression analysis revealed that hypertension, diabetes mellitus, obesity and smoking were significant risk factors for stroke. On the other hand, HDL-cholesterol and having NQO1 *1*2 heterozygote genotype were found to be protective factors against stroke.

Key words: Ischemic stroke, Turkish population, genetic polymorphism, CYP2E1, FMO3, NQO1, GSTM1, GSTP1, GSTT1, NOS3

ÖZ

TÜRK POPULASYONUNDA CYP2E1, FMO3, NQO1, GST VE NOS3 GENETİK POLİMORFİZMLERİNİN İSKEMİK İNME RİSKİ İLE İLİŞKİSİ

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Önemli bir sakatlık ve ölüm nedeni olan inme, serebral arterlerdeki kan akımının kesintisi ya da şiddetli bir şekilde azalması olarak tanımlanır. Karotid ateroskleroz inme için önemli bir risk faktörüdür ve oksidatif stres aterosklerozun patogenezinde önemli bir rol oynar. Çeşitli çevresel ve genetik risk faktörlerinin kombinasyonunun bu hastalığın gelişme yatkınlığını arttırdığı düşünülmektedir. Bu nedenle ilaç metabolize eden enzimlerin polimorfizmlerinin incelenmesi hastalığın moleküler etiyolojisinin belirlenmesi açısından önem taşımaktadır. Bu çalışmanın temel amacı, oksidatif strese neden olan (CYP2E1, FMO3 ve NOS3) ve oksidatif strese karşı koruyucu olan (GST ve NQO1) enzimlerin polimorfizmleri ile aterosklerozun patogenezi ve iskemik inme riski arasındaki olası ilişkileri incelemektir.

Çalışma grubu, aralarında akrabalık bulunmayan 245 iskemik inme hastası ve 145 sağlıklı kontrolden oluşmuştur. Yaş ve cinsiyet açısından hasta ve kontrol grupları arasında anlamlı bir fark yoktur. Hipertansiyon, diyabet, sigara kullanımı ve obeziteni inme hastalarında kontrollerden en az 2 kat daha fazla olduğu görülmüştür. Kontrollerle kıyaslandığında, inmeli hastalarda total kolesterol, trigliserit ve LDL-kolesterol seviyeleri yüksekken, HDL-kolesterol seviyesi düşük olarak bulunmuştur.

İskemik inme riski üzerine yapılan hasta-kontrol analizinde, CYP2E1*5B mutant aleli *5B hastalık riski ile ilişkili olarak bulunmuştur (eşitsizlik oranı [Odds Ratio]; OR=7.876, 95%CI=1.025-60.525, *P*=0.019). Bunun yanında, CYP2E1*5B genotip dağılımı açısından inme hastaları ve kontroller arasında önemli bir fark olduğu gözlenmiştir (OR=0.869, 95%CI=1.044-62.339, *P*=0.017). Öte yandan NQO1*2 polimorfizminde, NQO1 heterozigot (*1*2) ve NQO1 homozigot mutant (*2*2) genotipleri birlikte iskemik inmeye karşı koruyucu olarak bulunmuştur (OR=0.627, 95%CI=0.414-0.950, *P*=0.027). Hipertansiyonlu bireylerin inme riski FMO3 472GA grubunda en yüksek bulunmuştur (OR=6.110, *P*=0.000). Diyabetiklerde GSTP1 313AG genotipi en yüksek risk faktörü olarak bulunmuştur (OR=3.808 *P*=0.001). Diğer taraftan NQO1 *1*2 heterozigot genotipi sigara içenlerde inme riski ile 5 kat daha fazla ilişkili bulunmuştur (OR=5.000, *P*=0.000). Buna ek olarak GSTM1 present genotipi obez insanlarda 8 kat yüksek inme riski teşkil etmektedir (OR=8.068, *P*=0.001). Lojistik regresyon analizi hipertansiyon, diyabet, obezite ve sigara içmenin inme için önemli risk faktörleri olduğunu göstermiştir. Öte yandan HDL-kolesterol ve NQO1 *1*2 heterozigot genotipi inmeye karşı koruyucu faktörler olarak bulunmuştur.

Anahtar kelimeler: İskemik inme, Türk populasyonu, genetik polimorfizm, CYP2E1, FMO3, NQO1, GSTM1, GSTP1, GSTT1, NOS3

Dedicated to my family,

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CAD	Coronary artery disease
CDNB	1-chloro-2,4-dinitrobenzene
CNV	Copy number variations
CYP	Cytochrome P-450
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra acetic acid
FAD	Flavin adenine dinucleotide
FADOOH	Peroxyflavin
FMN	Flavin mononucleotide
FMO	Flavin containing monooxygenase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
HDL	High density lipoprotein
LDL	Low density lipoprotein
MI	Myocardial infarction
NOS	Nitric oxide synthase
NQO	NAD(P)H quinone oxidoreductase
$ONOO^-$	Peroxynitrite
OR	Odds ratio
ox-LDL	Oxidized low density lipoproteins
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism

TG	Triglyceride
TIA	Transient ischemic attack
VNTR	Varying number of tandem repeats
χ^2	Chi-square

CHAPTER 1

INTRODUCTION

1.1 Stroke

Stroke is the term employed to describe the acute neurological and irreversible manifestations of cerebrovascular disease which result from interruptions to blood flow in the brain. Brain tissue depends for its survival on a continuous flow of blood providing it with oxygen and glucose and other essential nutrients. The blood supply is obtained from four main vessels, the right and left carotid arteries at the side of the throat and the two vertebral arteries which run up through the bones in the neck. These two pairs of major blood vessels, linked by the anterior and posterior communicating arteries, converge on the lower surface of the brain. From this point, blood is transported through the brain via network of branch arteries. Arteries in the brain can be blocked by:

- the local development of clots,
- the formation of deposits on the artery walls which narrow the channel until it is completely closed,
- the lodging of emboli,
- thromboses formed elsewhere in the circulatory system following a heart attack, or during or after surgery for example, which are unable to pass through the narrowed tubes.

Weakness or numbness of face, arm or leg is the most common symptom of stroke. Other symptoms include confusion, difficulty speaking or understanding speech, difficulty seeing with one or both eyes; difficulty walking, dizziness, loss of balance or coordination; severe headache with no known cause; fainting or unconsciousness. The effects of a stroke depend on which part of the brain is injured and how severely it is affected. A very severe stroke can cause sudden death.

The incidence of stroke is heavily age-related. 50% of all strokes are in over the age of 75. Although stroke is perceived to be a disease of the elderly, it can occur at any age. Stroke is the third leading cause of death worldwide after coronary heart disease and other cardiovascular diseases, according to World Health Organization reports. Annually, 15 million people worldwide suffer a stroke. Of these, 5 million die and another 5 million are left permanently disabled, placing a burden on family and community.

1.1.1 Classification of Stroke

Stroke is a heterogenous disorder and accurate and reproducible definitions and classifications are essential for risk factors studies. Different stroke subtypes and phenotypes have different pathogenic mechanisms and an understanding of these is essential in planning and interpreting candidate gene studies. A pathophysiological classification of stroke is shown in Table 1.1. This divides stroke into its two main subtypes of ischemic stroke and hemorrhagic stroke, and subdivides each of these (Warlow *et al*., 2003).

Table 1. 1 Pathophysiological classification of stroke.

STROKE	
ISCHEMIC STROKE	**HEMORRHAGIC STROKE**
*Large artery	*Primary subarachnoid hemorrhage
*Cardioembolic	*Primary intracerebral hemorrhage
*Lacunar (small vessel disease)	

1.1.1.1 Ischemic Stroke

Ischemic stroke accounts for about 87 percent of all cases. Ischemic strokes occur as a result of an obstruction within a blood vessel supplying blood to the brain (Figure 1.1). The underlying condition for this type of obstruction is the development of fatty deposits lining the vessel walls. This condition is called atherosclerosis.

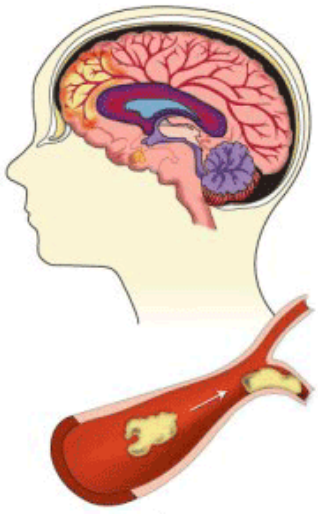


Figure 1. 1 Ischemic stroke occurs when a blood clot blocks the blood flow in an artery within the brain. (Taken from http://www.cdc.gov/stroke/types_of_stroke.htm)

Many different symptoms can occur, depending on which part of the brain is deprived of blood and oxygen. When the arteries that branch from the internal carotid artery are affected, blindness in one eye or abnormal sensations and weakness in one arm or leg or on one side of the body are most common. When the arteries that branch from the vertebral arteries in the back of the brain are affected, dizziness and vertigo, double vision, and generalized weakness of both sides of the body are more common. Many other symptoms, such as difficulty in speaking (for example, slurred speech) and loss of coordination, can occur.

1.1.1.2 Hemorrhagic Stroke

Hemorrhagic stroke accounts for about 13 percent of stroke cases. It results from a weakened vessel that ruptures and bleeds into the surrounding brain (Figure 1.2). The blood accumulates and compresses the surrounding brain tissue. The two types of hemorrhagic strokes are intracerebral hemorrhage or subarachnoid hemorrhage.

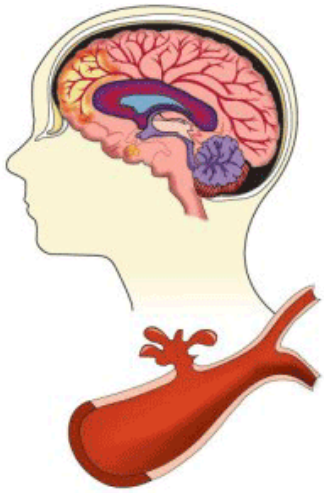


Figure 1. 2 Hemorrhagic stroke occurs when a blood vessel bursts within the brain. (Taken from http://www.cdc.gov/stroke/types_of_stroke).

Intracerebral hemorrhage is the most common type of hemorrhagic stroke. It occurs when an artery in the brain bursts, flooding the surrounding tissue with blood. Subarachnoid hemorrhage is bleeding in the area between the brain and the thin tissues that cover it.

1.1.2 Risk factors for stroke

The risk factors of stroke can be classified with respect to subtype of stroke, modifiability of risk factor and scientific certainty of relation between risk factor and stroke. The risk factors of stroke are given in Table 1.2. Hypertension has been defined as the major risk factor of ischemic stroke (63%), followed by hypercholesterolemia (37%), diabetes mellitus (35%), ischemic heart disease (23%), atrial fibrillation (20%) and smoking (17%) in Turkish population (Kumral *et al*., 1998).

1.1.2.1 Certain Risk Factors of Stroke

1.1.2.1.1 Non-modifiable Risk Factors

Age is important risk factor for stroke. According to study conducted by Wolfe (2000) the incidence of stroke approximately doubles with each successive decade over the age of 55 years. Another study reported that stroke was highly seen in people aged 65 years and older (McGruder *et al*., 2004). There is lots of evidence that parental history of stroke (Fiebach *et al*., 1989; Haheim *et al*., 1993; Welin *et al*., 1987; Kiely *et al*., 1993) especially maternal history of stroke (Welin *et al*., 1987) is an important factor for stroke. Blacks have higher risks of hypertension, diabetes and

Table 1. 2 Risk factors of stroke.

A- Certain Risk Factors of Stroke	**B- Uncertain Risk Factors of Stroke**
Non-modifiable Risk Factors	*Geographic location
*Age	*Socioeconomic factors
*Heredity and Race	*Alcohol abuse
*Sex (gender)	
*Prior stroke	
*Transient ischemic attacks (TIA)	
or heart attack	
Modifiable Risk Factors	
*High blood pressure	
*Cigarette smoking	
*Diabetes mellitus	
*Carotid or other artery disease	
*Atrial fibrillation	
*Other heart disease	
*Sickle cell disease	
*High blood cholesterol	
*Poor diet	
*Physical activity	
*Obesity	

obesity. For this reason, when compared to Caucasians, Africans and Americans have a much higher risk of death from stroke. Male gender is an important determinant of stroke. In most age groups, stroke incidence was higher in men than women (Alter *et al*., 1986; Boysen *et al*., 1988; Michel *et al*., 2010). However, more than half of total stroke deaths occur in women. Not only hormone dependent mechanisms but also hormone independent mechanisms protect women against cerebrovascular disease. Liu and *et al*., (2009) reported that estrogen enhances vasodilatation, improves endothelial dysfunction and increases blood flow after vascular occlusion. In addition estrogen inhibits platelet adherence and aggregation (Feuring *et al.*, 2002). Furthermore transient ischemic attack (TIA) was reported that an important risk factor especially for ischemic stroke (Gandolfo *et al*., 1988; Harmsen *et al*., 2006; Morte *et al*., 2008).

1.1.2.1.2Modifiable Risk Factors

Many modifiable risk factors for stroke such as hypertension, cigarette smoking, diabetes mellitus, etc. have been identified. One of the most important risk factor of stroke is hypertension which is a serious condition that leads to health problems namely coronary heart disease, heart failure and stroke. Furthermore, hypertension was found to be a major risk factor both hemorrhagic and ischemic stroke (Fiebach *et al*., 1989; Jamrozik *et al*., 1994; Can Demirdöğen *et al*., 2008; Can Demirdöğen *et al*., 2009, Türkanoğlu *et al*., 2010). In the literature cigarette smoking was shown to be related with stroke independently from hypertension and other risk factors (Wolf *et al*., 1988). On the other hand some authors showed diabetes as a strong risk factor of stroke (Bell, 1994; Stegmayr and Asplund, 1995; Goldstein *et al*., 2001).

1.1.2.2 Uncertain Risk Factors of Stroke

Geographic location, socioeconomic factors and alcohol abuse are considered as uncertain risk factors of stroke. Although stroke morbidity and mortality due to geographic differences have been reported in some studies (Takeya *et al*., 1984; Malmgren *et al*., 1987; Feinleib *et al*., 1993) nature of these differences as risk factors is controversial and unclear.

1.2 Free Radicals and Oxidative Stress

Free radicals are atoms, molecules, or parts of molecules possessing unpaired electrons (Karlsson, 1997). They are highly reactive and capable of initiating and participating in chain free radical reactions. In biological systems, free radicals and reactive non-radical species are constantly generated. Free radicals and reactive non-radical species containing oxygen are traditionally denoted as reactive oxygen species (ROS). ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO.) (Chiou and Tzeng, 2000). Superoxide can directly affect the function of ion channels in vascular endothelium, and vascular dysfunction is a distinguishing feature of many diseases, including coronary heart disease, stroke, and diabetes (Brzezinska *et al*., 2005). Although hydrogen peroxide is poorly reactive at physiological levels, it can affect some enzymes. The conversion of low-reactive oxygen intermediates to high-reactive species requires some transition metal ions, especially iron. Hydrogen peroxide reacts with the iron and produces hydroxyl radical, which can damage every organic molecule, by Fenton's reaction. In tissues, auto-oxidation of tissue component and some enzymatic reactions form free radicals. Radical overproduction may originate from a sustained activation of NADPH oxidase complex in leukocyte cytoplasmic membrane or from mitochondrial electron transport chain. Radicals are considered mediators of cellular injury in ischemia,

inflammation, acute hypertension, traumatic brain injury, diabetes, atherosclerosis, rheumatoid arthritis (Popov *et al*., 2003).

Normally, there is equilibrium between ROS generation and the antioxidant capacity of the organism in the biological systems. When the radical formation reaches to high levels, the antioxidant capacity may be inadequate to compensate for the increase in ROS. The condition occurring when the physiological balance between oxidants and antioxidants is disrupted in favor of the former with the potential damage for the organism is called as oxidative stress (Sies, 1985). As a result of this condition, oxidative damage, accumulation of toxic end products and development of pathological states could be observed. Oxidative stress plays a pivotal role in the pathogenesis of atherosclerosis that is the main cause of a group of cardiovascular diseases (Harrison *et al.*, 2003; Singh and Jialal, 2006; Tavori *et al*., 2009).

1.3 Atherosclerosis

Atherosclerosis is a progressive disease characterized by the accumulation of cholesterol deposits in arteries (Figure 1.3). In the arterial wall, certain cell type proliferation, gradually influence on the vessel lumen and reduce blood flow, is observed due to cholesterol deposition. These lesions are called as fatty streak lesions. When lesions grow enough to significantly reduce the blood flow through an artery, myocardial infarction or stroke occur.

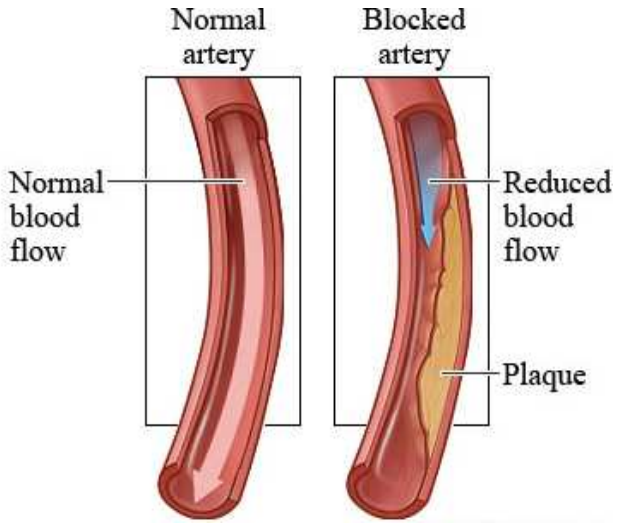


Figure 1. 3 A normal artery with normal blood flow and an artery containing plaque build up (atherosclerosis). (Taken from http://www.health.com/health/library)

Age, gender, hypertension, diabetes, cigarette smoking are important risk factors of atherosclerosis and these risk factors are associated with an increased production of ROS (Antoniades *et al*., 2003). Reactive oxygen species affect the vascular function via several mechanisms. These mechanisms can be summarized as follows. Firstly, ROS directly react with cell membranes and nuclei and damage to them. In the other mechanism, ROS interact with endogenous vasoactive mediators formed in endothelial cells by this way ROS regulate vasomotion and atherogenic process. In the third mechanism given in Figure 1.4, ROS provoke an oxidative modification from low density lipoprotein (LDL) to oxidized low density lipoprotein (ox-LDL) by peroxidizing lipid components. Circulating monocytes migration to the subendothelial space is stimulated by ox-LDL and also causes endothelial cell injury. The modified LDL is taken up by macrophages which become foam cells, leading to the formation of atherosclerotic plaque (Bonomoni *et al*., 2008).

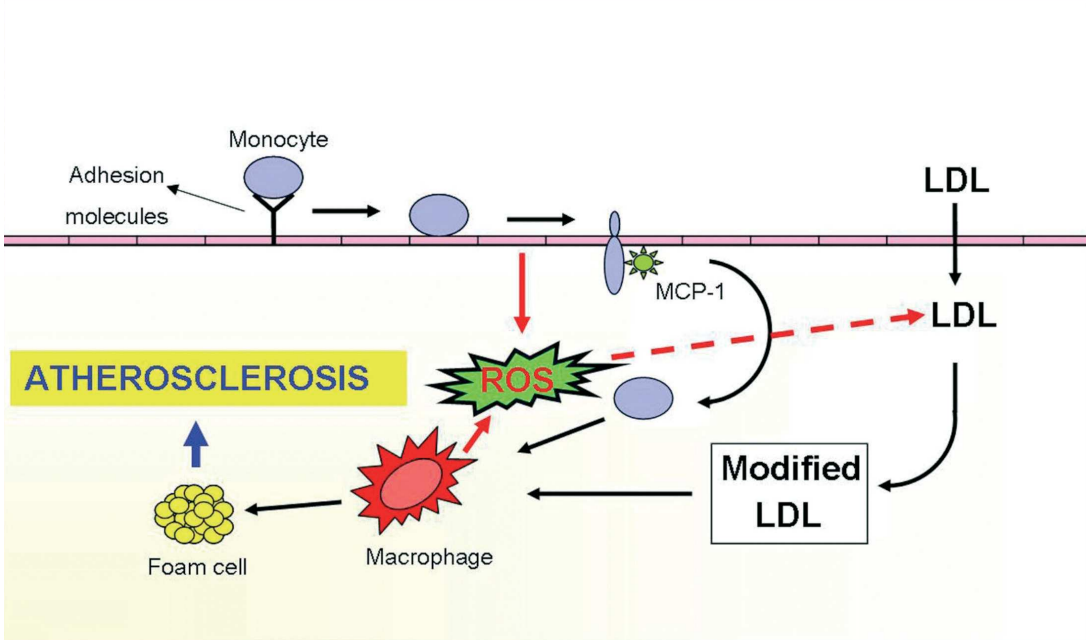


Figure 1. 4 Oxidative stress in atherosclerosis. An increase of free radical production is associated with atherosclerosis (Taken from Bonomoni *et al*., 2008).

Ox-LDL is an important mediator for atherosclerosis. There are different biological effects of ox-LDL such as inhibition of endothelial nitric oxide synthase, promotion of vasoconstriction and adhesion, stimulation of cytokines and increase platelet aggregation (Jialal and Grundy, 1992; Kaul *et al*., 2001; Stocker and Keaney, 2001; Keaney, 2005; Madamanchi *et al*., 2005). Native form of LDL is not atherogenic. LDL receptors do not recognize modified LDL (ox-LDL), but ox-LDL is taken up by the scavenger receptor pathway in macrophages leading to appreciable cholesterol ester accumulation and foam cell formation (Witzum and Steinberg, 1991). Foam cell formation is considered as the initiating step to develop atherosclerotic plaque.

1.4 Xenobiotic Metabolism

Xenobiotic metabolism is the group of metabolic pathways that modify the chemical structure of xenobiotics, which are compounds foreign to an organism's normal biochemistry, such as drugs and poisons. Many xenobiotics are lipophilic so their excretion from the body is difficult. As a result of this they have to be metabolized into more hydrophilic compounds before they can be eliminated from the body. This biotransformation or metabolism is divided into three phases (Figure 1.5).

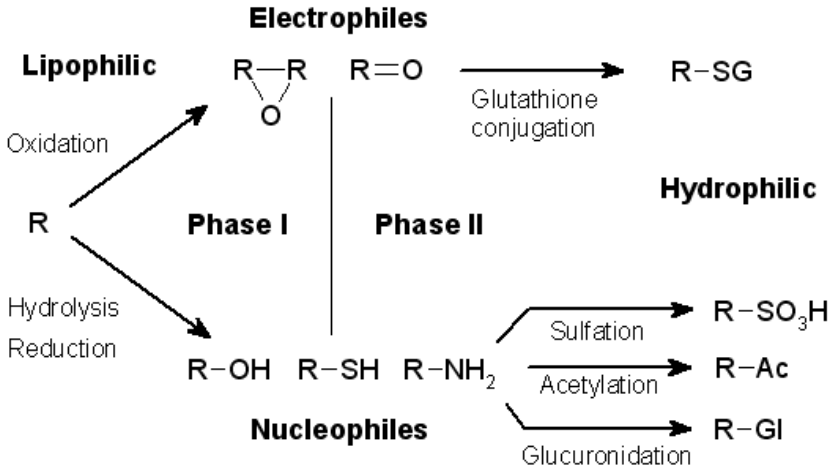


Figure 1. 5 Phase I and Phase II of the metabolism of a lipophilic xenobiotic (Taken from http://en.wikipedia.org/wiki/Xenobiotic_metabolism).

The phase I reactions convert xenobiotics to more chemically reactive compound by the action of oxidation, reduction and hydrolysis reactions and by insertion of -OH, -NH_2, -SH or –COOH groups. The phase I enzymes include mainly

cytochrome P450s (CYP450s), flavin-containing monooxygenases (FMOs), cyclooxygenases (COXs), monoamine oxidases (MAOs) and epoxide hydrolases (EHs). After phase I reactions, the modified compound undergoes a conjugation reaction like glucuronidation, sulfation and acetylation in phase II reactions that increase the water solubility of the compounds making them more easily excreted. The phase II reactions carried out by glutathione S-transferases (GSTs), UDP glucuronosyltransferases (UGTs), N-acetyltransferases (NATs), sulfotransferases (SULTs), etc. After then in phase III, the conjugated xenobiotics are pumped out of cells by efflux transporters.

Most of the chemicals need metabolic activation by phase I xenobiotic metabolizing enzymes in order to make DNA adducts that result in DNA damage. Furthermore some phase I enzymes play a role in the generation of ROS. On the other hand, especially phase II 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. Phase I and phase II enzymes are known to be polymorphic. Furthermore, it should be considered that polymorphisms may alter enzyme activity for this reason production or elimination of ROS may increase or decrease.

1.5 Genetic Polymorphisms

Genetic polymorphism is a difference in DNA sequence among individuals, groups or populations. Genetic polymorphisms can be classified according to functionality namely functional and non-functional polymorphisms and according to structure namely insertion/deletion, varying number of tandem repeats (VNTR), copy number variations (CNV), epigenetic variations of the human genome like DNA methylations, microRNA regulations and single nucleotide polymorphism (SNP). Genetic polymorphisms may be the result of chance processes, or may have been induced by external agents such as viruses or radiation. Genetic mutations are also

known as differences in DNA sequence but mutations are found in an individual that are rare, and may be unique to the individual. On the contrary polymorphisms are found in many individuals.

1.5.1 Insertion Deletion Polymorphism

Addition of one or more nucleotides into the DNA is called as an insertion. Insertions in the coding region of a gene may alter splicing of the mRNA, or cause a shift in the reading frame, both of which can significantly alter the gene product. Conversely deletions remove one or more nucleotides from the DNA. Like insertions, deletions can alter the reading frame of the gene. For example GSTM1 and GSTT1 isoforms have deletion polymorphism that causes lack of enzyme activity.

1.5.2 Variable Number of Tandem Repeats (VNTRs)

Tandem repeats are short lengths of DNA that are repeated multiple times within a gene. These sequences are also called variable number tandem repeats because different individuals within a population may have different numbers of repeats. There are two families of VNTRs namely microsatellites, repeats of sequences less than about 5 base pairs in length, and minisatellites, repeats of sequences more than about 5 base pairs in length.

1.5.3 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphism is a single base substitution with a frequency of more than 1% in at least one population. SNPs are the most simple form and most common source of genetic polymorphism in the human genome. There are two types of nucleotide base substitutions resulting in SNPs. The first one is transition substitution that occurs between purines (adenine, guanine) or between pyrimidines (cytosine, thymine). The second type is transversion substitution that occurs between a purine and a pyrimidine base.

SNPs may found within coding sequences of genes, non-coding regions of genes or in the intergenic regions. Coding region SNPs may have two effects namely synonymous and non- synonymous on the produced protein. Synonymous substitution does not change the amino acid sequence of the produced protein. Therefore this type of substitution is also called as a silent mutation. Non-synonymous substitution results in an alteration of the encoded amino acid. A non-synonymous polymorphism change may be either missense, which results in a different amino acid, or nonsense, which results in a premature stop codon. SNPs may also occur in regulatory regions of genes. These SNPs can change the amount or timing of a protein's production.

1.6 Genetic Studies on Stroke

Although the underlying molecular basis of genetic factors remains uncertain, studies reported that they appear to be important in multifactorial stroke pathogenesis (Flossmann *et al*., 2004; Jerrard-Dunne *et al*., 2003). Recently, a lot of studies that investigate the association between stroke and genetic variation of the DNA have been published (Nowak-Göttl *et al*., 1999; Chowdhury *et al*., 2001; Zee *et al*., 2004; Berger *et al*., 2007; Can Demirdöğen *et al*., 2008; Shi *et al*., 2008; Can Demirdöğen

et al., 2009; Türkanoğlu *et al*., 2010). Numerous polymorphisms in stroke have been determined as a consequence of rapid and cheaper genotyping technologies.
Studies that attempt to identify genetic variants that influence disease or phenotypic traits can be divided into two categories; linkage analysis studies, and association studies.

1.6.1 Linkage Studies

Linkage studies rely on the co-segregation of loci in pedigrees. Recombination between markers during meiosis occurs at a rate related to the distance between them. Therefore a disease/trait allele will be inherited in families along with a background section of the genome. By studying which genomic sections are commonly co-inherited with the disease/trait of interest in a family, the location of the variant of interest can be later refined (Dawn and Barrett, 2005). Linkage analysis is generally ‘genome-wide’ or ‘chromosome-wide’ and only identifies large regions of linkage, not specific genes or mutations. This method is most useful for variants that have a large effect. Linkage studies also have their limitations for late-onset conditions such as stroke, since it is not necessarily appropriate to assign young people as unaffected, when they may go on to develop the disease in the future.

1.6.2 Association Studies

Association studies compare the frequency of specific DNA sequence variants in groups of individuals in a case-control design. Association studies are more useful for variants that are common, but have small effects (Risch and Merikangas, 1996). This method looks for an association between the disease/trait and genetic variants in the population (Cordell and Clayton, 2005). Association studies can be either of candidate genes or genome-wide. Candidate gene studies require background

knowledge to inform the choice of genes to be studied. This decision may be based on prior evidence of association or linkage in the region, but are often selected with only tentative biological reasoning.

Association studies are powerful tools to identify genetic risk factors for stroke. Risch and Merikangas (1996) reported that association studies have greater statistical power to detect several genes of small effect. Therefore association studies considered that they are more efficient than linkage studies although they have important methodological challenges. Selection of control group is the most important challenge of association studies. Case and control groups must be chosen in similar population. Because the prevalence of some potential stroke risk polymorphism varies between different ethnic groups. That's why ethnicity is important to form case and control groups.

The other challenge of association studies is the selection of target genes and sequence variants. The etiological roles of genes in a disease state provide the selection of candidate genes. These genes may be selected from regions that have been identified through genome wide scans. On the other hand target genes can be chosen from among genes encode for specific proteins related to disease process. The types of polymorphism and its frequency in the population are important criteria for choosing specific polymorphism.

1.6.3 Genome-wide Association Studies

Genome-wide studies require no a priori expectation on which genes are associated with the disease or trait of interest. They usually involve genotyping of single nucleotide polymorphisms (SNPs) from across the entire genome. Genome-wide SNP chips have been developed that are either gene-centric; include large numbers of randomly selected SNPs from across the genome (Li *et al.*, 2008). SNP chips can now screen more than 1 million SNPs and the cost of genotyping has been rapidly decreasing, making genome-wide studies more affordable. However SNP

chips do not capture all genomic variation and so this approach may miss some important genetic associations, demonstrating the continued need for candidate gene studies.

1.7 Features and Polymorphisms of Phase I Xenobiotic Metabolizing Enzymes

The first step in the metabolism of xenobiotics including drugs is the catalysis by Phase I enzymes, mainly cytochrome P450s and flavin monooxygenases (FMO) (Adalı and Arınç, 1990; Arınç *et al.*, 1991; Arınç *et al.*, 1995; Adalı *et al*., 1996; Adalı *et al*., 1998; Adalı, 1998; Halpert *et al*., 1998; Adalı *et al*., 1999; Can Demirdöğen and Adalı, 2005; Aktaş *et al*., 2009). Molecules oxygenated by Phase I metabolism is usually conjugated with glucuronide, acetyl, sulfate or glutathione by different families of Phase II transferase enzymes.

1.7.1 Cytochrome P450s (CYP450s)

Cytochrome P450s are a super family of heme containing monooxygenases that metabolize a large number of compounds including xenobiotics and endogenous compounds. CYPs serve as a detoxification route and, in contrast, a metabolic activation route that yields free radicals including reactive oxygen species (ROS) and reactive metabolites which initiate toxic and carcinogenic events (Lu and Levin, 1974; Nebert and Gonzales, 1987; Arınç *et al*., 2000a; Arınç *et al*., 2000b; Arınç *et al*., 2005; Arınç *et al*., 2007). CYPs are located on the smooth endoplasmic reticulum of cells throughout the body, but the highest concentrations are found in the liver. The enzymes are divided into families based on amino acid sequence similarities, and each family can be further separated into subfamilies, which are designated by capital letters following the family designation (e.g., CYP3A). Individual enzymes

are subsequently indicated by arabic numerals (e.g., CYP3A4). An enzyme belongs to a family when the amino acid sequence possesses more than 40% homology, enzymes with more than 55% homology form a subfamily and individual enzymes can have to 97% homology between the sequences (Nebert and McKinnon, 1994; Nelson *et al.*, 1996). All of P450s has the ability to bind and activate two atoms of oxygen. The general reaction catalyzed by cytochrome P450s is summarized in Figure 1.6. This is an example of monooxygenation reaction. The heme iron binds two atoms of oxygen from the peroxide molecule. One of the two oxygen atoms is incorporated into substrate while the other is reduced to water.

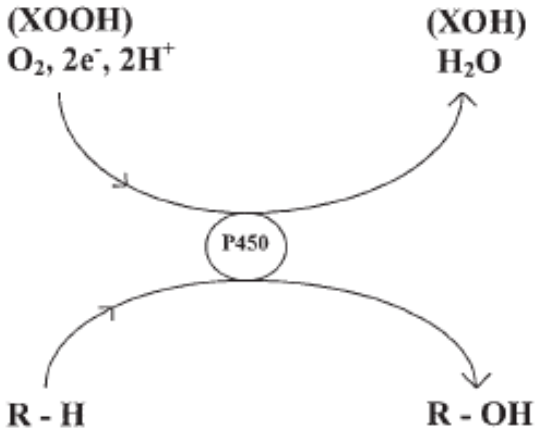


Figure 1. 6 P450 catalyzed reactions. R-H, substrate; R-OH, hydroxylated product; XOOH, peroxide (X=H or organic residue); XOH, hydroxylated by-product (Taken from Anzenbacher and Anzenbacherova, 2001).

Rodriguez-Antona and Ingelman-Sundberg (2006) reported that there are more than 57 active P450 genes and 58 pseudogenes in human. Some of the cytochrome P450s is responsible for the synthesis of steroid hormones, cholesterol, vitamin D and bile acids, and the metabolism of prostaglandins and eicosanoids. On the other

hand several families of cytochrome P450s (families 1-4) play a role in Phase I reactions of xenobiotic metabolism. These enzymes have interindividual variation due to genetic polymorphisms, environmental factors, physiological status and disease state (Al Omari and Murry, 2007; Ulusoy *et al*., 2007a; Ulusoy *et al*., 2007b).

1.7.1.1 Features of Cytochrome P4502E1 (CYP2E1)

CYP2E1, ethanol inducible form of cytochrome P450s, has important toxicological roles. The enzyme is localized mainly in the liver but is also found in extrahepatic tissues such as lung, kidney, brain, heart and bone marrow and induced in the brain after ethanol treatment or ischemia (Warner and Gustafsson, 1994; Tindberg *et al*., 1996). CYP2E1 is responsible for metabolism and bioactivation of not only low molecular weight substances including acetone, ethanol but also procarcinogens such as benzene, styrene and N-nitrosodimethylamine and drugs like acetaminophen, halothane, isoflurane, chlorzoxazone and phenacetin (Guengerich and Shimada, 1991; Arınç *et al*., 2000a; Arınç *et al*., 2000b; Arınç *et al*., 2007). That's why CYP2E1 enzyme is considered as an important determinant of human susceptibility to toxicity and carcinogenicity of industrial and environmental chemicals. In Figure 1.7, the metabolism of acetaminophen by CYP2E1 and GST is shown. Acetaminophen is converted to a highly reactive quinone metabolite by CYP2E1 enzyme. This form of acetaminophen can produce reactive oxygen species and covalently bind to cellular nucleophiles such as DNA, RNA and proteins, resulting in cell death. If P450 derived quinone metabolite is conjugated with glutathione by GST enzymes, it is found in inactive form. Therefore under conditions of high levels of CYP2E1 and/or low levels of glutathione, the active metabolite can bind to the cellular macromolecules resulting in toxicity.

As mentioned above CYP2E1converts a large number of low molecular weight chemicals to reactive intermediates that can bind to cellular macromolecules such as DNA, RNA and protein causing cell damage, hepatitis and cirrhosis. During the

P450 catalytic cycle, P450s use H^+ from NADPH to reduce O_2 leading to the production of H_2O_2 and superoxide anion radical (Figure 1.8). The process of uncoupling of the catalytic cycle can lead to escape of O_2^-. Therefore, CYP2E1 metabolism of a number of its substrates is known to lead to increased ROS (Caro and Cederbaum, 2004).

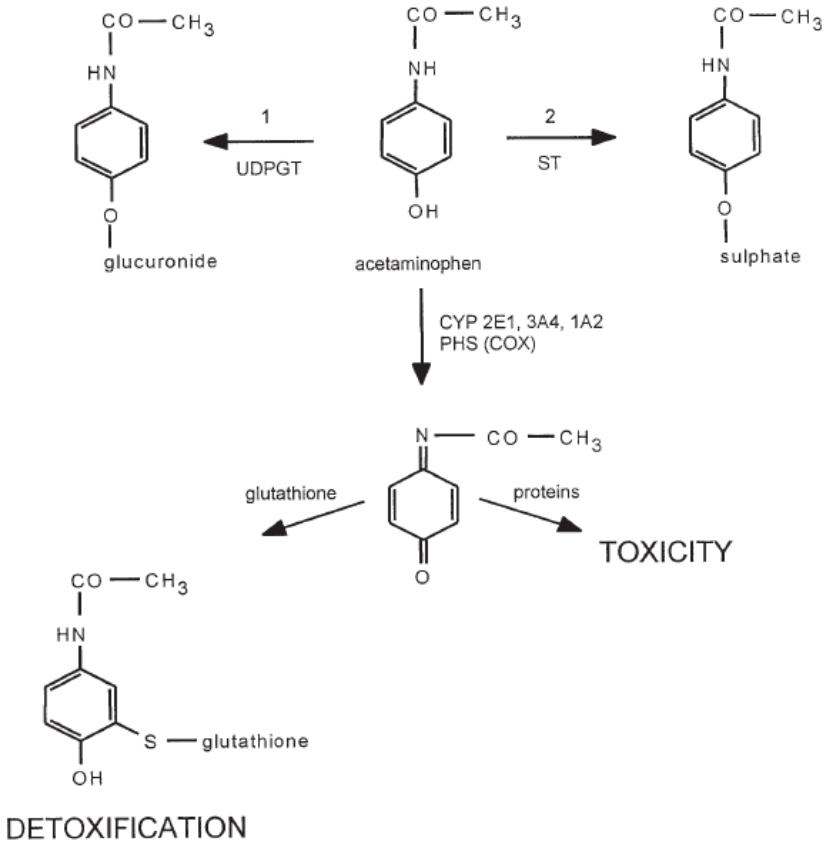


Figure 1.7 Metabolism of acetaminophen by CYP2E1 and GST (Taken from Anzenbacher and Anzenbacherova, 2001).

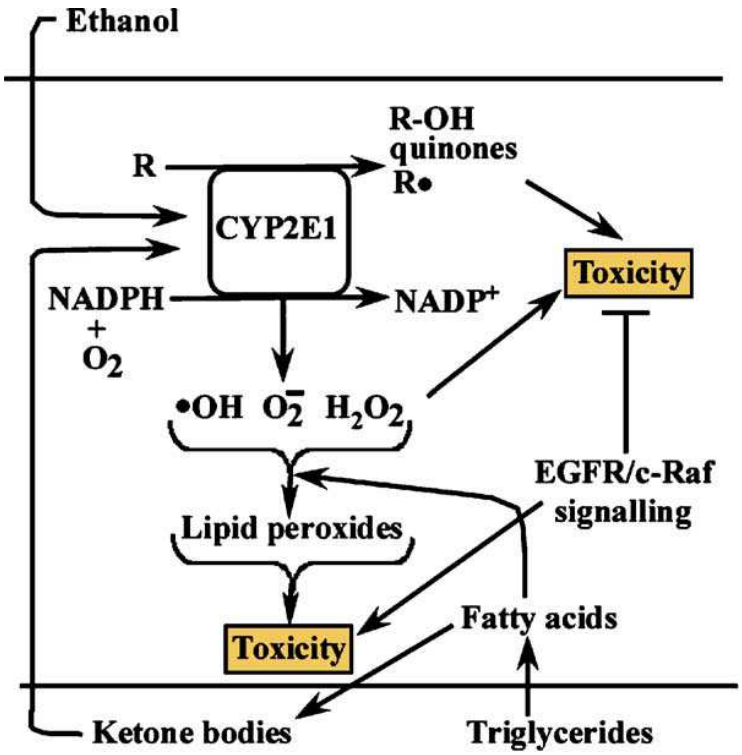


Figure 1. 8 Schematic of the influence of CYP2E1 in generation of ROS and cell toxicity (Taken from Gonzalez, 2005).

As can be seen from the Figure 1.8, expression of CYP2E1 is induced by ethanol. As a result of this, ROS production including $^{\cdot}OH$, O_2^{-}, H_2O_2 is elevated in cell. Fatty acids can be subjected to peroxidation leading to reactive species that can damage cellular macromolecules. Fatty acids can also be metabolized to ketone bodies that can induce expression of CYP2E1 and be metabolized by CYP2E1 leading to more ROS production. Therefore increased ROS production causes oxidative stress in the cell.

1.7.1.2 Polymorphisms of CYP2E1

The human *CYP2E1* gene is located on 10q24.3-qter. It has 18,745 bp long containing nine exons and eight introns. It produces a 493 amino acid protein. There are six single nucleotide polymorphisms in *CYP2E1* gene. Among *CYP2E1* polymorphisms, the most frequently studied ones are the *CYP2E1*5B* (*Rsa*I/*Pst*I RFLP; position:C-1053T/G-1293C) polymorphism located in the 5'-flanking region of the gene, which enhance the transcription and increase the level of CYP2E1 enzyme activity (Liu *et al*., 2009; Hayashi *et al*., 1991); and *CYP2E1*6* (*Dra*I RFLP; position T7632A) polymorphism located in intron 6 (Uematsu *et al*., 1991), which was shown to lower 'chlorzoxazone metabolic ratios' (Haufroid *et al*., 2002), and was correlated with single strand breaks in DNA (Vodicka *et al.*, 2001). The other important polymorphism of CYP2E1 gene is CYP2E1*7B (*Dde*I RFLP, position G-71T) polymorphism located in the promoter region. Fairbrother *et al.* (1998) reported that this polymorphism may be associated with the expression or regulation of the gene. Several case-control studies have described the influence of these polymorphisms with increased risk for various cancer types such as lung cancer, oral cancer, urothelial cancer, childhood acute lymphoblastic leukemia in different populations (El Zein *et al.*, 1997; Wu *et al.*, 1998; Farker *et al*., 1998; Liu *et al*., 2001, Ulusoy *et al.*, 2007a). According to study conducted by Salama *et al*. (2002) polymorphic *5B allele was found to be 2.5 times increased risk factor for atherosclerosis.

1.7.2 Flavin Containing Monooxygenases (FMOs)

1.7.2.1 Features of FMOs

The flavin containing monooxygenases (FMO, EC 1.14.13.8) are NADPH dependent enzyme family that catalyzes the oxygenation of wide variety of nucleophilic compounds containing sulfur, nitrogen or phosphorus atoms (Table 1.3) (Ziegler, 1988; Cashman, 1995; Adalı *et al*., 1999; Can Demirdöğen and Adalı, 2005; Aktaş *et al*., 2009). Trimethylamine (TMA), the simple dietary-derived aliphatic amine, is rapidly absorbed and metabolized by liver FMOs to N-oxide. In the human population, this major route of TMA metabolism is subject to genetic polymorphism. In the case of mutation the capacity to oxidize TMA to TMA N-oxide is diminished in the liver. Affected individuals excrete excessive amounts of free TMA in their urine, breath and sweat, which exhibits a bad odor reminiscent of rooting fish characteristic of the associated disorder trimethylaminuria (TMAU) or fish odor syndrome.

Six isoforms of FMOs are found in human but only five of them are functional. The sixth FMO gene was found to be pseudogene (Hines *et al*., 2002). Each FMO isoform has different substrate specificity, but among the family member they show overlapping substrate specificity. The FMO1, FMO2, FMO3, FMO4 and FMO6 genes are located around a 250 kb cluster at chromosome locus 1q23-24 (Shephard *et al*., 1993; Hernandez *et al*., 2004). The FMO5 gene is separately mapped to chromosome 1q21 (McCombie *et al*., 1996). FMO3 is functional form expressed in human liver and FMO3 gene consists of eight coding and one noncoding exons that translate into a protein with 531 amino acids (Lomri *et al*., 1992; Philips *et al*., 1995; Dolphin *et al*., 1997).

Eswaramoorthy and colleagues (2006) have been determined the structure of FMO of the yeast *Schizosaccharomyces pombe* (Figure 1.9). The protein has two structural domains namely big and small domain. There is a channel between two

domains. While FAD only interacts with big domain, NADPH binds to the small domain by means of its adenine group.

Table 1. 3 Substrates for FMO (Adapted from Hodgson et al., 1995, Ziegler, 1988, and Poulsen, 1981).

Chemical Class	Examples
Nitrogen-Containing Organics	
Primary amines	n-Octylamine
Secondary amines	
Acylic	N-Methylaniline, Disipramine
Cyclic	Desmethyltrifluperazine, Perazine
Tertiary amines	
Acylic	Chloropromazine, Imipramine
	Benzphetamine, Tamoxifen
Cyclic	Nicotine, Morphine, Cocaine, Atropine
Hydroxylamines	N-Hydroxyaminoazobenzene
Hydrazines	
Monosubstituted	Methylhydrazine, Phenylhydrazine
Disubstituted (1,1)	1,1-Dimethylhydrazine, N-Aminopyrrolidine
Disubstituted (1,2)	1,2-Dimethylhydrazine, Procarbazine
Sulfur-Containing Organics	
Thiols	Dithiothreitol, Cysteamine, Glutathione
	↑-Mercaptoethanol, Cysteine
Sulfides	Phorate, Aldicarb, Cimetidine, Ranitidine
Disulfides	Butyl disulfide, o-Dithiane
Thiocarbamides, etc	Thiourea, Methimazole
Dithioacids and Dithiocarbanates	Thioacetamide
Mercaptopurines and Mercaptopyrimidines	Dithiobenzoate
Phosphorus-Containing Organics	
Phosphines	Diethylphenylphosphine
Phosphonothioates	Fonofos
Selenium-Containing Organics	2-Selenylbenzanilide
Boronic Acids	
Inorganics	HS^-, S_8, I^-, IO^-, I_2, CNS^-

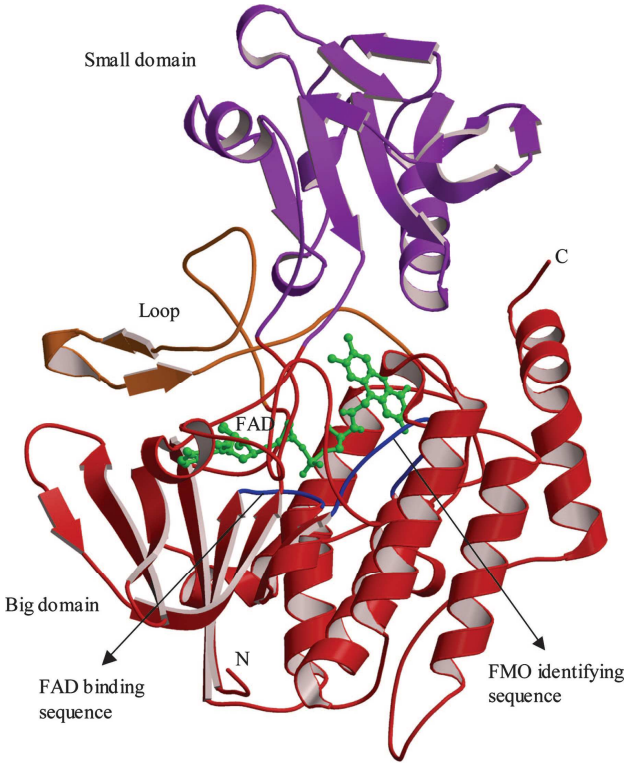


Figure 1.9 Structure of FMO of Schizosaccharomyces pombe. Ribbon representation of the protein and ball-and-stick model of FAD. The strand–turn–helix motifs and the loop interlinking the two domains are labeled. FAD is in the large domain and has no interaction with the small domain (Taken from Eswaramoorthy *et al*., 2006).

The catalytic cycle of FMO is depicted in Figure 1.10. FAD undergoes 2-electron reduction by NADPH in the first step of the catalytic cycle. In the second step reduced FAD ($FADH_2$) accepts molecular oxygen to generate peroxyflavin (FADOOH) and it is waiting for a suitable nucleophile in this state. When a suitable substrate binds to the FADOOH-protein complex, 1 atom of molecular oxygen is transferred to the substrate and substrate becomes oxygenated (SO) and 1 atom of molecular oxygen is used to form water. In the absence of an oxidisable substrate, the FADOOH intermediate decomposes, yielding H_2O_2.

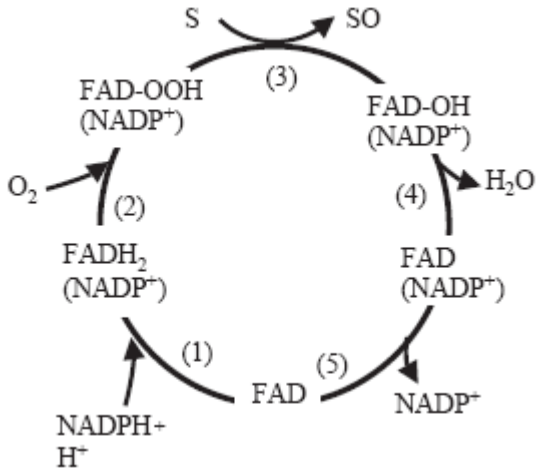


Figure 1. 10 Catalytic cycle of flavin-containing monooxygenase. (1) FAD reduced by NADPH (fast). (2) $FADH_2$ reacts with O_2 (fast). Flavin-hydroperoxide is stable: thought to be the form of in which FMO exists in the cell. (3) FAD-OOH reacts with any suitable nucleophile gaining access to active site. No substrate binding required. (4) One atom of O_2 is incorporated into substrate and the other into H_2O-FMO is a monooxygenase. (5) FAD-OH is converted to FAD via release of H_2O (slowest step in the cycle). The final step in the cycle is the release of $NADP^+$ (slow) (Taken from Krueger and Williams, 2005).

Krueger and Williams (2005) says about reactive oxygen species generation of FMO that "The FMO must have evolved a mechanism to protect nucleophilic sites (e.g., methionine, cysteine) from oxidative attack by the peroxyflavin. Furthermore, the structural features of the FAD pocket must be designed to minimize uncoupling/leakage of reactive oxygen species from the breakdown of FADOOH. As FMO is present at high concentrations in the endoplasmic reticulum of some tissues, a significant production of superoxide anion radical or hydrogen peroxide from decomposition of the FADOOH would be detrimental." Again Krueger and Williams (2005) reported that formation of hydrogen peroxide by FMO has an important role for controlling of the overall redox state of the cell.

1.7.2.2 Polymorphisms of FMO3

Fifteen nonsynonymous SNPs are identified in *FMO3* gene. Most of these SNPs have little or no effect on enzyme activity (Krueger and Williams, 2005; Koukouritaki and Hines, 2005). For example Koukouritaki *et al*., (2007) reported that g.11177C>A (N61K) SNP decrease enzyme activity against four different substrates dramatically. On the contrary Lattard *et al*., (2003) shown that g.21599T>C (L360P) SNP increases catalytic activity of FMO3 enzyme 2 to 5-fold. However these SNPs have limited significance for the general population because of their low frequency. The two most common SNPs were detected in exons 4 and 7 of *FMO3* gene. The G472A found in exon 4 and leads amino acid substitution from glutamate (Glu) to lysine (Lys) at position 158. The other mutation in exon 7 is A923G which causes a replacement of Glu to glycine (Gly) at position 308. Some studies showed that these two polymorphisms reduce FMO3 enzyme activity (Cashman and Zhang, 2006; Koukouritaki and Hines, 2005; Lattard *et al*, 2003; Shimizu *et al*., 2007).

1.8 Features and Polymorphisms of Phase II Xenobiotic Metabolizing Enzymes

1.8.1 NAD(P)H:Quinone Oxidoreductase 1 (NQO1)

1.8.1.1 Features of NQO1

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a phase II detoxification enzyme that catalyzes two electron reduction of various quinines utilizing NAD(P)H as an electron donor. Transformation of quinones to hydroquinones by NQO1 is an

important cellular defense mechanism against oxidative stress (Joseph *et al*., 2000). NQO1 is a homodimer and has a molecular weight of about 60 kDa. Each subunit consists of two domains namely large catalytic domain and smaller C-terminal domain (Figure 1.11). The catalytic domain of each monomer contains two FAD molecules.

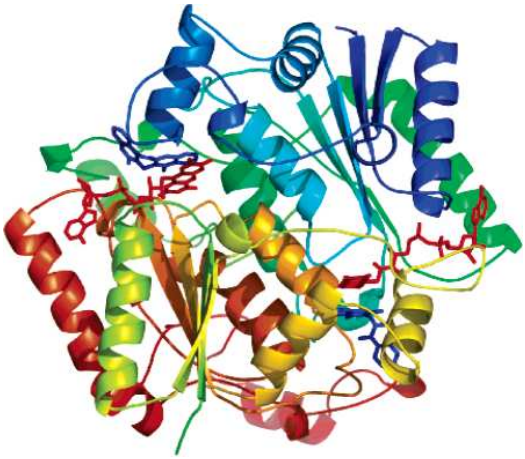


Figure 1. 11 Overall structure of the human NQO1 homodimer. FAD colored in red. (Taken from Asher *et al*., 2006).

NQO1 shows functions via ping-pong mechanism (Figure 1.12). In this mechanism NAD(P)H binds to NQO1 while it reduces the FAD cofactor. And then it is released to allow the quinone substrate to bind the enzyme and to be reduced. The cofactor (NAD(P)H) and the substrate (quinone) occupy the same site of NQO1, thus providing a molecular basis for this ping-pong mechanism (Li *et al*., 1995).

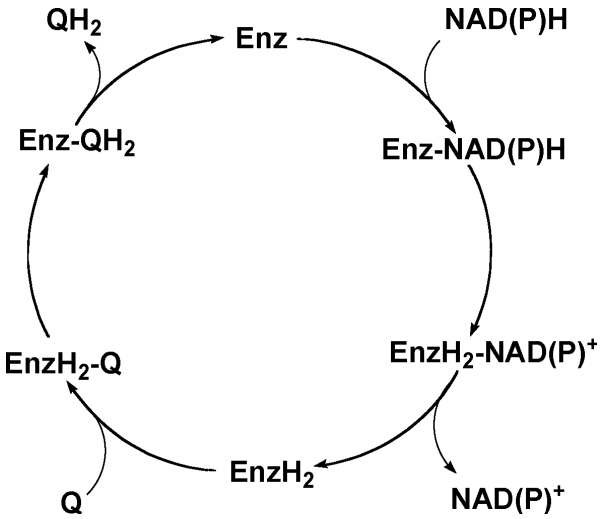


Figure 1. 12 Ping-pong mechanism of NQO1 (Taken from Colucci *et al*., 2008).

As mentioned earlier NQO1 reduces quinones to hydroquinones in a single two electron step and leads to the formation of more water soluble and, therefore more easily excreted hydroquinone metabolite. However, quinones have a broad chemical class and sometimes two electron reduction by NQO1 yields a more reactive hydroquinone metabolite (Ross *et al*., 2000). The reactive hydroquinone metabolites may generate aggressive oxygen species or some hydroquinones may rearrange to generate reactive alkylating agents. Therefore NQO1 has a potential causing oxidative stress through this mechanism (Figure 1.13).

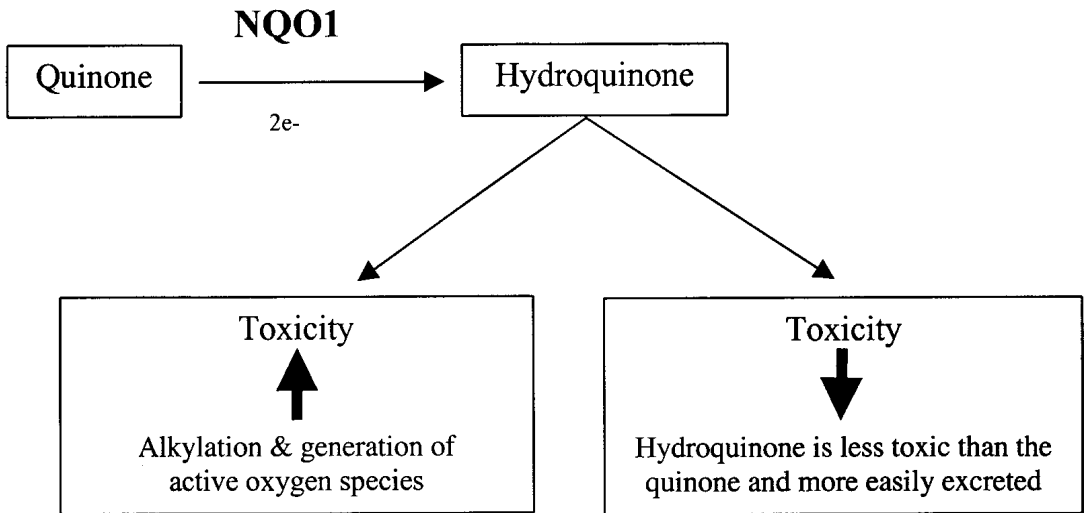


Figure 1. 13 Activation and deactivation resulting from NQO1-mediated reduction of quinones (Taken from Ross *et al.*, 2000).

1.8.1.2 Polymorphisms of NQO1

NQO1 gene has two SNPs namely NQO1*2 and NQO1*3 polymorphisms. NQO1*3 polymorphism, also known as C465T, causes a change the amino acid at position 139 from arginine (Arg) to tryptophan (Trp). Several studies reported that NQO1*3 polymorphism leads to the deletion of exon 4 and generates a protein lacking the quinone binding site because of alternative mRNA splicing (Gasdaska *et al.*,1995; Pan *et al.*, 2002). However the frequency of NQO1*3 polymorphism is very low. It ranges between 0% and 5% in different populations (Gaedigk *et al.*, 1998; Eguchi-Ishimae *et al.*, 2005).

The other polymorphism NQO1*2 is a single nucleotide change from cytosine (C) to thymine (T) at position 609 of the NQO1 cDNA coding for a proline (Pro) to serine (Ser) change at position 187 in the amino acid structure of the protein. Kuehl *et al.* (1995) reported that while 609TT mutant genotype causes loss of enzyme activity, heterozygote 609CT genotype nearly 3-fold decreases enzyme activity.

Therefore a mutation at this locus could increase susceptibility of oxidative stress. Several studies concluded that NQO1*2 polymorphism has an additive effect on oxidative damage, so it has great significance for cancer susceptibility and chemoprotection (Riley and Workman, 1992; Siegel *et al*., 1999). In addition Han et al. (2009) showed that 609T allele has significantly higher risk of carotid artery plaque development when compared to 609C allele. Furthermore strong association between lack of NQO1 activity and coronary heart disease was shown in study conducted by Martin *et al*. (2009). NQO1 609TT genotype frequency was found to be higher in both Alzheimer and Parkinson's disease patients. These data suggesting that NQO1*2 polymorphism was considered as a risk factor for these diseases (Bian *et al*., 2008; Shao *et al*., 2001). Numerous studies reported that there is an association between 609T mutant allele and different kinds of leukemia such as infant leukemia (Smith *et al*., 2002; Wiemels *et al*., 1999), childhood acute lymphoblastic leukemia (Krajinovic *et al*., 2002) and therapy treated leukemias (Larson *et al*., 1999).

1.8.2 Glutathione S-Transferases (GSTs)

1.8.2.1 Features of GSTs

The glutathione S-transferases are major phase II detoxification enzymes. GSTs catalyze nucleophilic attack by reduced glutathione on nonpolar compounds that contain an electrophilic carbon, nitrogen or sulphur atom (Keen and Jakoby, 1978, Armstrong, 1997, Hayes *et al*., 2005), resulting in the formation of oxidized glutathione (GSSG) (Figure 1.14).

GSTs are superfamily of polymorphic enzymes that catalyze detoxification of metabolites produced by oxidative stress within the cell and they are induced by reactive oxygen species (Hayes and Pulford, 1995; Hayes *et al*., 2005). Therefore,

GSTs are considered as one of the defense systems against the damaging effects of oxidative stress.

As mentioned previously in part 1.4 the enzymic detoxification of xenobiotics consists of three phases namely, phase I, phase II and phase III. Phase I reactions are catalyzed by cytochrome P-450 enzyme systems. In phase I and phase II reactions, nonpolar xenobiotics are converted to more polar compound and therefore less toxic metabolite, which can be eliminated easily from the cell via phase III system (Figure 1.15). Phase II enzymes catalyze the conjugation of activated xenobiotics to an endogenous water soluble substrate, such as reduced glutathione (GSH), UDP-glucuronic acid or glycine. Quantitatively, conjugation to GSH, which is catalyzed by the GSTs, is the major phase II reaction in many species.

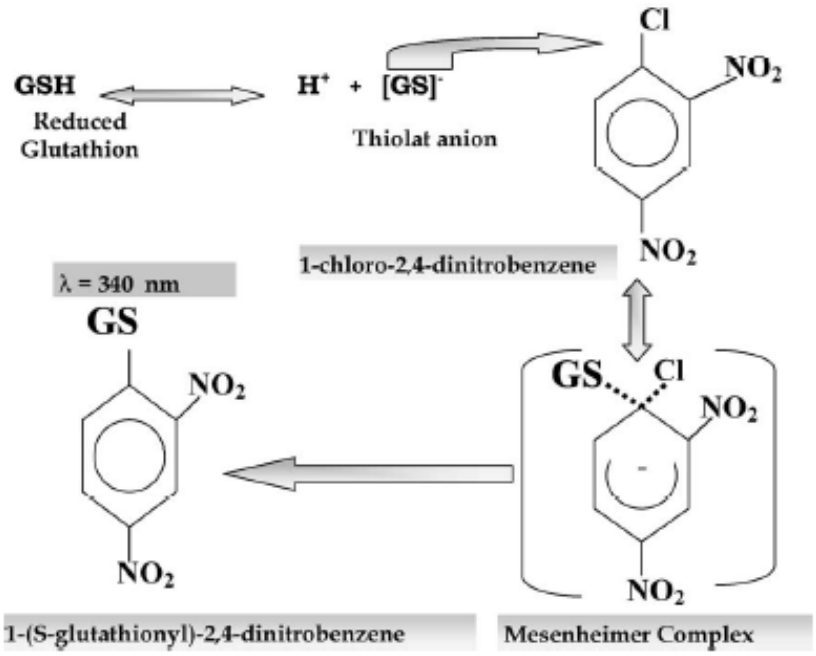


Figure 1. 14 General reaction catalyzed by GSTs.

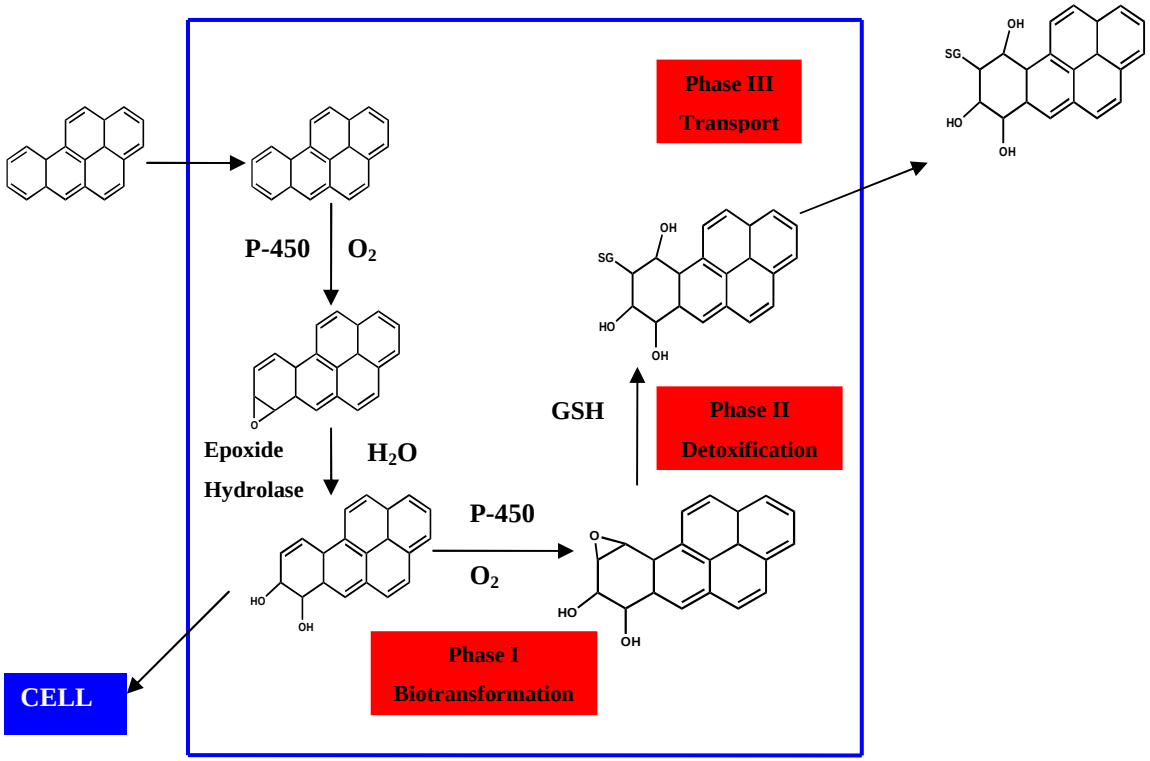


Figure 1. 15 Overview of enzymic biotransformation by phase I (cytochrome P450 and epoxide hydrolase), phase II (glutathione S-transferase) and phase III mechanisms (Taken from Türkanoğlu, 2007).

1.8.2.2 Polymorphisms of GSTs

Human GSTs are categorized into six main classes according to substrate specificity, chemical affinity, structure, amino acid sequence and kinetic behavior of the enzyme: GST-α (Alpha), GST-μ (Mu), GST-π (Pi), GST-θ (Theta), GST-Z (Zeta) and GST-Ω (Omega). The genes encoding the GST isoenzymes GSTM1 and GSTT1 have null alleles resulting from gene deletion and the null genotypes of GSTM1 and GSTT1 have reduced enzyme activity (Bruhn *et al*., 1998; Zhong *et al*., 2006). In Caucasians GSTT1 and GSTM1 null genotype frequencies vary from 13% to 26% and 42% to 60%, respectively (Garte *et al*., 2001). Ada *et al.* (2004) reported that in Turkish population the frequencies of GSTT1 null (17.3%) and GSTM1 null genotypes (51.9%) are similar to Caucasian populations. GST polymorphisms have been investigated in relation to various types of diseases, including diabetes mellitus, hypertension, Parkinson's disease, rheumatoid arthritis and particular types of cancer (Yalın *et al*., 2007; Wang *et al*., 2006; Keladaa *et al*., 2003; Park *et al*., 2004; Singh *et al*., 2008; Lewis *et al*., 2002; Kentaro *et al*., 2008) . GSTT1 present genotype was found as a protective factor against type 2 diabetes mellitus in Chinese population (Wang *et al*., 2006). On the other hand, in Turkish population, GSTT1 polymorphism did not influence the risk of diabetes. In the same study, GSTM1 null genotype frequency of diabetic patients was found to be significantly higher than that of control group (Yalın *et al*., 2007). Increased risk of hypertension has been observed in the GSTM1/GSTT1 double null genotypes or GSTM1 null genotype (Kentaro *et al*., 2008). According to a recent study, compared to the present genotypes, GSTT1 null and GSTM1 null genotype groups contained relatively more hypertensive stroke patients (Türkanoğlu *et al*., 2010).

The *GSTP1* gene polymorphism is a single nucleotide change from adenine (A) to guanine (G) at position 313 of the GSTP1 cDNA coding for an isoleucine to valine change at position 105 in the amino acid structure of the protein. The valine amino acid results in decreased enzyme activity (Ali Osman *et al.*, 1997). Zimniak *et al*.,

(1994) reported that GSTP1 313G allele has greater in vitro activity toward 1-chloro-2,4-dinitrobenzene (CDNB) which is a common substrate of GST isozymes. In male Turkish population, the genotype frequencies of GSTP1 313AA, 313AG and 313GG were found as 58.7%, 35.3% and 6%, respectively (Ada *et al*., 2007). Several studies showed that GSTP1 313GG mutant genotype has greater breast cancer risk (Helzlsouer *et al*., 1998; Egan *et al*., 2004; Gudmundsdottir *et al*., 2001). In addition Vilar *et al*., (2007) reported that GSTP1 313G allele shows a significant association with sporadic Parkinson's disease. On the other hand Zuntar *et al*., (2004) observed that Alzheimer disease patients had higher frequency of GSTP1 313G mutant genotype than controls. However they could not find significant association between GSTP1 A313G alleles and susceptibility to Alzheimer disease.

1.9 Features and Polymorphisms of Nitric Oxide Synthase (NOS)

1.9.1 Features of NOS

Nitric oxide synthase (NOS) produces endogenous nitric oxide (NO) from the amino acid L-arginine as shown in Figure 1.16. In mammals, there are 3 distinct genes that encode NOS isoenzymes namely neuronal (nNOS or NOS1), cytokine-inducible (iNOS or NOS2) and endothelial (eNOS or NOS3). The NOS3 gene is conserved through evolution, consists of 26 exons spanning 21 kilobase, and encodes an enzyme that generates NO in the vascular endothelium.

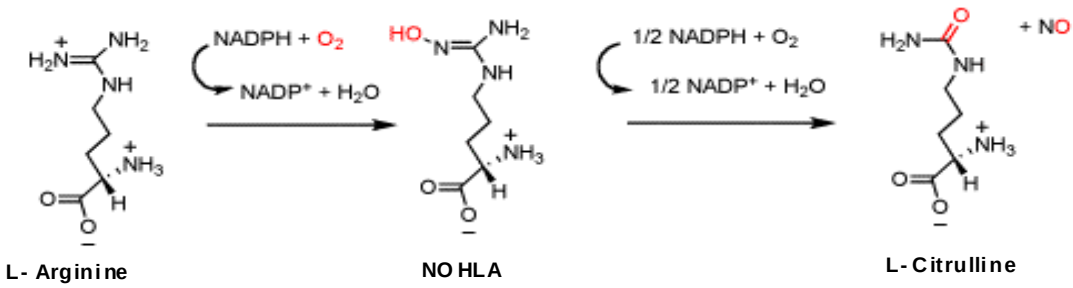


Figure 1.16 Reaction catalyzed by nitric oxide synthase (NOS) enzyme.

There are N-terminal oxygenase domain including single heme and tetrahydrobiopterin (BH_4) binding sites and C-terminal reductase domain including single binding sites for flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and NADPH, and a calmodulin (CaM) binding site between the oxygenase and reductase domains in the structure of NOS3 enzyme (Figure 1.17 A). Homodimerization of NOS3 enzyme is required for oxidation of L-arginine (Figure 1.17 B). The transfer of electrons from NADPH, FAD and FMN in the reductase domain of one monomer continues toward the heme domain of the other and is facilitated by calcium bound CaM. The ferric heme moiety accepts electrons to perform two step catalysis hydroxylation of L-arginine to generate N-hydroxy-L-arginine and its oxidation to L-citrulline and NO.

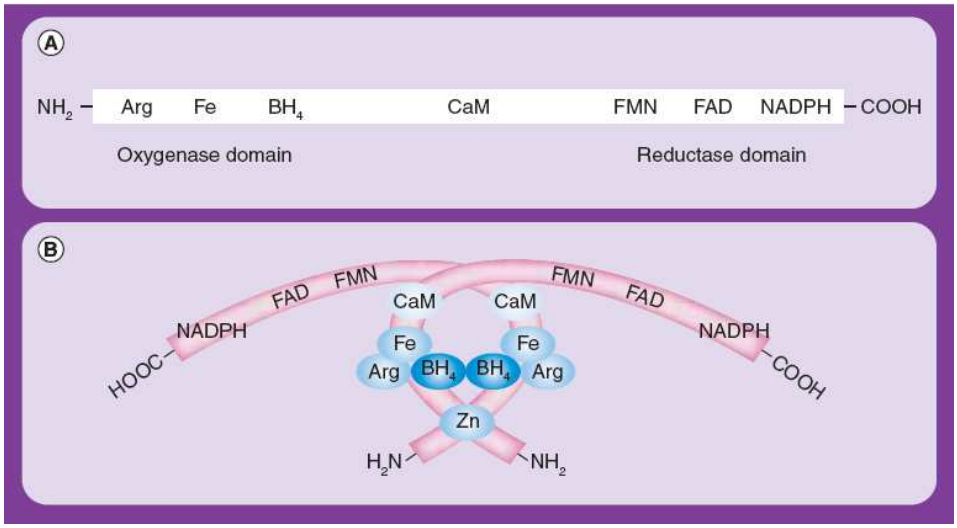


Figure 1. 17 A) Monomeric structure of NOS3 enzyme. **B)** Dimerization of NOS3 demonstrating the proximity of flavin groups (reductase domain) of one dimer to the oxygenase domain of the other dimer (Taken from Cooke *et al*., 2007).

Nitric oxide (NO) is an uncharged diatomic gas and a potent regulator of vasomotor tone and peripheral resistance. Studies showed that NO has various effects such as vasodilatation, inhibition of platelet adherence and aggregation, suppression of smooth muscle proliferation, and reduction of adherence of leucocytes to the endothelium (Cooke and Dzau, 1997; Moncada and Higgs, 1993, 2000; Radomski and Salas, 1995). Wilcox *et al.* (1997) reported that reduction in the activity of vascular NOS3 enzyme impairs endothelium-dependent vasodilatation in atherosclerotic vessels. According to study conducted by Willmot *et al*. (2005) extended ischemic area and reduction of blood flow in penumbra was observed in NOS3 deficient transgenic mice. Because of these characteristics, varieties in the *NOS3* gene have been considered to contribute to the development of ischemic stroke.

Under physiological conditions NOS3 produces NO that reacts with superoxide (O_2^-) radical produced by mitochondria and membrane bound oxidase system like xanthine oxidase (XO) to form peroxynitrite ($ONOO^-$). At low concentrations $ONOO^-$ causes vasorelaxation, decreases platelet aggregation, reduces leukocyte adhesion to the vessel wall like NO (Figure 1.18) (Wever *et al*., 1998).

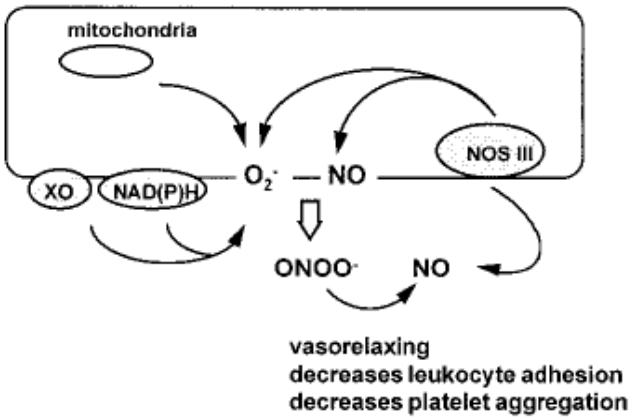


Figure 1. 18 Functions of NO under physiological conditions (Taken from Wever *et al*., 1998).

In the atherosclerosis condition NOS3 produces less NO. On the other hand superoxide formation is increased by both NOS3 and XO enzymes and also NADPH oxidases (Figure 1.19). Furthermore myeloperoxidases (MPO) and lipooxygenase (lipox) produce more superoxide in this case. When NOS2 is induced, the production of NO and superoxide is increased by NOS2. As a result of this situation high concentrations of peroxynitrite ($ONOO^-$) which has been associated with cellular toxicity may be observed in the cell (Wever *et al*., 1998).

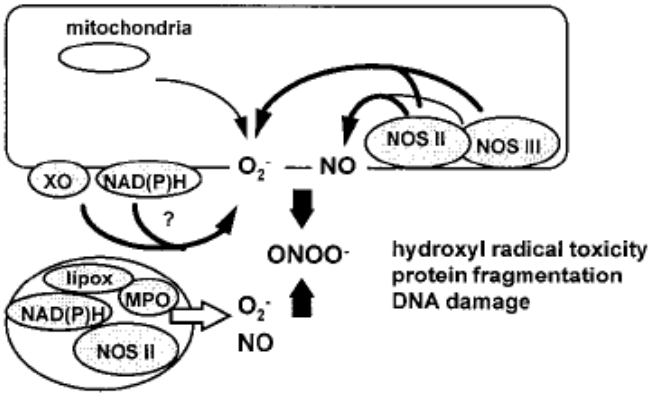


Figure 1.19 Formation of high concentrations of peroxynitrite ($ONOO^-$) in atherosclerosis condition (Taken from Wever *et al*., 1998).

1.9.2 Polymorphisms of NOS3

Several SNPs have been identified in the promoter, exons, and introns of the *NOS3* gene. G894T in exon 7 of the *NOS3* gene leads to a change of Glu to Asp at site 298. It has been demonstrated that this polymorphism alters NOS3 activity and may be associated with a reduction in the basal NO production (Tesauro *et al*., 2000). The mutant T allele was reported to be associated with hypertension, coronary artery disease (CAD) (Hingorani *et al.,* 1999), stroke (Hassan *et al*., 2004), and the number of stenotic vessels. Another *NOS3* gene polymorphism, T-786C located in the promoter region of the *NOS3* gene, results from replacement of a thymidine by a cytosine at nucleotide −786. It was reported that the T-786C polymorphism may change *NOS3* gene expression and result in a significant reduction in the NOS3 gene promoter activity (Nakayama *et al*., 1999). The T-786C polymorphism was also demonstrated to be associated with an increased predisposition to CAD in Chileans and Koreans (Jaramilo *et al*., 2008; Kim *et al*., 2007), to ischemic stroke in young black women (Howard *et al*., 2005), to coronary spasm in Japanese, and to myocardial infarction (MI) in Koreans (Nakayama *et al*., 1999; Jo *et al*., 2006), but not to increase the predisposition to MI and CAD in other Caucasians (Poirier *et al*.,

1999; Granath *et al*., 2001). The other *NOS3* gene polymorphism is the variable number of tandem repeat (VNTR) polymorphism located in intron 4 of *NOS3* (eNOS4b/a polymorphism) which is significantly associated with plasma NO concentration. In repeats of a 27-bp consensus sequence, there are two alleles, a common large allele and a smaller allele. The larger allele (eNOS4b allele), designated "b-insertion" has five tandem repeats, and the smaller allele (eNOS4a allele) "a-deletion" has four repeats. It has been reported that eNOS4 VNTR polymorphism may be responsible for plasma nitric oxide levels (Tsukada *et al*., 1998).

1.10 Aim of The Study

Stroke is the third leading cause of death behind the diseases of heart and cancer and causes more serious chronic disabilities than any other diseases. Atherosclerosis which causes thickening and hardening of the vascular wall due to plaque deposition in the inner lining of the arteries is a major cause of stroke. Oxidative stress plays an important role in the pathogenesis of atherosclerosis and carotid atherosclerosis is a risk factor for stroke.

The condition occurring when the physiological balance between oxidants and antioxidants is disrupted in favor of the former with potential damage for the organism is called as oxidative stress. In principle, oxidative stress can be caused by increased production of free radicals or diminished antioxidants. The tissue level of antioxidants critically influences susceptibility of various tissues to oxidative stress. Enhanced oxidative stress and oxidative damage to tissues are general features of some chronic diseases such as Alzheimer disease, Parkinson's disease, cancer, atherosclerosis and diabetes mellitus (Yoritaka *et al*., 1996, Berlett and Stadtman, 1997, Beal, 2002).

In the present study, we studied polymorphisms of enzymes (CYP2E1, FMO3 and NOS3) causing oxidative stress and enzymes (GST and NQO1) protecting against oxidative stress which is important factor in the pathogenesis of

atherosclerosis. The main objective of this study was to determine the usefulness of genetic polymorphisms as biomarkers for the determination of susceptibility to ischemic stroke in Turkish population. To achieve this aim, this study is designed to follow the steps given below:

***** obtaining total blood and serum samples from ischemic stroke patients and healthy controls,

***** isolation of genomic DNA in intact form from blood samples,

***** amplification of three regions in *CYP2E1*, two regions of *FMO3*, one region of *NQO1*, three regions of *GST* and three regions of *NOS3* genes by PCR,

***** digestion of the amplified fragments with restriction endonucleases to determine the genotype of each individual,

***** comparison of vascular risk factors and genotype and allele frequencies between ischemic stroke and control groups,

* comparison of genotype and allele frequencies between subgroups of patients and controls defined by age, gender, and presence of one of the risk factors, using statistical methods, in order to determine risk factors for ischemic stroke.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Subjects and Blood Sample Collection

A total of 245 blood samples were obtained from consecutive unrelated adult Caucasian patients with acute hemispheric ischemic stroke and 145 blood samples were obtained from symptom-free Caucasian controls from the same geographic region (central Anatolia, Turkey) with the collaboration of Gülhane Military Medical Academy, Department of Neurology, Ankara. Patients had an anterior circulation stroke resulted from carotid artery atherosclerotic disease. The cerebral infarction was initially diagnosed on the basis of neurological examination and brain computer tomography (CT) scan and then transthoracic echocardiographic examination, Holter study and Transcranial Doppler emboli detection procedure to rule out emboli source. In order to be considered eligible, the patients should meet following criteria: having anterior circulation stroke, no other major illnesses, including autoimmune diseases, neoplasms, coagulopathies, hepatic or renal failure, no known embolic source (aortic arch, cardiac or carotid), no family history of hematological, autoimmune or chronic inflammatory diseases, no history of myocardial infarction within 3 weeks or of transient ischemic attack or stroke at any time. Control subjects selected randomly from neurology outpatient clinics did not have stroke or transient ischemic attack at any time. All exclusion criteria were applied to the controls

exactly plus not having carotid stenosis (lumen narrowing) >50% or ulcerated carotid plaque.

All subjects underwent bilateral carotid Doppler ultrasound (CUSG) and transthoracic echocardiographic studies. A detailed history of conventional vascular risk factors and conditions was taken from each participant. Hypertension was defined as systolic blood pressure > 140 mm Hg and /or diastolic blood pressure > 90 mm Hg and/or use of antihypertensive drugs. Diabetes was defined as fasting glucose ≥ 6.99 mmol/L and/or use of pharmacological treatment. Smoking status of an individual was assigned ″yes″ if the individual is currently smoking or have quitted less than 3 months ago.

Routine laboratory tests, including electrocardiogram, chest X-ray, complete blood count, leukocyte differential, erythrocyte sedimentation rate, routine biochemistry tests including fasting glucose, lipid profile (triglycerides, total cholesterol, LDL, HDL, VLDL-C), creatinine, sodium, potassium, bilirubin, and liver function tests, routine urine tests and rheumatologic screening tests were performed for all participants.

All laboratory measurements were done blinded to clinical characteristics. Informed consent was obtained from all participants before study entry. Copy of the informed consent forms are given in Appendix A and Appendix B. The study was approved by the Ethical Committee of the Medical Academy (see Appendix C) and was carried out according to the principles of the Declaration of Helsinki.

4-5 mL of blood samples from ischemic stroke patients and controls were taken in EDTA-containing vacuumed tubes and stored at -20°C till use for DNA isolation. Blood samples were kept in 4°C while they were in active use.

2.1.2 Chemicals and Enzymes

Agarose (A-9539), bromophenol blue (B-5525), ethidium bromide (E-7637), ethylene diamine tetra acetic acid disodium salt (EDTA; E-5134), sodium chloride (NaCl; S-3014), sodium dodecyl sulfate (SDS; L-4390), 2-amino-2(hydroxymethyl)-

1,3-propandiol (Tris; T-1503), 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. Sucrose (7653) and Triton X-100 (11869) were the products of Merck & Co., Inc., Whitehouse Station, NJ, USA.

Taq DNA Polymerase (supplied together with MgCl2 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), DdeI (#ER1882), HinfI (#ER0801), BanII (#ER0281), PdiI (#ER1522), BsmAI (#ER0031), DraII (#ER0261), which were supplied with their buffers, were purchased from MBI Fermentas, USA.

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 Iontek (Iontek, Istanbul, Turkey) and Alpha DNA (Alpha DNA, Canada, USA). 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
FMO3 G472A	5'-GCTAGCATAGAAAAGAGGGA-3' 5'-CGAGAGTCACCCGAGTACCCG-3'	Park *et al.*, 1999
FMO3 A923G	5'-GTCTCTGTTTTCCATACAG-3' 5'-CTTCGCAATCCATGAGCCTC-3'	Park *et al.*, 1999
NQO1*2	5'-CCTCTCTGTGCTTTCTGTATCC-3' 5'-GATGGACTTGCCCAAGTGATG-3'	Eguchi-Ishimae *et al.*, 2005
GSTP1 A313G	5'-GAGGAAACTGAGACCCACTGAG-3' 5'-AGCCCCTTTCTTTGTTCAGCC-3'	Zhong *et al.*, 2006
GSTM1	5'-GAACTCCCTGAAAAGCTAAAGC-3' 5'-GTTGGGCTCAAATATACGGTGG-3'	Abdel-Rahman *et al.*, 1996
GSTT1	5'-TTCCTTACTGGTCCTCACATCTC-3' 5'-TCACCGGATCATGGCCAGCA-3'	
CYP1A1 exon7 (internal control)	5'-GAACTGCCACTTCAGCTGTCT-3' 5'-CAGCTGCATTTGGAAGTGCTC-3'	
NOS3 G894T	5'-AAGGCAGGAGACAGTGGATGGA-3' 5'-CCCAGTCAATCCCTTTGGTGCTCA-3'	Güldiken *et al.*, 2009
NOS3 T-786C	5'-ATGCTCCCACCAGGGCATCA-3' 5'-GTCCTTGAGTCTGACATTAGGG-3'	Jaramillo *et al.*, 2008
NOS3 INTRON4 VNTR	5'-TTATCAGGCCCTATGGTAGT-3' 5'-AACTCCGCTCAGCTGTCCT-3'	Kunnas *et al.*, 2002

2.2 Methods

Preparation of Tris-HCl pH 8.0, EDTA pH 8.0, TKME pH 7.6, saturated NaCl, TE pH 8.0 buffers used in human genomic DNA isolation from whole blood, TBE pH 8.3, ethidium bromide, gel loading dye solutions used in agarose gel electrophoresis, and solutions used in PCR amplification procedure are described in Appendix D.

2.2.1 Isolation of Genomic DNA from Human Whole Blood Samples

Genomic DNA was isolated according to the method of Lahiri and Schnabel (1993), with some modifications. In this method, 750 µL of whole blood which was taken into EDTA-containing vacuumed tubes was treated with an equal volume of low-salt buffer containing 10 mM Tris-HCl pH 7.6, 10 mM KCl, 2 mM EDTA, 4 mM $MgCl_2$ (TKME buffer), and 20 µL of Triton X-100. The cells were lysed by inversions and the suspension is centrifuged at 1000 g for 10 min at room temperature. The pellet was washed three more times with TKME buffer and the final pellet was resuspended in 0.2 mL of TKME buffer. 20 µL 10% SDS was added, and the whole suspension was mixed thoroughly and incubated for 10 min at 58°C. Then 75 µL saturated NaCl (~6 M) was added, the tube was mixed well and centrifuged at 14000 g for 10 min, at 4°C. The supernatant, which contained the DNA was precipitated using two volume ice-cold absolute ethanol. The tubes were stored at -20°C for at least 30 min and DNA was precipitated to pellet by centrifugation at 10000g for 10 min at 4°C. Supernatant was removed, then air dried and DNA containing pellet was resuspended in 0.1 mL of 10 mM Tris-HCl pH 8.0, and 1 mM EDTA pH 8.0. (TE) and incubated 37°C for 1.5-2 hours.

2.2.2 Spectrophotometric Quantification of Genomic DNA

For the determination of the concentration of DNA in the sample, absorbance values at 260 nm and 280 nm were measured spectrophotometrically. 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 50µg/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 and lower values showed either RNA or protein contamination, respectively.

2.2.3 Qualification of Genomic DNA by Agarose Gel Electrophoresis

Intactness of DNA samples was performed by agarose gel electrophoresis using a horizontal agarose gel electrophoresis unit (Scie-Plas HU13W, Warwickshire, England). 0.5% agarose gel was prepared by 0.5X TBE buffer pH8.3 using microwave oven. The solution was cooled to approximately 60°C on a magnetic stirrer with continuous stirring for homogenous cooling. When cooled enough, ethidium bromide was added from a stock solution of 10 mg/mL in water to a final concentration of 0.5µg/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. 5 µL 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. Single band in agarose gel electrophoresis shows pure DNA preparations; however RNA contaminated preparations yield two bands. A smear shows that the DNA is degraded.

2.2.4 Genotyping of Single Nucleotide Polymorphisms

The present study was designed to study genetic polymorphisms of *CYP2E1*, *FMO3, NOS3* (leading to oxidative stress) and *GST, NQO1* (protecting cells against oxidative stress) genes. Three SNPs of CYP2E1, namely *5B, *6 and *7B, two SNPs of FMO3, namely G472A and A923G, two SNPs of NOS3, namely G894T and T-786C and also one insertion/deletion (VNTR) polymorphism of NOS3, one SNP of GSTP1, namely A313G and GSTM1 and GSTT1 null genotypes, one SNP of NQO1, namely NQO1*2 polymorphisms were studied. For SNP determination PCR-RFLP technique was used. GSTM1 and GSTT1 null genotypes were determined at the same time by multiplex PCR protocol and NOS3 VNTR polymorphism were identified by PCR reaction. The details of these methods were explained 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 for CYP2E1*5B

The 5'-flanking region of *CYP2E1* gene was amplified to study CYP2E1*5B (C-1053T/G-1293C) single nucleotide polymorphism using primer sequences given in Table 2.1. In order to obtain a single band belonging to the region in 5'-flanking

region of *CYP2E1* gene, PCR mixture and conditions were optimized before (Ulusoy *et al.*, 2007b) in our laboratory and were given in Table 2.2. The amplification program consisted of 35 cycles of denaturating at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. The final extension was performed at 72°C for 6 min. The amplified PCR products were analyzed on 2% agarose gel as described in section 2.2.3. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

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

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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		~200 ng
Taq DNA Polymerase	5U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.1.2Restriction Endonuclease Digestion of PCR Products for Determination of CYP2E1*5B SNPs

C-1053T/G-1293C SNPs which are in complete linkage disequilibrium are found in the 5'-flanking region of CYP2E1 gene. Hence, the PCR products were digested separately by *RsaI* for C-1053T and *PstI* for G-1293C SNPs. The

components of restriction enzyme digestion mixture are given in Table 2.3. Both of the *RsaI* and *PstI* digestion mixtures were incubated at 37°C for 18 hours for complete digestion and then analyzed on 2.5 % 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 DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.3 Constituents of reaction mixture for restriction endonuclease (*RsaI* and *PstI*) digestion of PCR products for the determination of C-1053T and G-1293C SNPs of *CYP2E1*5B* polymorphism.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		10 µL	
Restriction enzyme	10 U/µL	1 µL	10 U
Sterile apyrogen H_2O		16 µL	

2.2.4.2 Genotyping of CYP2E1*6 Polymorphism

2.2.4.2.1Polymerase Chain Reaction for CYP2E1*6

The intron 6 region of *CYP2E1* gene was amplified to study CYP2E1*6 (T7632A SNP) polymorphism according to the previously optimized PCR conditions (Ulusoy *et al.*, 2007b) as presented in Table 2.4. The amplification program consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of denaturating at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C

for 1 min. The final extension was performed at 72°C for 6 min. The amplified PCR products were analyzed on 2% agarose gel. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

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

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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		~200 ng
Taq DNA Polymerase	5U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.2.2 Restriction Endonuclease Digestion of PCR Products for Determination of CYP2E1*6 SNP

DraI restriction enzyme was used to digest PCR products of CYP2E1 intron 6 region. The optimized reaction mixture is described in Table 2.5. The digestion mixture was incubated at 37°C for 18 hours for complete digestion and then analyzed on 2.5% 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 DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.5 Constituents of reaction mixture for *DraI* restriction endonuclease digestion of PCR products for the determination of T7632A SNP of *CYP2E1*6* polymorphism.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		20 µL	
DraI	10 U/µL	0.6 µL	6 U
Sterile apyrogen H_2O		6.4 µL	

2.2.4.3 Genotyping of CYP2E1*7B Polymorphism

2.2.4.3.1 Polymerase Chain Reaction for CYP2E1*7B

The promoter region of *CYP2E1* gene was amplified to study CYP2E1*7B (G-71T SNP) polymorphism. The components for the optimized PCR are given in Table 2.6. The amplification program consisted of an initial denaturation step at 94°C for 5 min followed by 30 cycles of denaturating at 94°C for 1 min, annealing at 62°C for 1.5 min and extension at 72°C for 2 min. The final extension was performed at 72°C for 10 min. The amplified PCR products were analyzed on 2% agarose gel. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

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

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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		~200 ng
Taq DNA Polymerase	5U/µL	0.5 µL	2.5 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.3.2Restriction Endonuclease Digestion of PCR Products for Determination of CYP2E1*7B SNP

PCR products were digested with *DdeI* restriction enzyme in an optimized reaction mixture as described in Table 2.7. The digestion mixture was incubated at 60°C for 18 hours for complete digestion and then analyzed on 2.5% 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 DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.7 Constituents of reaction mixture for *DdeI* restriction endonuclease digestion of PCR products for the determination of G-71T SNP of *CYP2E1*7B* polymorphism.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		20 µL	
DdeI	10 U/µL	0.4 µL	4 U
Sterile apyrogen H_2O		6.6 µL	

2.2.4.4 Genotyping of FMO3 G472A Single Nucleotide Polymorphism

2.2.4.4.1Polymerase Chain Reaction for FMO3 G472A SNP

To detect G472A single nucleotide polymorphisms, exon 4 region of *FMO3* gene was amplified according to the optimized PCR mixture and conditions given in Table 2.8. The optimized amplification program consisted of an initial denaturation step at 94°C for 30 sec followed by 30 cycles of denaturating at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 3 min. The amplified PCR products were visualized on 2% agarose gels stained with ethidium bromide. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.8 Components of PCR mixture for FMO3 G472A SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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		~200 ng
Taq DNA Polymerase	5U/µL	0.3 µL	1.5 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.4.2Restriction Endonuclease Digestion of PCR Products for Determination of FMO3 G472A SNP

PCR products were digested with *Hinf*I restriction enzyme in an optimized mixture as described in Table 2.9. The digestion mixture was incubated at 37°C for 18 hours for complete digestion. The digestion products were visualized on 3% agarose gels, and genotypes were determined according to the banding pattern. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.9 Constituents of reaction mixture for *Hinf*I restriction endonuclease digestion of PCR products for the determination of FMO3 G472A SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		20 µL	
HinfI	10 U/µL	0.2 µL	2 U
Sterile apyrogen H_2O		6.8µL	

2.2.4.5 Genotyping of FMO3 A923G Single Nucleotide Polymorphism

2.2.4.5.1Polymerase Chain Reaction for FMO3 A923G SNP

The exon 7 region of *FMO3* gene was amplified to study A923G polymorphism according to the optimized PCR conditions as presented in Table 2.10. The optimized amplification program consisted of an initial denaturation step at 94°C for 30 sec followed by 30 cycles of denaturating at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 3 min. The amplified PCR products were analyzed on 2% agarose gel. 10 µL PCR products was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.10 Components of PCR mixture for FMO3 A923G SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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	3 µL	30 pmol
Forward Primer	10 pmol/µL	3 µL	30 pmol
Template DNA	Varies		~200 ng
Taq DNA Polymerase	5U/µL	0.3 µL	1.5 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.5.2Restriction Endonuclease Digestion of PCR Products for Determination of FMO3 A923G SNP

PCR products were digested with *Dra*II restriction enzyme in a mixture as described in Table 2.11. The digestion mixture was incubated at 37°C for 18 hours for complete digestion. The digestion products were visualized on 2.5% agarose gels, and genotypes were determined according to the banding pattern. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.11 Constituents of reaction mixture for *DraII* restriction endonuclease digestion of PCR products for the determination of FMO3 A923G SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		20 µL	
DraII	10 U/µL	0.4 µL	4 U
Sterile apyrogen H_2O		6.6µL	

2.2.4.6 Genotyping of NQO1*2 Single Nucleotide Polymorphism

2.2.4.6.1Polymerase Chain Reaction for NQO1*2

The exon 6 region of *NQO1* gene was amplified to study NQO1*2 (C609T SNP) polymorphism according to the optimized PCR conditions as presented in Table 2.12. The optimized amplification program consisted of an initial denaturation step at 95°C for 5 min followed by 30 cycles of denaturating at 95°C for 1 min, annealing at 59°C for 1.5 min and extension at 72°C for 2 min. The final extension was performed at 72°C for 10 min. The amplified PCR products were analyzed on 2% agarose gel. 10 µL PCR products was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.12 Components of PCR mixture for NQO1*2 SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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		~200 ng
Taq DNA Polymerase	5U/µL	0.4 µL	2 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.6.2Restriction Endonuclease Digestion of PCR Products for Determination of NQO1*2 SNP

PCR products were digested with *Hinf*I restriction enzyme in a mixture as described in Table 2.13. The digestion mixture was incubated at 37°C for 24 hours for complete digestion. The digestion products were visualized on 3% agarose gels, and genotypes were determined according to the banding pattern. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.13 Constituents of reaction mixture for *HinfI* restriction endonuclease digestion of PCR products for the determination of NQO1*2 SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		20 µL	
HinfI	10 U/µL	0.4 µL	4 U
Sterile apyrogen H_2O		6.6µL	

2.2.4.7 Genotyping of GSTP1 A313G Single Nucleotide Polymorphism

2.2.4.7.1 Polymerase Chain Reaction for GSTP1 A313G SNP

The exon 5 region of *GSTP1* gene was amplified to study A313G polymorphism. The components for the optimized PCR reaction are given in Table 2.14. The amplification program consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of denaturating at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec. The final extension was performed at 72°C for 5 min. The amplified PCR products were analyzed on 2% agarose gel. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.14 Components of PCR mixture for GSTP1 A313G SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 25 µL reaction mixture
Amplification buffer	10 X	2.5 µL	1X
$MgCl_2$	25 mM	1.5 µL	1.5 mM
dNTP mixture	10 mM	0.5 µL	200 µM
Reverse Primer	10 pmol/µL	2 µL	20 pmol
Forward Primer	10 pmol/µL	2 µL	20 pmol
Template DNA	Varies		~200 ng
Taq DNA Polymerase	5U/µL	0.3 µL	1.5 U
Sterile apyrogen H_2O		to 25 µL	

2.2.4.7.2Restriction Endonuclease Digestion of PCR Products for Determination of GSTP1 A313G SNP

BsmAI restriction enzyme was used to digest PCR products of GSTP1 exon 5 region. The reaction mixture is described in Table 2.15. The digestion mixture was incubated at 37°C for 24 hours for complete digestion and then analyzed on 3% 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 DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.15 Constituents of reaction mixture for *BsmAI* restriction endonuclease digestion of PCR products for the determination of GSTP1 A313G SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	6 µL	2 X
PCR product		15 µL	
BsmAI	10 U/µL	0.4 µL	4 U
Sterile apyrogen H_2O		8.6µL	

2.2.4.8 Genotyping of GSTM1 and GSTT1 Null by Multiplex Polymerase Chain Reaction

GSTM1 and GSTT1 genotypes were simultaneously determined using multiplex polymerase chain reaction approach (Abdel-Rahman *et al*., 1996). As an internal control, CYP1A1 exon 7 gene, which is never deleted, was co-amplified in order to distinguish the null genotypes from aborted PCR. Table 2.16 summarizes the components of the optimized PCR mixture. The standard PCR protocol consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of melting at 94°C for 2 min, annealing at 59°C for 1 min and extension at 72°C for 1 min. The final extension was performed at 72°C for 10 min. The amplified PCR products were analyzed on 2.5% agarose gel. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.16 Components of PCR mixture for GSTM1 and GSTT1 null.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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	3 µL	30 pmol
Forward Primer	10 pmol/µL	3 µL	30 pmol
Template DNA	Varies		~200 ng
Taq DNA Polymerase	5U/µL	0.4 µL	2 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.9 Genotyping of NOS3 G894T Single Nucleotide Polymorphism

2.2.4.9.1Polymerase Chain Reaction for NOS3 G894T SNP

To detect G894T single nucleotide polymorphisms exon 7 region of *NOS3* gene was amplified according to the optimized PCR conditions given in Table 2.17. The amplification program consisted of an initial denaturation step at 95°C for 5 min followed by 35 cycles denaturating at 94°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 1 min. The final extension was performed at 72°C for 5 min. The amplified PCR products were visualized on 2% agarose gels stained with ethidium bromide. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.17 Components of PCR mixture for NOS3 G894T SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	5 µL	1X
$MgCl_2$	25 mM	5 µL	2.5 mM
dNTP mixture	10 mM	0.5 µL	100 µM
Reverse Primer	10 pmol/µL	3 µL	30 pmol
Forward Primer	10 pmol/µL	3 µL	30 pmol
Template DNA	Varies		~200 ng
Taq DNA Polymerase	5U/µL	0.25 µL	1.25 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.9.2Restriction Endonuclease Digestion of PCR Products for Determination of NOS3 G894T SNP

BanII restriction enzyme was used to digest PCR products of NOS3 exon 7 region. The optimized reaction mixture is described in Table 2.18. The digestion mixture was incubated at 37°C for 16 hours for complete digestion and then analyzed on 3% 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 DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.18 Constituents of reaction mixture for *BanII* restriction endonuclease digestion of PCR products for the determination of NOS3 G894T SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		10 µL	
BanII	10 U/µL	0.2 µL	2 U
Sterile apyrogen H_2O		16.8µL	

2.2.4.10 Genotyping of NOS3 T-786C Single Nucleotide Polymorphism

2.2.4.10.1 Polymerase Chain Reaction for NOS3 T-786C SNP

The promoter region of *NOS3* gene was amplified to study T-786C polymorphism. The components for the optimized PCR mixture and conditions are given in Table 2.19. The amplification program consisted of an initial denaturation step at 98°C for 3 min followed by 30 cycles of denaturating at 94°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 2 min. The final extension was performed at 72°C for 10 min. The amplified PCR products were analyzed on 2% agarose gel. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.19 Components of PCR mixture for NOS3 T-786C SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	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	3 µL	30 pmol
Forward Primer	10 pmol/µL	3 µL	30 pmol
Template DNA	Varies		~200 ng
Taq DNA Polymerase	5U/µL	0.2 µL	1 U
Sterile apyrogen H_2O		to 50 µL	

2.2.4.10.2 Restriction Endonuclease Digestion of PCR Products for Determination of NOS3 T-786C SNP

PCR products were digested with *PdiI* restriction enzyme in a mixture as described in Table 2.20. The digestion mixture was incubated at 37°C for 16 hours for complete digestion. The digestion products were visualized on 3% agarose gels, and genotypes were determined according to the banding pattern. 30 µL of digestion product was mixed with 6 µL of gel loading dye and applied to the wells of the gel. 5 µL DNA ladder was applied to the one well. The gel was run until the bromophenol blue reached to the bottom of the gel, visualized under UV and photographed.

Table 2.20 Constituents of reaction mixture for *PdiI* restriction endonuclease digestion of PCR products for the determination of NOS3 T-786C SNP.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 30µL reaction mixture
Buffer	10 X	3 µL	1 X
PCR product		20 µL	
PdiI	10 U/µL	0.4 µL	4 U
Sterile apyrogen H_2O		6.6µL	

2.2.4.11 Genotyping of NOS3 intron4 VNTR Polymorphism

2.2.4.11.1 Polymerase Chain Reaction for NOS3 intron 4 VNTR

In order to determine VNTR polymorphism intron 4 region of *NOS3* gene was amplified according to the optimized PCR conditions given in Table 2.21. The standard PCR protocol consisted of an initial denaturation step at 94°C for 30 sec followed by 41 cycles of melting at 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 1 min. The final extension was performed at 72°C for 7 min. The amplified PCR products were visualized on 3% agarose gels stained with ethidium bromide. 10 µL PCR product was mixed by 2 µL of gel loading dye and applied to the wells of the gel. 5 µL of DNA ladder 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.

Table 2.21 Components of PCR mixture for NOS3 intron 4 VNTR.

Constituent	Stock Concentration	Volume to be added	Final Conc. in 50 µL reaction mixture
Amplification buffer	10 X	5 µL	1X
$MgCl_2$	25 mM	2 µL	1 mM
dNTP mixture	10 mM	1 µL	200 µM
Reverse Primer	10 pmol/µL	5 µL	50 pmol
Forward Primer	10 pmol/µL	5 µL	50 pmol
Template DNA	Varies		~200 ng
Taq DNA Polymerase	5U/µL	0.2 µL	1 U
Sterile apyrogen H_2O		to 50 µL	

2.2.5 Statistical Analysis

PASW Statistics 18 software package (SPSS, Chicago, IL, USA) was used for the statistical analyses. Continuous variables were expressed as mean ± SD. Normality of the sample distribution of each continuous variable was tested with the Kolmogorov-Smirnov test. Differences of continuous variables were evaluated by the Independent Samples t-test or Mann-Whitney U test, depending on the shape of the distribution curves. Categorical variables were expressed as proportions and compared using χ^2 test. Allele frequencies were determined by the gene counting method and departure from the Hardy-Weinberg equilibrium was evaluated by the χ^2 test. Comparisons of genotype distribution and allele frequencies were assessed by χ^2 statistics with 2 and 1 df, respectively.

In order to determine the effects of vascular risk factors, lipid parameters and genotypes in the prediction of ischemic stroke, logistic regression analyses with backward selection method was used. Age and sex were also included as covariates. 2-tailed probability values with 95% confidence intervals were estimated for each odds ratio. The Hosmer-Lemeshow goodness of fit test was used for calibration. A *P* value of less than 0.05 was evaluated as statistically significant.

CHAPTER 3

RESULTS

3.1 Study Population

The study population consisted of 245 ischemic stroke patients and 145 healthy controls. Clinical parameters of blood like serum total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol levels were measured by GATA Biochemistry laboratory. Besides, conventional risk factors including hypertension, diabetes, obesity and cigarette smoking were recorded for ischemic stroke patients and controls by GATA Neurology Department. The results of clinical laboratory tests and some risk factors of acute ischemic stroke patients and control subjects are summarized in Table 3.1. There was no statistically significant difference in mean age of the patient (64.5±13.3) and control groups (62.1±14.1, P=0.061). There were 104 female and 141 male among stroke patients. Number of males were found to be higher in patient group (57.6%) when compared to control group (50.3%; P=0.167). As expected, the conventional risk factors of acute ischemic stroke such as hypertension, diabetes, and smoking were found to be higher in patient group compared to controls. In addition, in stroke patients the prevalence of obesity (22.9%) was significantly higher than that of control (6.2%; P=0.000). The relative risk of ischemic stroke for hypertensive and obese subjects were 3-fold and 4.5-fold, respectively. Diabetes and smoking exhibited more than 2-fold relative risk for ischemic stroke. LDL-cholesterol level was significantly higher in stroke patients. Besides, triglyceride level of stroke patients and controls were almost the same. On

the other hand, HDL-cholesterol level was significantly lower in ischemic stroke patients (1.1±0.3 mmol/L) when compared to the control group (1.2 ± 0.3 mmol/L, *P*=0.001).

Table 3.1 Clinical laboratory data and conventional risk factors of acute ischemic stroke patients and controls.

Parameter	**Patients (n=245)**	**Controls (n=145)**	***P***	**OR(95%CI)**
Age (years)[a]	64.5±13.3	62.1±14.1	0.061	
Male, n (%)[b]	141 (57.6)	73 (50.3)	0.167	1.337 (0.885-2.020)
Hypertension, n (%)[b]	163 (66.5)	55 (37.9)	0.000	3.253 (2.121-4.989)
Diabetes mellitus, n (%)[b]	84 (34.3)	25 (17.2)	0.000	2.504 (1.511-4.151)
Obesity, n (%)[b]	56 (22.9)	9 (6.2)	0.000	4.477 (2.141-9.361)
Smokers, n (%)[b]	69 (28.2)	21 (14.5)	0.001	2.315 (1.349-3.972)
Total cholesterol (mmol/L)[c]	4.8±1.3	4.6±1.2	0.112	
Triglycerides (mmol/L)[c]	1.4±0.2	1.3±0.2	0.174	
HDL-cholesterol (mmol/L)[c]	1.1±0.3	1.2±0.3	0.001	
LDL-cholesterol (mmol/L)[c]	2.9±1.0	2.7±1.0	0.007	

Values are either number of subjects, percentage or mean ± SD
[a] Mann Whitney U test is applied
[b] Chi-square test is applied
[c] Independent Samples T-test is applied

3.2 Polymorphisms of Phase I Enzymes

3.2.1 CYP2E1 Polymorphisms

3.2.1.1 Analysis of Genotypes of *CYP2E1*5B* Polymorphism

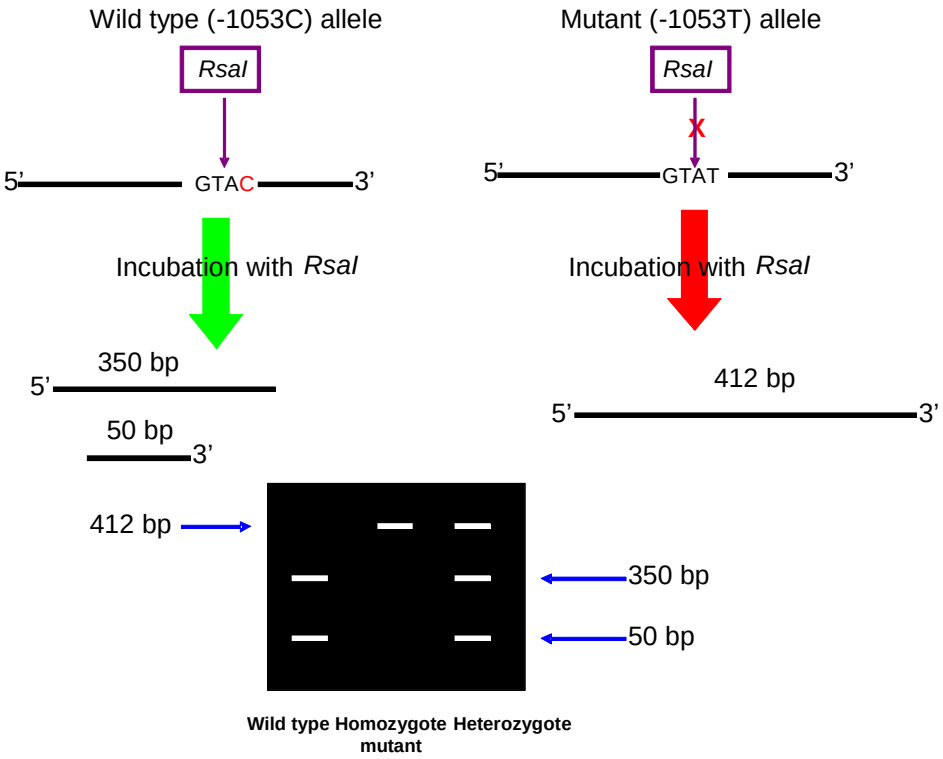
The 412 bp amplified region in the 5'-flanking region of *CYP2E1* gene included two single nucleotide substitutions: G-1293C and C-1053T that are in complete linkage disequilibrium with each other. These two SNPs are associated and always inherited together, because of complete linkage disequilibrium. For example, if -1053 is occupied with C, -1293 is always occupied with G, but never C. So, in wild types, *1A designates the position -1293 occupied by G and -1053 with C. Similarly, mutated alleles, with C at position -1293 and T at position -1053, are designated as *5B. The old designation for wild type allele was c1 and for mutated allele was c2. Figure 3.1 represents the sequence of amplified region in 5'-flanking region, showing the location of primers, both SNPs and restriction endonuclease recognition sites.

CACCCGTGAG CCAGTCGAGT CTACATTGTC AGTTCTCACC TCGAGGGGTG CCAAAAACCA
GAGGGAAGCA AAGGCCCCTG AAGCCTCTGC CAGAGGCCAA CGCCCCTTCT TGGTTCAGGA
G-1293C
GAGGTGCAGT GTTAGGTGCA GCACAACCAA TGACTTGCTT ATGTGGCTAA TAAATTGTCA
PstI
AGAGAAAAAC TGGGTTAGAA TGCAATATAT AGTATGTAGT CTCATTTTTG TATAAATACA
AGTATAAGAAT GGCATAACTC AAAATCCACA AGTGATTTGG CTGGATTGTA AATGACTTTT
ATTTTCTTCA GGCATAACTC AAAATCCACA AGTGATTTGG CTGGATTGTA AATGACTTTT
C-1053T
GTACAAAATT GCAACCTATG AATTAAGAAC TTCTATATAT TGCCAGTTAG AAGACAGAAT
RsaI
GAAAAACATT CTCTTCATTC TAA.....

Figure 3.1 Sequence of amplified fragment in 5'-flanking region of *CYP2E1* gene that includes G-1293C/C-1053T single nucleotide polymorphisms. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzymes *PstI* and *RsaI*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

The PCR products were digested separately by two restriction endonucleases, *RsaI* for C-1053T and *PstI* for G-1293C SNPs as described in section 2.2.4.1.2. -1053 position of *CYP2E1* gene is occupied by C in wild type alleles, while it is substituted to T in mutated alleles. In wild types, with C in position -1053, the PCR products bear a recognition site for restriction endonuclease *RsaI* (recognition site: 5'-GT↓AC-3'). Digestion with *RsaI* cuts the PCR product with wild type allele, producing two fragments of approximately 350 bp and 50 bp (Figure 3.2). However, in the mutated allele, -1053 position is occupied with T, so there is no suitable sequence that *RsaI* can recognize and cut PCR product. In this case, digestion of PCR products with *RsaI* yields one fragment of 412 bp, the PCR product itself. The heterozygotes would contain in total 3 bands in lengths of 412, 350, and 50 bp.

In the case of *CYP2E1*5B* variant allele, the 412 bp amplified region in 5'-flanking of *CYP2E1* gene includes a single nucleotide polymorphism at nucleotide position of -1293 which is occupied by G for wild type and occupied by C for the mutant allele. When analysing the *PstI* restriction endonuclease digestion results in agarose gel as given in Figure 3.3, wild type individual would yield an undigested single band of 412 bp since *PstI* has no recognition site (recognition site for *PstI* is 5'-CTGCA↓G-3') in the PCR product. However, in the mutated allele, that bears C at position -1293, there is recognition site for *PstI*. Therefore *PstI* digestion will result in two bands of approximately 300 and 100 bp for the mutant type. As a result, heterozygotes would contain three bands with 412, 300 and 100 bp. Figure 3.2 and Figure 3.3 show schematic representation and real agarose gel photograph of restriction endonuclease digestion products for the SNPs of CYP2E1. The genotype distribution and allele frequencies of *CYP2E1*5B* polymorphism are given in Table 3.2.



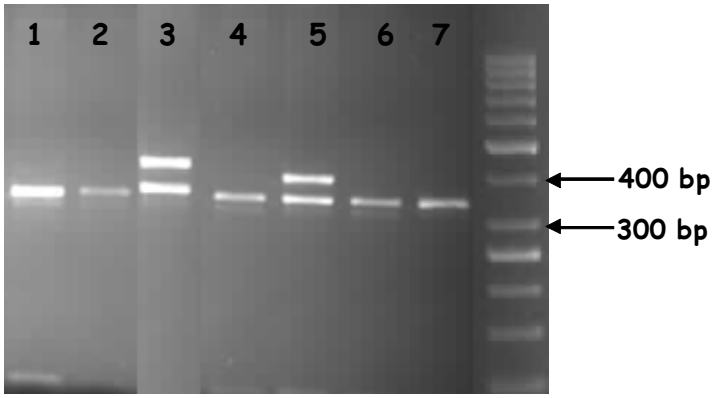
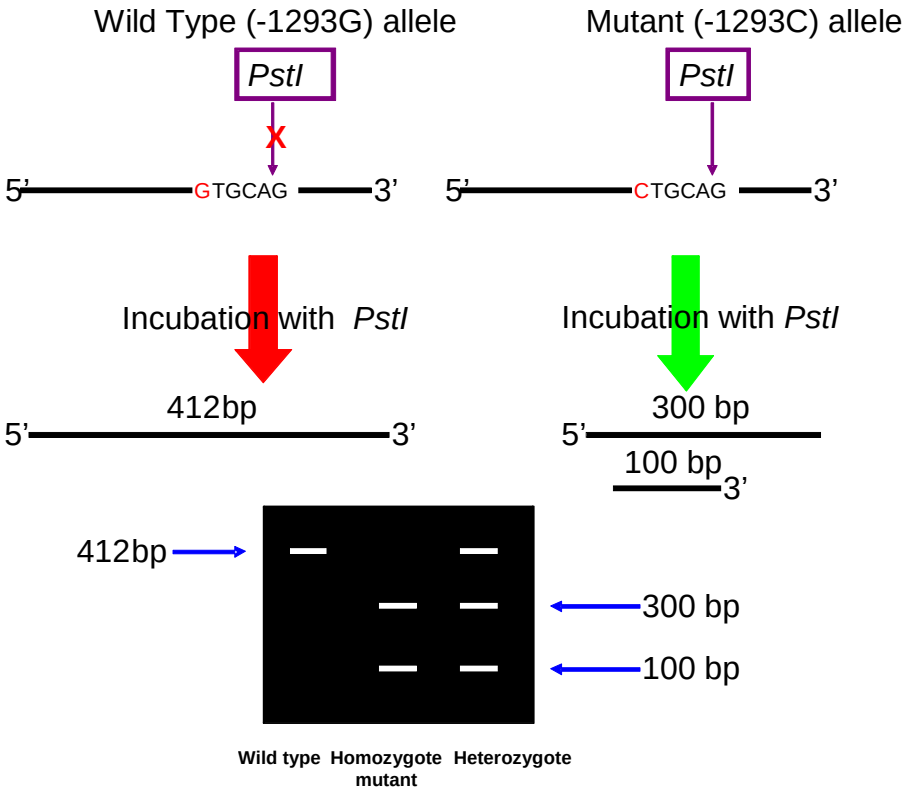


Figure 3.2 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*RsaI*) digestion products for the C-1053T SNP of CYP2E1*5B. In the gel photo lanes 3 and 5, heterozygote (*1A/*5B); lanes 1, 2, 4, 6, 7, homozygous wild type (*1A/*1A). All lanes also contain a 50 bp band which is not observable in the photo.



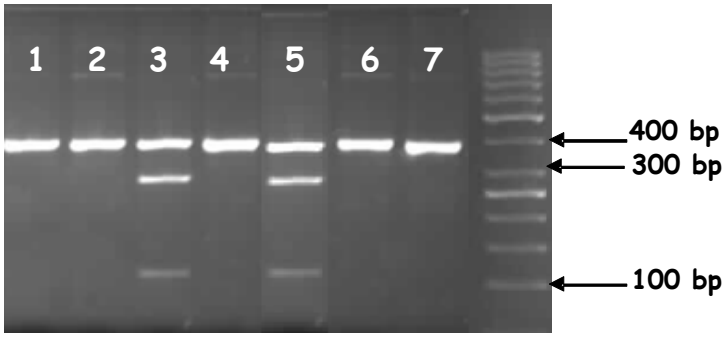


Figure 3.3 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*PstI*) digestion products for the G-1293C SNP of CYP2E1*5B. In the gel photo lanes 3 and 5, heterozygote (*1A/*5B); lanes 1, 2, 4, 6, 7, homozygous wild type (*1A/*1A).

Table 3.2 Genotype distribution and allele frequencies of *CYP2E1*5B* SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
CYP2E1*5B Genotypes, n(%)				
*1A*1A (c1c1)	232 (94.7)	144 (99.3)		
*1A*5B (c1c2)	13 (5.3)	1 (0.7)	0.869[a](1.044-62.339)	0.017
*5B*5B (c2c2)	0	0		
Allele frequency				
*1A (c1)	0.973	0.996	7.876[b](1.025-60.525)	0.019
*5B (c2)	0.027	0.004		

[a] *5B*5B+*1A*5B vs *1A*1A

[b] *5B vs *1A

In this study, a total of 245 ischemic stroke patients and 145 control subjects were investigated for CYP2E1*5B polymorphism. Among them, 232 patients and 144 controls were homozygous wild type (*1A*1A) while, 13 patients and 1 control were heterozygote (*1A*5B). None of the patients and controls had mutant genotype (*5B*5B). There was significant difference in the genotype frequencies of CYP2E1*5B between stroke patients and controls (*P*= 0.017).

The *1A (c1) allele frequency was found to be 0.973 and 0.996 in stroke patients and controls, respectively. While *5B (c2) allele frequency of stroke patients and controls was found to be 0.027 and 0.004, respectively. In the presence of mutant allele, the risk of having stroke was approximately 8 times significantly higher when compared to wild type allele (*P*= 0.019).

3.2.1.2 Analysis of Genotypes of *CYP2E1*6* Polymorphism

CYP2E1*6 polymorphism is a base substitution from T to A at position 7632 in the intron 6 of *CYP2E1* gene. This position is occupied by T in wild type alleles and

A in mutants. In CYP2E1*6 polymorphism, *6 is used to designate mutated allele and *1A is used to designate wild type allele. The old designation for mutated and wild type alleles are C and D, respectively. Figure 3.4 represents the partial nucleotide sequence of the amplified region, with primers, the SNP and recognition sites of *DraI*. PCR products belonging to the T7632A SNP region of CYP2E1 were subjected to digestion with *DraI* restriction enzyme. The amplified fragment contained two recognition sites as shown in Figure 3.4, one of which included the SNP in its sequence. The wild type alleles that contain 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. Therefore, as the PCR product contains an additional recognition sequence for the restriction enzyme, independently from the presence or absence of SNP, 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, as given in Figure 3.5, the 900 bp fragment is further cut into 600 bp and 300 bp fragments, as a second recognition site in the 900 bp fragment is present. In mutated allele, however, due to base substitution T to A is present, the corresponding recognition site is absent, and further digestion does not occur, resulting two fragments of 900 and 100 bp. Accordingly, the expected banding patterns upon digestion of amplified region in intron 6 of *CYP2E1* gene with *Dra*I restriction enzyme is as follows: In homozygous wild types, presence of two recognition sequences would yield three bands of 600, 300 and 100 bp, while in homozygous mutants, as one recognition sequence around SNP is lost due to base substitution, only two bands of 900 and 100 bp, are expected. The heterozygotes would contain in total four bands in lengths of 900, 600, 300 and 100 bp. The schematic and real agarose gel photo is given in Figure 3.5. Distribution of genotypes and frequencies of allele for CYP2E1*6 polymorphism in stroke patients and controls are given in Table 3.3.

GTGGTCTTAA GGCTCGTCAG TTCTGAAAG CAGGTATTAT AGGCTCTGAA GTTATTTCCC
CCAAGAAAGT CGACATGTGA TGGATCCAG GTCAGACCCT GGGCTTTTCT TGTTCTTTCC
TTCTTCTTCT TCTTTTTATT TATTTATTTT TTTTTTTGAG GGGACAGGGT CTCAC.....

T7632A

ACCACCACAC CCAGCTGATT AAAAATTTAA AAAAATTATT TTGGCTGGGC ACAGTGGCTG

DraI

ATACCTGTAA TCCTGGCACT TT..... ....
TAGGGGAACC ATGGAATCAA AAAATGTTTT AAATTATTAT TTAGTAGGAG GTTCCAATAT

DraI

AGACAAAAGG AAAATAAATA TGATTGACAT GTATATATCG ATTGCCAAAT TGAACGTTTA
TTAACATTTT GCGATACTTC CATCAGAGCT CTAAAAAGA .....

Figure 3.4 Sequence of amplified fragment in intron 6 region of *CYP2E1* gene that includes T7632A single nucleotide polymorphism. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *DraI*, 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).

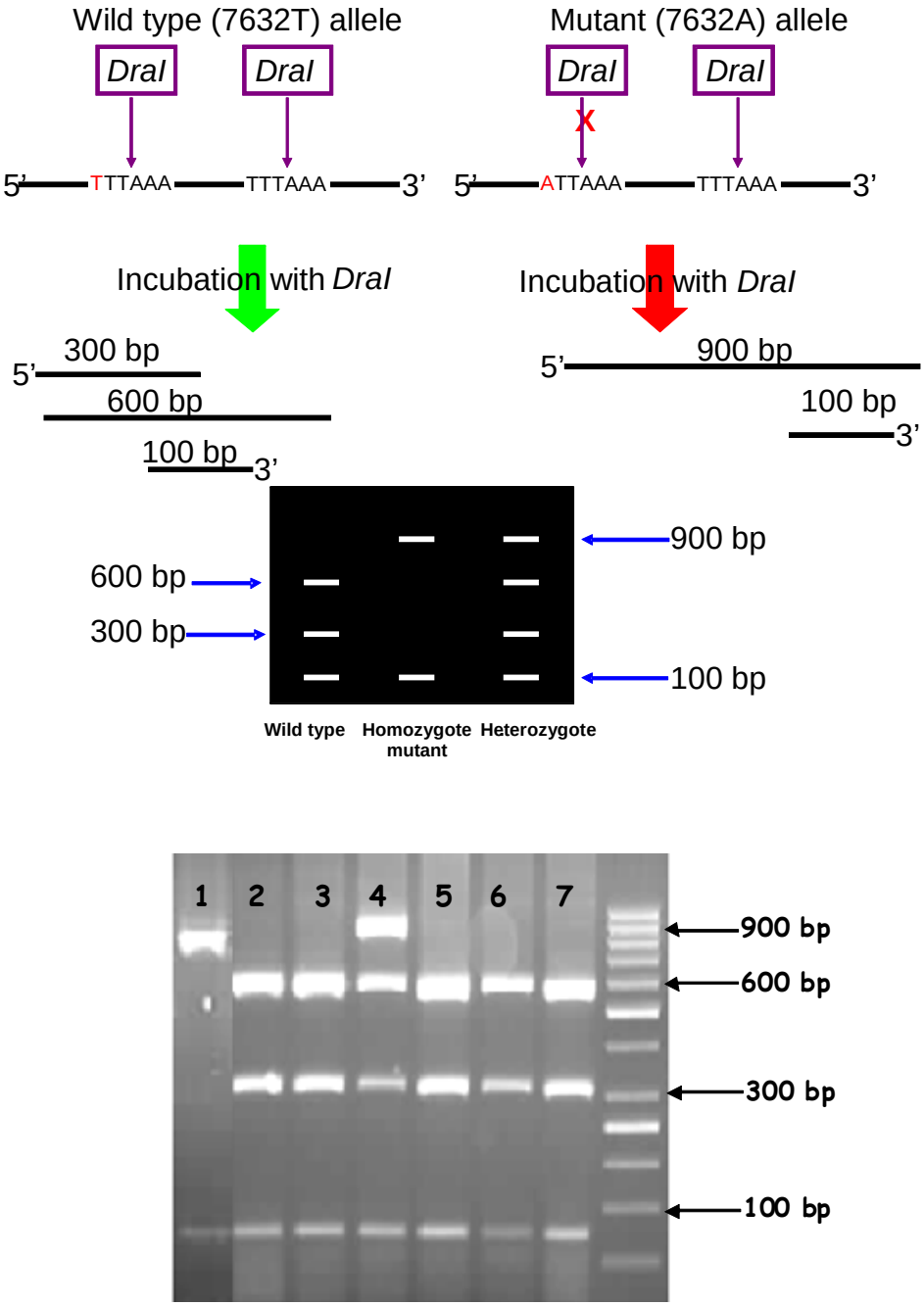


Figure 3.5 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*DraI*) digestion products for the T7632A SNP of CYP2E1*6. In the gel photo lanes 2, 3, 5, 6, 7, homozygous wild type (*1A/*1A); lane 4; heterozygote (*1A/*6); lane 1, homozygous mutated (*6/*6).

Table 3.3 Genotype distribution and allele frequencies of *CYP2E1*6* SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
CYP2E1*6 Genotypes, n(%)				
*1A*1A (DD)	212 (86.6)	126 (86.9)		
*1A*6 (DC)	29 (11.8)	19 (13.1)	1.032[a] (0.563-1.892)	0.918
*6*6 (CC)	4 (1.6)	0		
Allele frequency				
*1A (D)	0.924	0.934	1.164[b] (0.657-2.067)	0.601
*6 (C)	0.076	0.066		

[a]*6*6+*1A*6 vs *1A*1A

[b]*6 vs *1A

For the CYP2E1*6 polymorphism, 86.6% patients and 86.9% controls had homozygous *1A*1A genotype. The percentage of *1A*6 heterozygous individuals were 11.8% and 13.1% in the stroke patients and controls, respectively. On the other hand, there were only 1.6% homozygous mutated individuals among stroke patients and not any homozygous mutated individual among controls. Both wild type and mutated allele frequencies were similar in patient and control groups. So, there was no significant difference between patients and controls with respect to genotype distribution and allele frequencies.

3.2.1.3 Analysis of Genotypes of *CYP2E1*7B* Polymorphism

CYP2E1*7B polymorphism is at position -71 near the TATA box of the *CYP2E1* gene. The sequence of the PCR product, showing the primers, the single nucleotide polymorphism and the recognition site of restriction enzyme *DdeI* is presented in Figure 3.6. In CYP2E1*7B polymorphism, *7B is used to designate mutated allele and *1A is used to designate wild type allele. The old designation for mutated and wild type alleles are T and G, respectively. PCR products belonging to the G-71T SNP region of CYP2E1 were subjected to digestion with *DdeI* restriction enzyme. Wild type alleles possess the nucleotide G at position -71, and a suitable recognition sequence is present for *DdeI* restriction enzyme (recognition sequence: 5'-C↓TNAG-3'). So wild type alleles result in 310 and 50 bp bands. On the other hand, in mutated individuals, the nucleotide at position -71 is T, which leads to absence of a suitable recognition site for the restriction enzyme. Homozygous mutated individuals yield undigested PCR product of 360 bp long. Therefore, heterozygous individuals have three fragments 360, 310 and 50 bp long. Figure 3.7 shows schematic representation and real agarose gel photo. Table 3.4 summarizes the genotype distribution and allele frequencies of CYP2E1*7B polymorphism.

GCATGGGGAT GTGGCTGGAG TTCCCCGTTG TCTAACCAGT GCCAAAGGGC AGGACGGTAC

CTCACCCCAC GTTCTTAACT ATGGGTTGGC AACATGTTCC TGGATGTGTT TGCTGGCACA

GTGACAGGTG CTAGCAACCA GGGTGTTGAC ACAGTCCAAC TCCATCCTCA CCAGGTCACT

GGCTGGAACC CCTGGGGGCC ACCATTGCGG GAATCAGCCT TTGAAACGAT GGCCAACAGC

AGCTAATAAT AAACCAGTAA TTTGGGATAG ACGAGTAGCA AGAGGGCATT GGTTGGTGGG

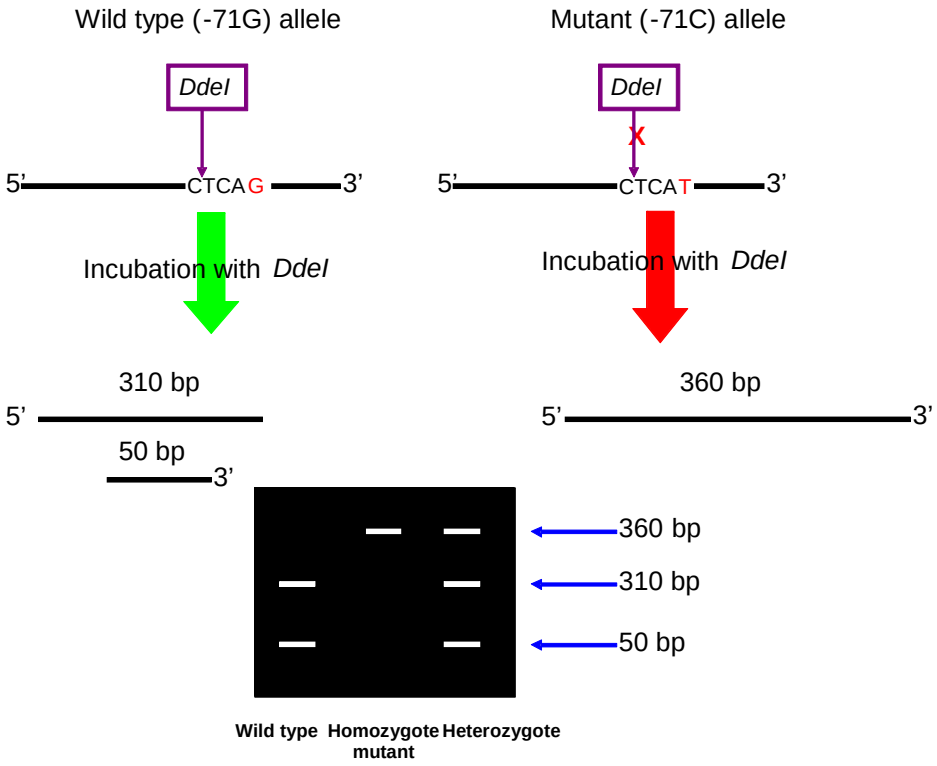
G-71T

TCACCCTCCT TCTCAGAACA CATTATAAAA ACCTTCCGTT TCCACAGGAT TGTCTCCCGG

DdeI

GCTGGCAGCA GGGCCCCAGC .....

Figure 3.6 Sequence of amplified fragment covering the G-71T single nucleotide polymorphism of *CYP2E1*. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *DdeI*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).



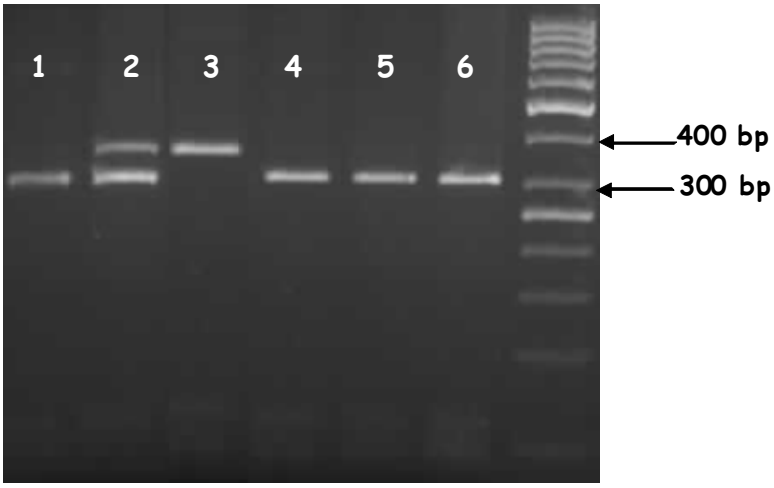


Figure 3.7 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*DdeI*) digestion products for the G-71T SNP of CYP2E1*7B. In the gel photo Lanes 1, 4-6 homozygous wild type (*1A/*1A); lane 2; heterozygote (*1A/*7B); lane 3, homozygous mutated (*7B/*7B).

Table 3.4 Genotype distribution and allele frequencies of *CYP2E1*7B* SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
CYP2E1*7B Genotypes, n(%)				
*1A*1A (GG)	210 (85.7)	123 (84.8)		
*1A*7B (GT)	31 (12.7)	20 (13.8)	0.932[a] (0.523-1.661)	0.811
*7B*7B (TT)	4 (1.6)	2 (1.4)		
Allele frequency				
*1A (G)	0.920	0.917	0.958[b] (0.564-1.629)	0.875
*7B (T)	0.080	0.083		

[a]*7B*7B+*1A*7B vs *1A*1A

[b]*7B vs *1A

In this study, a total of 245 ischemic stroke patients and 145 control subjects were investigated for CYP2E1*7B polymorphism. Among them, 210 patients and 123 controls were homozygous wild type (*1A*1A) while, 31 patients and 20 controls were heterozygote (*1A*7B). Four and 2 homozygous mutated (*7B*7B) individuals were found in stroke patients and controls, respectively. Therefore, there is no significant difference in the CYP2E1*7B genotype frequencies between stroke patients and controls. The *1A allele frequency was almost the same in stroke patients (0.920) and controls (0.917; *P*=0.875). When compared to stroke patients (0.080), the variant *7B allele frequency was found to be nearly same in control group (0.083).

3.2.1.4 Combination Analysis of CYP2E1 Polymorphisms

In this study, three different CYP2E1 polymorphisms namely CYP2E1*5B, CYP2E1*6 and CYP2E1*7B were studied. The effect of the combination of these polymorphisms on stroke risk was analyzed and results were given in Figure 3.8. The patients and controls according to genotypes were grouped under two categories such as “risk” and “no risk”. For CYP2E1 polymorphisms, while mutated allele was considered as risky allele, wild type allele was non-risky allele in this study. Furthermore the subjects carrying mutated allele were grouped under “risk” title and subjects carrying wild type allele were grouped under “no risk” title. In addition heterozygote genotype was thought as risky genotype and included in risky group. Co-presence of CYP2E1*5B and CYP2E1*6 polymorphisms was found to increase the risk of stroke almost 4.9-fold, which was not statistically significant (*P*=0.101). In risky control group, there was no subjects with any of CYP2E1*5B-CYP2E1*7B, CYP2E1*6-CYP2E1*7B and CYP2E1*5B-CYP2E1*6-CYP2E1*7B haplotypes.

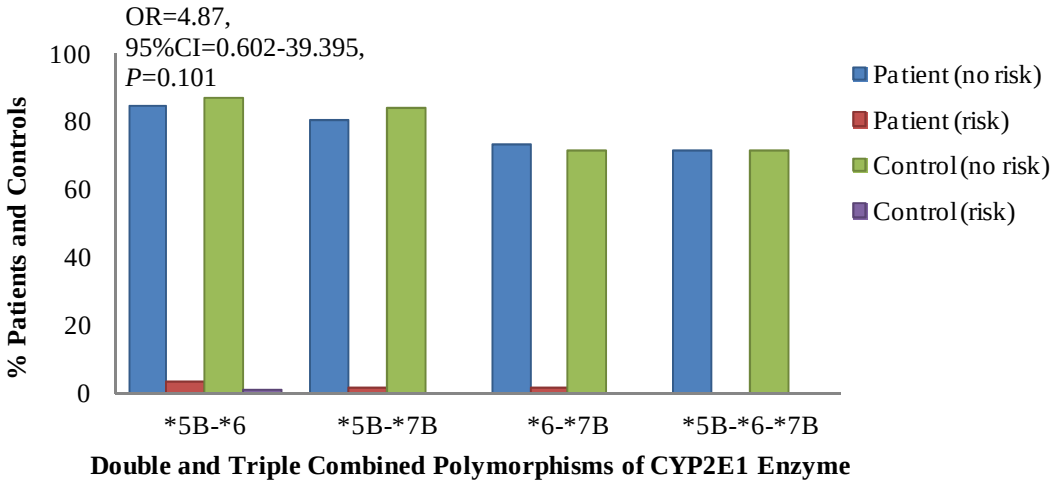


Figure 3.8 The double and triple combination of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B SNPs.

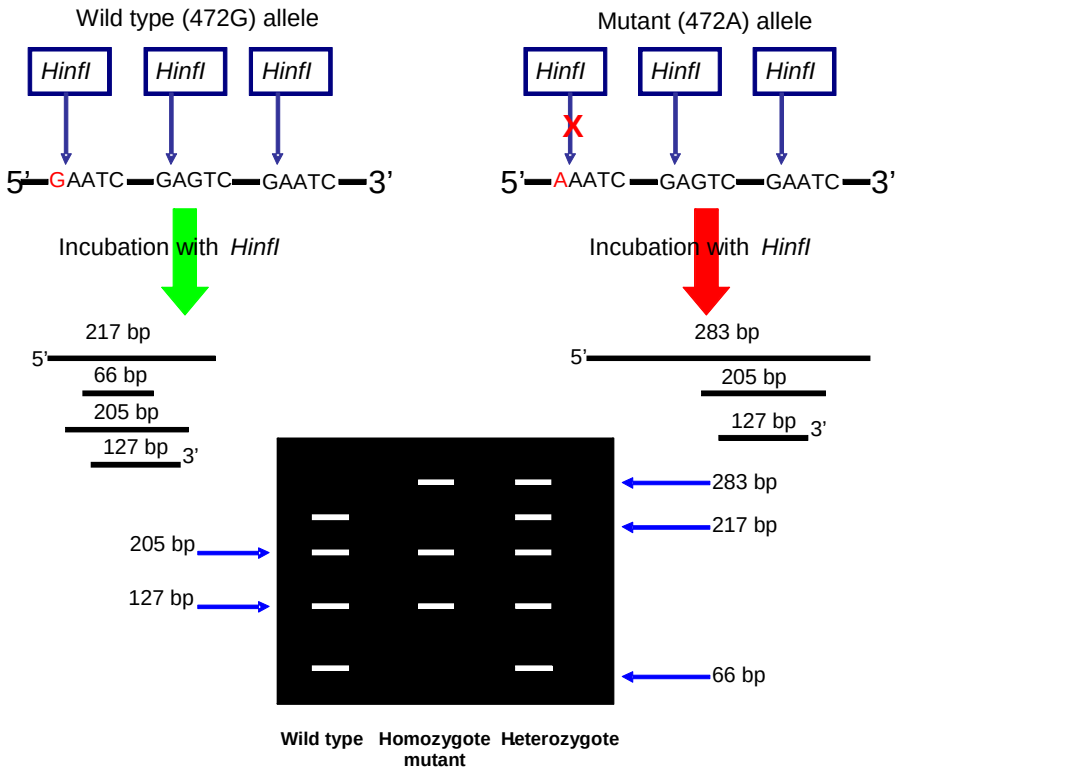
3.2.2 FMO3 Polymorphisms

3.2.2.1 Analysis of Genotypes of FMO3 G472A Polymorphism

The 615 bp amplified region in the exon 4 of FMO3 gene included a single nucleotide polymorphism at nucleotide position of 472. This position is occupied by G in wild type alleles and A in mutants. Figure 3.9 represents the partial nucleotide sequence of the amplified region, with primers, the SNP and recognition sites of *HinfI*. The amplified fragment contains three recognition sites for the restriction enzyme *HinfI* (recognition sequence for *HinfI* is: 5'-G↓ANTC-3'), one of them is present only if the nucleotide at position 472 is occupied by G (wild type allele), the other two are present in any condition. Upon digestion with the restriction enzyme *HinfI*, the PCR product is cut into three bands of 283 bp, 205 bp and 127 bp long, independently from the SNP. As shown in Figure 3.10, wild type individuals, the nucleotide at position 472 is G, which creates an additional recognition site for *HinfI*, hence upon digestion with the restriction enzyme, the 283 bp band is further cut into 217 bp and 66 bp bands. On the other hand, mutant type alleles possess the nucleotide A at position 472, and a suitable recognition sequence is not present for *HinfI* restriction enzyme in this case. So, mutated alleles result in 283 bp, 205 bp and 127 bp bands. Therefore, heterozygote individuals would yield five fragments such as 283 bp, 217 bp, 205 bp, 127 bp and 66 bp long. The schematic representation and real agarose gel photo is given in Figure 3.10. The genotype distribution and allele frequencies of FMO3 G472A polymorphism are given in Table 3.5. There were 109 (44.5%) homozygous wild type, 57 (23.3%) heterozygous and 79 (32.2%) homozygous mutated individuals in patient group. The mutated (A) allele frequency was 0.439 while the wild type allele (G) frequency was 0.561 in patients. In control group of 145 individuals, 39.4% were homozygous wild type (GG), 30.3% heterozygous (GA) and 30.3% homozygous mutant type (AA). It was observed that among the controls, G and A allele frequency were found to be 0.545 and 0.455, respectively.

TATCTGCCAA AACCATTTGC TAGCATAGAA AAGAGGGATT TCTTTCTGTA TTTCTCTTAG
ACATTTGTAT CCAGTGTAAA TAAACATCCT GATTTTGCAA CTACTGGCCA GTGGGATGTT
ACCACTGAAA GGGATGGTAA AAAAGAATCG GCTGTCTTTG ATGCTGTAAT GGTTTGTTCC
HinfI G472A
GGACATCATG TGTATCCCAA CCTACCAAAA GAGTCCTTTC CAGGTAAGGC CAAAATTTAA
HinfI
GCTGCTAGCC ACATAACTGA CAAAAATGAA TATCTTGATA ATGTCTTCTT TTTTCTAAAA
GTATAAGCAG GTTAAATTAA AATATACTTC TGTTATATCT AATATGCTTG GTGTGTTAAA
ATAGCACATT ATTGTGACTG CATCTATTCA CAAGGTCGCT TCTGTTAAAG TCTTTGTTTA
AATATATGAC TCAAACTGCC ATGTATTTCT CACTTTTCAC TCAGGACTAA ACCACTTTAA
HinfI
AGGCAAATGC TTCCACAGCA GGGACTATAA AGAACCAGGT GTATTCAATG GAAAGCGTGT
CCTGGTGGTT GGCCTGGGGA ATTCGGGCTG TGATATTGCC ACAGAACTCA GCCGCACAGC
AGAACAGGTA CTACTCCCCG GGTACTCGGG TGACTCTCGT TACTGACAGA AGAGTTATTA

Figure 3.9 Sequence of amplified fragment covering the G472A single nucleotide polymorphism of *FMO3*. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *HinfI*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).



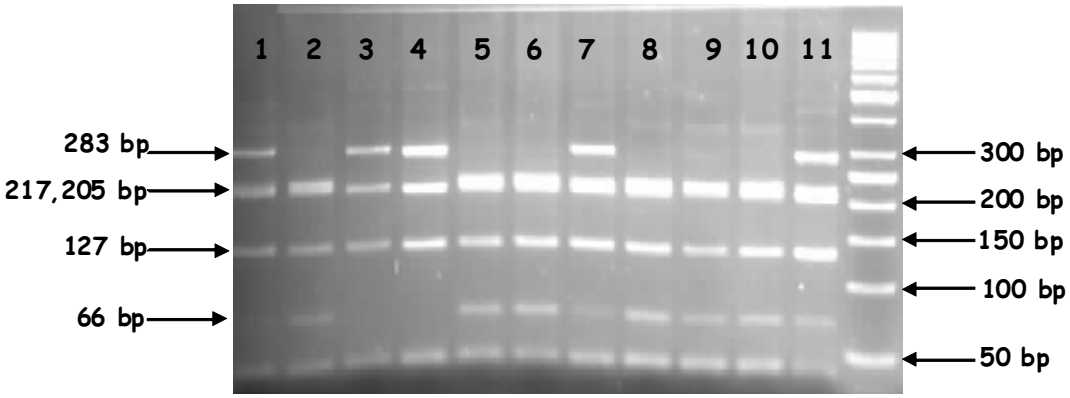


Figure 3.10 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*HinfI*) digestion products for the G472A SNP of FMO3. In the gel photo lanes 2, 5, 6, 8-10 homozygous wild type (472GG); lanes 7 and 11; heterozygote (472GA); lanes 1, 3, 4 homozygous mutated (472AA).

Table 3.5 Genotype distribution and allele frequencies of FMO3 G472A SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
FMO3G472A Genotypes, n(%)				
GG	109 (44.5)	57 (39.4)		
GA	57 (23.3)	44 (30.3)	0.808 [a] (0.532-1.227)	0.31
AA	79 (32.2)	44 (30.3)		
Allele frequency				
G	0.561	0.545	0.936 [b] (0.699-1.253)	0.656
A	0.439	0.455		

[a]GA+AA vs GG

[b]A vs

3.2.2.2 Analysis of Genotypes of FMO3 A923G Polymorphism

FMO3 A923G polymorphism is a base substitution from A to G at position 923 in exon 7 and the PCR region involving the SNP was 408 bp long. The sequence of the PCR product, showing the primers, the single nucleotide polymorphism and the recognition site of restriction enzyme *DraII* is presented in Figure 3.11. PCR products belonging to the A923G SNP region of FMO3 were subjected to digestion with *DraII* restriction enzyme. When analysing the *DraII* restriction endonuclease digestion results in agarose gel given in Figure 3.12, wild type individual would yield an undigested single band of 408 bp since *DraII* has no recognition site (recognition site for *DraII* is 5'-RG↓GNCCY-3') in the PCR product. However, in the mutated allele, that bears G at position 923, there is recognition site for *DraII*. Therefore *DraII* digestion will result in two bands of 293 and 115 bp for the mutant type. As a result, heterozygotes would contain three bands with 408, 293 and 115 bp. Figure 3.12 shows schematic representation and real agarose gel photograph. The genotype distribution and allele frequencies of FMO3 A923G polymorphism are given in Table 3.6.

TACACTTCCA ATAATTGTCT CTGTTTTCCA TACAGAGTCC TGAGGAAAGA GCCTGTATTT
AACGATGAGC TCCCAGCAAG CATTCTGTGT GGCATTGTGT CCGTAAAGCC TAACGTGAAG

A923G
GAATTCACAG AGACCTCGGC CATTTTTGAG GATGGGACCA TATTTGAGGG CATTGACTGT

DraII
GTAATCTTTG CAACAGGGTA TAGTTTTGCC TACCCCTTCC TTGATGAGTC TATCATCAAA
A GCAGAAACA ATGAGATCAT TTTATTTAAAG GAGTATTTCC TCCTCTACTT GAGAAGTCAA
CCATAGCAGT GATTGGCTTT GTCCAGTCCC TTGGGGCTGC CATTCCCACA GTTGACCTCC
AGTCCCGCTG GGCAGCACAA GTAATAAAGG GTAAGTCAAT AAAGAGGCTC ATGGATTGCG

AAGATGAATG

Figure 3.11 Sequence of amplified fragment covering the A923G single nucleotide polymorphism of *FMO3*. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *DraII*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

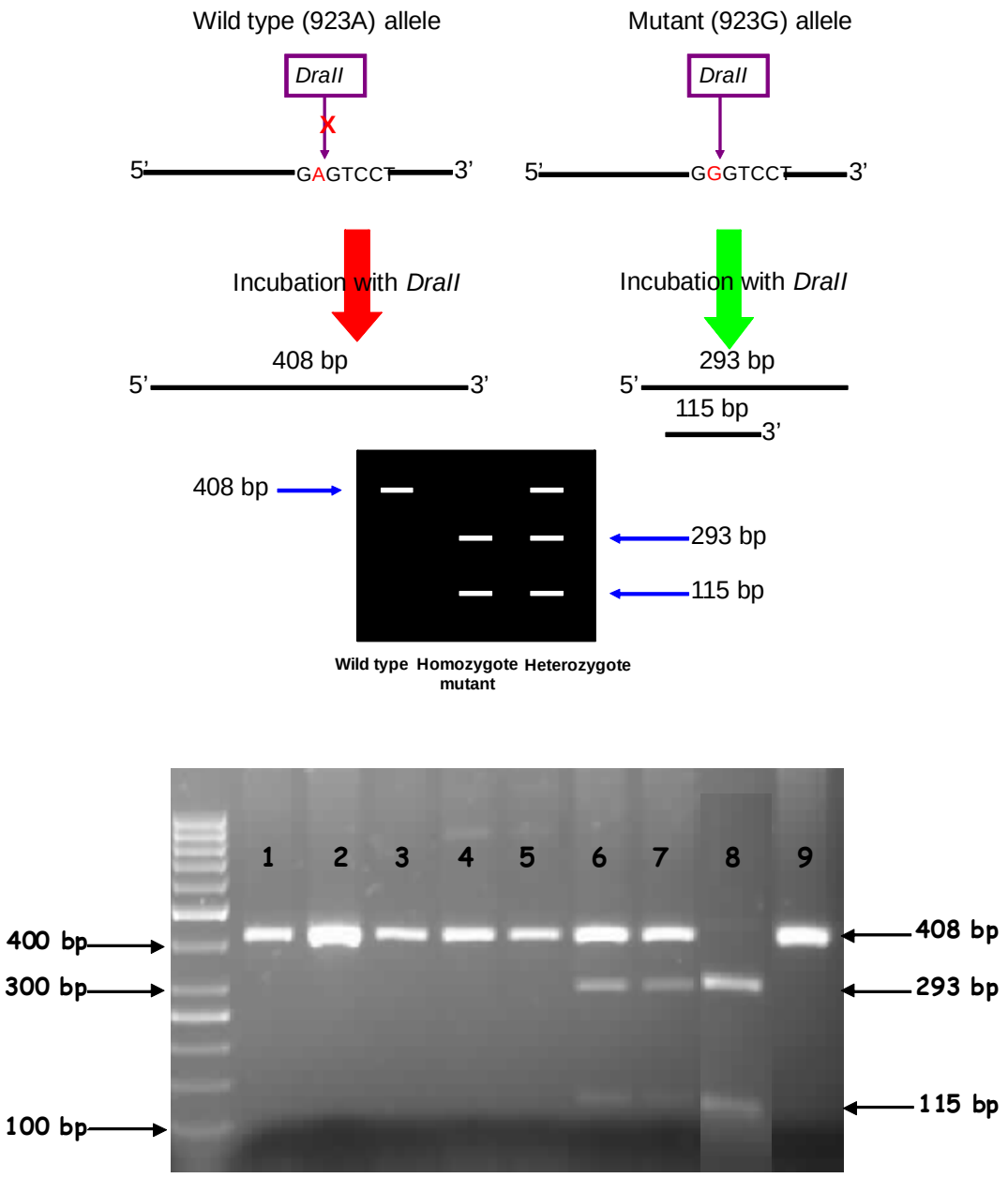


Figure 3.12 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*DraII*) digestion products for the A923G SNP of FMO3. In the gel photo lanes 1-5, 9 homozygous wild type (923AA); lanes 6, 7; heterozygote (923AG); lane 8, homozygous mutated (923GG).

Table 3.6 Genotype distribution and allele frequencies of FMO3 A923G SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
FMO3A923G Genotypes, n(%)				
AA	214 (87.3)	129 (89.0)		
AG	30 (12.3)	16 (11.0)	1.168[a] (0.615-2.219)	0.635
GG	1 (0.4)	0		
Allele frequency				
A	0.935	0.945	1.196[b] (0.645-2.221)	0.569
G	0.065	0.055		

[a]AG+GG vs AA
[b]G vs A

For the FMO3 A923G polymorphism, 87.3% patients and 89.0% controls had homozygous wild type (AA) genotype. The percentage of heterozygous (AG) individuals was 12.3% and 11.0% in the stroke patients and controls, respectively. On the other hand, there were only 0.4% homozygous mutated (GG) individuals among stroke patients and not any homozygous mutated individual among controls. Both wild type and mutated allele frequencies were similar in patient and control groups. So, there was no significant difference between patients and controls with respect to genotype distribution and allele frequencies.

3.2.2.3 Combination Analysis of FMO3 Polymorphisms

Figure 3.13 shows the analyses of combinations of two SNPs of FMO3 polymorphism as risk factor for the development of stroke. Again patients and controls were grouped under “risk” and “no risk” titles according to genotypes. For

FMO3 polymorphisms while mutated allele was considered as risky allele, wild type allele was non-risky allele in this study. Furthermore the subjects carrying mutated allele were grouped under “risk” title and subjects carrying wild type allele were grouped under “no risk” title. In addition heterozygote genotype was thought as risky genotype and included in risky group. The percentage of double combined SNPs of FMO3 was lower in risky patient group (12.2%) when compared to non-risky patient group (44.1%). Similarly in risky control group (11.0%) the percentage of double combined SNPs was found to be lower than non-risky control group (39.3%).

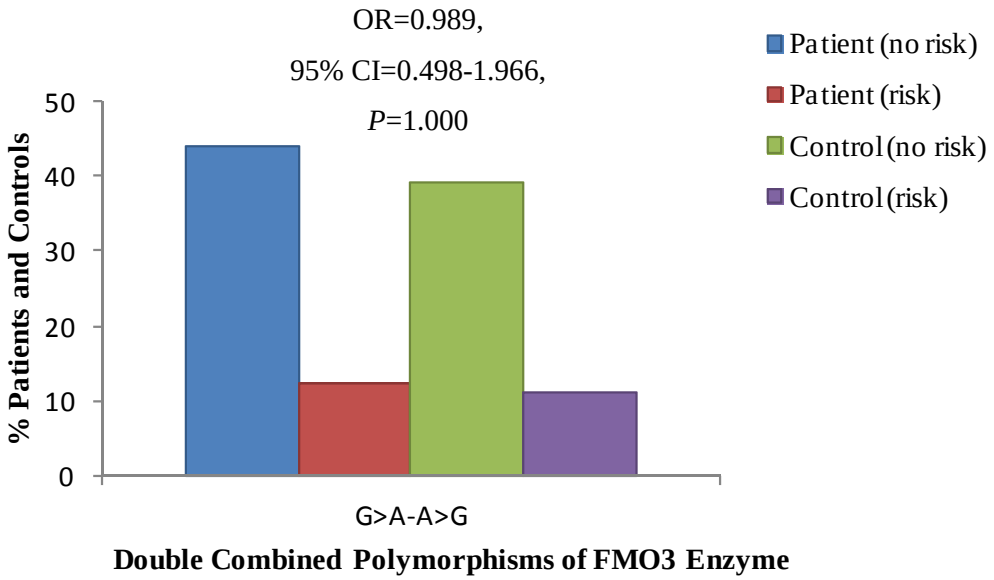


Figure 3.13 The double combination of FMO3 G472A and FMO3 A923G SNPs.

3.3 Polymorphisms of Phase II Enzymes

3.3.1 NQO1 Polymorphism

3.3.1.1 Analysis of Genotypes of NQO1*2 Polymorphism

NQO1*2 single nucleotide polymorphism is a C to T base substitution at position 609 of NQO1 cDNA. In NQO1*2 polymorphism, *2 is used to designate mutated allele and *1 is used to designate wild type allele. The old designation for mutated and wild type alleles are T and C, respectively. The amplified PCR product in exon 6 bears two recognition sites for the restriction enzyme *HinfI* (recognition sequence for *HinfI* is: 5'-G↓ANTC-3'), one of them is present only when nucleotide at position 609 is occupied by T (mutated allele), the other is present in any condition. Figure 3.14 presents the sequence of 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 *HinfI*. Upon digestion with the restriction enzyme *HinfI*, the PCR product is cut into two bands of 210 bp and 40 bp long, independently from the SNP. Wild type alleles possess the nucleotide C at position 609, and a suitable recognition site is not present for *HinfI* restriction enzyme in this case. So, wild type alleles result in 210 bp and 40 bp bands as shown in Figure 3.15. On the other hand, in mutated individuals, the nucleotide at position 609 is T, which creates an additional recognition site for *HinfI*, hence upon digestion with the restriction enzyme, the 210 bp band is further cut into 150 bp and 60 bp bands. Schematic representation and real agarose gel photo is given in Figure 3.15. Genotype distribution and allele frequencies of NQO1*2 polymorphism are given in Table 3.7.

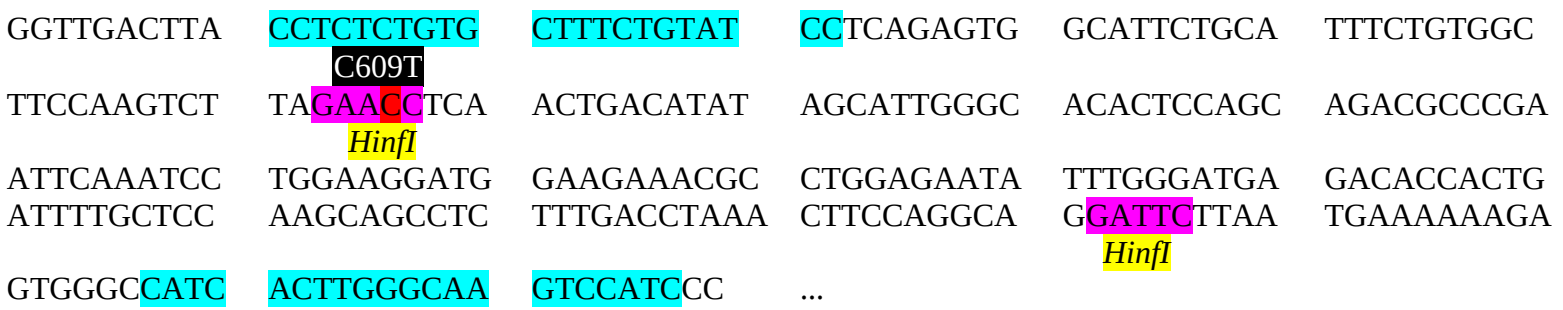
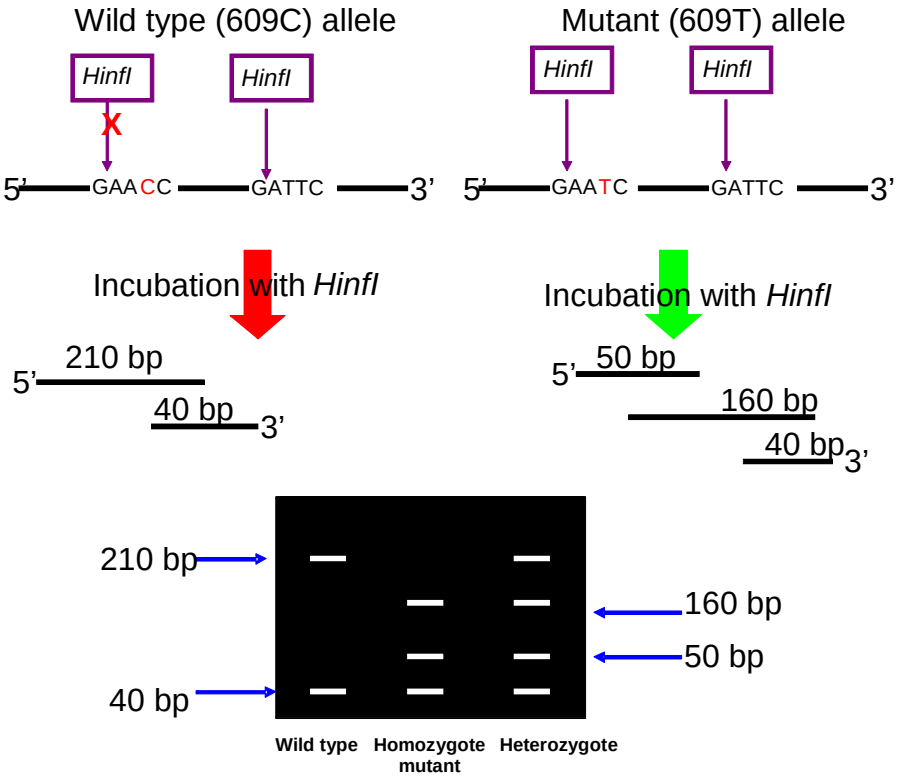


Figure 3.14 Sequence of amplified fragment covering the NQO1*2 single nucleotide polymorphism. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *HinfI*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).



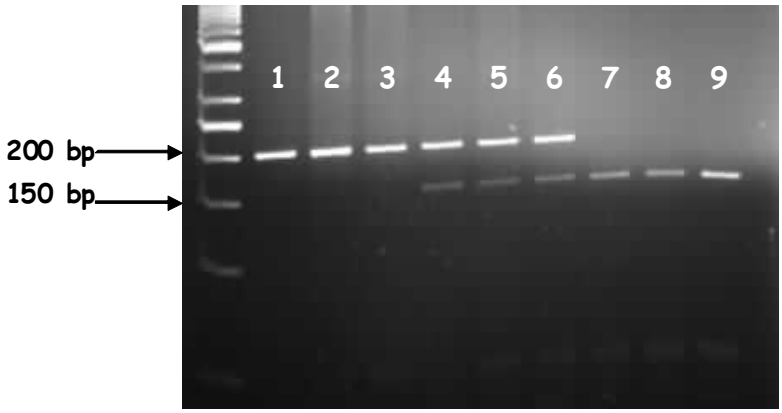


Figure 3.15 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*HinfI*) digestion products for the C609T SNP of NQO1. In the gel photo lanes 1-3 homozygous wild type (*1/*1); lanes 4-6; heterozygote (*1/*2); lanes 7-9, homozygous mutated (*2/*2). All lanes also contain a 40 bp band which is not observable in the photo.

Table 3.7 Genotype distribution and allele frequencies of NQO1*2 SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
NQO1*2				
Genotypes, n(%)				
*1*1 (CC)	153 (62.4)	74 (51.0)		
*1*2 (CT)	81 (33.1)	66 (45.5)	0.627[a] (0.414-0.950)	0.027
*2*2 (TT)	11 (4.5)	5 (3.5)		
Allele frequency				
*1 (C)	0.789	0.737	0.739[b] (0.527-1.038)	0.080
*2 (T)	0.211	0.263		

[a]CT+TT vs CC
[b]T vs C

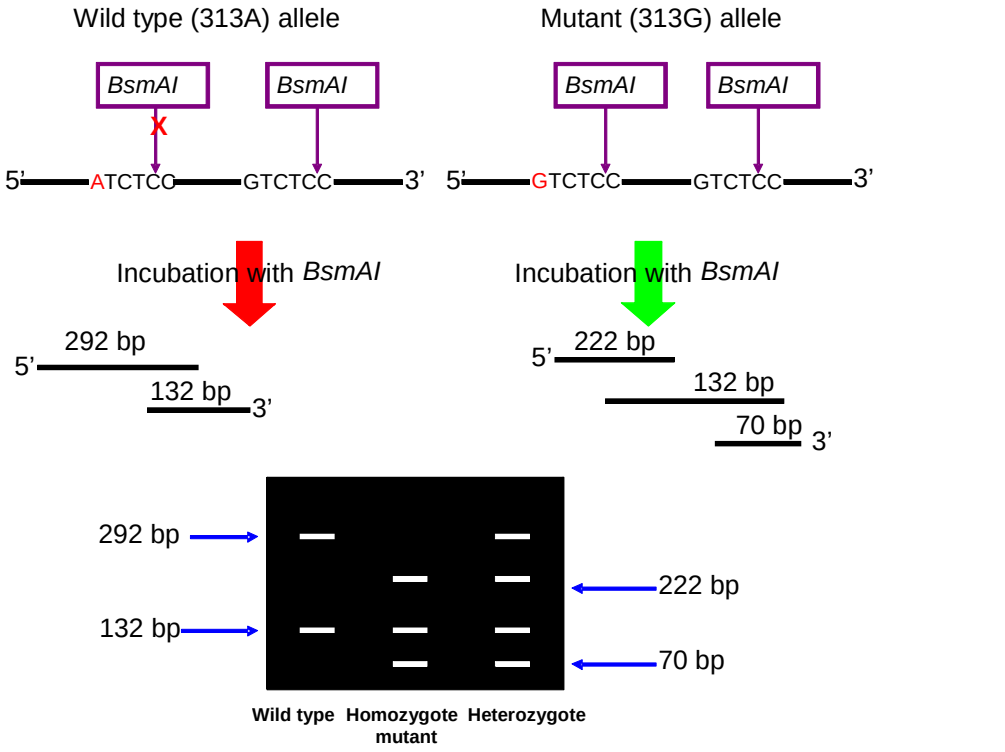
In this study, a total of 245 ischemic stroke patients and 145 control subjects were investigated for NQO1*2 polymorphism. Among them, 153 patients and 74 controls were homozygous wild type while, 81 patients and 66 controls were heterozygote. Eleven patients and 5 controls had homozygous mutated genotype. Therefore, there is significant difference in the genotype frequencies of NQO1*2 polymorphism between stroke patients and controls. The *1 allele frequency was found to be 0.789 in stroke patients and 0.737 in controls. While *2 allele frequency of stroke patients and controls were 0.211 and 0.263, respectively.

3.3.2 GST Polymorphisms

3.3.2.1 Analysis of Genotypes of GSTP1 A313G Polymorphism

GSTP1 A313G single nucleotide polymorphism is a A to G base substitution at position 313 of GSTP1 cDNA coding for an isoleucine to valine change at position 105 in the amino acid structure of the protein. The amplified PCR product in exon 5 bears two recognition sites for the restriction enzyme *BsmAI* (recognition site for *BsmAI* is 5'-GTCTCN↓-3') , one of them is present only when nucleotide at position 313 is occupied by G (mutated allele), the other is present in any condition. Figure 3.16 presents the sequence of approximately 424 bp long PCR product in exon 5 of *GSTP1*, highlighting the sequence of primers, location of SNP and the recognition sequence of the restriction enzyme *BsmAI*. Upon digestion with the restriction enzyme *BsmAI*, the PCR product is cut into two bands of 132 bp and 292 bp long, independently from the SNP. Wild type alleles possess the nucleotide A at position 313, and a suitable recognition site is not present for *BsmAI* restriction enzyme in this case. So, wild type alleles result in 132 bp and 292 bp bands as shown in Figure 3.17. On the other hand, in mutated individuals, the nucleotide at position 313 is G, which creates an additional recognition site for *BsmAI*, hence upon digestion with the restriction enzyme, the 292 bp band is further cut into 222 bp and 70 bp bands. Figure 3.17 shows schematic representation and real agarose gel photo. Genotype distribution and allele frequencies of GSTP1 A313G polymorphism are given in Table 3.8.

Figure 3.16 Sequence of amplified fragment covering the A313G single nucleotide polymorphism of *GSTP1*. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *BsmAI*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).



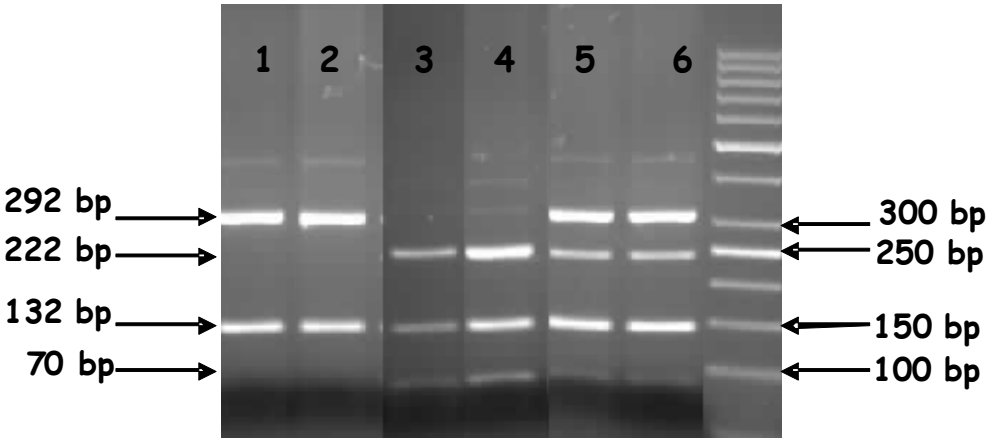


Figure 3.17 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*BsmAI*) digestion products for the A313G SNP of GSTP1. In the gel photo lanes 1, 2; homozygous wild type (313AA); lanes 5, 6; heterozygote (313AG); lanes 3, 4; homozygous mutated (313GG).

Table 3.8 Genotype distribution and allele frequencies of GSTP1 A313G SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
GSTP1 Genotypes, n(%)				
AA	133 (54.3)	73 (50.3)		
AG	88 (35.9)	50 (34.5)	0.854[a] (0.566-1.288)	0.451
GG	24 (9.8)	22 (15.2)		
Allele frequency				
A	0.722	0.676	0.801[b] (0.584-1.098)	0.167
G	0.278	0.324		

[a]AG+GG vs AA
[b]G vs A

There were 133 (54.3%) homozygous wild type, 88 (35.9%) heterozygous and 24 (9.8%) homozygous mutated individuals in patient group. The mutated (G) allele frequency was 0.278 while the wild type allele (A) frequency was 0.722 in patients. In control group of 145 individuals, 50.3% were homozygous wild type (AA), 34.5% heterozygous (AG) and 15.2% homozygous mutant type (GG). It was found that among the controls A and G allele frequency were found to be 0.676 and 0.324, respectively. Therefore, distribution of genotype and allele frequencies was not statistically different between stroke patients and controls.

3.3.2.2 Analysis of Genotypes of GSTM1 and GSTT1

GSTM1 and GSTT1 genes possess null polymorphisms which are resultant of deletion of the genes, so that enzyme is not expressed. Null polymorphisms of both genes were detected by multiplex PCR, where amplified PCR products were selected from the regions coding for the gene. Figure 3.18 presents the sequences amplified for GSTM1 and GSTT1 from chromosomes 1 and 22, respectively, where the genes located. Figure also shows the sequence of amplified region of CYP1A1 gene in chromosome 15. CYP1A1 gene 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 the 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.

Both for GSTM1 and GSTT1, 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 that the individual is homozygous mutated, and does not express the enzyme. Figure 3.19 presents schematic representation and an example for the agarose gel electrophoresis result of multiplex PCR. The multiplex PCR results in a band of 215 bp for GSTM1, 480 bp band for GSTT1, and 312 bp band for CYP1A1, the internal control. Table 3.9 summarizes the genotype distribution of both GSTM1 and GSTT1 polymorphisms in ischemic stroke patients and controls.

GSTT1

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

CYP1A1

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

GSTM1

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

Figure 3.18 Sequence of amplified fragments of GSTT1, CYP1A1 and GSTM1 genes, in order. The turquoise highlighted sequences are forward and reverse primers (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

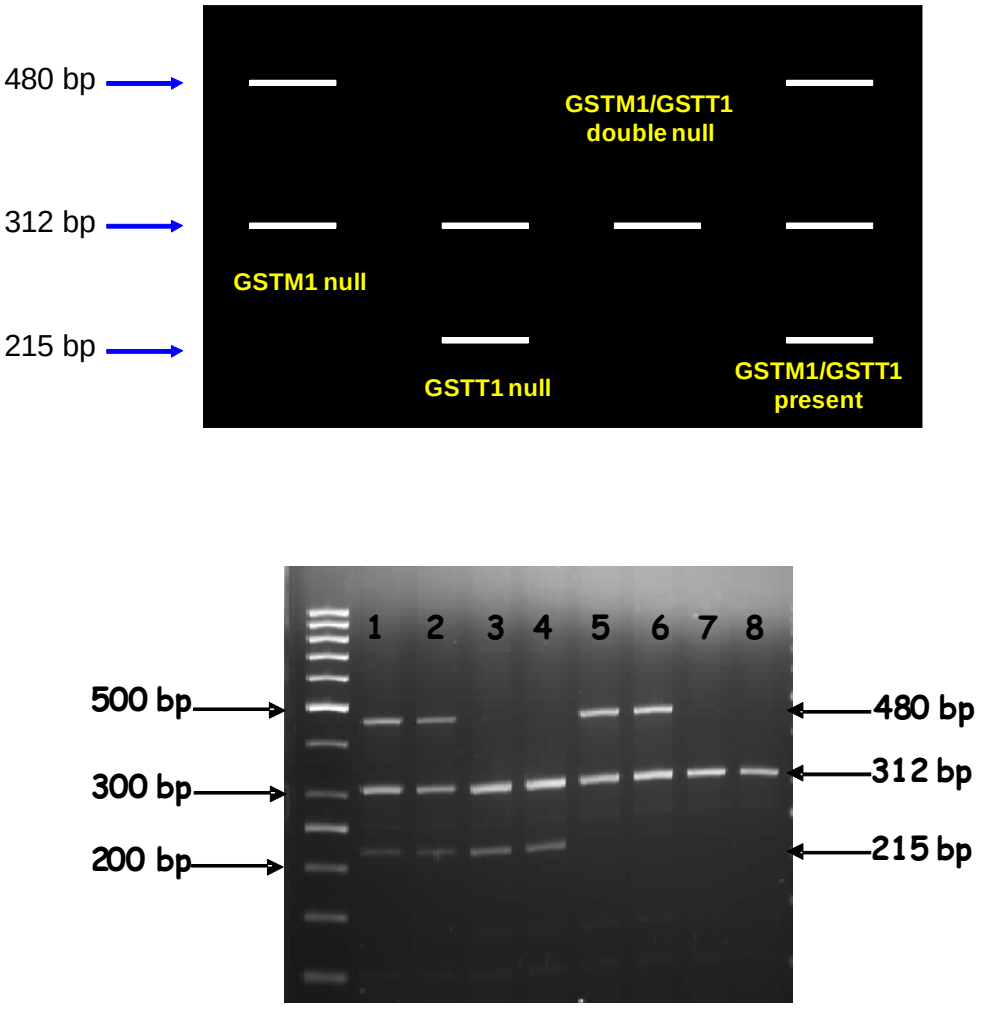


Figure 3.19 Schematic representation and real agarose gel electrophoresis of GSTM1 and GSTT1 PCR products. In the gel photo, lanes 1, 2; GSTM1 present/GSTT1 present, lanes 3, 4; GSTM1 present/ GSTT1 null, lanes 5, 6; GSTM1 null/GSTT1 present, lanes 7,8; GSTM1 null/GSTT1 null.

Table 3.9 Genotype distributions of GSTM1 and GSTT1 polymorphisms in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
GSTM1 Genotypes, n(%)				
Present	113 (46.1)	56 (38.6)	0.735[a] (0.484-1.117)	0.148
Null	132 (53.9)	89 (61.4)		
GSTT1 Genotypes, n(%)				
Present	177 (72.2)	110 (75.9)	1.207[b] (0.753-1.936)	0.432
Null	68 (27.8)	35 (24.1)		

[a,b]null vs present

For the GSTM1 polymorphism, 46.1% patients and 38.6% controls had GSTM1 present genotype. The percentage of null genotypes was 53.9% and 61.4% in the stroke patients and controls, respectively. On the other hand, for GSTT1 present genotype, patient group had lower frequency (72.2%) when compared to control group (75.9%). The GSTT1 null genotype frequency was found to be 27.8% in patients, while 24.1% in controls. But there was no significant difference between patient and control groups with respect to GSTM1 and GSTT1 genotype distribution.

3.3.2.3 Combination Analysis of GST Polymorphisms

Figure 3.20 presents the analyses of combinations of three polymorphisms of GST enzyme as risk factors for the development of ischemic stroke. The percentage of GSTM1 and GSTT1 double combined polymorphism was found to be higher in

patient non-risky group (36.3%) than risky patient group (18.0%). Similarly non-risky control group (33.8%) had greater percentage of double combined GSTM1 and GSTT1 polymorphisms when compared to risky control group (19.3%). Therefore double combined GSTM1 and GSTT1 polymorphisms were not found to be significant risk factor for stroke. In addition, we observed that in terms of GSTM1 and GSTP1 double combined polymorphisms there was no significant difference in risky and non-risky patient and control groups. When combinations of two SNPs on the same individual was considered as risk factor, combination of GSTT1 and GSTP1 A313G polymorphisms was found to increase the risk of stroke 1.2-fold (95% CI: 0.618-2.307, *P*=0.598), but the results were statistically insignificant. When triple combined GST polymorphisms were taken into consideration there was no significant risk for stroke.

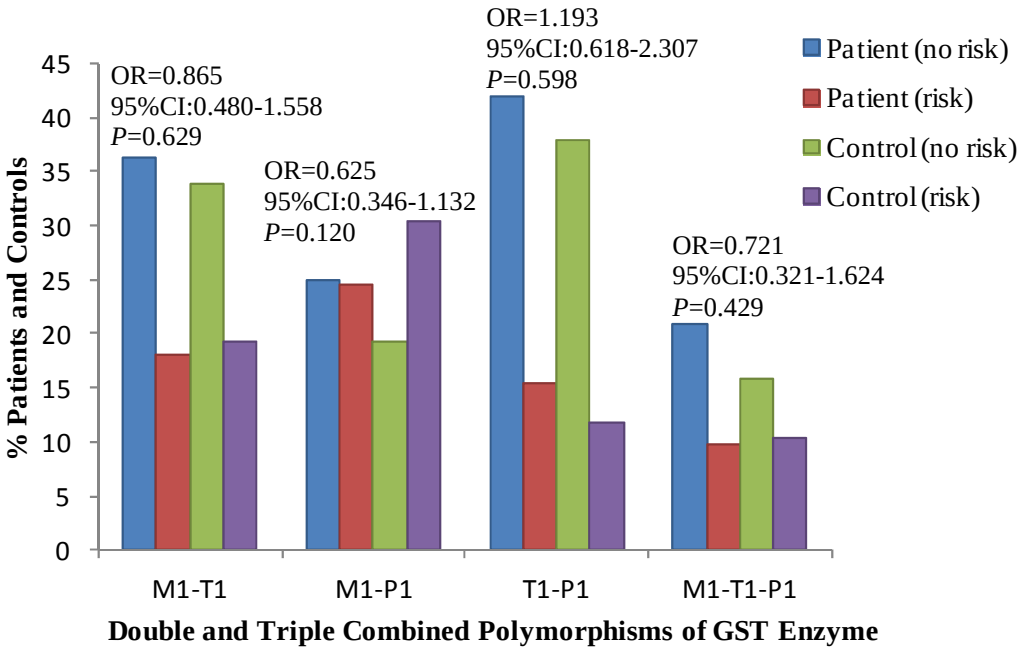


Figure 3.20 The double and triple combination of GSTP1, GSTM1 and GSTT1 SNPs.

3.4 NOS3 Polymorphisms

3.4.1 Analysis of Genotypes of NOS3 G894T Polymorphism

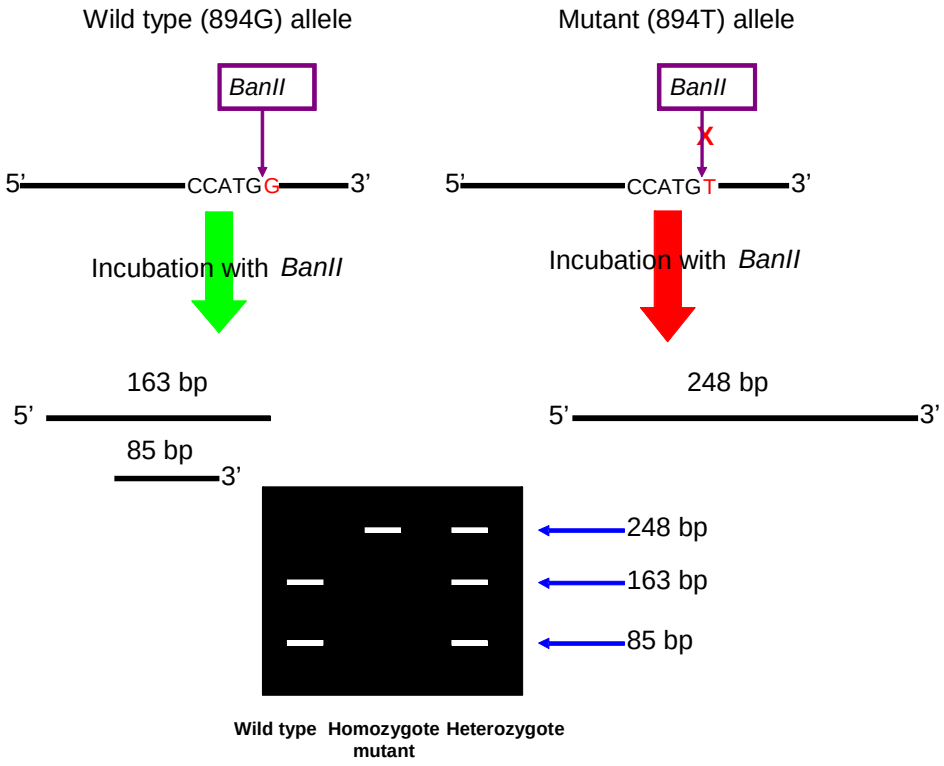
NOS3 G894T single nucleotide polymorphism is a G to T base substitution at position 894 of NOS3 cDNA coding for a glutamic acid to aspartate change at position 298 in the amino acid structure of the protein. The amplified PCR product in exon 7 bears a recognition site for the restriction enzyme *BanII*, it is present only when nucleotide at position 894 is occupied by G. Figure 3.21 presents the sequence of approximately 248 bp long PCR product in exon 7 of *NOS3*, highlighting the sequence of primers, location of SNP and the recognition sequence of the restriction enzyme *BanII*. Upon digestion with the restriction enzyme *BanII*, the wild type individuals have the recognition site for *BanII* (recognition site for *BanII* is 5'-GRGCY↓C-3'), the PCR product is cut into two bands of 163 bp and 85 bp long. Mutant type alleles possess the nucleotide T at position 894, and a suitable recognition site is not present for *BanII* restriction enzyme in this case. So, mutant type alleles result in a 248 bp single band. Schematic representation and real agarose gel photo is given in Figure 3.22. Table 3.10 summarizes the genotype distribution and allele frequencies of NOS3 G894T polymorphism.

GTGGTCACGG AGACCCAGCC AATGAGGGAC CCTGGAGATG AAGGCAGGAG ACAGTGGATG
GAGGGGTCCC TGAGGAGGGC ATGAGGCTCA GCCCCAGAAC CCCCTCTGGC CCACTCCCCA
CAGCTCTGCA TTCAGCACGG CTGGACCCCA GGAAACGGTC GCTTCGACGT GCTGCCCCTG

G894T
CTGCTGCAGG CCCCAGATGA GCCCCCAGAA CTCTTCCTTC TGCCCCCCGA GCTGGTCCTT

BanII
GAGGTGCCCC TGGAGCACCC CACGTGAGCA CCAAAGGGAT TGACTGGGTG GGATGGAGGG

Figure 3.21 Sequence of amplified fragment covering the G894T single nucleotide polymorphism of *NOS3*. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *BanII*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).



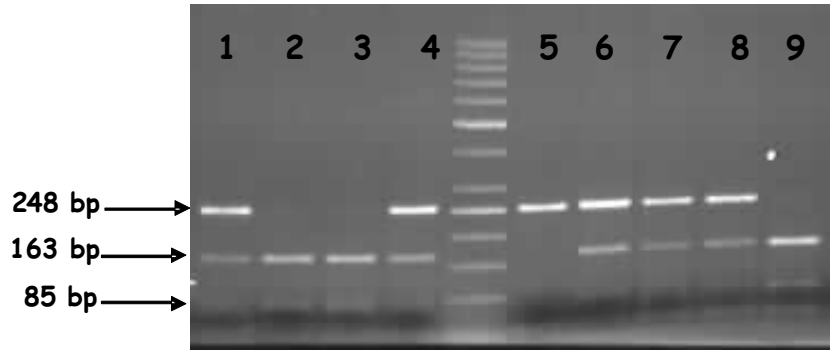


Figure 3.22 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*BanII*) digestion products for the G894T SNP of NOS3. In the gel photo lanes 2-3, 9; homozygous wild type (894GG); lanes 1, 4, 6-8; heterozygote (894GT); lane 5, homozygous mutated (894TT).

Table 3.10 Genotype distribution and allele frequencies of NOS3 G894T SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
NOS3G894T Genotypes, n(%)				
GG	82 (33.5)	55 (37.9)		
GT	156 (63.7)	80 (55.2)	1.215[a] (0.792-1.863)	0.373
TT	7 (2.8)	10 (6.9)		
Allele frequency				
G	0.653	0.655	1.009[b] (0.744-1.370)	1.000
T	0.347	0.345		

[a]TT+GT vs GG
[b]T vs G

In this study, a total of 245 ischemic stroke patients and 145 control subjects were investigated for NOS3 G894T polymorphism. Among them, 82 patients and 55 controls were homozygous wild type while, 156 patients and 80 controls were heterozygote. Seven patients and 10 controls had homozygous mutated genotype. Therefore, there is no significant difference in the genotype frequencies of NOS3 G894T polymorphism between stroke patients and controls. The G allele frequency was found to be nearly same in stroke patients (0.653) and controls (0.655). T allele frequency of stroke patients was also same as controls.

3.4.2 Analysis of Genotypes of NOS3 T-786C Polymorphism

T-786C polymorphism is a base substitution from T to C at position -786 and the PCR region involving the SNP is 236 bp long. Figure 3.23 presents the sequence of PCR product in promoter region of *NOS3*, highlighting the sequence of primers,

location of SNP and the recognition sequence of the restriction enzyme *PdiI*. In the wild type allele, with T at position -786, has no *PdiI* cleavage site (recognition site for *PdiI* is 5'-GCC↓GGC-3'), whereas PCR product is cleaved into two pieces of 203 and 33 bp long in the mutated allele, with C at position -786. Schematic representation and real agarose gel photo is given in Figure 3.24. Table 3.11 summarizes the genotype distribution and allele frequencies of NOS3 T-786C polymorphism.

There were 146 (59.6%) homozygous wild type, 93 (38.0%) heterozygous and 6 (2.4%) homozygous mutated individuals in patient group. The mutated (C) allele frequency was 0.215 while the wild type allele (T) frequency was 0.785 in patients. In control group of 145 individuals, 56.6% were homozygous wild type (TT), 39.3% heterozygous (TC) and 4.1% homozygous mutant type (CC). It was observed that among the controls T and C allele frequency were found to be 0.762 and 0.238, respectively. But there was no significant difference between patient and control groups with respect to genotype and allele frequency distribution of NOS3 T-786C polymorphism.

TGTTTGTCTG TCTGTCTGCT GCTCCTAGTC TCTGCCTCTC CCAGTCTCTC AGCTTCCGTT
TCTTTCTTAA ACTTTCTCTC AGTCTCTGAG GTCTCGAAAT CACGAGGCTT CGACCCCTGT
GGACCAGATG CCCAGCTAGT GGCCTTTCTC CAGCCCCTCA GATGACACAG AACTACAAAC
CCCAGCATGC ACTCTGGCCT GAAGTGCCTG GAGAGTGCTG GTGTACCCCA CCTGCATTCT
GGGAACTGTA GTTTCCCTAG TCCCCCATGC TCCCACCAGG GCATCAAGCT CTTCCCTGGC

T-786C

TGGCTGACCC TGCCTCAGCC CTAGTCTCTC TGCTGACCTG CGGCCCCGGG AAGCGTGCGT

PdiI

CACTGAATGA CAGGGTGGGG GTGGAGGCAC TGGAAGGCAG CTTCCTGCTC TTTTGTGTCC
CCCACTTGAG TCATGGGGGT GTGGGGGTTC CAGGAAATTG GGGCTGGGAG GGGAAGGGAT
ACCCTAATGT CAGACTCAAG GACAAAAAGT CACTACATCC TTGCTGGGCC TCTATCCCCA

Figure 3.23 Sequence of amplified fragment covering the T-786C single nucleotide polymorphism of *NOS3*. The turquoise highlighted sequences are forward and reverse primers, red highlighted nucleotide shows location of SNP, and the pink highlighted sequences show the recognition sites for restriction enzyme *PdiI*, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

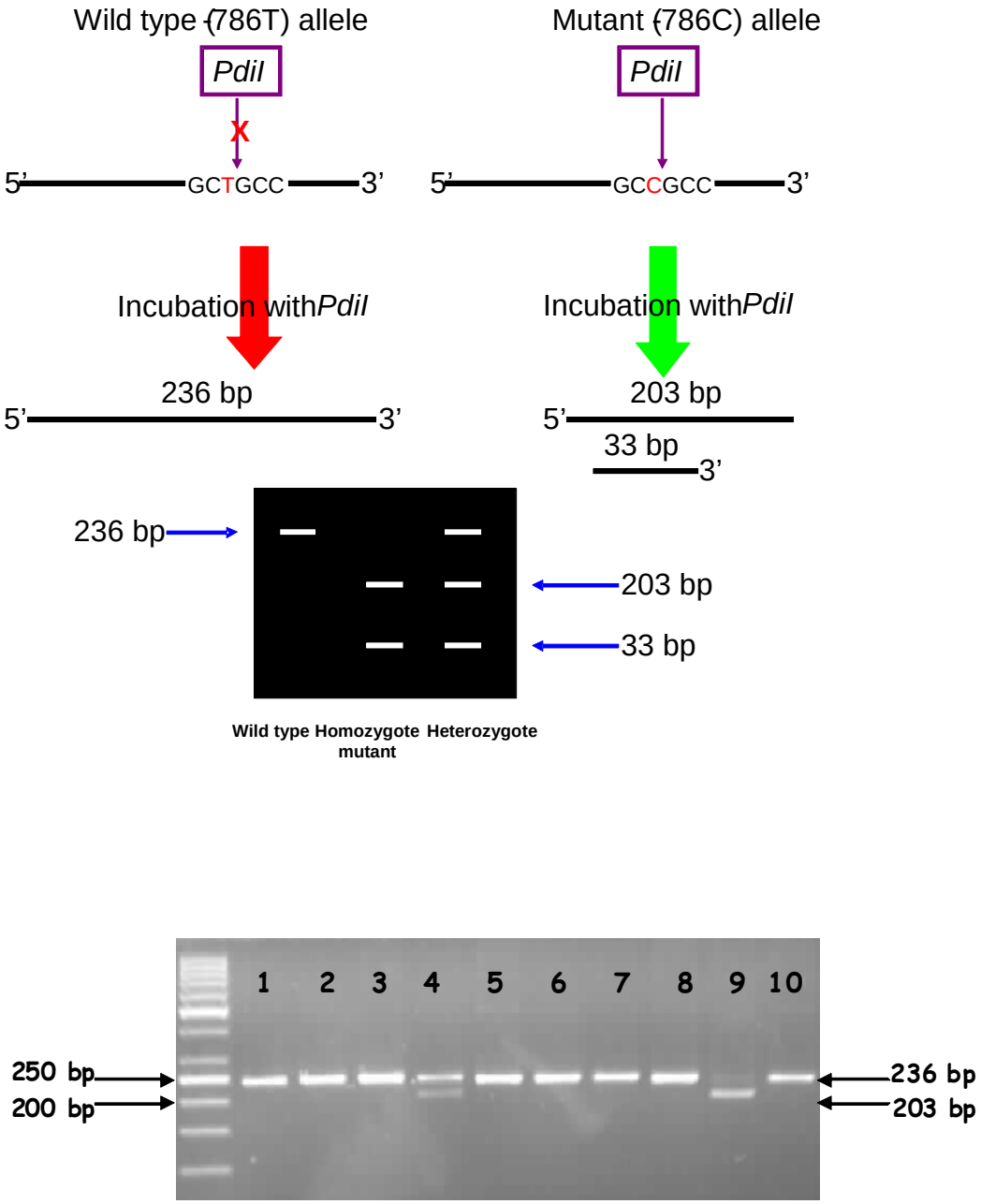


Figure 3.24 Schematic representation (upper part) and agarose gel electrophoresis (lower part) of restriction endonuclease (*PdiI*) digestion products for the T-786C SNP of NOS3. In the gel photo lanes 1-3, 5-8, 10; homozygous wild type (-786TT); lane 4; heterozygote (-786TC); lane 9, homozygous mutated (-786CC). Lanes 4 and 9 also contain a 33 bp band which is not observable in the photo.

Table 3.11 Genotype distribution and allele frequencies of NOS3 T-786C SNP in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
NOS3T-786C Genotypes, n(%)				
TT	146 (59.6)	82 (56.6)		
TC	93 (38.0)	57 (39.3)	0.883[a] (0.582-1.338)	0.556
CC	6 (2.4)	6 (4.1)		
Allele frequency				
T	0.785	0.762	0.873[b] (0.618-1.234)	0.443
C	0.215	0.238		

[a]TC+CC vs TT
[b]C vs T

3.4.3 Analysis of Genotypes of NOS3 intron 4 VNTR Polymorphism

The variable number of tandem repeat (VNTR) polymorphism is located in intron 4 of *NOS3* (eNOS4b/a polymorphism). In repeats of a 27-bp consensus sequence, there are two alleles, a common large allele and a smaller allele. The larger allele (eNOS4b allele), designated “b-insertion” has five tandem repeats, and the smaller allele (eNOS4a allele) “a-deletion” has four repeats. Figure 3.25 presents the sequence of approximately 194 bp long PCR product in intron 4 of *NOS3*, highlighting the sequence of primers and repeated sequence. When the amplified PCR product has insertion, it gives a single 194 bp band. On the other hand, if the amplified PCR product has deletion polymorphism, it gives a single 167 bp band. Schematic representation and real agarose gel photo is given in Figure 3.26. The genotype distributions and allele frequencies of NOS3 intron 4 VNTR polymorphism are given in Table 3.12.

CTGCCCCACC CTCAGCACCC AGGGGAACCT CAGCCCAGTA GTGAAGACCT GGTTATCAGG
CCCTATGGTA GTGCCTTGGC TGGAGGAGGG GAAAGAAGTC TAGACCTGCT GCAGGGGTGA
GGAAGTCTAG ACCTGCTGCA GGGGTGAGGA AGTCTAGACC TGCTGCAGGG GTGAGGAAGT
CTAGACCTGC CTAGACCTGC TGCGGGGGTG AGGAAGTCTA GACCTGCTGC GGGGGTGAGG
ACAGCTGAGC GGAGCTTCCC TGGGCGGTGC TGTCAGTAGC AGGAGCAGCC TCCTGGAAAA

Figure 3.25 Sequence of amplified fragment covering the intron 4 VNTR polymorphism of *NOS3*. The turquoise highlighted sequences are forward and reverse primers.Yellow, red, green and pink highlighted sequences show 27 bp repeated sequence, as indicated (the nucleotide sequence is taken from http://www.ncbi.nlm.nih.gov).

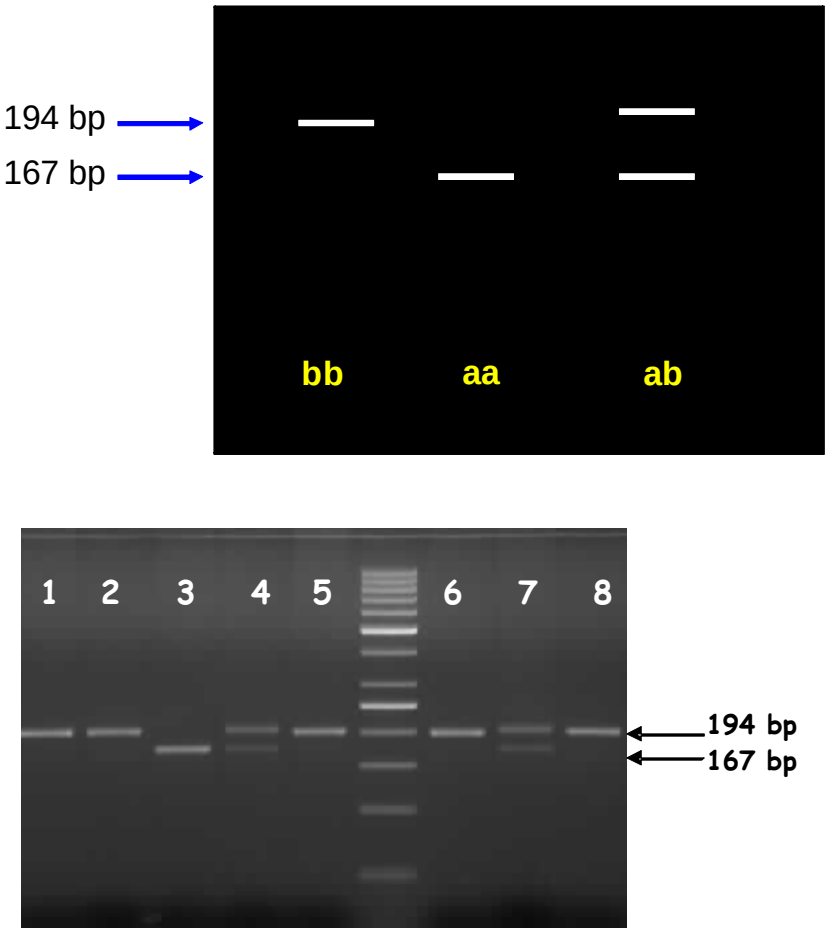


Figure 3.26 Schematic representation and real agarose gel electrophoresis of PCR products for NOS3 VNTR. In the gel photo lanes 1, 2, 5, 6, 8; larger “bb” genotype; lanes 4, 7; heterozygote “ab” genotype; lane 3; small “aa” genotype.

Table 3.12 Genotype distribution and allele frequencies of NOS3 intron 4 VNTR in ischemic stroke patients and controls.

	Patients (n=245)	**Controls (n=145)**	**OR(95%CI)**	***P***
NOS3VNTR Genotypes, n(%)				
aa	2 (0.8)	3 (2.1)		
ab	71 (29.0)	33 (22.7)	1.285[a] (0.807-2.047)	0.288
bb	172 (70.2)	109 (75.2)		
Allele frequency				
a	0.153	0.134	1.163[b] (0.766-1.766)	0.477
b	0.847	0.866		

[a]aa+ab vs bb
[b]a vs b

The frequency of homozygous aa genotype was 0.8% and 2.1% in stroke patients and controls, respectively. For heterozygous ab genotype, patient group had higher frequency (29.0%) when compared to control group (22.7%). The homozygous bb genotype frequency of patients (70.2%) was lower than that of controls (75.2%). On the other hand, there were no significant difference between patient and control group with respect to a and b allele frequencies.

3.4.4 Combination Analysis of NOS3 Polymorphisms

In Figure 3.27, the percentage of the double and triple combinations of NOS3 G894T, NOS3 T-786C and NOS3 VNTR polymorphisms are given in risky and non-risky patient and control groups. Risky and non-risky groups were created just as

CYP2E1 polymorphism. We observed that if the risky genotypes of NOS3 G894T and NOS3 T-786C polymorphisms are found on the same subjects at the same time, double combined risky genotype had 1-fold increased risk of stroke; but this was statistically insignificant (*P*=0.798). Co-presence of NOS3 G894T and NOS3 VNTR polymorphisms increased the risk approximately 2-fold, which was not statistically significant (*P*=0.092). On the other hand combination of NOS3 T-786C and NOS3 VNTR polymorphisms was found to increase the risk of stroke development 1-fold (OR=1.098; 95% CI=0.628-1.922; *P*=0.742). We also analyzed triple combinations of NOS3 polymorphisms and found that surprisingly triple combined mutant was found to be protective against stroke (OR=0.612; 95% CI=0.321-1.168; *P*=0.135).

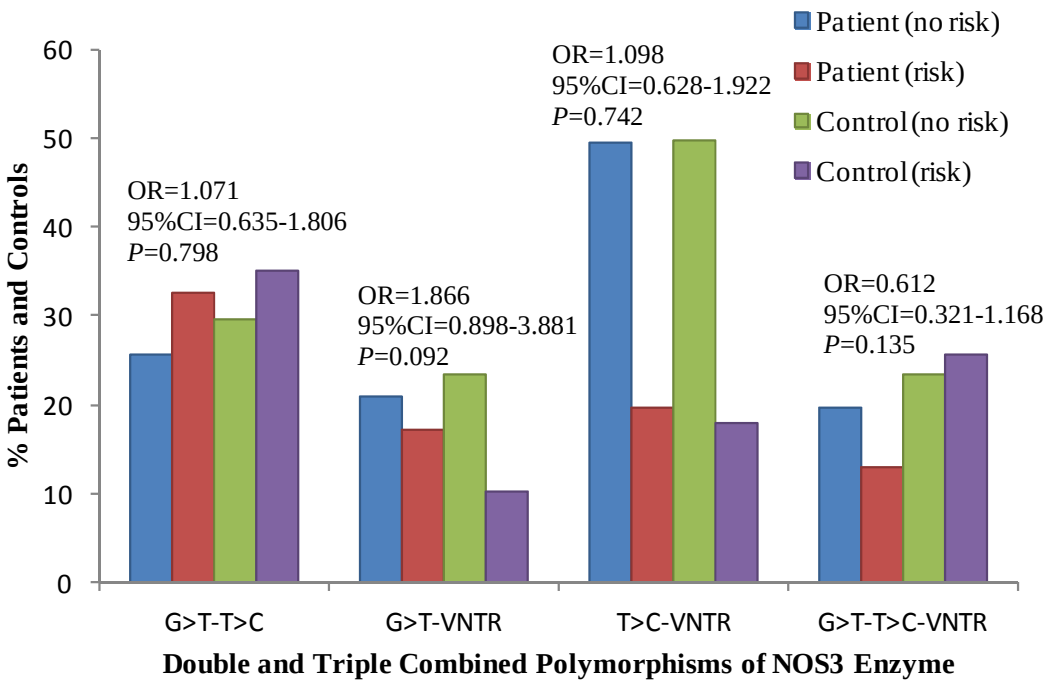


Figure 3.27 The double and triple combination of NOS3 G894T, NOS3 T-786C and NOS3 VNTR SNPs**.**

3.5 Distribution of Genotypes in Different Certain Risk Factor Groups

In this section the genotype distribution of CYP2E1, FMO3, NQO1, GST and NOS3 enzymes SNPs were analyzed in different groups composed of certain risk factors of ischemic stroke.

3.5.1 Distribution of CYP2E1 Genotypes in Different Certain Risk Factor Groups

3.5.1.1 Distribution of CYP2E1 Genotypes in Hypertensive-Normotensive Group

The genotype distribution of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B polymorphisms in hypertensive stroke patient and control and normotensive stroke patient and control groups are given in Table 3.13. For CYP2E1*5B wild type (*1A*1A) genotype, hypertensive ischemic stroke patients (93.3%) had lower frequency than normotensive ischemic stroke patients (97.6%). Hypertensive patient group had 11 heterozygous (*1A*5B). On the other hand normotensive stroke patients had 2 heterozygous (*1A*5B) individuals. There was no homozygous mutated (*5B*5B) individual in normotensive patient group. In hypertensive and normotensive control groups, wild typed individuals had almost same frequency.

For CYP2E1*6 polymorphism, there were 87.2% wild type (*1A*1A), 11.0% heterozygous (*1A*6) and 1.8% homozygous mutated (*6*6) individuals in hypertensive stroke patients. While the frequency of wild type (*1A*1A) was 83.6%, the frequency of heterozygous genotype (*1A*6) was 16.4% in hypertensive control group. In the normotensive group, stroke patients (85.4%) had lower frequency of wild type genotype when compared to controls (88.9%). Among normotensives, 11 patients and 10 controls were heterozygote. On the other hand, there was no mutated

genotype in normotensive controls and in patients just 1 individual had mutated genotype.

In terms of CYP2E1*7B genotype frequencies, there were no statistically differences both in hypertensive stroke patients and controls (*P*=0.245) and in normotensive stroke patients and controls (*P*=0.228).

Table 3.13 CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotype frequencies in hypertensive and normotensive groups.

Genotypes n(%)	Hypertensive (n=218)			Normotensive (n=172)		
	Stroke (n=163)	Controls (n=55)	*P*	Stroke (n=82)	Controls (n=90)	*P*
CYP2E1*5B						
*1A*1A	152 (93.3)	55 (100)		80 (97.6)	89 (98.9)	
*1A*5B	11 (6.7)	0	NA[a]	2 (2.4)	1 (1.1)	0.506[a]
*5B*5B	0	0		0	0	
CYP2E1*6						
*1A*1A	142 (87.2)	46 (83.6)		70 (85.4)	80 (88.9)	
*1A*6	18 (11.0)	9 (16.4)	0.517[b]	11 (13.4)	10 (11.1)	0.490[b]
*6*6	3 (1.8)	0		1 (1.2)	0	
CYP2E1*7B						
*1A*1A	138 (84.7)	50 (90.9)		72 (87.8)	73 (81.1)	
*1A*7B	23 (14.1)	5 (9.1)	0.245[c]	8 (9.8)	15 (16.7)	0.228[c]
*7B*7B	2 (1.2)	0		2 (2.4)	2 (2.2)	

[a] *5B*5B+*1A*5B vs *1A*1A

[b]*6*6+*1A*6 vs *1A*1A

[c]*7B*7B+*1A*7B vs *1A*1A

NA: Not Applicable

3.5.1.2 Distribution of CYP2E1 Genotypes in Diabetic-Nondiabetic Group

Table 3.14 summarizes the genotype distribution of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B polymorphisms in diabetic stroke patient and control and non-diabetic stroke patient and control groups. The CYP2E1*5B wild type genotype frequency was found to be 94% in diabetic stroke patients and 100% in diabetic controls. In the diabetic patients, 5 individuals had heterozygote genotype. The percentage of wild type and heterozygote genotypes were 95.0% and 5.0% in non-diabetic stroke patients, respectively. 99.2% of the non-diabetic controls had wild type. The distribution of CYP2E1*6 wild type and heterozygote genotypes were approximately same in diabetic and non-diabetic stroke patients and in diabetic and non-diabetic controls. Therefore, there was no significant difference with respect to genotype distribution of CYP2E1*6 polymorphism in diabetic/non-diabetic group. For CYP2E1*7B polymorphism, wild type genotype frequency was found to be 84.5% in diabetic patients and 86.3% in non-diabetic patients, respectively. In diabetic group, the heterozygote genotype percentage (14.3%) was found to be higher than that of control (12%). When compared to non-diabetic stroke patients (11.8%), the heterozygote genotype frequency was found to be higher in that of control (14.2%). However, none of the P values were significant.

Table 3.14 CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotype frequencies in diabetic and non-diabetic groups.

Genotypes n(%)	Diabetic (n=109)			Non-diabetic (n=281)		
	Stroke (n=84)	Controls (n=25)	*P*	Stroke (n=161)	Controls (n=120)	*P*
CYP2E1*5B						
*1A*1A	79 (94.0)	25 (100)		153 (95.0)	119 (99.2)	
*1A*5B	5 (6.0)	0	NA[a]	8 (5.0)	1(0.8)	0.051[a]
*5B*5B	0	0		0	0	
CYP2E1*6						
*1A*1A	74 (88.1)	22 (88.0)		138 (85.7)	104 (86.7)	
*1A*6	10 (11.9)	3 (12.0)	0.990[b]	19 (11.8)	16 (13.3)	0.819[b]
*6*6	0	0		4 (2.5)	0	
CYP2E1*7B						
*1A*1A	71 (84.5)	21 (84.0)		139 (86.3)	102 (85.0)	
*1A*7B	12 (14.3)	3 (12.0)	0.949[c]	19 (11.8)	17 (14.2)	0.751[c]
*7B*7B	1 (1.2)	1 (4.0)		3 (1.9)	1 (0.8)	

[a] *5B*5B+*1A*5B vs *1A*1A

[b]*6*6+*1A*6 vs *1A*1A

[c]*7B*7B+*1A*7B vs *1A*1A

NA: Not Applicable

3.5.1.3 Distribution of CYP2E1 Genotypes in Smoker-Nonsmoker Group

In smoker stroke patient and control and non-smoker stroke patient and control groups, the CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotype distributions is given in Table 3.15. Among smoker patients, there were 65 wild type and 4 heterozygote individuals for CYP2E1*5B polymorphism. Non-smoker patient group had 94.9% wild type and 5.1% heterozygote genotype. The entire non-smoker

control group had wild type. For CYP2E1*6 polymorphism, the frequency of wild type was higher in smoker stroke patients (84.1%) than smoker controls (76.2%). On the other hand wild type percentage was very close to each other in non-smoker stroke patient (87.5%) and non-smoker control groups (88.7%). In the similar manner the frequency of heterozygote genotype was found to be same in non-smoker patients (11.4%) and controls (11.3%). However the smoker patients (13.0%) had lower frequency for heterozygote genotype than smoker controls (23.8%). Both smoker stroke patients and non-smoker stroke patients had 2 homozygous mutated individuals. None of the controls had homozygous mutated individual. When Table 3.15 was examined in terms of CYP2E1*7B polymorphism, the frequency of wild type individual was found to be lower in smoker stroke patients (78.3%) than non-smoker stroke patients (88.6%). The frequency of wild type was very close in smoker (85.7%) and non-smoker control groups (84.7%). When compared to smoker stroke patients (18.8%), the heterozygote genotype frequency was found to be higher in that of control (14.3%). On the other hand the percentage of heterozygote genotype was lower in non-smoker patients (10.2%) than non-smoker controls (13.7%). The numbers of homozygous mutated individuals were same in smoker and non-smoker patient groups and non-smoker control group. But significant differences were not observed both between smoker patient and control and non-smoker patient and control groups with respect to genotype distribution of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B polymorphisms.

Table 3.15 CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotype frequencies in smoker and non-smoker groups.

Genotypes n(%)	Smoker (n=90)			Non-smoker (n=300)		
	Stroke (n=69)	Controls (n=21)	*P*	Stroke (n=176)	Controls (n=124)	*P*
CYP2E1*5B						
*1A*1A	65 (94.2)	20 (95.2)		167 (94.9)	124 (100)	
*1A*5B	4 (5.8)	1 (4.8)	0.856[a]	9 (5.1)	0	NA[a]
*5B*5B	0	0		0	0	
CYP2E1*6						
*1A*1A	58 (84.1)	16 (76.2)		154 (87.5)	110 (88.7)	
*1A*6	9 (13.0)	5 (23.8)	0.409[b]	20 (11.4)	14 (11.3)	0.751[b]
*6*6	2 (2.9)	0		2 (1.1)	0	
CYP2E1*7B						
*1A*1A	54 (78.3)	18 (85.7)		156 (88.6)	105 (84.7)	
*1A*7B	13 (18.8)	3 (14.3)	0.455[c]	18 (10.2)	17 (13.7)	0.315[c]
*7B*7B	2 (2.9)	0		2 (1.2)	2 (1.6)	

[a] *5B*5B+*1A*5B vs *1A*1A

[b]*6*6+*1A*6 vs *1A*1A

[c]*7B*7B+*1A*7B vs *1A*1A

NA: Not Applicable

3.5.1.4 Distribution of CYP2E1 Genotypes in Obese- Nonobese Group

The genotype distribution of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B polymorphisms in obese stroke patient and control and non-obese stroke patient and control groups are summarized in Table 3.16. When compared to obese stroke patients (96.4%), the wild type frequency of CYP2E1*5B was found to be higher in obese controls (100%). Only 2 obese stroke patients had heterozygote genotype. We

observed that among non-obese, 178 patients and 135 controls were wild type. In addition 11 patients had heterozygote genotype. For the genotype distribution of CYP2E1*6 polymorphism, there was no statistically significant difference between patients and controls in obese/non-obese group. Not only obese stroke patients and controls but also non-obese stroke patients and controls had similar frequencies of CYP2E1*7B wild type, heterozygote and homozygote mutated genotype.

Table 3.16 CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotype frequencies in obese and non-obese groups.

Genotypes n(%)	Obese (n=65)			Non-obese (n=325)		
	Stroke (n=56)	Controls (n=9)	*P*	Stroke (n=189)	Controls (n=136)	*P*
CYP2E1*5B						
*1A*1A	54 (96.4)	9 (100)		178 (94.2)	135 (99.3)	
*1A*5B	2 (3.4)	0	NA[a]	11 (5.8)	1 (0.7)	NA[a]
*5B*5B	0	0		0	0	
CYP2E1*6						
*1A*1A	52 (92.9)	7 (77.8)		160 (84.7)	119 (87.5)	
*1A*6	3 (5.3)	2 (22.2)	0.147[b]	26 (13.7)	17 (12.5)	0.468[b]
*6*6	1 (1.8)	0		3 (1.6)	0	
CYP2E1*7B						
*1A*1A	47 (83.9)	8 (88.9)		163 (86.2)	115 (84.6)	
*1A*7B	8 (14.3)	1 (11.1)	0.702[c]	23 (12.2)	19 (14.0)	0.670[c]
*7B*7B	1 (1.8)	0		3 (1.6)	2 (1.4)	

[a] *5B*5B+*1A*5B vs *1A*1A

[b]*6*6+*1A*6 vs *1A*1A

[c]*7B*7B+*1A*7B vs *1A*1A

NA: Not Applicable

3.5.2 Distribution of FMO3 Genotypes in Different Certain Risk Factor Groups

3.5.2.1 Distribution of FMO3 Genotypes in Hypertensive-Normotensive Group

In hypertensive/ normotensive stroke patient and control groups the FMO3 G472A and FMO3 A923G genotype distribution are given in Table 3.17. The FMO3 G472A wild type genotype (GG) frequency was found to be 40.5% in hypertensive stroke patients and 47.3% in hypertensive controls. In the hypertensive patients, 41 individuals had heterozygote (AG) and 56 individuals had homozygote mutated (AA) genotype. The percentage of wild type, heterozygote and homozygote mutated genotypes were 52.4%, 19.6% and 28% in normotensive stroke patients, respectively. In the normotensive controls, the frequencies of wild type, heterozygote and homozygote mutated genotypes were 34.4%, 34.4% and 31.2%, respectively. Therefore, there was a significant difference of genotype distribution of FMO3 G472A polymorphism between normotensive stroke patients and controls (P=0.017). For FMO3 A923G polymorphism, wild type (AA) genotype frequency was found to be 87.1% in hypertensive patients and 87.8% in normotensive patients. In hypertensive patient group, the heterozygote (AG) genotype percentage (12.3%) was found to be higher than that of control (7.3%). When compared to normotensive stroke patients (12.2%), the heterozygote genotype frequency was found to be higher in normotensive control (13.3%). Only 1 homozygote mutated (GG) individual was found in the hypertensive stroke patient group. Nevertheless, statistically significant differences were not observed both between hypertensive stroke patients and controls and normotensive stroke patients and controls in terms of genotype distribution of FMO3 A923G polymorphism.

Table 3.17 FMO3 G472A and FMO3 A923G genotype frequencies in hypertensive and normotensive groups.

Genotypes n(%)	Hypertensive (n=218)			Normotensive (n=172)		
	Stroke (n=163)	Controls (n=55)	*P*	Stroke (n=82)	Controls (n=90)	*P*
FMO3 G472A						
GG	66 (40.5)	26 (47.3)		43 (52.4)	31 (34.4)	
GA	41 (25.2)	13 (23.6)	0.379[a]	16 (19.6)	31 (34.4)	0.017[a]
AA	56 (34.3)	16 (29.1)		23 (28.0)	28 (31.2)	
FMO3 A923G						
AA	142 (87.1)	51 (92.7)		72 (87.8)	78 (86.7)	
AG	20 (12.3)	4 (7.3)	0.259[b]	10 (12.2)	12 (13.3)	0.823[b]
GG	1 (0.6)	0		0	0	

[a]GA+AA vs GG

[b]AG+GG vs AA

3.5.2.2 Distribution of FMO3 Genotypes in Diabetic-Nondiabetic Group

Table 3.18 summarizes the genotype distribution of FMO3 G472A and FMO3 A923G polymorphisms in diabetic/non-diabetic group. For FMO3 G472A wild type genotype, diabetic ischemic stroke patients (47.6%) had higher frequency than non-diabetic ischemic stroke patients (42.9%). Diabetic patient group had 20 heterozygous and 24 homozygous mutated individuals. On the other hand non-diabetic stroke patients had 37 heterozygous and 55 homozygote mutated individuals. Both wild type and heterozygote genotype frequencies were found to be higher in diabetic controls when compared to non-diabetic controls. Moreover homozygous mutated genotype frequency was higher in non-diabetic control group than diabetic control group. For FMO3 A923G polymorphism, there were 94% wild

type, 4.8% heterozygous and 1.2% homozygous mutated individuals in diabetic stroke patients. While the frequency of wild type was 92%, the frequency of heterozygous genotype was 8% in diabetic control group. In the non-diabetic group, stroke patients (83.9%) had lower frequency of wild type genotype when compared to controls (88.3%). Among non-diabetics, 26 patients and 14 controls were heterozygote. On the other hand, there was no mutated genotype in non-diabetic patients and controls. Neither FMO3 G472A nor FMO3 A923G polymorphisms showed significantly different genotype distribution in diabetic/non-diabetic stroke patient and control groups.

Table 3.18 FMO3 G472A and FMO3 A923G genotype frequencies in diabetic and non-diabetic groups.

Genotypes n(%)	Diabetic (n=109)			Non-diabetic (n=281)		
	Stroke (n=84)	Controls (n=25)	*P*	Stroke (n=161)	Controls (n=120)	*P*
FMO3 G472A						
GG	40 (47.6)	11 (44.0)		69 (42.9)	46 (38.3)	
GA	20 (23.8)	8 (32.0)	0.750[a]	37 (23.0)	36 (30.0)	0.445[a]
AA	24 (28.6)	6 (24.0)		55 (34.1)	38 (31.7)	
FMO3 A923G						
AA	79 (94.0)	23 (92.0)		135 (83.9)	106 (88.3)	
AG	4 (4.8)	2 (8.0)	0.714[b]	26 (16.1)	14 (11.7)	0.287[b]
GG	1 (1.2)	0		0	0	

[a]GA+AA vs GG

[b]AG+GG vs AA

3.5.2.3 Distribution of FMO3 Genotypes in Smoker-Nonsmoker Group

The genotype distribution of FMO3 G472A and FMO3 A923G polymorphisms in smoker/non-smoker groups are summarized in Table 3.19. When compared to smoker stroke patients (53.6%), the wild type frequency of FMO3 G472A was found to be lower in smoker controls (42.8%). Only 7 smoker stroke patients had heterozygote genotype and 25 smoker stroke patients had homozygous mutated genotype. We observed that among non-smokers, 72 patients and 48 controls were wild type. In addition 50 patients and 38 controls had heterozygote genotype. The homozygote mutated genotype percentage was found to be same in non-smoker stroke patients and controls. For the genotype distribution of FMO3 A923G polymorphism, there was no statistically significant difference between patients and controls in smoker/non-smoker group.

Table 3.19 FMO3 G472A and FMO3 A923G genotype frequencies in smoker and non-smoker groups.

Genotypes n(%)	Smoker (n=90)			Non-smoker (n=300)		
	Stroke (n=69)	Controls (n=21)	*P*	Stroke (n=176)	Controls (n=124)	*P*
FMO3 G472A						
GG	37 (53.6)	9 (42.8)		72 (40.9)	48 (38.8)	
GA	7 (10.2)	6 (28.6)	0.387[a]	50 (28.4)	38 (30.6)	0.702[a]
AA	25 (36.2)	6 (28.6)		54 (30.7)	38 (30.6)	
FMO3 A923G						
AA	62 (89.9)	19 (90.5)		152 (86.4)	110 (88.7)	
AG	7 (10.1)	2 (9.5)	0.934[b]	23 (13.2)	14 (11.3)	0.547[b]
GG	0	0		1 (0.6)	0	

[a]GA+AA vs GG

[b]AG+GG vs AA

3.5.2.4 Distribution of FMO3 Genotypes in Obese- Nonobese Group

Table 3.20 summarizes the genotype distribution of FMO3 G472A and FMO3 A923G polymorphisms in obese/non-obese patient and control groups. When Table 3.20 was examined in terms of FMO3 G472A polymorphism, the frequency of wild type individual was found to be higher in obese stroke patients (50.0%) than non-obese stroke patients (42.9%). However the frequency of wild type was lower in obese control group (33.3%) than non-obese control group (39.7%). When compared to obese stroke patients (12.5%), the heterozygote genotype frequency was found to be higher in that of control (22.3%). On the other hand the percentage of heterozygote genotype was lower in non-obese patients (26.5%) than non-obese controls (30.9%). Among obese group, stroke patients and controls had 21 and 4 homozygote mutated individuals, respectively. The frequency of homozygous mutated genotype was very close in non-obese patient (30.6%) and non-obese control group (29.4%). For FMO3 A923G polymorphism, the frequency of wild type was higher in obese stroke patients (92.9%) than obese controls (88.9%). On the other hand wild type percentage was found to be lower in non-obese stroke patient group (85.7%) than non-obese control group (89.0%). In the non-obese group, 26 stroke patients and 15 controls had heterozygote genotype. In the similar manner the obese patients (4) had high number of heterozygote genotyped individuals when compared to obese controls (1). One of the non-obese stroke patients had homozygous mutated genotype. But there were no significant differences both between obese stroke patients and controls and non-obese stroke patients and controls with respect to FMO3 G427A and FMO3 A923G genotype distributions.

Table 3.20 FMO3 G472A and FMO3 A923G genotype frequencies in obese and non-obese groups.

Genotypes n(%)	Obese (n=65)			Non-obese (n=325)		
	Stroke (n=56)	Controls (n=9)	*P*	Stroke (n=189)	Controls (n=136)	*P*
FMO3 G472A						
GG	28 (50.0)	3 (33.3)		81 (42.9)	54 (39.7)	
GA	7 (12.5)	2 (22.3)	0.353[a]	50 (26.5)	42 (30.9)	0.569[a]
AA	21 (37.5)	4 (44.4)		58 (30.6)	40 (29.4)	
FMO3 A923G						
AA	52 (92.9)	8 (88.9)		162 (85.7)	121 (89.0)	
AG	4 (7.1)	1 (11.1)	0.678[b]	26 (13.8)	15 (11.0)	0.388[b]
GG	0	0		1 (0.5)	0	

[a]GA+AA vs GG

[b]AG+GG vs AA

3.5.3 Distribution of NQO1 Genotypes in Different Certain Risk Factor Groups

3.5.3.1 Distribution of NQO1 Genotypes in Hypertensive-Normotensive Group

The genotype distribution of NQO1*2 polymorphism is given in Table 3.21. Hypertensive ischemic stroke patients (62.6%) had higher frequency of wild type (*1*1) genotype than hypertensive controls (52.7%). While hypertensive patient group had 55 heterozygous (*1*2) and 6 homozygous mutated (*2*2) individuals, hypertensive control group had 25 heterozygote and 1 homozygote mutated individuals. In the normotensive group, wild type genotype frequency was found to

be higher in patients (62.2%) when compared to controls (50%). On the contrary in normotensive patients (31.7%) the heterozygote genotype percentage was lower than that of controls (45.6%). In addition normotensive stroke patients had 5 and normotensive controls had 4 homozygote mutated individuals. Statistically significant differences were not observed in hypertensive/ normotensive stroke patient and control groups in terms of genotype distribution of NQO1*2 polymorphism.

Table 3.21 NQO1*2 genotype frequencies in hypertensive and normotensive groups.

Genotypes n(%)	Hypertensive (n=218)			Normotensive (n=172)		
	Stroke (n=163)	Controls (n=55)	*P*	Stroke (n=82)	Controls (n=90)	*P*
NQO1*2						
*1*1 (CC)	102 (62.6)	29 (52.7)		51 (62.2)	45 (50.0)	
*1*2 (CT)	55 (33.7)	25 (45.5)	0.197[a]	26 (31.7)	41 (45.6)	0.108[a]
*2*2 (TT)	6 (3.7)	1 (1.8)		5 (6.1)	4 (4.4)	

[a]CT+TT vs CC

3.5.3.2 Distribution of NQO1 Genotypes in Diabetic-Nondiabetic Group

Table 3.22 summarizes the genotype distribution of NQO1*2 polymorphism in diabetic/non-diabetic group. The wild type genotype frequency was found to be 66.7% in diabetic stroke patients and 48% in diabetic controls. In the diabetic patients, 25 individuals had heterozygote and 3 individuals had homozygote mutated genotype. In addition there were 13 heterozygote and no homozygote mutated genotype in diabetic control group. The percentage of wild type and heterozygote

genotypes were 60.2% and 34.8% in non-diabetic stroke patients, respectively. Similarly, in non-diabetic controls the percentage of wild type (51.7%) was found to be higher than the percentage of heterozygote genotype (44.2%). On the other hand, there were 8 patients and 5 controls with mutated genotype in non-diabetic group. Genotype distribution of NQO1*2 polymorphism was not found statistically different both in diabetic stroke patients and controls and non-diabetic stroke patients and controls.

Table 3.22 NQO1*2 genotype frequencies in diabetic and non-diabetic groups.

Genotypes n(%)	Diabetic (n=109)			Non-diabetic (n=281)		
	Stroke (n=84)	Controls (n=25)	*P*	Stroke (n=161)	Controls (n=120)	*P*
NQO1*2						
*1*1 (CC)	56 (66.7)	12 (48.0)		97 (60.2)	62 (51.7)	
*1*2 (CT)	25 (29.7)	13 (52.0)	0.091[a]	56 (34.8)	53(44.2)	0.151[a]
*2*2 (TT)	3 (3.6)	0		8 (5.0)	5 (4.1)	

[a]CT+TT vs CC

3.5.3.3 Distribution of NQO1 Genotypes in Smoker-Nonsmoker Group

The genotype distribution of NQO1*2 polymorphism in smoker/non-smoker groups is summarized in Table 3.23. Thirty eight smoker stroke patients, 15 smoker controls, 115 non-smoker stroke patients and 59 non-smoker controls had wild type. The heterozygote genotype frequency was higher in smoker patients (39.1%) than that of controls (31.6%). Among smokers, only 4 stroke patients had homozygote mutated genotype. The heterozygote genotype frequency in non-smoker stroke patients (30.3%) was found to be lower than non-smoker controls (48.4%). Moreover

the percentage of homozygote mutated genotype was almost same in non-smoker patients (4.5%) and non-smoker controls (4.0%). We observed statistically significant difference in genotype distribution of NQO1*2 polymorphism between non-smoker stroke patient and non-smoker control groups.

Table 3.23 NQO1*2 genotype frequencies in smoker and non-smoker groups.

Genotypes n(%)	Smoker (n=90)			Non-smoker (n=300)		
	Stroke (n=69)	Controls (n=21)	*P*	Stroke (n=176)	Controls (n=124)	*P*
NQO1*2						
*1*1 (CC)	38 (55.1)	15 (68.4)		115 (65.2)	59 (47.6)	
*1*2 (CT)	27 (39.1)	6 (31.6)	0.182[a]	54 (30.3)	60 (48.4)	0.002[a]
*2*2 (TT)	4 (5.8)	0		7 (4.5)	5 (4.0)	

[a]CT+TT vs CC

3.5.3.4 Distribution of NQO1 Genotypes in Obese- Nonobese Group

In obese/non-obese patient and control groups the NQO1*2 genotype distribution is given in Table 3.24. When compared to obese stroke patients (64.3%), the wild type frequency of NQO1*2 was found to be lower in obese controls (55.6%). Nineteen obese stroke patients had heterozygote genotype and 1 obese stroke patients had homozygous mutated genotype. In addition 4 heterozygote individuals were found in obese control group. We observed that among non-obese, 117 patients and 69 controls were wild type. The frequency of heterozygote genotype was lower in non-obese patients (32.8%) than that of control (45.6%). Conversely the homozygote mutated genotype frequency was found to be higher in non-obese stroke

patients (5.3%) when compared to non-obese controls (3.7%). In terms of NQO1*2 genotype distribution there was no significant difference in obese and non-obese groups.

Table 3.24 NQO1*2 genotype frequencies in obese and non-obese groups.

Genotypes n(%)	Obese (n=65)			Non-obese (n=325)		
	Stroke (n=56)	Controls (n=9)	*P*	Stroke (n=189)	Controls (n=136)	*P*
NQO1*2						
*1*1 (CC)	36 (64.3)	5 (55.6)		117 (61.9)	69 (50.7)	
*1*2 (CT)	19 (33.9)	4 (44.4)	0.614[a]	62 (32.8)	62 (45.6)	0.058[a]
*2*2 (TT)	1 (1.8)	0		10 (5.3)	5 (3.7)	

[a]CT+TT vs CC

3.5.4 Distribution of GST Genotypes in Different Certain Risk Factor Groups

3.5.4.1 Distribution of GST Genotypes in Hypertensive-Normotensive Group

In hypertensive/ normotensive stroke patient and control groups the GSTM1, GSTT1 and GSTP1 A313G genotype distribution are given in Table 3.25. The GSTM1 present genotype frequency was found to be similar in hypertensive stroke patients (47.2%) and hypertensive controls (47.3%). The GSTM1 null genotype frequency was higher in hypertensive patients (57.8%) when compared to controls (52.7%). On the other hand in normotensive group while present genotype had higher percentage in patients (43.9%) than controls (33.3%), null genotype had lower percentage in patients (56.1%) than controls (66.7%). Among hypertensive stroke

patients, 116 individuals had GSTT1 present and 47 individuals had GSTT1 null genotype. When compared to normotensive stroke patients (74.4%), normotensive controls (72.2%) had approximately same frequency of GSTT1 present genotype. In the similar manner normotensive patients and controls had very close percentage for GSTT1 null genotype. For GSTP1 A313G polymorphism, wild type (AA) genotype frequency was found to be 54.0% in hypertensive patients and 54.9% in normotensive patients. In hypertensive group, the heterozygote (AG) genotype percentage (36.2%) was found to be lower than that of control (38.2%). When compared to normotensive stroke patients (35.4%), the heterozygote genotype frequency was found to be lower in normotensive controls (32.2%). There were 16 and 8 individuals with homozygote mutated (GG) genotype in hypertensive patients and hypertensive controls, respectively. The homozygote mutated genotype frequency was found to be lower in normotensive patients (9.8%) than normotensive controls (15.6%). None of the genotype distributions of GST polymorphisms had statistically significant difference both between hypertensive stroke patients and controls and normotensive stroke patients and controls.

Table 3.25 GSTM1, GSTT1 and GSTP1 **g**enotype frequencies in hypertensive and normotensive groups.

Genotypes n(%)	Hypertensive (n=218)			Normotensive (n=172)		
	Stroke (n=163)	Controls (n=55)	*P*	Stroke (n=82)	Controls (n=90)	*P*
GSTM1						
Present	77 (47.2)	26 (47.3)	0.997[a]	36 (43.9)	30 (33.3)	0.155[a]
Null	86 (57.8)	29 (52.7)		46 (56.1)	60 (66.7)	
GSTT1						
Present	116 (71.2)	45 (81.8)	0.120[b]	61 (74.4)	65 (72.2)	0.748[b]
Null	47 (28.8)	10 (18.2)		21 (25.6)	25 (27.8)	
GSTP1 A313G						
AA	88 (54.0)	26 (47.3)		45 (54.9)	47 (52.2)	
AG	59 (36.2)	21 (38.2)	0.389[c]	29 (35.4)	29 (32.2)	0.727[c]
GG	16 (9.8)	8 (14.5)		8 (9.8)	14 (15.6)	

[a,b]null vs present

[c]AG+GG vs AA

3.5.4.2 Distribution of GST Genotypes in Diabetic-Nondiabetic Group

Table 3.26 summarizes the genotype distribution of GSTM1, GSTT1 and GSTP1 A313G polymorphisms in diabetic/non-diabetic group. For GSTM1 present genotype, diabetic ischemic stroke patients (46.4%) had similar frequency with non-diabetic ischemic stroke patients (46.0%). While diabetic patient group had 45 null genotyped individuals, non-diabetic patient group had 87 individuals. Present genotype frequency was found to be lower in diabetic controls (32%) when compared to non-diabetic controls (40%). Moreover null genotype frequency was higher in diabetic control group (68%) than non-diabetic control group (60%). For GSTT1 polymorphism, there were 72.6% present and 27.4% null genotypes in

diabetic stroke patients. While the frequency of present genotype was 76%, the frequency of null genotype was 24% in diabetic control group. In the non-diabetic group, stroke patients (72%) had lower frequency of present genotype when compared to controls (75.8%). Among non-diabetics, 45 patients and 29 controls had null genotype. We observed that there was no statistically significant difference in genotype distribution of GSTP1 A313G polymorphism in diabetic/non-diabetic patient and control groups.

Table 3.26 GSTM1, GSTT1 and GSTP1 **g**enotype frequencies in diabetic and non-diabetic groups.

Genotypes n(%)	Diabetic (n=109)			Non-diabetic (n=281)		
	Stroke (n=84)	Controls (n=25)	*P*	Stroke (n=161)	Controls (n=120)	*P*
GSTM1						
Present	39 (46.4)	8 (32.0)	0.201[a]	74 (46.0)	48 (40.0)	0.319[a]
Null	45 (53.6)	17 (68.0)		87 (54.0)	72 (60.0)	
GSTT1						
Present	61 (72.6)	19 (76.0)	0.737[b]	116 (72.0)	91 (75.8)	0.475[b]
Null	23 (27.4)	6 (24.0)		45 (28.0)	29 (24.2)	
GSTP1 A313G						
AA	38 (45.3)	14 (56.0)		95 (59.0)	59 (49.2)	
AG	37 (44.0)	8 (32.0)	0.344[c]	51 (31.7)	42 (35.0)	0.101[c]
GG	9 (10.7)	3 (12.0)		15 (9.3)	19 (15.8)	

[a,b]null vs present

[c]AG+GG vs AA

3.5.4.3 Distribution of GST Genotypes in Smoker-Nonsmoker Group

The genotype distribution of GSTM1, GSTT1 and GSTP1 A313G polymorphisms in smoker/non-smoker groups are summarized in Table 3.27. When compared to smoker stroke patients (44.9%), the present genotype frequency of GSTM1 was found to be lower in smoker controls (28.6%). Thirty eight smoker stroke patients had null genotype and 15 smoker controls had null genotype. We observed that among non-smokers, 82 patients and 50 controls had present genotype. In addition 94 patients and 74 controls had null genotype. For the genotype distribution of GSTT1 polymorphism, there was no statistically significant difference between patients and controls in smoker/non-smoker group. When Table 3.27 was examined in terms of GSTP1 A313G polymorphism, 37 smoker stroke patients, 12 smoker controls, 96 non-smoker stroke patients and 61 non-smoker controls had wild type. The heterozygote genotype frequency was higher in smoker patients (37.7%) than that of controls (33.3%). On the contrary in smoker stroke patients the frequency of homozygote mutated genotype was found to be lower (8.7%) than smoker controls (9.5%). The heterozygote genotype frequency in non-smoker stroke patients (35.3%) and non-smoker controls (34.7%) was found to be very close to each other. Moreover the percentage of homozygote mutated genotype was lower in non-smoker patients (10.2%) than non-smoker controls (16.1%).

Table 3.27 GSTM1, GSTT1 and GSTP1 genotype frequencies in smoker and non-smoker groups.

Genotypes n(%)	Smoker (n=90)			Non-smoker (n=300)		
	Stroke (n=69)	Controls (n=21)	*P*	Stroke (n=176)	Controls (n=124)	*P*
GSTM1						
Present	31 (44.9)	6 (28.6)	0.182[a]	82 (46.6)	50 (40.3)	0.281[a]
Null	38 (55.1)	15 (71.4)		94 (53.4)	74 (59.7)	
GSTT1						
Present	44 (63.8)	15 (71.4)	0.518[b]	133 (75.6)	95 (76.6)	0.835[b]
Null	25 (36.2)	6 (28.6)		43 (24.4)	29 (23.4)	
GSTP1 A313G						
AA	37 (53.6)	12 (57.2)		96 (54.5)	61 (49.2)	
AG	26 (37.7)	7 (33.3)	0.777[c]	62 (35.3)	43 (34.7)	0.361[c]
GG	6 (8.7)	2 (9.5)		18 (10.2)	20 (16.1)	

[a,b]null vs present

[c]AG+GG vs AA

3.5.4.4 Distribution of GST Genotypes in Obese- Nonobese Group

Table 3.28 summarizes the genotype distribution of GSTM1, GSTT1 and GSTP1 A313G polymorphisms in obese/non-obese patient and control groups. In obese stroke patients (46.4%) and non-obese stroke patients (46.0%) the frequency of GSTM1 present genotype was found to be close to each other. However the frequency of present genotype was lower in obese control group (22.2%) than non-obese control group (39.7%). When compared to obese stroke patients (53.6%), the GSTM1 null genotype frequency was found to be higher in that of control (77.8%). On the other hand the percentage of null genotype was lower in non-obese patients (54.0%) than non-obese controls (60.3%). Among obese group, stroke patients and

controls had 33 and 5 GSTT1 present genotyped individuals, respectively. The frequency of present genotype was almost close in non-obese patient (76.2%) and non-obese control group (77.2%). While the GSTT1 null genotype percentage was 41.1% in obese stroke patients, it was found to be 44.4% in obese controls. The null genotype frequencies were nearly close to each other in non-obese patients (23.8%) and controls (22.8%). For GSTP1 A313G polymorphism, the frequency of wild type was higher in obese stroke patients (53.6%) than obese controls (44.4%). On the other hand wild type percentage was found to be higher in non-obese stroke patient group (54.5%) than non-obese control group (50.7%). In the non-obese group, 67 stroke patients and 46 controls had heterozygote genotype. In the similar manner the obese patients (21) had high number of heterozygote genotyped individuals when compared to obese controls (4). The homozygote mutated genotype frequency was found to be 8.9% in obese patients and 11.2% in obese controls. On the other hand non-obese controls (15.5%) had higher frequency of homozygote mutated genotype than non-obese patients (10.1%). We observed that there were no significant differences between obese/non-obese stroke patients and controls with respect to genotype distribution of GSTM1, GSTT1 and GSTP1 A313G polymorphisms.

Table 3.28 GSTM1, GSTT1 and GSTP1 **g**enotype frequencies in obese and non-obese groups.

Genotypes n(%)	Obese (n=65)			Non-obese (n=325)		
	Stroke (n=56)	Controls (n=9)	*P*	Stroke (n=189)	Controls (n=136)	*P*
GSTM1						
Present	26 (46.4)	2 (22.2)	0.173[a]	87 (46.0)	54 (39.7)	0.256[a]
Null	30 (53.6)	7 (77.8)		102 (54.0)	82 (60.3)	
GSTT1						
Present	33 (58.9)	5 (55.6)	0.849[b]	144 (76.2)	105 (77.2)	0.831[b]
Null	23 (41.1)	4 (44.4)		45 (23.8)	31 (22.8)	
GSTP1 A313G						
AA	30 (53.6)	4 (44.4)	0.611[c]	103 (54.5)	69 (50.7)	0.503[c]
AG	21 (37.5)	4 (44.4)		67 (35.4)	46 (33.8)	
GG	5 (8.9)	1 (11.2)		19 (10.1)	21 (15.5)	

[a,b]null vs present

[c]AG+GG vs AA

3.5.5 Distribution of NOS3 Genotypes in Different Certain Risk Factor Groups

3.5.5.1 Distribution of NOS3 Genotypes in Hypertensive-Normotensive Group

The genotype distribution of NOS3 G894T, NOS3 T-786C and NOS3 intron 4 VNTR polymorphisms are given in Table 3.29. For NOS3 G894T wild type (GG) genotype, hypertensive ischemic stroke patients (32.5%) had lower frequency than hypertensive controls (45.4%). While hypertensive patient group had 107 heterozygous (GT) and 3 homozygous mutated (TT) individuals, hypertensive control group had 26 heterozygote (GT) and 4 homozygote mutated (TT) individuals.

In the normotensive group, wild type and heterozygote genotype frequencies were very close in patients and controls. In addition normotensive stroke patients had 4 and normotensive controls had 6 homozygote mutated individuals.

For NOS3 T-786C polymorphism, there were 60.1% wild type (TT), 38.7% heterozygous (TC) and 1.2% homozygous mutated (CC) individuals in hypertensive stroke patients. While the frequency of wild type was 56.4%, the frequencies of heterozygous and homozygous mutated genotype were 38.2% and 5.4% in hypertensive control group, respectively. In the normotensive group, stroke patients (58.5%) had higher frequency of wild type genotype when compared to controls (56.7%). Among normotensive, 30 patients and 36 controls were heterozygote. On the other hand, there were 4 patients and 3 controls with mutated genotype in normotensive group.

In terms of NOS3 VNTR polymorphism genotype frequencies, there were no statistically differences in hypertensive stroke patients and controls (P=0.696). Moreover, in normotensive stroke patients and controls had significantly NOS3 VNTR genotype distribution (P=0.038).

Table 3.29 NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotype frequencies in hypertensive and normotensive groups.

Genotypes n(%)	Hypertensive (n=218)			Normotensive (n=172)		
	Stroke (n=163)	Controls (n=55)	*P*	Stroke (n=82)	Controls (n=90)	*P*
NOS3 G894T						
GG	53 (32.5)	25 (45.4)		29 (35.4)	30 (33.3)	
GT	107 (65.6)	26 (47.3)	0.083[a]	49 (59.8)	54 (60.0)	0.779[a]
TT	3 (1.8)	4 (7.3)		4 (4.8)	6 (6.7)	
NOS3 T-786C						
TT	98 (60.1)	31 (56.4)		48 (58.5)	51 (56.7)	
TC	63 (38.7)	21 (38.2)	0.624[b]	30 (36.6)	36 (40.0)	0.804[b]
CC	2 (1.2)	3 (5.4)		4 (4.9)	3 (3.3)	
NOS3 VNTR						
aa	1 (0.6)	1 (1.8)		1 (1.2)	2 (2.2)	
ab	42 (25.8)	15 (27.3)	0.696[c]	29 (35.4)	18 (20.0)	0.038[c]
bb	120 (73.6)	39 (70.9)		52 (63.4)	70 (77.8)	

[a]TT+GT vs GG

[b]TC+CC vs TT

[c]aa+ab vs bb

3.5.5.2 Distribution of NOS3 Genotypes in Diabetic-Nondiabetic Group

Table 3.30 summarizes the genotype distribution of NOS3 G894T, NOS3 T-786C and NOS3 intron 4 VNTR polymorphisms in diabetic/non-diabetic group. The NOS3 G894T wild type genotype frequency was found to be 36.9% in diabetic stroke patients and 40% in diabetic controls. In the diabetic patients, 51 individuals had heterozygote and 2 individuals had homozygote mutated genotype. In addition there were 14 heterozygote and 1 homozygote mutated genotype in diabetic control

group. The percentage of wild type and heterozygote genotypes were 33% and 64% in non-diabetic stroke patients, respectively. Similarly, in non-diabetic controls the percentage of wild type (37.5%) was found to be lower than the percentage of heterozygote genotype (55.0%). On the other hand, there were 5 patients and 9 controls with mutated genotype in non-diabetic group.

The distribution of NOS3 T-786C wild type and heterozygote genotypes were approximately same in diabetic stroke patients and controls. But the homozygote mutated genotype frequency was lower in diabetic stroke patients than that of controls. NOS3 T-786C wild type, heterozygote genotypes had similar frequency in non-diabetic patients and controls. Therefore there was no significant difference with respect to genotype distribution of NOS3 T-786C polymorphism in diabetic/non-diabetic group. For NOS3 VNTR polymorphism, “aa” genotype frequency was found to be 1.2% in diabetic patients and 0.6% in non-diabetic patients, respectively. In diabetic group, the heterozygote “ab” genotype percentage was found to be lower in patients (28.6%) than that of controls (32%). When compared to non-diabetic stroke patients (29.2%), the “ab” heterozygote genotype frequency was found to be lower in that of control (20.8%). While “bb” genotype frequency was same in diabetic patients and non-diabetic patients, it was found to be higher in non-diabetic control group than diabetic control group. However, none of the P values were significant.

Table 3.30 NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotype frequencies in diabetic and non-diabetic groups.

Genotypes n(%)	Diabetic (n=109)			Non-diabetic (n=281)		
	Stroke (n=84)	Controls (n=25)	*P*	Stroke (n=161)	Controls (n=120)	*P*
NOS3 G894T						
GG	31 (36.9)	10 (40.0)		54 (33.0)	45 (37.5)	
GT	51 (60.7)	14 (56.0)	0.779[a]	105 (64.0)	66 (55.0)	0.309[a]
TT	2 (2.4)	1 (4.0)		5 (3.0)	9 (7.5)	
NOS3 T-786C						
TT	52 (61.9)	15 (60.0)		94 (58.4)	67 (55.8)	
TC	29 (34.5)	8 (32.0)	0.864[b]	64 (39.8)	49 (40.8)	0.669[b]
CC	3 (3.6)	2 (8.0)		3 (1.8)	4 (3.4)	
NOS3 VNTR						
aa	1 (1.2)	1 (4.0)		1 (0.6)	2 (1.7)	
ab	24 (28.6)	8 (32.0)	0.555[c]	47 (29.2)	25 (20.8)	0.170[c]
bb	59 (70.2)	16 (64.0)		113 (70.2)	93 (77.5)	

[a]TT+GT vs GG
[b]TC+CC vs TT
[c]aa+ab vs bb

3.5.5.3 Distribution of NOS3 Genotypes in Smoker-Nonsmoker Group

In smoker/non-smoker patient and control groups the NOS3 G894T, NOS3 T-786C and NOS3 intron 4 VNTR genotype distributions is given in Table 3.31. Among smoker patients, there were 28 wild type, 39 heterozygote and 2 homozygote mutated individuals for NOS3 G894T polymorphism. Non-smoker patient group had 30.7% wild type, 66.5% heterozygote and 2.8% homozygote mutated genotype. The wild type frequency was lower in smoker controls (28.6%) than non-smoker controls (39.5%). On the contrary the heterozygote and homozygote mutated genotype

frequencies were found to be higher in smoker controls when compared to non-smoker controls. For NOS3 T-786C polymorphism, the frequency of wild type was lower in smoker stroke patients (58.0%) than smoker controls (66.7%). On the other hand wild type percentage was found to be higher in non-smoker stroke patient (60.2%) and non-smoker control groups (54.9%). The frequency of heterozygote genotype was found to be 39.1% in smoker patient, 33.3% in smoker control group, 37.5% in non-smoker patient and 40.3% in non-smoker control group. Smoker stroke patients had 2 homozygous mutated individuals but smoker controls had no homozygous mutated individuals. In the non-smoker group 4 patients and 6 controls had homozygote mutated genotype. When Table 3.31 was examined in terms of NOS3 VNTR polymorphism, 1 smoker stroke patient, 1 non-smoker stroke patient and 3 non-smoker controls had “aa” genotype. The heterozygote “ab” genotype frequency was higher in smoker patients (36.3%) than that of controls (19.0%). On the contrary in smoker stroke patients the frequency of “bb” genotype was found to be lower (62.3%) than smoker controls (81.0%). The heterozygote “ab” genotype frequency in non-smoker stroke patients (26.1%) and non-smoker controls (23.4%) was found to be very close to each other. Similarly the percentage of “bb” genotype was almost same in non-smoker patients (73.3%) and non-smoker controls (74.2%). None of the NOS3 polymorphisms had significantly different genotype distribution both in smoker stroke patients and controls and in non-smoker stroke patients and controls.

Table 3.31 NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotype frequencies in smoker and non-smoker groups.

Genotypes n(%)	Smoker (n=90)			Non-smoker (n=300)		
	Stroke (n=69)	Controls (n=21)	*P*	Stroke (n=176)	Controls (n=124)	*P*
NOS3 G894T						
GG	28 (40.6)	6 (28.6)		54 (30.7)	49 (39.5)	
GT	39 (56.5)	13 (61.9)	0.320[a]	117 (66.5)	67 (54.0)	0.113[a]
TT	2 (2.9)	2 (9.5)		5 (2.8)	8 (6.5)	
NOS3 T-786C						
TT	40 (58.0)	14 (66.7)		106 (60.2)	68 (54.9)	
TC	27 (39.1)	7 (33.3)	0.476[b]	66 (37.5)	50 (40.3)	0.352[b]
CC	2 (2.9)	0		4 (2.3)	6 (4.8)	
NOS3 VNTR						
aa	1 (1.4)	0		1 (0.6)	3 (2.4)	
ab	25 (36.3)	4 (19.0)	0.113[c]	46 (26.1)	29 (23.4)	0.862[c]
bb	43 (62.3)	17 (81.0)		129 (73.3)	92 (74.2)	

[a]TT+GT vs GG

[b]TC+CC vs TT

[c]aa+ab vs bb

3.5.5.4 Distribution of NOS3 Genotypes in Obese- Nonobese Group

The genotype distribution of NOS3 G894T, NOS3 T-786C and NOS3 intron 4 VNTR polymorphisms in obese/non-obese groups are summarized in Table 3.32. When compared to obese stroke patients (26.8%), the wild type frequency of NOS3 G894T was found to be lower in obese controls (22.2%). Thirty eight obese stroke patients had heterozygote genotype and 3 obese stroke patients had homozygous mutated genotype. In addition 7 heterozygote individuals were found in obese

control group. We observed that among non-obese, 67 patients and 53 controls were wild type. The frequency of heterozygote genotype was higher in non-obese patients than that of control. Conversely the homozygote mutated genotype frequency was found to be lower in non-obese stroke patients when compared to non-obese controls. For the genotype distribution of NOS3 T-786C polymorphism, there was no statistically significant difference between patients and controls in obese/non-obese group. We observed that for NOS3 VNTR polymorphism, “aa” genotype frequency was found to be 1.8% in obese patients and 0.5% in non-obese patients, respectively. In obese group, the heterozygote “ab” genotype percentage was found to be lower in patients (23.2%) than that of controls (44.4%). When compared to non-obese stroke patients (30.7%), the “ab” heterozygote genotype frequency was found to be lower in non-obese controls (21.3%). The homozygote “bb” genotype frequency was higher in obese stroke patients (75.0%) than obese controls (55.6%). On the contrary non-obese stroke patients (68.8%) had lower “bb” genotype frequency than that of control (76.5%). In terms of genotype distributions of NOS3 G894T, NOS3 T-786C and NOS3 intron4 VNTR polymorphisms, there were no statistically significant difference both between obese stroke patients and controls and non-obese stroke patients and controls.

Table 3.32 NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotype frequencies in obese and non-obese groups.

Genotypes n(%)	Obese (n=65)			Non-obese (n=325)		
	Stroke (n=56)	Controls (n=9)	*P*	Stroke (n=189)	Controls (n=136)	*P*
NOS3 G894T						
GG	15 (26.8)	2 (22.2)		67 (35.5)	53 (39.0)	
GT	38 (67.9)	7 (77.8)	0.772[a]	118 (62.4)	73 (53.7)	0.517[a]
TT	3 (5.3)	0		4 (2.1)	10 (7.3)	
NOS3 T-786C						
TT	39 (69.6)	5 (55.6)		107 (56.6)	77 (56.6)	
TC	17 (30.4)	3 (33.3)	0.402[b]	76 (40.2)	54 (39.7)	1.000[b]
CC	0	1 (11.1)		6 (3.2)	5 (3.7)	
NOS3 VNTR						
aa	1 (1.8)	0		1 (0.5)	3 (2.2)	
ab	13 (23.2)	4 (44.4)	0.226[c]	58 (30.7)	29 (21.3)	0.126[c]
bb	42 (75.0)	5 (55.6)		130 (68.8)	104 (76.5)	

[a]TT+GT vs GG

[b]TC+CC vs TT

3.6 Effects of Conventional Vascular Risk Factors in Different CYP2E1, FMO3, NOS3, GSTM1, GSTT1, GSTP1 and NQO1 Genotypes of Ischemic Stroke Patients and Controls

In this section among CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NQO1*2, GSTM1, GSTT1, GSTP1, NOS3 G894T, NOS3 T-786C, and NOS3 VNTR genotypes the effects of conventional risk factors such as hypertension, diabetes, smoking and obesity on ischemic stroke were analyzed.

3.6.1 Effects of Conventional Vascular Risk Factors in CYP2E1*5B, CYP2E1*6 and CYP2E1*7B Genotypes of Ischemic Stroke Patients and Controls

Table 3.33 shows the effects of hypertension, diabetes, smoking and obesity together with CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotypes on the risk of stroke. In CYP2E1*5B genotypes group, the proportion of stroke patients to controls was found to be higher in hypertensive, diabetic, smoker and obese group than normotensive, non-diabetic, non-smoker and non-obese group. For CYP2E1*5B *1A*1A genotyped individuals, the risk of having stroke was 3 times higher in hypertensives when compared to normotensives. Similarly in diabetics, smokers and obese group *1A*1A genotype had 2 to 4-fold increased risk for stroke.

When Table 3.33 was examined in terms of CYP2E1*6 genotypes, in diabetics and smokers *1A*1A genotype had 2.5 times higher risk factor than non-diabetics and non-smokers. The risk ratio was increase up to 3.5 in hypertensives and also to 5.5 in obese group when compared to normotensives and non-obese group. For CYP2E1*7B genotypes, if the person had *1A*1A genotype the risk of having stroke was 2.7 times higher in hypertensives when compared to normotensives. On the other hand, hypertensives having *1A*7B genotype were found to be 8.6-fold higher statistically significant risk factor for stroke. Both diabetic (OR=2.481, *P*=0.000) and smoker (OR=2.019, *P*=0.017) groups had the higher risk of stroke for *1A*1A genotype when compared to non-diabetic and non-smoker groups. In addition, for individuals carrying *1A*1A genotype, the risk of having stroke is almost 4 times higher in obese group when compared to non-obese group (*P*=0.000).

Table 3.33 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotypes and stroke-control status.

Genotypes		Hypertensive	Normotensive	OR	*P*	Diabetic	Non-diabetic	OR	*P*	Smoker	Non-smoker	OR	*P*	Obese	Non-obese	OR	*P*
All	stroke	163	82	3.253	0.000	84	161	2.504	0.000	69	176	2.314	0.001	56	189	4.477	0.000
	control	55	90			25	120			21	124			9	136		
CYP2E1*5B																	
*1A*1A	stroke	152	80	3.074	0.000	79	153	2.457	0.000	65	167	2.413	0.001	54	178	4.550	0.000
	control	55	89			25	119			20	124			9	135		
*1A*5B	stroke	11	2	NA	NA	5	8	NA	NA	4	9	NA	NA	2	11	NA	NA
	control	0	1			0	1			1	0			0	1		
*5B*5B	stroke	0	0	NA	NA	0	0	NA	NA	0	0	NA	NA	0	0	NA	NA
	control	0	0			0	0			0	0			0	0		
CYP2E1*6																	
*1A*1A	stroke	142	70	3.527	0.000	74	138	2.534	0.000	58	154	2.589	0.001	52	160	5.525	0.000
	control	46	80			22	104			16	110			7	119		
*1A*6	stroke	18	11	1.818	0.315	10	19	2.807	0.154	9	20	1.260	0.725	3	26	0.980	1.000
	control	9	10			3	16			5	14			2	17		
*6*6	stroke	3	1	NA	NA	0	4	NA	NA	2	2	NA	NA	1	3	NA	NA
	control	0	0			0	0			0	0			0	0		
CYP2E1*7B																	
*1A*1A	stroke	138	72	2.798	0.000	71	139	2.481	0.000	54	156	2.019	0.017	47	163	4.144	0.000
	control	50	73			21	102			18	105			8	115		
*1A*7B	stroke	23	8	8.625	0.000	12	19	3.578	0.069	13	18	4.092	0.043	8	23	6.608	0.057
	control	5	15			3	17			3	17			1	19		
*7B*7B	stroke	2	2	NA	NA	1	3	0.333	0.540	2	2	NA	NA	1	3	NA	NA
	control	0	2			1	1			0	2			0	2		

Note: Values are number of subjects. Comparisons are by chi-square test.
OR: odds ratio. NA: Not applicable.

3.6.2 Effects of Conventional Vascular Risk Factors in FMO3 G472A and FMO3 A923G Genotypes of Ischemic Stroke Patients and Controls

As shown in Table 3.34, among FMO3 G472A genotypes, risk of ischemic stroke in hypertensives was found to be highest within GA genotype when compared to normotensives (OR=6.110, *P*=0.000). Moreover, in hypertensives (OR=4.261, *P*=0.000) and diabetics, AA genotype (OR=2.763, *P*=0.038) was found to be significant risk factor for ischemic stroke. We found that both in smokers and obese individuals, AA genotype had same risk for ischemic stroke. On the other hand, in GG genotype group, the risk of having stroke was 2.7 times higher in smokers when compared to non-smokers. For obesity, GG (OR=6.222, *P*=0.001) and AA (OR=3.620, *P*=0.020) genotypes were found to be significant risk factors of stroke.
For FMO3 A923G genotypes, hypertensives having AA and AG genotypes had 3-fold and 6-fold greater risk of stroke than normotensives, respectively. While AA genotype was found to be 2.6 and 2.3 times higher risk of stroke in diabetics and smokers, this ratio was increased up to 4.8-fold in obese group.

Table 3.34 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to FMO3 G472A and FMO3 A923G genotypes and stroke-control status.

Genotypes		Hypertensive	Normotensive	OR	*P*	Diabetic	Non-diabetic	OR	*P*	Smoker	Non-smoker	OR	*P*	Obese	Non-obese	OR	*P*
All	stroke	163	82	3.253	0.000	84	161	2.504	0.000	69	176	2.314	0.001	56	189	4.477	0.000
	control	55	90			25	120			21	124			9	136		
FMO3 G472A																	
GG	stroke	66	43	1.830	0.066	40	69	2.424	0.021	37	72	2.740	0.013	28	81	6.222	0.001
	control	26	31			11	46			9	48			3	54		
GA	stroke	41	16	6.110	0.000	20	37	2.432	0.059	7	50	0.886	0.840	7	50	2.940	0.176
	control	13	31			8	36			6	38			2	42		
AA	stroke	56	23	4.261	0.000	24	55	2.763	0.038	25	54	2.932	0.027	21	58	3.620	0.020
	control	16	28			6	38			6	38			4	40		
FMO3 A923G																	
AA	stroke	142	72	3.016	0.000	79	135	2.696	0.000	62	152	2.361	0.002	52	162	4.854	0.000
	control	51	78			23	106			19	110			8	121		
AG	stroke	20	10	6.000	0.007	4	26	1.076	1.000	7	23	2.130	0.377	4	26	2.307	0.462
	control	4	12			2	14			2	14			1	15		
GG	stroke	1	0	NA	NA	1	0	NA	NA	0	1	NA	NA	0	1	NA	NA
	control	0	0			0	0			0	0			0	0		

Note: Values are number of subjects. Comparisons are by chi-square test.
OR: odds ratio. NA: Not applicable.

3.6.3 Effects of Conventional Vascular Risk Factors in NQO1*2 Genotypes of Ischemic Stroke Patients and Controls

In each genotype group of NQO1*2 polymorphism, the proportion of stroke patients to controls was increased in hypertensives when compared to normotensives. As seen in Table 3.35, both *1*1 (OR=3.103, *P*=0.000) and *1*2 (OR=3.469, *P*=0.000) genotypes were found to be significant risk factor of stroke in hypertensives. Among diabetics, for *1*1 genotype, the risk of having stroke was 2.9 times higher than non-diabetics (*P*=0.001). *1*2 genotype had 5-fold increased risk of stroke in smoker subjects when compared to non-smoker subjects (*P*=0.000). While *1*1 genotype had 4.2-fold greater risk in obese people than non-obese people (*P*=0.002), *1*2 genotype had approximately 4.7-fold increased risk of stroke in obese individuals (*P*=0.003).

Table 3.35 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to NQO1*2 genotypes and stroke-control status.

Genotypes		Hypertensive	Normotensive	OR	*P*	Diabetic	Non-diabetic	OR	*P*	Smoker	Non-smoker	OR	*P*	Obese	Non-obese	OR	*P*
All	stroke	163	82	3.253	0.000	84	161	2.504	0.000	69	176	2.314	0.001	56	189	4.477	0.000
	control	55	90			25	120			21	124			9	136		
NQO1*2																	
*1*1	stroke	102	51	3.103	0.000	56	97	2.982	0.001	38	115	1.299	0.445	36	117	4.246	0.002
	control	29	45			12	62			15	59			5	69		
*1*2	stroke	55	26	3.469	0.000	25	56	1.820	0.123	27	54	5.000	0.000	19	62	4.750	0.003
	control	25	41			13	53			6	60			4	62		
*2*2	stroke	6	5	4.800	0.196	3	8	NA	NA	4	7	NA	NA	1	10	NA	NA
	control	1	4			0	5			0	5			0	5		

Note: Values are number of subjects. Comparisons are by chi-square test.
OR: odds ratio. NA: Not applicable.

3.6.4 Effects of Conventional Vascular Risk Factors in GSTM1, GSTT1 and GSTP1 A313G Genotypes of Ischemic Stroke Patients and Controls

Table 3.36 shows the effects of hypertension, diabetes, smoking and obesity together with GSTM1, GSTT1 and GSTP1 genotypes on the risk of stroke. For GSTM1 genotypes while present genotype had 2.4-fold greater risk, null genotype had 3.8 times higher risk in hypertensives when compared to normotensives. When we examined diabetic people in the GSTM1 present genotype group, the risk of having stroke was more than 3.2-fold higher in diabetics when compared to non-diabetics (P=0.005). Moreover, in diabetics, null genotype had 2 times greater risk of stroke than non-diabetics (P=0.014). For smokers, GSTM1 present genotype with 3.150 odds ratio was found to be significant risk factor (P=0.013). Among obese people with GSTM1 present genotype, the risk of stroke was significantly higher than non-obese people (OR=8.068, P=0.001). On the other hand, GSTM1 null genotype was found to be 3.4-times significant risk factor in obese group when compared to non-obese group (P=0.003). GSTT1 present and null genotypes had 2.7-fold and 5.5-fold greater risk of stroke in hypertensives when compared to normotensives, respectively. In the diabetics, only GSTT1 present genotype was found to be significant risk factor (OR=2.518, P=0.001). In addition, GSTT1 present genotype had 2.1 times higher risk in smokers compared to non-smokers (P=0.022). In obese individuals, the risk of having stroke was higher both in the GSTT1 present and null genotypes than non-obese individuals (for present genotype OR=4.812, P=0.000, for null genotype OR=3.961, P=0.014). When Table 3.36 was examined in terms of GSTP1 A313G genotypes, AA, AG and GG genotypes had 3.5-, 2.8- and 3.5- fold significantly greater risk of stroke in hypertensives when compared to normotensives, respectively. Both in diabetics and smokers, only AG genotype was found to be significant risk factor for stroke (for diabetics OR=3.808, P=0.001; for smokers OR=2.576, P=0.039). Among obese subjects, for AA genotype the risk of having stroke was 5 times higher than non-obese subjects (P=0.001). Similarly AG genotype had 3.6-fold greater risk in obese people than non-obese people (P=0.020).

Table 3.36 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to GSTM1, GSTT1 and GSTP1 A313G genotypes and stroke-control status.

Genotypes		Hypertensive	Normotensive	OR	*P*	Diabetic	Non-diabetic	OR	*P*	Smoker	Non-smoker	OR	*P*	Obese	Non-obese	OR	*P*
All	stroke	163	82	3.253	0.000	84	161	2.504	0.000	69	176	2.314	0.001	56	189	4.477	0.000
	control	55	90			25	120			21	124			9	136		
GSTM1																	
Present	stroke	77	36	2.467	0.006	39	74	3.162	0.005	31	82	3.150	0.013	26	87	8.068	0.001
	control	26	30			8	48			6	50			2	54		
Null	stroke	86	46	3.868	0.000	45	87	2.190	0.014	38	94	1.994	0.041	30	102	3.445	0.003
	control	29	60			17	72			15	74			7	82		
GSTT1																	
Present	stroke	116	61	2.746	0.000	61	116	2.518	0.001	44	133	2.095	0.022	33	144	4.812	0.000
	control	45	65			19	91			15	95			5	105		
Null	stroke	47	21	5.595	0.000	23	45	2.470	0.074	25	43	2.810	0.039	23	45	3.961	0.014
	control	10	25			6	29			6	29			4	31		
GSTP1 A313G																	
AA	stroke	88	45	3.535	0.000	38	95	1.685	0.137	37	96	1.959	0.066	30	103	5.024	0.001
	control	26	47			14	59			12	61			4	69		
AG	stroke	59	29	2.809	0.004	37	51	3.808	0.001	26	62	2.576	0.039	21	67	3.604	0.020
	control	21	29			8	42			7	43			4	46		
GG	stroke	16	8	3.500	0.039	9	15	3.800	0.065	6	18	3.333	0.155	5	19	5.526	0.101
	control	8	14			3	19			2	20			1	21		

Note: Values are number of subjects. Comparisons are by chi-square test.
OR: odds ratio. NA: Not applicable.

3.6.5 Effects of Conventional Vascular Risk Factors in NOS3 G894T, NOS3 T-786C and NOS3 VNTR Genotypes of Ischemic Stroke Patients and Controls

The effect of hypertension, diabetes, smoking and obesity together with NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotypes on the ischemic stroke risk is given in Table 3.37. When compared to normotensives, in hypertensives the risk of having stroke was found to be 2.193 times and 4.535 times higher for NOS3 G894T GG and GT genotypes, respectively. On the other hand, GG genotype had 2.6-fold and 4.2-fold greater stroke risk in diabetics and smokers than non-diabetics and non-smokers. We observed that the person carrying GG genotype together with obesity had approximately 6-fold increase risk for stroke (*P*=0.010). In addition, GT genotyped obese people were found to be 3 times more likely to stroke than non-obese people (*P*=0.003). For NOS3 T-786C genotypes, in hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker and obese/non-obese groups, TT and TC genotypes were determined as a significant risk factor of stroke. As seen in Table 3.37, the probability of having stroke varied from 2 to 5 times for TT genotype in the conventional risk factors groups except smokers. In diabetics and smokers, the TC genotype had 2.4- and 2.9- fold greater risk for stroke than non-diabetics and non-smokers, respectively. We found that among people with TC genotype, hypertension and obesity significantly increase the risk of having stroke (for hypertension OR=3.600, *P*=0.000; for obesity OR=4.026, *P*=0.022). When NOS3 VNTR "bb" genotype was taken into consideration, in the hypertensives, the risk of having stroke was 4-fold higher than normotensives (P=0.000). On the other hand, "bb" genotype was found to be significant risk factor in diabetic (OR=3.034, *P*=0.000) and obese (OR=6.720, *P*=0.000) groups when compared to non-diabetic and non-obese groups. In the smokers "ab" genotype had 3.9-fold significantly greater risk of stroke than non-smokers, respectively.

Table 3.37 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotypes and stroke-control status.

Genotypes		Hypertensive	Normotensive	OR	*P*	Diabetic	Non-diabetic	OR	*P*	Smoker	Non-smoker	OR	*P*	Obese	Non-obese	OR	*P*
All	stroke	163	82	3.253	0.000	84	161	2.504	0.000	69	176	2.314	0.001	56	189	4.477	0.000
	control	55	90			25	120			21	124			9	136		
NOS3 G894T																	
GG	stroke	53	29	2.193	0.026	31	54	2.607	0.018	28	54	4.234	0.002	15	67	5.932	0.010
	control	25	30			10	45			6	49			2	53		
GT	stroke	107	49	4.535	0.000	51	105	1.938	0.052	39	117	1.717	0.124	38	118	3.358	0.003
	control	26	54			14	66			13	67			7	73		
TT	stroke	3	4	1.125	1.000	2	5	3.600	0.322	2	5	1.600	0.682	3	4	NA	NA
	control	4	6			1	9			2	8			0	10		
NOS3 T-786C																	
TT	stroke	98	48	3.358	0.000	52	94	2.226	0.015	40	106	1.832	0.078	39	107	5.613	0.000
	control	31	51			15	67			14	68			5	77		
TC	stroke	63	30	3.600	0.000	29	64	2.472	0.037	27	66	2.922	0.017	17	76	4.026	0.022
	control	21	36			8	49			7	50			3	54		
CC	stroke	2	4	0.500	0.558	3	3	2.000	0.558	2	4	NA	NA	0	6	NA	NA
	control	3	3			2	4			0	6			1	5		
NOS3 VNTR																	
aa	stroke	1	1	2.000	0.709	1	1	2.000	0.709	1	1	NA	NA	1	1	NA	NA
	control	1	2			1	2			0	3			0	3		
ab	stroke	42	29	1.737	0.191	24	47	1.595	0.325	25	46	3.940	0.014	13	58	1.625	0.426
	control	15	18			8	25			4	29			4	29		
bb	stroke	120	52	4.142	0.000	59	113	3.034	0.000	43	129	1.804	0.061	42	130	6.720	0.000
	control	39	70			16	93			17	92			5	104		

Note: Values are number of subjects. Comparisons are by chi-square test.
OR: odds ratio. NA: Not applicable.

3.7 Logistic Regression Analysis

In order to determine the effects of vascular factors, lipid parameters, and CYP2E1, FMO3, NQO1, GSTP1, GSTM1, GSTT1, NOS3 genotypes in the prediction of ischemic stroke were evaluated by using logistic regression analysis with backward selection method. Different combinations of parameters in the overall study population or in different subgroups were used to set up different binary logistic regression models.

Model 1

Age, sex, hypertension, smoking status, diabetes, obesity, lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes were added as covariates in model 1 and logistic regression analysis showed that hypertension (OR=2.847, 95%CI=1.735-4.674, *P*=0.000), smoking (OR=2.723, 95%CI=1.474-5.032, *P*=0.001), obesity (OR=2.887, 95%CI=1.302-6.402, *P*=0.009), LDL-cholesterol (OR=1.362, 95%CI=1.060-1.750, *P*=0.016) to be the strongest determinants of ischemic stroke (Table 3.38). On the other hand HDL-cholesterol (OR=0.300, 95%CI=0.130-0.693, *P*=0.005) and NQO1 heterozygote (*1*2) genotype (OR=0.562, 95%CI=0.348-0.908, *P*=0.018) were found to be preventive factors for ischemic stroke. The model predicted 69.5% of cases correctly and Hosmer-Lemeshow goodness of fit test pointed out that the calibration of the model was satisfactory ($\chi 2$=2.7; 8 degrees of freedom; *P*=0.952).

Table 3.38 Logistic regression analysis of vascular risk factors (age, sex, hypertension, smoking status, diabetes, obesity), lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes (Model 1)

Parameters	**OR**	**95% CI**	***P***
Hypertension	2.847	1.735-4.674	0.000
Smoking	2.723	1.474-5.032	0.001
Obesity	2.887	1.302-6.402	0.009
LDL-cholesterol	1.362	1.060-1.750	0.016
HDL-cholesterol	0.300	0.130-0.693	0.005
NQO1*1*2	0.562	0.348-0.908	0.018

Model 2

In model 2 only elderly (aged>59 years) subjects were used for logistic regression analysis. Covariates were chosen same as model 1, except that age was not included. According to results hypertension (OR=2.685, 95%CI=1.407-5.124, *P*=0.003), smoking (OR=3.873, 95%CI=1.529-9.815, *P*=0.004), obesity (OR=2.706, 95%CI=1.081-6.770, *P*=0.033), LDL-cholesterol (OR=3.619, 95%CI=1.450-9.031, *P*=0.006) were found to be the strongest determinants of ischemic stroke (Table 3.39). In addition NQO1 heterozygote (*1*2) genotype (OR=0.483, 95%CI=0.266-0.878, *P*=0.017) had preventive effect against ischemic stroke. The model predicted 72.3% of cases correctly and Hosmer-Lemeshow goodness of fit test pointed out that the calibration of the model was satisfactory ($\chi 2$=7.772; 8 degrees of freedom; *P*=0.456).

Table 3.39 Logistic regression analysis of vascular risk factors (sex, hypertension, smoking status, diabetes, obesity), lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes in elderly (aged>59) group (Model 2)

Parameters	**OR**	**95% CI**	***P***
Hypertension	2.685	1.407-5.124	0.003
Smoking	3.873	1.529-9.815	0.004
Obesity	2.706	1.081-6.770	0.033
LDL-cholesterol	3.619	1.450-9.031	0.006
NQO1*1*2	0.483	0.266-0.878	0.017

Model 3

In this model only younger **(**aged<60 years) subjects were analyzed and sex, hypertension, smoking status, diabetes, obesity, lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes were added as covariates. In this case the strongest determinants of stroke such as smoking, obesity, diabetes, LDL-cholesterol were not found to be effective. On the other hand logistic regression analysis revealed hypertension (OR=3.992, 95%CI=1.492-10.682, *P*=0.006) and total cholesterol (OR=1.597, 95%CI=1.098-2.325, *P*=0.014) as significant predictors of stroke (Table 3.40). We observed that HDL- cholesterol was found to have protective effects. 69.1% of cases were predicted correctly by the model and the calibration was satisfactory ($\chi 2$=13.552; 8 degrees of freedom; *P*=0.094).

Table 3.40 Logistic regression analysis of vascular risk factors (sex, hypertension, smoking status, diabetes, obesity), lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes in younger (aged<60) group (Model 3)

Parameters	**OR**	**95% CI**	***P***
Hypertension	3.992	1.492-10.682	0.006
Total cholesterol	1.597	1.098-2.325	0.014
HDL-cholesterol	0.168	0.035-0.797	0.025

Model 4

Within female subjects when age, hypertension, smoking status, diabetes, obesity, lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes were selected as covariates (Table 3.41) for model 4, hypertension, diabetes, smoking status and LDL-cholesterol were found to be significant determinants of stroke. As the model 1 and model 3 HDL-cholesterol had protective effect against stroke and also NOS3 894TT genotype had protective effect against stroke in model 4. The model correctly predicted 74.4% of the cases and the Hosmer-Lemeshow goodness of fit test demonstrated that the calibration of the model was satisfactory ($\chi 2$=6.614; 8 degrees of freedom; *P*=0.579) for logistic regression.

Table 3.41 Logistic regression analysis of vascular risk factors (age, hypertension, smoking status, diabetes, obesity), lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes in female group (Model 4)

Parameters	**OR**	**95% CI**	***P***
Hypertension	5.432	2.411-12.242	0.000
Diabetes mellitus	3.502	1.400-8.759	0.007
Smoking	9.406	2.131-41.506	0.003
LDL-cholesterol	2.329	1.483-3.659	0.000
HDL-cholesterol	0.130	0.033-0.506	0.003
NOS3 894TT	0.017	0.001-0.334	0.007

Model 5

In model 5 the same analysis that analyzed in model 4 was repeated in a male group. Hypertension and obesity was found to be strongest determinants of stroke (Table 3.42). NQO1 *1*2 heterozygote genotype and HDL-cholesterol was found to be significant protector of stroke. 72.9% of cases were predicted correctly by the model and the calibration was satisfactory ($\chi 2$=2.891; 8 degrees of freedom; *P*=0.941).

Table 3.42 Logistic regression analysis of vascular risk factors (age, hypertension, smoking status, diabetes, obesity), lipid parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NOS3 G894T, NOS3 T-786C, NOS3 VNTR, GSTM1, GSTT1, GSTP1 A313G and NQO1*2 genotypes in male group (Model 5)

Parameters	**OR**	**95% CI**	***P***
Hypertension	2.981	1.535-5.789	0.001
Obesity	6.138	1.349-27.935	0.019
HDL-cholesterol	0.302	0.094-0.971	0.044
NQO1*1*2	0.463	0.239-0.898	0.023

CHAPTER 4

DISCUSSION

Stroke, being the third leading cause of death in the world, is described as interruption or severe reduction of blood flow in cerebral arteries (Sarti *et al*., 2000; Crack and Taylor, 2005). Carotid stenosis, resulting from atherosclerosis, is a risk factor for stroke (Nagai *et al*., 2001). Oxidative stress, the condition occurring when the physiological balance between oxidants and antioxidants is disrupted, plays an important role in the pathogenesis of atherosclerosis, cancer and diabetes. Indeed, production of reactive oxygen species is increased in ischemic stroke, leading to oxidative stress that contributes to brain damage (Crack and Taylor, 2005; Alexandrova *et al*., 2004).

Oxidative stress contributes to the initiation and development of stroke via different interrelated mechanisms such as excitotoxicity resulting in cellular enzyme activation and ROS generation, mitochondrial dysfunction accompanied by excessive radical production, activation and oxidative damage of endothelium resulting in reduced bioavailability of nitric oxide, lipid peroxidation of plasma and cellular components including those in the arterial vessel wall.

ROS is very important in the development of oxidative stress condition. As mentioned above in biological systems, equilibrium normally exists between ROS generation and antioxidant capacity of the organism. At high rates of radical generation, the antioxidant capacity may be insufficient to compensate for the increase in ROS and to reset the original balance and oxidative stress may occur. There are various polymorphic enzymes that play role in the production and

elimination of ROS. These polymorphisms are important either in increase or decrease enzyme activity. In this study, it is proposed that greatly reduced activity of ROS detoxifying enzymes or induced activity of ROS producing enzymes due to genetic polymorphisms increases oxidative stress which may contribute to ischemic stroke. In this context, understanding the effect of polymorphisms of enzymes playing role in either in the production (like CYP2E1, FMO3 and NOS3) or elimination (like GST and NQO1) of reactive oxygen species in ischemic stroke development is of crucial importance. Therefore, in present study, it was aimed to study three genetic polymorphisms of CYP2E1, two genetic polymorphisms of FMO3, one genetic polymorphism of NQO1, three genetic polymorphisms of GST and three genetic polymorphisms of NOS3 enzymes as risk factors for ischemic stroke. In addition, roles of conventional risk factors such as gender, hypertension, smoking, diabetes mellitus, obesity and lipid parameters such as total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol were also evaluated.

Demographic characteristics and lipid parameters

The risk factors of ischemic stroke were given in details in the chapter 1, section 1.1.2. Stroke is a late onset disorder and age is one of the most significant determinants of stroke (McGruder *et al*., 2004; Wolfe, 2000). In the present study, there was no difference in mean age of patients (64.5±13.3) and controls (62.1±14.1, P=0.061). One of the most difficult parts of this study was to create control group. Because there were lots of criteria (Chapter 2 section 2.1.1) in order to select control subjects and it was very difficult to find any elderly individual who carrying all of these criteria. In order to minimize the effect of age on results and to make meaningful conclusions, the mean age of patients and controls were matched.

The other non-modifiable risk factor of stroke is male gender. Alter *et al*. (1986), Boysen *et al*. (1988), Michel *et al*. (2010) were reported that in most age groups male gender had higher stroke incidence than female gender. In this study 141 male and 104 female subjects were found in the patient group. Males had 1.3 times greater risk of stroke in the present study. The male gender prevalence of patients and controls

were 57.6% and 50.3%, respectively. There was not a statistically significant difference between the patient and control groups with respect to gender (*P*=0.167).

Hypertension (Fiebach *et al*., 1989; Jamrozik *et al*., 1994), diabetes mellitus (Bell, 1994; Stegmayr and Asplund, 1995; Goldstein *et al*., 2001) and smoking (Wolf *et al*., 1988) are modifiable risk factors of stroke. Among risk factors, the most important one is hypertension for stroke and controlling hypertension reduces by 40% the risk of first or recurrent stroke (Lawes *et al.*, 2004). In the previous studies carried out in our laboratory, hypertension and diabetes mellitus were found to be significant determinants of ischemic stroke (Can Demirdöğen *et al*., 2008; Can Demirdöğen *et al*., 2009; Türkanoğlu *et al*., 2010). In this study obesity (OR=4.477, *P*=0.000) and smoking (OR=2.315, *P*=0.001) in addition to hypertension (OR=3.253, *P*=0.000) and diabetes mellitus (OR=2.504, *P*=0.000) were also found to be significant risk factors for stroke. Our findings were consistent with Kumral *et al*.'s (1998) study reported that in Turkish population hypertension, diabetes and smoking increase the risk of stroke.

The Prospective Study Collaboration group did meta analysis of data from 45 observational cohorts in 1995. This study comprised of 450.000 individuals in which 13.397 fatal strokes. And they reported that there was no association between total cholesterol concentration and risk of fatal stroke (Prospective studies collaboration, 1995). According to another meta-analysis study results cholesterol concentration was found to be associated with fatal ischemic stroke before the age of 70 years; however, after 70 years no association was found (Lewington *et al.*, 2007). The stroke subtypes could be important to find out association between lipid parameters and stroke risk. Because there are different mechanisms that underlying the pathogenesis stroke subtypes. Iso *et al*. (1989) showed that the association of cholesterol with risk of ischemic stroke in their study. In the same study, the inverse relationship was seen between cholesterol and hemorrhagic stroke. There were contradictory data about cholesterol for risk of stroke in the literature. In present study, total cholesterol levels of patients (4.8±1.3) was found to be higher than controls (4.6±1.2, *P*=0.112) but this difference was not statistically significant.

Triglyceride level was found to be significant predictor of stroke and coronary heart disease in the studies conducted by Labreuche *et al.* (2009) and Tanne *et al*. (2001).

In addition, according to a study performed in the Asia-Pacific region serum triglycerides were important and independent predictor of coronary heart disease and stroke (Patel *et al.,* 2004). On the other hand, our results do not support these findings. In our study, there was no statistically significant difference with respect to triglyceride levels between patients (1.4±0.2) and controls (1.3±0.2, *P*=0.174).

Oxidized LDL is an important mediator for atherosclerosis. Aviram (1993) reported that inhibition of LDL modifications may prevent the development of atherosclerotic lesion. Inhibition of oxidation of LDL by HDL is a known fact today. HDL is known as a protective factor against atherosclerosis which is important step in the pathogenesis of stroke. High levels of HDL-cholesterol were shown as a significant protective factor of stroke and coronary heart disease (Wannamethee *et al.,* 2000; Tanne *et al.,* 2001, Türkanoğlu *et al.,* 2010). We expected to see same results in present study. HDL-cholesterol transport cholesterol to the liver for excretion or reutilization, therefore it is known as good cholesterol. In this study HDL-cholesterol levels of patients (1.1±0.3) were significantly lower than controls (1.2±0.3; *P*=0.001). Also, our findings promote the protective effect of HDL-cholesterol against ischemic stroke.

Several clinical studies shown that intensive lowering of LDL-cholesterol levels in patients at risk of cardiovascular disease (CVD) decreases the further risk of CVD (Sever *et al.,* 2003; Cannon *et al.,* 2004; LaRosa *et al.,* 2005). In addition, a recent meta-analysis shown that intensive lowering of LDL-cholesterol cause 16% reduction in ischemic stroke but no significant results were obtained for hemorrhagic stroke (Cholesterol Treatment Trialists' (CTT) Collaboration, 2010). In this study, ischemic stroke patients (2.9±1.0) had significantly higher LDL-cholesterol level than controls (2.7±1.0; *P*=0.007) as expected.

Genotype Distributions of CYP2E1, FMO3, NQO1, GSTP1, GSTM1, GSTT1 and NOS3 Polymorphisms in Turkish Population and Other Ethnicities

Genetic polymorphisms show variability in different ethnicities and also the same gene frequency could be different in the same population. In these aspects determination and comparison of polymorphism frequencies in different populations

is of crucial importance. There are lots of population studies on genetic polymorphism frequencies in different populations and also genetic epidemiological studies provide information about the frequencies of genetic polymorphism of healthy subjects in various populations.
In the present study, 145 healthy subjects were used to determine 12 different genetic polymorphisms frequencies in Turkish population and results obtained were compared with other Turkish population studies and different ethnic groups' studies.

The comparison of CYP2E1 genotype distributions in Turkish population with different ethnic groups

Table 4.1 shows the comparison of genotype and allele frequencies distributions of CYP2E1*5B polymorphism in different populations. The result of genotype distribution of CYP2E1*5B polymorphism of Turkish population was found to be very similar when compared to other Turkish population studies. While the *5B variant allele frequency was found to be 0.004 in the present study, the same allele frequency was reported to be 0.019 (Ömer *et al*., 2001; Ulusoy *et al*., 2007b), 0.021 (Aydın-Sayitoglu *et al*., 2006) within Turkish population. There are a number of CYP2E1*5B polymorphism studies that conducted in different regions of Asian continent. Similar genotype frequencies for this polymorphism were reported for Chinese, Japanese, Taiwanese and Kazakh populations. However, these genotype frequencies were found to be different from Turkish population. The mutant *5B allele frequency was ranging between 0.201-0.280 in Asian populations. Our genotype distribution of CYP2E1*5B polymorphism was very similar with European Caucasian populations like Swedish, German, Italian, Spanish and French. The prevalence of *5B*5B mutant genotype frequency was very low in these European populations and the *5B variant allele frequency was between 0.023-0.053 in these populations. CYP2E1*5B genotype distribution of American populations was different from Turkish population. The mutant allele frequency in control individuals in this study (0.004) was lower than in most of the American populations, including African-American, European-American, Mexican-American, Brazilian and Chilean. This allele frequency was ranging between 0.010-0.155 in these populations.

Table 4.1 Comparison of the genotype and allele frequencies distributions of CYP2E1*5B polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	*1A*1A (c1c1)	*1A*5B (c1c2)	*5B*5B (c2c2)	*1A (c1)	*5B (c2)	Reference
Turkish (This study)	145	99.3	0.7	0	0.9965	0.0035	
Turkish	153	96.1	3.9	0	0.9805	0.0195	Ömer *et al.*, 2001
Turkish	140	95.7	4.3	0	0.9785	0.0215	Aydın-Sayitoglu *et al.*, 2006
Turkish	207	96.1	3.9	0	0.9805	0.0195	Ulusoy *et al.*, 2007b
Asian							
Chinese	122	51.6	43.5	4.9	0.7335	0.2665	Persson *et al.*, 1999
Chinese	196	61.7	31.6	6.6	0.7750	0.2240	Gao *et al.*, 2002
Chinese	181	53.6	41.4	5	0.7430	0.2570	Wang *et al.*, 2003
Japanese	612	63.9	32	4.1	0.7990	0.2010	Oyama *et al.*, 1997
Japanese	196	61.2	34.7	4.1	0.7855	0.2145	Ogawa *et al.*, 2003
Kazakh	240	53.3	37.5	9.2	0.7205	0.2795	Qin *et al.*, 2008
Taiwanase	120	67	32	12	0.8300	0.2800	Stephens *et al.*, 1994
Taiwanase	320	61.8	35.4	2.8	0.7950	0.2050	Hildesheim *et al.*, 1995
Taiwanase	231	58	35.1	6.9	0.7555	0.2445	Wang *et al.*, 1999
European							
French	172	91.6	4.7	0	0.9395	0.0235	Bouchardy *et al.*, 2000
German	373	94.3	5.7	0	0.9715	0.0285	Brockmöller *et al.*, 1996
German	297	94.9	4.4	0.7	0.9710	0.0290	Neuhaus *et al.*, 2004
Italian	114	91	9	0	0.9550	0.0450	Ingelman-Sundberg *et al.*, 1993
Spanish	390	96.1	5.9	0	0.9905	0.0295	Bolufer *et al.*, 2007
Swedish	148	90	9.4	0.6	0.9470	0.0530	Persson *et al.*, 1993
American							
African-American	126	98	2	0	0.9900	0.0100	Stephens *et al.*, 1994
African-American	114	86.8	12.3	0.9	0.9295	0.0705	Wu *et al.*, 1997
Brazilian	221	89.1	10.4	0.5	0.9430	0.0570	Canalle *et al.*, 2004
Brazilian	191	90	9	1	0.9450	0.0550	Nishimoto *et al.*, 2000
Chilean	148	71	27	2	0.8450	0.1550	Quinones *et al.*, 2001
European-American	449	92	7	1	0.9550	0.0450	Stephens *et al.*, 1994
Mexican-American	92	70.7	28.3	1	0.8485	0.1515	Wu *et al.*, 1997

Table 4.2 represents the comparison of genotype and allele frequencies distributions of CYP2E1*6 polymorphism in Turkish and other populations. As seen in Table 4.2, three different studies reported the genotype distributions of CYP2E1*6 polymorphism in Turkish population and similar results were obtained from all of these studies. While the frequency of *6 variant allele was found to be 0.066 in the present study, the same allele frequency was reported to be 0.077 (Kayaaltı *et al.*,

2010), 0.083 (Ulusoy *et al*., 2007b) and 0.082 (Ömer *et al*., 2001) within Turkish population.

Table 4.2 Comparison of the genotype and allele frequencies distributions of CYP2E1*6 polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	*1A*1A (DD)	*1A*6 (DC)	*6*6 (CC)	*1A (D)	*6 (C)	Reference
Turkish (This study)	145	86.9	13.1	0	0.9345	0.0655	
Turkish	163	85.3	14.1	0.6	0.9235	0.0765	Kayaaltı *et al.,* 2010
Turkish	207	84	15.5	0.5	0.9175	0.0825	Ulusoy *et al.,* 2007b
Turkish	153	84.3	15	0.7	0.9180	0.0820	Ömer *et al.,* 2001
Asian							
Chinese	122	48.4	46.7	4.9	0.7175	0.2825	Persson *et al.,* 1999
Chinese Han	103	55.3	36.9	7.8	0.7375	0.2625	Wang *et al.,* 2009
Japanese	76	56.6	28.9	14.5	0.7105	0.2895	Uematsu *et al.,* 1994
Kazakh	107	72	27.1	0.9	0.8555	0.1445	Wang *et al.,* 2009
North Indians	227	64.8	32.2	3	0.8090	0.1910	Mittal *et al.,* 2005
Taiwanase	119	55	43	3	0.7650	0.2450	Stephens *et al.,* 1994
Taiwanase	320	57.2	38.4	4.4	0.7640	0.2360	Hildesheim *et al.,* 1995
Uygur	149	66.4	29.6	4	0.8120	0.1880	Wang *et al.,* 2009
European							
British	155	83.2	16.1	0.7	0.9125	0.0875	Yang *et al.,* 2001
Caucasian	1360	85.4	13.8	0.8	0.9230	0.0770	Garte *et al.,* 2001
French	172	87.8	11.6	0.6	0.9360	0.0640	Bouchardy *et al.,* 2000
German	373	87.3	12.4	0.3	0.9350	0.0650	Brockmöller *et al.,* 1996
German	236	83.1	16.5	0.4	0.9135	0.0865	Neuhaus *et al.,* 2004
Italian	114	83	17	0	0.9150	0.0850	Ingelman-Sundberg *et al.,* 1993
Swedish	152	81	18.4	0.6	0.9020	0.0980	Persson *et al.,* 1993
American							
African-American	114	84	16	1	0.9200	0.0900	Stephens *et al.,* 1994
Brazilian	251	86.9	12.7	0.4	0.9325	0.0675	Rossini *et al.,* 2006
Chilean	129	63.6	31	5.4	0.7910	0.2090	Quinones *et al.,* 2001
European-American	142	80	19	1	0.8950	0.1050	Stephens *et al.,* 1994
Mexican	104	72.1	24	3.9	0.8410	0.1590	Konishi *et al.,* 2003

Different genotype distribution of CYP2E1*6 polymorphism was observed between Turkish population and Asian populations. The mutant *6 allele frequency was higher in Asian populations than Turkish population. European populations had same genotype distributions with Turkish population. The *6 mutant allele frequency ranged between 0.064-0.098 in European populations as reported in Table 4.2. Just as Asian populations, American populations had different genotype distribution when compared to Turkish population. They had higher *6 mutant allele frequency than Turkish population.

Table 4.3 shows the genotype and allele frequencies distributions of CYP2E1*7B polymorphism in Turkish population and other populations including British, German and Swedish. There were limited number of studies about CYP2E1*7B polymorphism frequencies of different populations in the literature. In the present study, the genotype frequencies of CYP2E1*7B polymorphism were found as 84.8% *1A*1A, 13.8% *1A*7B and 1.4% *7B*7B in the healthy subjects. These were similar to the results obtained for other Turkish population studies (Ulusoy *et al*., 2007b; Kayaaltı *et al*., 2010). In addition, these results were consistent with the European populations' studies. The mutant *7B allele frequency was found to be 0.052, 0.072, 0.037 and 0.041 in British (Yang *et al*., 2001), German (Thier *et al*., 2002), German (Neuhaus *et al*., 2004) and Swedish (Ernstgard *et al*., 2004) populations, respectively.

Table 4.3 Comparison of the genotype and allele frequencies distributions of CYP2E1*7B polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	*1A*1A (GG)	*1A*7B (GT)	*7B*7B (TT)	*1A (G)	*7B (T)	Reference
Turkish (This study)	145	84.8	13.8	1.4	0.9170	0.0830	
Turkish	207	86.9	12.6	0.5	0.9320	0.0680	Ulusoy *et al.,* 2007b
Turkish	163	86.5	13.5	0	0.9325	0.0675	Kayaaltı *et al.,* 2010
European							
British	155	90.3	9	0.7	0.9480	0.0520	Yang *et al.,* 2001
German	56	85.7	14.3	0	0.9285	0.0715	Thier *et al.,* 2002
German	299	92.6	7.4	0	0.9630	0.0370	Neuhaus *et al.,* 2004
Swedish	37	91.9	8.1	0	0.9595	0.0405	Ernstgard *et al.,* 2004

The comparison of FMO3 genotype distributions in Turkish population with different ethnic groups

In this study, G472A and A923G SNPs located in exon 4 and 7 of FMO3 gene, respectively were investigated. The genotype and allele frequencies of G472A polymorphism in Turkish and other ethnic groups are summarized in Table 4.4. In the present study, the variant 472A allele frequency in 145 healthy subjects was found as 0.455. According to findings of Mao *et al*. (2009), 472A allele frequency was 0.358 in Turkish population. These results could be considered as similar. However, Mao *et al*. (2009) was not reported the genotype frequencies of this polymorphism in Turkish population. As can be seen from Table 4.4, our FMO3 G472A genotype distributions were different from Asian, European, American, African and Australian populations. The frequency of mutant 472A allele was higher than both Asian and European populations. On the other hand, same allele frequency (0.455, this study) was found to be very similar with American populations including African-American (0.449, Park *et al*., 2002; 0.480, Hao *et al.*, 2007), Canadian (0.426, Cashman *et al.,* 2000) and Caucasian-American (0.423, Park *et al.,* 2002). In

addition, in African and Australian populations variant 472A allele frequency was found to be 0.400 (Cashman *et al.*, 2001) and 0.437 (Cashman *et al.*, 2000), respectively.

Table 4.4 Comparison of the genotype and allele frequencies distributions of FMO3 G472A polymorphism in Turkish population control groups and previously published data in other populations control groups

Population	N	GG	GA	AA	G	A	Reference
Turkish (This study)	145	39.4	30.3	30.3	0.5455	0.4545	
Asian							
Asian	66	71	27	2	0.8450	0.1550	Cashman *et al.*, 2001
Han Chinese	256	56.6	37.1	6.3	0.7515	0.2485	Hao *et al.*, 2007
Korean	93	66.7	31.2	2.1	0.8230	0.1770	Bae *et al.*, 2006
Korean	219	67.1	27.9	5	0.8105	0.1895	Park *et al.*, 2002
European							
Caucasian	179	39	44	17	0.6100	0.3900	Cashman *et al.*, 2001
German	120	57.5	27.5	15	0.7125	0.2875	Poetsch *et al.*, 2010
Hispanic	85	42	45	13	0.6450	0.3550	Cashman *et al.*, 2001
American							
African-American	188	32	46.2	21.8	0.5510	0.4490	Park *et al.*, 2002
African-American	50	26	52	22	0.5200	0.4800	Hao *et al.*, 2007
Canadian	169	32	50.8	17.2	0.5740	0.4260	Cashman *et al.*, 2000
Caucasian-American	52	32.7	50	17.3	0.5770	0.4230	Park *et al.*, 2002
Other							
African	90	33	52	14	0.5900	0.4000	Cashman *et al.*, 2001
Australian	39	30.7	51.3	18	0.5635	0.4365	Cashman *et al.*, 2000

The other FMO3 SNP analyzed in this study was A923G, and genotype and allele frequencies distributions of this SNP in Turkish population and comparison of different populations is presented in Table 4.5. In the present study the frequency of the mutant 923G allele in 145 Turkish controls was found to be 0.055. The genotype

distribution of A923G polymorphism in Turkish population was similar with African-American population. The frequency of 923G allele was ranging between 0.150-0.183 in Asian population and 0.120-0.170 in European population. On the other hand, 923G mutant allele frequency was similar with African-American (0.045, Park *et al.,* 2002) and African (0.040, Cashman *et al.,* 2001) populations.

Table 4.5 Comparison of the genotype and allele frequencies distributions of FMO3 A923G polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	AA	AG	GG	A	G	Reference
Turkish (This study)	145	89	11	0	0.9450	0.0550	
Asian							
Asian	66	73	26	2	0.8600	0.1500	Cashman *et al.,* 2001
Han Chinese	256	69.5	27.7	2.8	0.8335	0.1665	Hao *et al.,* 2007
Korean	219	68	27.4	4.6	0.8170	0.1830	Park *et al.,* 2002
European							
Caucasian	179	72	24	5	0.8400	0.1700	Cashman *et al.,* 2001
German	120	78.3	16.6	5	0.8660	0.1330	Poetsch *et al.,* 2010
Hispanic	85	78	20	2	0.8800	0.1200	Cashman *et al.,* 2001
American							
African-American	188	91.5	8	0.5	0.9550	0.0450	Park *et al.,* 2002
African-American	50	100	0	0	1.0000	0.0000	Hao *et al.,* 2007
Caucasian-American	52	59.6	40.4	0	0.7980	0.2020	Park *et al.,* 2002
Other							
African	90	92	8	0	0.9600	0.0400	Cashman *et al.,* 2001

*The comparison of NQO1*2 genotype distributions in Turkish population with different ethnic groups*

The prevalence of NQO1*2 polymorphism in Turkish population and different ethnic groups is shown in Table 4.6. In the present study, the genotype frequencies of

NQO1*2 polymorphism were found as 51% *1*1, 45.5% *1*2 and 3.5% *2*2 in the healthy subjects. These results were also similar with the results found for Turkish population (Sirma *et al.,* 2004) where the genotype frequencies of *1*1, *1*2 and *2*2 were 53.8%, 42.7% and 3.5%, respectively. The Asian populations (22.4%, Kelsey *et al.*, 1997; 10.6%, Naoe *et al.*, 2000; 15.1%, Sunaga *et al.*, 2002; 12.7%, Eguchi-Ishimae *et al.*, 2005; 17.8%, Hamajima *et al.*, 2002; 18.8%, Kelsey *et al.*, 1997) had higher frequency of mutant *2*2 genotype than Turkish population. The mutant *2 allele frequency was found as 0.263 for Turkish population in this study. Some of the European populations including Greek (0.201, Stavropoulou *et al.*, 2011), Caucasian (0.242, Kelsey *et al.*, 1997) and Spanish (0.222, Bolufer *et al.*, 2007) populations had very similar mutant allele frequency with Turkish population. Besides, the mutant *2 allele frequency of African-American people (0.221) and Turkish people (0.263, this study) was found to be very close to each other according to Kelsey *et al.*'s (1997) study results. On the other hand, the *2 variant allele frequency was considerably high in Mexican-American population (0.416, Kelsey *et al.*, 1997), while the same allele frequency was found to be lower in Canadian (0.169, Begleiter *et al.*, 2006) and US white (0.180, Kiffmeyer *et al.*, 2004) people.

Table 4.6 Comparison of the genotype and allele frequencies distributions of NQO1*2 polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	*1*1 (CC)	*1*2 (CT)	*2*2 (TT)	*1 (C)	*2 (T)	Reference
Turkish (This study)	145	51	45.5	3.5	0.7375	0.2625	
Turkish	286	53.8	42.7	3.5	0.7515	0.2485	Sirma *et al.*, 2004
Asian							
Chinese	49	28.6	50	22.4	0.5360	0.4740	Kelsey *et al.*, 1997
Japanese	150	34	55.3	10.6	0.6165	0.3825	Naoe *et al.*, 2000
Japanese	152	34.2	50.7	15.1	0.5955	0.4045	Sunaga *et al.*, 2002
Japanese	197	44.7	42.6	12.7	0.6600	0.3400	Eguchi-Ishimae *et al.*, 2005
Japanese	640	37.5	44.7	17.8	0.5985	0.4015	Hamajima *et al.*, 2002
Korean	69	33.3	47.8	18.8	0.5720	0.4270	Kelsey *et al.*, 1997
European							
Greek	380	61.8	36.3	1.9	0.7995	0.2005	Stavropoulou *et al.*, 2011
Caucasian	114	56.1	39.5	4.4	0.7585	0.2415	Kelsey *et al.*, 1997
Caucasian	205	65.9	30.2	3.9	0.8100	0.1900	Harth *et al.*, 2000
Caucasian	239	68.2	27.6	4.2	0.8200	0.1800	Park *et al.*, 2003
Spanish	447	59.9	35.8	4.3	0.7780	0.2220	Bolufer *et al.*, 2007
Swedish	530	69.4	28.9	1.7	0.8385	0.1615	Alexandrie *et al.*, 2004
American							
Canadian	349	68.5	29.2	2.3	0.8310	0.1690	Begleiter *et al.*, 2006
African-American	136	61	33.8	5.2	0.7790	0.2210	Kelsey *et al.*, 1997
Mexican-American	161	32.3	52.2	15.5	0.5840	0.4160	Kelsey *et al.*, 1997
US white	258	67.5	29	3.5	0.8200	0.1800	Kiffmeyer *et al.*, 2004

The comparison of GSTP1, GSTM1 and GSTT1 genotype distributions in Turkish population with different ethnic groups

The GSTP1 A313G polymorphism genotype and allele frequencies distributions in different ethnic groups are shown in Table 4.7. Genotype frequencies of GSTP1 A313G polymorphism were obtained 50.3% 313AA, 34.5% 313AG and 15.2% 313GG in control group of this study. These genotype distributions were found a

little bit different from other Turkish population studies. However, the mutant 313G allele frequency of present study was found to be 0.324 and very similar allele frequencies were reported in different Turkish population studies (0.378, Ateş *et al.*, 2005; 0.327, Yalın *et al.*, 2007; 0.290, Altaylı *et al.*, 2009). The genotype distribution of Turkish population was different from Asian and European populations. However, the mutant 313G allele frequency was almost same with Indian's (0.323, Ramprasath *et al.*, 2011). Similarly the same allele frequency was found as 0.344 in Austrian's (Harris *et al.*, 1998), 0.365 in Caucasian's (Ramos *et al.*, 2011) and 0.353 in Argentinean's (Galván *et al.*, 2011).

Table 4.7 Comparison of the genotype and allele frequencies distributions of GSTP1 A313G polymorphism in Turkish population control groups and previously published data in other populations control groups

Population	N	AA	AG	GG	A	G	Reference
Turkish (This study)	145	50.3	34.5	15.2	0.6755	0.3245	
Turkish	204	44.1	36.3	19.6	0.6225	0.3775	Ateş *et al.*, 2005
Turkish	98	44.9	44.9	10.2	0.6735	0.3265	Yalın *et al.*, 2007
Turkish	128	48.4	45.3	6.3	0.7105	0.2895	Altaylı *et al.*, 2009
Asian							
Indian	270	43.7	48.1	8.2	0.6775	0.3225	Ramprasath *et al.*, 2011
Taiwanese	736	69.9	26.7	3.4	0.8325	0.1675	Yeh *et al.*, 2007
European							
Austrian	199	40.2	50.8	9	0.6560	0.3440	Harris *et al.*, 1998
Bulgarian	126	54	38.9	7.1	0.7345	0.2655	Vlaykova *et al.*, 2007
Caucasian	15	47	33	20	0.6350	0.3650	Ramos *et al.*, 2011
Caucasian (Portuguese)	95	64.2	34.7	1.1	0.8155	0.1845	Vilar *et al.*, 2007
Italian	133	48.9	36.1	6.8	0.6695	0.2485	Gravina *et al.*, 2011
Other							
Argentinean	102	41.2	47	11.8	0.6470	0.3530	Galván *et al.*, 2011

The genotype distribution of GSTM1 polymorphism in various populations is given in Table 4.8. This polymorphism was highly studied in Turkish population.

The present and null GSTM1 genotype frequencies were found as 38.6% and 61.4% in this study, respectively. The present and null allele frequencies were reported as 43.8% and 56.2% in a smaller group in the previous study (Türkanoğlu *et al.,* 2010). In addition, the GSTM1 null allele frequency was found as 50.8% (Altaylı *et al.,* 2009), 32.7% (Yalın *et al.,* 2007), 43.1% (Ateş *et al.,* 2005), 51.9% (Ada *et al.,* 2004) within Turkish population. In the Asian populations like Chinese, Iranian, Japanese, Korean and Taiwanese had similar null allele frequency with Turkish population. On the other hand, GSTM1 null allele frequency was found to be lower in Indian population (24.6%, Joseph *et al.,* 2004; 20.7%, Ramprasath *et al.,* 2011) than Turkish population. The same allele frequency was very similar with European populations and null allele frequency was ranging between 40.2%- 58.3%. As can be seen from the Table 4.8 GSTM1 null genotype frequency of this study showed no big difference with American populations including Brazilian (45.7%, Canalle *et al.,* 2004), Canadian (51.3%, Garte *et al.,* 2001), US white (53.5%, Kiffmeyer *et al.,* 2004; Chen *et al.,* 1997; 54% Davies *et al.,* 2002). On the other hand, GSTM1 null allele frequency was lower in US black population (27.7% Chen *et al.,* 1997; 32% Davies *et al.,* 2002) than Turkish population (61.4%, this study).

The distribution of GSTT1 genotypes in different ethnic groups of healthy subjects are provided in Table 4.9. In the present study, the genotype frequencies of GSTT1 polymorphism were found as 75.9% GSTT1 present and 24.1% GSTT1 null in 145 healthy subjects, and similar frequencies as 21% (Türkanoğlu *et al.,* 2010), 22.4% (Yalın *et al.,* 2007), 26% (Ateş *et al.,* 2005), 17.3% (Ada *et al.,* 2004). However, Altaylı *et al.* (2009) reported much lower GSTT1 null allele frequency of 7%, which was different from other studies on Turkish population. Except Indian population the other Asian population studies reported much higher GSTT1 null allele frequencies than Turkish population. GSTT1 null allele frequency of Turkish population and various European populations including Dutch, French, German, Italian, Portuguese and Spanish were similar. Among the American populations US black population had high null allele frequency (24.1%, Chen *et al.,* 1997; 28%, Davies *et al.,* 2002) and these frequencies were very similar with this study's result (24.1%, this study).

Table 4.8 Comparison of the genotype frequency distributions of GSTM1 polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	Present	Null	Reference
Turkish (This study)	145	38.6	61.4	
Turkish	105	43.8	56.2	Türkanoğlu *et al.*, 2010
Turkish	128	49.2	50.8	Altaylı *et al.*, 2009
Turkish	98	67.3	32.7	Yalın *et al.*, 2007
Turkish	204	56.9	43.1	Ateş *et al.*, 2005
Turkish	133	48.1	51.9	Ada *et al.*, 2004
Asian				
Chinese	284	48.6	51.4	Zhang *et al.*, 2010
Indian	118	75.4	24.6	Joseph *et al.*, 2004
Indian	270	79.3	20.7	Ramprasath *et al.*, 2011
Iranian	131	59.5	40.5	Saadat and Saadat, 2001
Iranian	236	46.6	53.4	Mohammadynejad *et al.*, 2011
Japanese	150	48.7	51.3	Naoe *et al.*, 2000
Japanese	100	49	51	Harada *et al.*, 2001
Japanese	152	63.2	36.8	Sunaga *et al.*, 2002
Korean	165	47.9	52.1	Garte *et al.*, 2001
Taiwanese	736	44.1	55.9	Yeh *et al.*, 2007
European				
Danish	537	46.4	53.6	Garte *et al.*, 2001
Dutch	419	49.6	50.4	Garte *et al.*, 2001
French	1184	46.6	53.4	Garte *et al.*, 2001
German	734	48.4	51.6	Garte *et al.*, 2001
Italian	133	47.4	52.6	Gravina *et al.*, 2011
Italian	810	50.6	49.4	Garte *et al.*, 2001
Portuguese	501	41.7	58.3	Garte *et al.*, 2001
Portuguese	102	59.8	40.2	Ramalhinho *et al.*, 2011
Spanish	451	48.6	51.4	Bolufer *et al.*, 2007
American				
Brazilian	221	54.3	45.7	Canalle *et al.*, 2004
Canadian	304	48.7	51.3	Garte *et al.*, 2001
US black	203	72.4	27.6	Chen *et al.*, 1997
US black	201	68	32	Davies *et al.*, 2002
US white	267	46.5	53.5	Kiffmeyer *et al.*, 2004
US white	213	46.5	53.5	Chen *et al.*, 1997
US white	532	46	54	Davies *et al.*, 2002

Table 4.9 Comparison of the genotype frequency distributions of GSTT1 polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	Present	Null	Reference
Turkish (This study)	145	75.9	24.1	
Turkish	105	79	21	Türkanoğlu *et al.*, 2010
Turkish	128	93	7	Altaylı *et al.*, 2009
Turkish	98	77.6	22.4	Yalın *et al.*, 2007
Turkish	204	74	26	Ateş *et al.*, 2005
Turkish	133	82.7	17.3	Ada *et al.*, 2004
Asian				
Chinese	284	48.2	51.8	Zhang *et al.*, 2010
Indian	118	91.5	8.5	Joseph *et al.*, 2004
Indian	270	82.2	17.8	Ramprasath *et al.*, 2011
Iranian	236	72.5	27.5	Mohammadynejad *et al.*, 2011
Iranian	131	68.7	31.3	Saadat and Saadat, 2001
Japanese	150	46	54	Naoe *et al.*, 2000
Japanese	152	61.2	38.8	Sunaga *et al.*, 2002
Korean	165	48.5	51.5	Garte *et al.*, 2001
Singaporean	243	48.1	51.9	Garte *et al.*, 2001
Taiwanese	736	50.9	49.1	Yeh *et al.*, 2007
European				
Dutch	419	77.1	22.9	Garte *et al.*, 2001
French	512	83.2	16.8	Garte *et al.*, 2001
German	487	80.5	19.5	Garte *et al.*, 2001
Italian	133	77.4	22.6	Gravina *et al.*, 2011
Italian	553	83.7	16.3	Garte *et al.*, 2001
Portuguese	102	82.4	17.6	Ramalhinho *et al.*, 2011
Spanish	455	86.6	13.4	Bolufer *et al.*, 2007
American				
Brazilian	221	80.5	19.5	Canalle *et al.*, 2004
Canadian	274	82.2	17.2	Garte *et al.*, 2001
US black	203	75.9	24.1	Chen *et al.*, 1997
US black	201	72	28	Davies *et al.*, 2002
US white	270	82.5	17.5	Kiffmeyer *et al.*, 2004
US white	532	84	16	Davies *et al.*, 2002
US white	213	85	15	Chen *et al.*, 1997

The comparison of NOS3 genotype distributions in Turkish population with different ethnic groups

The comparison of NOS3 G894T polymorphism in Turkish population and various ethnic populations is represented in Table 4.10. In the present study, the genotype frequencies of NOS3 G894T polymorphism were found as 37.9% 894GG, 55.2% 894GT and 6.9% 894TT in 145 healthy subjects. Different genotype distributions were observed in Turkish population. For example, high mutant 894TT genotype frequency reported in the studies conducted by Yemişçi *et al.* (2009) and Bayazit *et al.* (2009). On the other hand, similar mutant 894TT genotype frequency was obtained from study (4.9%) performed Güldiken *et al.* (2008). In this study, mutant 894T allele frequency of our population (0.345) was found to be highly similar with those of Caucasian (0.362, Tanus-Santos *et al.,* 2002), French (0.393, Elbaz *et al.,* 2000) and Greek people (0.312, Andrikopoulos *et al.,* 2008; 0.300, Vasilakou *et al.,* 2008; 0.338, Kitsios and Zintzaras, 2010). Mutant allele frequencies in Asian populations (0.115, Cheng *et al.,* 2008; 0.064, Tamemoto *et al.,* 2008; 0.052, Shin *et al.,* 2010; 0.126, Moe *et al.,* 2008) were found to be considerably lower than that of determined in our study. Moreover, the same allele frequency also was found to be lower in American populations than Turkish populations.

Table 4.10 Comparison of the genotype and allele frequencies distributions of NOS3 G894T polymorphism in Turkish population control groups and previously published data in other populations control groups

Population	N	GG	GT	TT	G	T	Reference
Turkish (This study)	145	37.9	55.2	6.9	0.6550	0.3450	
Turkish	81	27.2	58	14.8	0.5620	0.4380	Yemişçi *et al.*, 2009
Turkish	133	49.3	45.8	4.9	0.7220	0.2780	Güldiken *et al.*, 2009
Turkish	159	56.6	33.3	10.1	0.7325	0.2675	Bayazit *et al.*, 2009
Asian							
Chinese	309	78.6	19.7	1.6	0.8845	0.1145	Cheng *et al.*, 2008
Japanese	283	88	11.3	0.7	0.9365	0.0635	Tamemoto *et al.*, 2008
Korean	115	89.6	10.4	0	0.9480	0.0520	Shin *et al.*, 2010
Singaporean	207	77.3	20.3	2.4	0.8745	0.1255	Moe *et al.*, 2008
European							
Caucasian	47	36.2	55.3	8.5	0.6385	0.3615	Tanus-Santos *et al.*, 2002
French	460	35.4	50.4	14.1	0.6060	0.3930	Elbaz *et al.*, 2000
Greek	727	48.4	40.9	10.7	0.6885	0.3115	Andrikopoulos *et al.*, 2008
Greek	161	47	46	7	0.7000	0.3000	Vasilakou *et al.*, 2008
Greek	302	44.7	43.1	12.2	0.6625	0.3375	Kitsios and Zintzaras, 2010
Italian	67	28	61	11	0.5850	0.4150	Colomba *et al.*, 2008
Spanish	136	27.2	52.2	20.6	0.5330	0.4670	Sole´-Padulle´s *et al.*, 2004
American							
Brazilian	230	40.4	53	6.5	0.6690	0.3300	Piccoli *et al.*, 2008
Brazilian	102	52	44	4	0.7400	0.2600	Sandrim *et al.*, 2006
Chilean	112	66	32	2	0.8200	0.1800	Jaramillo *et al.*, 2010

The genotype and allele frequencies distributions of NOS3 T-786C polymorphism in different ethnic groups of healthy subjects are provided in Table 4.11. In this study, the frequency of -786C allele in 145 healthy subjects was found as 0.238. This result was almost similar to the -786C allele frequency found in the studies on Turkish population (0.374, Sinici *et al.*, 2010; 0.298, Yemişçi *et al.*, 2009). The mutant -786C allele frequency of Turkish population was found to be higher than Asian population (0.101, Cheng *et al.*, 2008; 0.100, Shin *et al.*, 2010; 0.141, Moe *et al.*, 2008). On the contrary, European populations (0.468, Tanus-Santos *et al.*, 2002; 0.394, Kitsios and Zintzaras, 2010; 0.540, Colomba *et al.*, 2008; 0.428, Venturelli *et al.*, 2005) had high mutant allele frequency when compared to

Turkish population. The allele frequency of -786C found in this study was similar to the ones found in American populations (0.375, Sandrim *et al.*, 2006; 0.230, Jaramillo *et al.*, 2010).

Table 4.11 Comparison of the genotype and allele frequencies distributions of NOS3 T-786C polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	TT	TC	CC	T	C	Reference
Turkish (This study)	145	56.6	39.3	4.1	0.7625	0.2375	
Turkish	71	38	49.3	12.7	0.6265	0.3735	Sinici *et al.*, 2010
Turkish	79	44.3	51.9	3.8	0.7025	0.2975	Yemişçi *et al.*, 2009
Asian							
Chinese	309	80.9	18.1	1	0.8995	0.1005	Cheng *et al.*, 2008
Korean	115	80.9	18.3	0.8	0.9005	0.0995	Shin *et al.*, 2010
Singaporean	207	73.4	25.1	1.5	0.8595	0.1405	Moe *et al.*, 2008
European							
Caucasian	47	21.3	63.8	14.9	0.5320	0.4680	Tanus-Santos *et al.*, 2002
Greek	289	35	51.2	13.8	0.6060	0.3940	Kitsios and Zintzaras, 2010
Italian	67	21	50	29	0.4600	0.5400	Colomba *et al.*, 2008
Italian	360	32.8	48.9	18.3	0.5725	0.4275	Venturelli *et al.*, 2005
American							
Brazilian	102	37	51	12	0.6250	0.3750	Sandrim *et al.*, 2006
Chilean	112	59	38	4	0.7800	0.2300	Jaramillo *et al.*, 2010

The genotype and allele frequencies of NOS3 intron 4 VNTR polymorphism in healthy subjects from various ethnic populations are given in Table 4.12. The genotype distribution of this polymorphism was very similar within Turkish population studies. The small “a” allele frequency was reported as 0.135 in this study. This allele frequency was found to be very similar with Asian (0.113, Shin *et al.*, 2010), European (0.171, Tanus-Santos *et al.*, 2002; 0.150, Vasilakou *et al.*, 2008;

0.115, Colomba *et al.*, 2008) and American (0.135, Sandrim *et al.*, 2006) populations.

Table 4.12 Comparison of the genotype and allele frequencies distributions of NOS3 intron 4 VNTR polymorphism in Turkish population control groups and previously published data in other populations control groups.

Population	N	aa	ab	bb	a	b	Reference
Turkish (This study)	145	2.1	22.7	75.2	0.1345	0.8655	
Turkish	71	2.8	25.4	71.8	0.1550	0.8450	Sinici *et al.*, 2010
Turkish	81	16	29.6	54.3	0.3080	0.6910	Yemişçi *et al.*, 2009
Turkish	181	2.1	19.9	79	0.1205	0.8895	Bayazit *et al.*, 2008
Asian							
Korean	115	0.8	20.9	78.3	0.1125	0.8875	Shin *et al.*, 2010
European							
Caucasian	47	4.3	25.5	70.2	0.1705	0.8295	Tanus-Santos *et al.*, 2002
Greek	161	3	24	73	0.1500	0.8500	Vasilakou *et al.*, 2008
Greek	303	4	33.3	62.7	0.2065	0.7935	Kitsios and Zintzaras, 2010
Italian	67	0	23	77	0.1150	0.8850	Colomba *et al.*, 2008
American							
Brazilian	102	4	19	77	0.1350	0.8650	Sandrim *et al.*, 2006
Chilean	112	1	14	85	0.0800	0.9200	Jaramillo *et al.*, 2010

This study revealed information on the genotype distribution of CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NQO1*2, GSTP1 A313G, GSTM1 null, GSTT1 null, NOS3 G894T, NOS3 T-786C and NOS3 intron 4 VNTR polymorphisms of 145 controls in Turkish population. Many of them show very similar results with previously published work on Turkish population. On the other hand, some of them show different genotype and allele frequencies within the Turkish population studies.

In summary, according to our results some of the genotype and allele frequencies (CYP2E1*5B, CYP2E1*6, NQO1*2, GSTM1, GSTT1, NOS3 T-786C, NOS3 intron 4 VNTR) were found to be very similar within the Turkish population studies. On the other hand, some of them (CYP2E1*7B, GSTP1 A313G, NOS3 G894T) showed different genotype and allele frequencies in various Turkish populations' studies. These differences should result from the different population size, mean age and the demographic origin of the study populations that were used. In general, allele frequencies of Turkish population in this study were found to be very similar with white populations including Canadian, Danish, French, German, Greek, Portuguese, Spanish etc.

Analysis of CYP2E1, FMO3, NQO1, GSTP1, GSTM1, GSTT1 and NOS3 genetic polymorphisms in patients with ischemic stroke and healthy controls

This study focused on seven genes, *CYP2E1*, *FMO3*, *NQO1*, *GSTP1*, *GSTM1*, *GSTT1* and *NOS3*, which are important in reactive oxygen species production or elimination. Case-control analyses were done on 245 ischemic stroke patients and 145 healthy controls, and the effect of these seven genes, and a total twelve polymorphisms, were investigated as risk factors (Table 3.2-3.12). Moreover, the combined haplotypes of these genotypes were examined with respect to stroke risk (Figure 3.8, 3.13, 3.20, 3.27). In addition, the risk of these genes was investigated in different subgroups such as hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker and obese/non-obese (Table 3.13-3.32). The effects of conventional risk factors in different genotypes were analyzed in detail by population stratification study. And also, further analyses were done to determine the effects of vascular factors, lipid parameters and these seven genes' genotypes in the prediction of ischemic stroke.

Association of CYP2E1 genotypes with stroke risk

Cytochrome P450 enzymes play an important role in the body's defense system against xenobiotic exposure because of their responsibility for the oxidative

metabolism of endogenous and exogenous compounds (Gonzalez, 1989). Several P450 enzymes show genetic polymorphism and these polymorphisms may alter the enzyme activity. CYP2E1, ethanol-inducible enzyme, catalyzes the oxidation of xenobiotic substrates and has the ability to activate many xenobiotic compounds to their toxic metabolites (Fahn and Cohen, 1992; Jenner, 1998). Especially these toxic metabolites are free radicals. Therefore, CYP2E1 is a dangerous enzyme to form oxidative stress condition, and its enhanced activity due to polymorphisms could increase the risk of ischemic stroke. In the literature, there was not any study for the association of genetic polymorphisms of CYP2E1 enzyme on the risk of development ischemic stroke. However, mutant *5B allele of CYP2E1*5B polymorphism was shown as a risk factor for atherosclerosis (Salama *et al.,* 2002) which play important role in development of stroke. On the other hand, the associations were found between CYP2E1 polymorphisms and various cancer types such as lung cancer, oral cancer, urothelial cancer, nasopharyngeal carcinoma and hepatocellular carcinoma (Kato *et al.,* 1992; Ladero *et al.,* 1996; Hildesheim *et al.,* 1995; El Zein *et al.,* 1997; Wu *et al.,* 1998; Farker *et al*., 1998; Liu *et al*., 2001).

The genotype and allele frequencies of three SNPs of *CYP2E1* gene namely CYP2E1*5B, CYP2E1*6 and CYP2E1*7B were determined in 245 ischemic stroke patients and 145 healthy controls in present study. These results were given in Chapter 3 Table 3.2-3.4. As can be seen from these tables no significant differences were found between patients and controls with respect to all of these polymorphisms of *CYP2E1* gene' genotype and allele frequencies but only CYP2E1*5B polymorphism mutant *5B allele frequency was found to be significantly different in patients (0.027) and controls (0.004, P=0.019) as expected.

The effects of combination of double and triple combined SNPs on ischemic stroke risk were also analyzed (Figure 3.8) and co-presence of CYP2E1*5B and CYP2E1*6 polymorphisms was found to increase the risk of stroke almost 4.9-fold, which was not statistically significant (P=0.101).

As mentioned earlier hypertension, diabetes mellitus, smoking and obesity were found to be significant risk factors of stroke in present study. Therefore genotype distribution of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B polymorphisms were

analyzed in subgroups of these risk factors and no differences were observed in patient and control groups (Table 3.13-3.16).

The conventional vascular risk factors were analyzed in terms of proportion of ischemic stroke patients to controls for mutant and heterozygous genotype and homozygous wild type genotype groups. The results of this analysis were given in Table 3.33 but in here these results were summarized in Table 4.13. The risk of having stroke in hypertensive individuals was 3-fold higher than normotensives within the wild type *1A*1A genotype of CYP2E1*5B polymorphism group. For CYP2E1*6 wild type and CYP2E1*7B wild type the same risk was 3.5-fold and 2.7-fold higher in hypertensives than normotensives, respectively. As mentioned previous chapter hypertension increased the risk of stroke 3.2-fold. CYP2E1*5B and CY2E1*7B polymorphisms may have a protective effect against stroke because they decrease the risk of stroke among hypertensives that carry *1A*1A wild type genotype. On the other hand, hypertensive individuals with CYP2E1*7B heterozygote *1A*7B genotype was 8.6 times more prone to develop stroke. It shows that *7B mutant allele increases the effect of hypertension on ischemic stroke risk as expected. Similar trend was observed for another vascular risk factor diabetes mellitus. CYP2E1*5B, CYP2E1*6 wild types and CYP2E1*7B heterozygote genotypes increase the risk of stroke among smokers. Some studies reported that CYP2E1 activates many chemicals in cigarette smoking such as butadiene, benzene and nitrosamines (Yamazaki *et al.*, 1992; Raunio *et al.*, 1995). Therefore, it generates higher amounts of reactive oxygen species. Similar results were obtained for obese people.

Table 4.13 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to CYP2E1*5B, CYP2E1*6 and CYP2E1*7B genotypes and stroke-control status. (The table was derived from Table 3.33)

Subgroup	Polymorphism-Genotype	OR	*P*
Hypertensive/Normotensive	CYP2E1*5B- *1A*1A	3.074	0.000
	CYP2E1*6- *1A*1A	3.527	0.000
	CYP2E1*7B- *1A*1A	2.798	0.000
	CYP2E1*7B- *1A*7B	8.625	0.000
Diabetic/Non-diabetic	CYP2E1*5B- *1A*1A	2.457	0.000
	CYP2E1*6- *1A*1A	2.534	0.000
	CYP2E1*7B- *1A*1A	2.481	0.000
Smoker/Non-smoker	CYP2E1*5B- *1A*1A	2.413	0.001
	CYP2E1*6- *1A*1A	2.589	0.001
	CYP2E1*7B- *1A*1A	2.019	0.017
	CYP2E1*7B- *1A*7B	4.092	0.043
Obese/Non-obese	CYP2E1*5B- *1A*1A	4.550	0.000
	CYP2E1*6- *1A*1A	5.525	0.000
	CYP2E1*7B- *1A*1A	4.144	0.000

Association of FMO3 genotypes with stroke risk

As mentioned previously in Chapter 1 section 1.7.2.1, FMO enzyme is found at high concentrations in some tissues and in these tissues the production of hydrogen peroxide and superoxide anion could be detrimental. In order to provide overall redox state, the level of ROS is important. If redox state is disrupted oxidative stress condition occurs. It is known that FMO3 G472A and A923G variants decrease the enzyme activity. Reduce enzyme activity may cause reduction of the production of ROS. Therefore, the mutant genotype may be protective factor against ischemic stroke.

FMO3 enzyme has a wide range of substrate specificity including some drugs such as morphine, cocaine, nicotine, chlorpromazine, imipramine, tamoxifen, cimetidine, ranitidine, and chemicals such as phorate, aldicarb and fonofons. And also catecholamines produced in response to stress are substrate for FMO3 enzyme and they are metabolized by a minor pathway of FMO3. Catecholamines modulate heart rate and blood pressure. Polymorphism of the *FMO3* gene decreases the enzyme activity. Therefore, the catabolism of catecholamines is decreased, which impact on blood pressure homeostasis. High blood pressure is one of the important risk factors of stroke. So the variation of FMO3 enzyme could play an important role in the pathogenesis of stroke. FMO3 G472A and FMO3 A923G polymorphisms were studied and genotype and allele frequencies of these polymorphisms were given in Table 3.5 and Table 3.6. According to our knowledge, this is the first study investigating association of FMO3 G472A and A923G polymorphisms with ischemic stroke risk. Moreover, there are restricted association studies of FMO3 polymorphism with any disease state in the literature. Poetsch *et al.* (2010) reported that G472A polymorphism of FMO3 may be considered as an additional genetic risk factor of sudden infant death syndrome (SIDS) in children. In addition according to a study conducted in Korea, the genotype and allele frequencies of the FMO3 G472A and FMO3 A923G polymorphisms were not found to be significantly different in control and colorectal cancer patients (Bae *et al.*, 2006). In this study, we did not observe the significant differences between patients and controls with respect to genotype and allele frequencies of both FMO3 G472A and A923G polymorphisms. The combination of these two polymorphisms (Figure 3.13) had almost 1 odds ratio which means that there is no association between ischemic stroke and double combined mutant genotypes.

Within the normotensives significant difference were found in genotype frequencies of FMO3 G472A polymorphism between patients and controls. Among other subgroups no significant differences were observed for genotype distributions of these two polymorphisms. The effects of conventional vascular risk factors and genotypes on ischemic stroke development were analyzed and results were given in Table 3.34. The significant findings are revealed in Table 4.14.

Table 4.14 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to FMO3 G472A and FMO3 A923G genotypes and stroke-control status. (The table was derived from Table 3.34)

Subgroup	Polymorphism-Genotype	OR	*P*
Hypertensive/Normotensive	FMO3-472GA	6.110	0.000
	FMO3-472AA	4.261	0.000
	FMO3-923AA	3.016	0.000
	FMO3-923AG	6.000	0.007
Diabetic/Non-diabetic	FMO3-472GG	2.424	0.021
	FMO3-472AA	2.763	0.038
	FMO3-923AA	2.696	0.000
Smoker/Non-smoker	FMO3-472GG	2.740	0.013
	FMO3-472AA	2.932	0.027
	FMO3-923AA	2.361	0.002
Obese/Non-obese	FMO3-472GG	6.222	0.001
	FMO3-472AA	3.620	0.020
	FMO3-923AA	4.854	0.000

As seen in Table 4.14 while FMO3 G472A heterozygote 472GA genotype increased the risk of stroke 6 times together with hypertension, mutant 472AA genotype had 4-fold greater risk in hypertensive subjects. On the other hand, with respect to FMO3 A923G polymorphism wild type 923AA and heterozygote 923AG genotypes significantly increased the risk of stroke 3 times and 6 times in hypertensive individuals, respectively. FMO3 472GG wild type and 472AA homozygous mutant genotypes had 2.4 and 2.7-fold greater risk in diabetic/non-diabetic group. In addition, the risk of having stroke of diabetics in FMO3 923AA wild type group was 2.7 times elevated. Both in smoker/non-smoker group and obese/non-obese groups like diabetic/non-diabetic group FMO3 472GG, 472AA and 923AA genotypes significantly increased the risk of having stroke. According to

these findings, wild type genotypes of FMO3 G472A and A923G polymorphisms have greater risk than mutant genotypes of these polymorphisms, as expected.

Association of NQO1 genotypes with stroke risk

NQO1 plays a key role in cellular antioxidant defense against oxidative stress (Ross *et al.,* 2000). Riley and Workman (1992) reported that NQO1 reduce oxidative stress by preventing participation of quinines and their derivatives in redox cycling. In addition, recent studies showed that highly expressed and inducible endogenous NQO1 in cardiovascular cells may act as a potential superoxide scavenger (Zhu *et al.,* 2007; Siegel *et al.,* 2004). NQO1*2 polymorphism is located in exon 6 and strongly effects the enzyme activity. While this SNP decreases NQO1 protein levels and activity in heterozygotes, it results in a complete loss of NQO1 protein levels and activity in homozygous genotypes. Considering previous results, in this study T allele carriers may have low enzyme activity, decreased ROS removal, increased oxidative stress. So these individuals could be more prone to develop atherosclerosis and stroke. According to study on type 2 diabetic patients conducted by Han *et al*. (2009), atherosclerotic plaque prevalence was found to be higher in the T allele carriers than non-T allele carriers. On the contrary, animal studies showed that when compared to rats carrying 609CC genotype; as a consequence of decreased ability for coagulation reduce the availability of coagulation proteins in 609T polymorphism carrying rats (Ernster *et al.,* 1972). Therefore, individuals carrying the NQO1 609T polymorphism may have lower concentration of blood coagulation factors that are important factors as associated with the development of arterial atherosclerosis. Consequently, variant genotype of NQO1*2 polymorphism may reduce ischemic stroke risk. Shyu *et al.* (2010) reported that NQO1 609CT heterozygote genotype was found to be statistically significant protective factor against ischemic stroke (OR=0.47).

The effect of NQO1*2 polymorphism on ischemic stroke risk was analyzed in this study (Table 3.7). Although the significant difference was found to be genotype frequencies of NQO1*2 polymorphism between patients and controls (P=0.027), no significant difference was observed in stroke patient and control groups with respect

to allele frequencies (*P*=0.080). Our findings were consistent with the results of Shyu *et al*.' (2010) study. The odds ratio calculated as 609CT+609TT vs. 609TT genotype and variant genotypes were found to be 0.6-fold reduce ischemic stroke risk. Only one polymorphic region of *NQO1* gene was investigated in present study, therefore combination analysis was not done for this gene. In subgroup analyses the genotype distribution of NQO1*2 polymorphism was found as significantly different between patients and controls in non-smoker group (*P*=0.002). Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to NQO1*2 genotypes and stroke-control status were given in Table 3.35. The statistically significant results of this analysis were summarized in Table 4.15.

Table 4.15 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to NQO1*2 genotypes and stroke-control status (The table was derived from Table 3.35)

Subgroup	Polymorphism-Genotype	OR	*P*
Hypertensive/Normotensive	NQO1*2- *1*1	3.103	0.000
	NQO1*2- *1*2	3.469	0.000
Diabetic/Non-diabetic	NQO1*2- *1*1	2.982	0.001
Smoker/Non-smoker	NQO1*2- *1*2	5.000	0.000
Obese/Non-obese	NQO1*2- *1*1	4.246	0.002
	NQO1*2- *1*2	4.750	0.003

The wild type *1*1 and heterozygote *1*2 genotypes of NQO1*2 significantly increased the stroke risk 3 and almost 3.5 times together with hypertension, respectively. Furthermore, *1*1 wild type was found to be significant risk predictor for stroke in diabetic (OR=2.982, *P*=0.001) and obese (OR=4.246, *P*=0.002) people. On the other hand, *1*2 heterozygote genotype was 5 times and 4.7 times more prone to develop stroke in smoker and obese subjects, respectively. As can be seen

from the Table 4.15, generally *1*1 wild type was found to be significant risk factor for stroke.

Association of GSTP1, GSTM1 and GSTT1 genotypes with stroke risk

Glutathione S-transferases catalyze the detoxification of metabolites produced by oxidative stress within the cell and they are induced by ROS. Therefore GSTs are considered as one of the defense systems against the damaging effects of oxidative stress which is important factor for stroke development. GSTs are a super family of polymorphic enzymes and polymorphisms affect activity of GST isozymes. So studying polymorphism of GST isozymes is important to illuminate association of GSTs and stroke risk. GSTP1 A313G SNP is found in exon 5 and this polymorphism cause aminoacid substitution resulting in decreased enzyme activity. Different studies reported about association of GSTP1 A313G polymorphism with various diseases in the literature. However, this is the first study investigating association between ischemic stroke and GSTP1 A313G polymorphism. Wang *et al.* (2007) reported that variant genotypes of GSTP1 (313AG, 313GG) had higher risk for carotid atherosclerosis. On the other hand, Nomani *et al.* (2011) observed no association between GSTP1 genotypes and coronary artery disease. Several studies showed that GSTP1 313GG mutant genotype has greater breast cancer risk (Helzlsouer *et al*., 1998; Egan *et al*., 2004; Gudmundsdottir *et al*., 2001). In addition, 313G variant allele was found to be associated with Parkinson's and Alzhemier diseases (Vilar *et al*., 2007; Zuntar *et al*., 2004). According to our study, there were no significant difference between patients and controls with respect to GSTP1 A313G genotype and allele frequencies (Table 3.8). Our findings showed that any genotypes of GSTP1 A313G polymorphism were not significant risk factor for stroke development.

The genes encoding the GST isoenzymes GSTM1 and GSTT1 have null alleles resulting from gene deletion and the null genotypes of GSTM1 and GSTT1 have reduced enzyme activity (Bruhn *et al.,* 1998; Zhong *et al.,* 2006). There were conflicting results about GST polymorphisms in the literature. While GSTT1 present genotype had protective effect against type 2 diabetes mellitus in Chinese population

(Wang *et al*., 2006), investigations showed that GSTT1 polymorphism did not affect the development of diabetes in Turkish population (Yalın *et al.*, 2007). In addition GSTM1 null genotype frequency of diabetic Turkish patients was found to be significantly higher than that of control group (Yalın *et al*., 2007). Kentaro *et al*. (2008) reported that both GSTM1/GSTT1 null and GSTM1 null genotypes had greater risk of hypertension.

In the present study, we expected to observe a significantly higher prevalence of null genotypes of GSTM1 and GSTT1 among stroke patients. However, no significant differences were found between patients (GSTM1 null=53.9%, GSTT1 null=27.8%) and controls (GSTM1 null=61.4%, GSTT1 null=24.1%) with respect to null genotype frequencies (Table 3.9). The frequency of GSTM1 null genotype previously found to be 50.6% in patients (n=172) and 56.2% in controls (n=105) in a smaller subgroup of the study population of the present investigation (Türkanoğlu *et al.*, 2010). In the same study, GSTT1 null genotype frequency was reported as 19.8% in patients and 21% in controls. A very large difference was not found between previous and present results of the study.

The effect of the double and triple combined polymorphism of *GSTP1, GSTM1* and *GSTT1* genes on ischemic stroke risk were also analyzed (Figure 3.20) but no statistically significant results were obtained. In addition, genotype and allele frequencies of GSTP1, GSTM1 and GSTT1 polymorphisms did not show differences in subgroup analyses. The study population was stratified according to GSTP1 A313G, GSTM1 null and GSTT1 null genotype frequencies (Table 3.36) and significant results were summarized in Table 4.16 in this chapter. Hypertension is an important risk factor of ischemic stroke and it increases the stroke risk 3.2 times in this study. In hypertensive group while GSTM1 present genotype increased the risk of having stroke almost 2.5 times, GSTM1 null genotype had approximately 4-fold greater risk for stroke. On the other hand, GSTT1 present and GSTT1 null genotypes had increased risk of stroke 2.7-fold and 5.5-fold in hypertensive/normotensive group, respectively. These results showed that the harmful impact of hypertension on ischemic stroke decreased by both GSTM1 and GSTT1 present genotypes. On the other hand, we observed that null genotypes contribute the effect of hypertension on stroke development. Among the GSTP1 A313G genotypes wild type 313AA was

found to be predictor of stroke in hypertensive subjects (OR=3.535, *P*=0.000). Similarly 313GG genotype increased the risk of stroke 3.5 times and 313AG heterozygote genotype was 2.8 times more prone to develop stroke in hypertensive individuals. GSTM1 present and GSTM1 null genotypes were also found significant predictor of stroke in diabetic/non-diabetic, smoker/non-smoker and obese/non-obese group. While the risk of having stroke of diabetics in GSTT1 present genotype group was 2.5 times elevated, in GSTP1 313AG heterozygote genotype was found to be 3.8-fold greater risk factor for stroke. GSTT1 present genotype increased the risk of having stroke 2 times together with smoking and we observed that this effect reached to 3-fold if the subjects have GSTT1 null genotype. In addition, in the same group GSTP1 313AG heterozygote genotype was found to be increase the risk of stroke 2.5 times. The effect of GSTT1 present genotype (OR=4.812, *P*=0.000) was higher than GSTT1 null genotype (OR=3.961, *P*=0.014) in obese people. GSTP1 313AA wild type (OR=5.024, *P*=0.001) and GSTP1 313AG heterozygote (OR=3.604, *P*=0.020) genotypes were also found to be significant predictor of stroke in obese group.

Table 4.16 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to GSTM1, GSTT1 and GSTP1 A313G genotypes and stroke-control status (The table was derived from Table 3.36)

Subgroup	Polymorphism-Genotype	OR	*P*
Hypertensive/Normotensive	GSTM1-Present	2.467	0.006
	GSTM1-Null	3.868	0.000
	GSTT1-Present	2.746	0.000
	GSTT1-Null	5.595	0.000
	GSTP1-313AA	3.535	0.000
	GSTP1-313AG	2.809	0.004
	GSTP1-313GG	3.500	0.039
Diabetic/Non-diabetic	GSTM1-Present	3.162	0.005
	GSTM1-Null	2.190	0.014
	GSTT1-Present	2.518	0.001
	GSTP1-313AG	3.808	0.001
Smoker/Non-smoker	GSTM1-Present	3.150	0.013
	GSTM1-Null	1.994	0.041
	GSTT1-Present	2.095	0.022
	GSTT1-Null	2.810	0.039
	GSTP1-313AG	2.576	0.039
Obese/Non-obese	GSTM1-Present	8.068	0.001
	GSTM1-Null	3.445	0.003
	GSTT1-Present	4.812	0.000
	GSTT1-Null	3.961	0.014
	GSTP1-313AA	5.024	0.001
	GSTP1-313AG	3.604	0.020

Association of NOS3 genotypes with stroke risk

NOS3 gene catalyzes the generation of NO which mediates vascular relaxation in response to vasoactive substances and shear stress. In addition it mediates inhibition of platelet adherence and aggregation, suppression of smooth muscle

proliferation, and reduction of adherence of leucocytes to the endothelium. These properties of NOS3 make it a biologically reasonable candidate in order to study as a susceptibility gene in ischemic stroke. Different polymorphisms of NOS3 have been identified and the most studied one is NOS3 G894T polymorphism. However, the results of association of G894T polymorphism with ischemic stroke are quite contradictory. Recently, the positive association has been reported between NOS3 G894T polymorphism and ischemic stroke risk (Luka *et al.,* 2011; Berger *et al.,* 2007, Elbaz *et al.,* 2000). On the other hand, in some studies no association between NOS3 G894T polymorphism and stroke was found (Majumdar *et al.,* 2010, Guldiken *et al.,* 2009; Szolnoki *et al.,* 2005; MacLeod *et al.,* 1999; Markus *et al.,* 1998). In this study, we also observed no significant association between NOS3 G894T polymorphism and ischemic stroke risk (Table 3.10).

The second polymorphism studied in present study was NOS3 T-786C. Cheng *et al.* (2008) reported that -786CC genotype was more prone to develop stroke in Chinese population. On the other hand, some studies showed that -786TT genotype prevalence was higher ischemic stroke patients when compared to controls (Howard *et al.,* 2005, Majumdar *et al.,* 2010) and our findings were very similar with these two studies. We could not find any association between T-786C polymorphism and ischemic stroke risk (Table 3.11).

The last polymorphism analyzed in this study was NOS3 intron4 VNTR polymorphism. Again there were controversial results about this polymorphism and stroke association in the literature. Intron 4bb genotype was shown as an important genetic risk factor for early-onset ischemic stroke in Chinese population (Shi *et al.,* 2008). On the contrary, according to some studies 4a allele was found to be risk factor for stroke (Hou *et al.,* 2001; Majumdar *et al.,* 2010). In present study, 4a allele was found to be risk factor for stroke but this result was not statistically significant (Table 3.12). In addition any significant results were not obtained from the combination of these polymorphisms. In the subgroup analyses only in normotensive group NOS3 intron4 VNTR genotype frequencies were found to be significantly different between patients and controls (P=0.038). The population stratification analyses' results were shown in Table 3.37 and significant results were summarized in Table 4.17 in this chapter.

Table 4.17 Stratification of hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker, obese/non-obese individuals according to NOS3 G894T, NOS3 T-786C and NOS3 VNTR genotypes and stroke-control status (The table was derived from Table 3.37)

Subgroup	Polymorphism-Genotype	OR	*P*
Hypertensive/Normotensive	NOS3/894GG	2.193	0.026
	NOS3/894GT	4.535	0.000
	NOS3/-786TT	3.358	0.000
	NOS3/-786TC	3.600	0.000
	NOS3VNTR/bb	4.142	0.000
Diabetic/Non-diabetic	NOS3/894GG	2.607	0.018
	NOS3/-786TT	2.226	0.015
	NOS3/-786TC	2.472	0.037
	NOS3VNTR/bb	3.034	0.000
Smoker/Non-smoker	NOS3/894GG	4.234	0.002
	NOS3/-786TC	2.922	0.017
	NOS3VNTR/ab	3.940	0.014
Obese/Non-obese	NOS3/894GG	5.932	0.010
	NOS3/894GT	3.358	0.003
	NOS3/-786TT	5.613	0.000
	NOS3/-786TC	4.026	0.022
	NOS3VNTR/bb	6.720	0.000

For the NOS3 G894T polymorphism, among the hypertensive group, the risk of having stroke was increased in both 894GG wild type (OR=2.193, *P*=0.026) and 894GT heterozygote (OR=4.535, *P*=0.000) genotypes. The 894GG wild type were found to be significant predictor of stroke in diabetic (OR=2.607, *P*=0.018), smoker (OR=4.234, *P*=0.002) and obese (OR=5.932, *P*=0.010) people. In addition, in obese group, 894GT heterozygote genotype was also found to be 3-fold increased the risk of having stroke. When Table 4.17 was examined with respect to NOS3 T-786C polymorphism, we observed that -786TT wild type and -786TC heterozygote genotypes were found to be risky genotypes in hypertensive, diabetic and obese people. In smoker group only -786TC heterozygote genotype was approximately 3

times more prone to develop stroke. For NOS3 intron4 VNTR polymorphism “bb” larger genotype had increased risk of stroke in hypertensive (OR=4.142, *P*=0.000), diabetic (OR=3.034, *P*=0.000) and obese (OR=6.720, *P*=0.000) groups. The risk of having stroke in smoker individuals was almost 4-fold (*P*=0.014) higher than non-smokers within the NOS3 intron4 VNTR “ab” heterozygote genotype group.

Logistic regression analyses

Logistic regression analyses with backward selection method were used to determine the effects of vascular factors, lipid parameters and genotypes in the stroke susceptibility. Five different models were prepared by combination of various parameters in order to determine the risk factors of stroke. According to first model, while hypertension, smoking, obesity and LDL-cholesterol were found to be significant determinant of stroke, HDL-cholesterol and NQO1*2 polymorphism heterozygote genotype were found as a significant protective factor for stroke in our study population. The same analysis was done among elderly (aged>59 years) people and again strong determinants of stroke was found as hypertension, smoking, obesity and LDL-cholesterol. However, only NQO1 609CT heterozygote genotype had preventive effect. In the third analysis, same model was applied on younger (aged<60 years) people. In this case only hypertension and total cholesterol were found to be significant predictors of stroke for younger people. Furthermore, HDL-cholesterol was found to be preventive factor against stroke. Logistic regression analysis was done on female subjects and hypertension, diabetes mellitus, smoking and LDL-cholesterol were significant and the strongest determinants of ischemic stroke. On the other hand, HDL-cholesterol and NOS3 894TT genotype was found as significant risk factors for ischemic stroke in females. In the last model, same analysis was repeated in a male group. According to this analysis, only hypertension and obesity was found to be significant risk factor for stroke, however HDL-cholesterol and NQO1 609CT heterozygote genotype had preventive effect against ischemic stroke. As a conclusion in the present study, hypertension, smoking and LDL-cholesterol were strong determinants of stroke. And also HDL-cholesterol and NQO1 609CT heterozygote genotype were found to be preventive factor of ischemic

stroke in our study population. These findings overlap with our expectations because these factors are well defined determinants in stroke risk and protection. Surprisingly NQO1 609CT heterozygote genotype was found as a significant protector against stroke in different logistic regression models. Actually Shyu *et al.* (2010) reported that NQO1 609CT heterozygote genotype was found to be statistically significant protective factor against ischemic stroke. NQO1 may show protective effect due to its role in the blood coagulation pathway.

CHAPTER 5

CONCLUSION

Ischemic stroke is a multifactorial disease leading severe long-term disability and it is the third leading cause of death in developed countries. Although many studies have been reported to elucidate etiological and pathological mechanisms of disease, the genetic and molecular basis of disease remains poorly understood. Recent studies have shown that reactive oxygen species (ROS) causing oxidative stress play a pivotal role in the pathogenesis of atherosclerosis that is the main cause of a group of cardiovascular diseases including ischemic stroke. Therefore this study focused on the genetic polymorphisms of the enzymes that function in either production or elimination of ROS, with the hypothesis that the development of ischemic stroke could be associated with these polymorphisms either alone or in combination. For this aim, three polymorphisms of CYP2E1 (CYP2E1*5B, CYP2E1*6 and CYP2E1*7B), two polymorphisms of FMO3 (G472A, A923G), one polymorphism of NQO1 (NQO1*2), three polymorphisms of GST (GSTP1 A313G, GSTM1 null, GSTT1 null) and three polymorphisms of NOS3 (G894T, T-786C and intron4 VNTR) were studied in present study. In addition vascular factors and lipid parameters were evaluated with respect to risk of stroke.

The study population was comprised of 245 ischemic stroke patients and 145 healthy controls. The age and gender were not different in patient and control group. Hypertension, diabetes mellitus, obesity and smoking were found to be significant risk factors for stroke and these results were also confirmed with logistic regression analyses in different models. As expected, total cholesterol, triglyceride and LDL-cholesterol levels were higher in patients when compared to controls. On the other

hand, HDL-cholesterol level was found to be lower in patients than controls and logistic regression analysis showed that high HDL-cholesterol level had preventive effect on stroke.

The two study groups did not differ in terms of CYP2E1*6 and CYP2E1*7B genotype distributions. On the other hand, CYP2E1*5B polymorphism had significantly different genotype distribution between stroke patients and controls (*P*=0.017). The double and triple combination of CYP2E1 polymorphisms were analyzed and observed that co-presence of CYP2E1*5B and CYP2E1*6 polymorphisms was found to increase the risk of stroke almost 4.9-fold, which was not statistically significant (*P*=0.101).

In the case control analysis of both FMO3 G472A and FMO3 A923G polymorphisms the significant difference was not observed between patients and controls in terms of distribution of genotype and allele frequencies. The combination of FMO3 G472A and A923G variant genotypes had no effect on ischemic stroke risk because the odds ratio of this analysis was found as almost 1 (OR=0.989, 95% CI=0.498-1.966, *P*=1.000).

For NQO1*2 polymorphism, significant difference was found between two study groups with respect to genotype distribution. The variant genotype was found as a protector for stroke (OR=0.627, 95% CI=0.414-0.950, *P*=0.027). Logistic regression analysis also showed that NOQ1*1*2 heterozygote genotype had protective effect on ischemic stroke especially in male and elderly (aged>59 years) subjects.

GSTP1 A313G, GSTM1 null and GSTT1 null polymorphisms of genotype and allele frequencies were not different in patient and control groups. While the combination of GSTM1 null-GSTT1 null (OR=0.865, 95% CI=0.480-1.558, *P*=0.629), GSTM1 null and GSTP1 313AG+GSTP1 313GG (OR=0.625, 95% CI=0.346-1.132, *P*=0.120) and GSTM1 null-GSTT1 null-GSTP1 313AG+GSTP1 313GG (OR=0.721, 95% CI=0.321-1.624, *P*=0.429) had protective effect on stroke, co-presence of GSTT1 null and GSTP1 313AG+GSTP1 313GG genotypes increased the risk of having stroke (OR=1.193, 95% CI=0.618-2.307, *P*=0.598). However, these results were not found to be statistically significant.

Genotype and allele distributions of NOS3 G894T, NOS3 T-786C and NOS3 intron4 VNTR polymorphisms were found to be not significantly different between patient and control groups. In addition no significant association was found between combined genotypes of NOS3 and ischemic stroke risk.

The conventional vascular risk factors were analyzed in terms of proportion of ischemic stroke patients to controls for mutant and heterozygous genotype and homozygous wild type genotype groups. Wild types of CYP2E1*5B, CYP2E1*6 and CYP2E1*7B polymorphisms were found to be significant risk factors in hypertensive (OR=3.074, *P*=0.000; OR=3.527, *P*=0.000; OR=2.798, *P*=0.000, respectively), diabetic (OR=2.457, *P*=0.000; OR=2.534, *P*=0.000; OR=2.481, *P*=0.000, respectively), smoker (OR=2.413, *P*=0.001; OR=2.589, *P*=0.001; OR=2.019, *P*=0.017, respectively) and obese (OR=4.550, *P*=0.000; OR=5.525, *P*=0.000; OR=4.144, *P*=0.000, respectively) subjects. In addition heterozygote genotype of CYP2E1*7B was found increase the risk of stroke in hypertensive and smoker individuals 8.6 times and 4 times, respectively.

For FMO3 G472A polymorphism, heterozygote 472GA and homozygous mutant 472AA genotypes had increased the risk of having stroke 6-fold and 4-fold in hypertensive subjects, respectively. FMO3 472GG and 472AA genotypes had greater risk of stroke in diabetic, smoker and obese subjects. Analysis of FMO3 A923G polymorphism showed that 923AA wild type genotype was significant risk factor in hypertensive (OR=3.016, *P*=0.000), diabetic (OR=2.696, *P*=0.000), smoker (OR=2.361, *P*=0.002) and obese (OR=4.854, *P*=0.000) people. In addition the risk of hypertensive individuals having stroke was higher for 923AG heterozygote genotype (OR=6.000, *P*=0.007) compared to other genotypes.

In hypertensives, NQO1*2 wild type and heterozygote genotypes increased the risk of stroke 3.1 times and 3.4 times, respectively. The proportion of diabetic stroke patients to diabetic controls compared to the proportion of non-diabetic patients to non-diabetic controls was high in NQO1*2 wild type genotype (OR=2.982, *P*=0.001). On the other hand the risk of having stroke was found to be higher in obese subjects for same genotype (OR=4.246, *P*=0.002). While NQO1*2 heterozygote genotype increased the risk of stroke in smokers and obese individuals 5 times and 4.7 times, respectively.

Interestingly, all of the genotypes of GSTP1 A313G, GSTM1 null and GSTT1 null polymorphisms were found as significant risk factors for stroke in hypertensive subjects. However, the effects of GSTM1 present and GSTT1 present genotypes on stroke risk were lower than the effects of GSTM1 null and GSTT1 null genotypes. GSTM1 null and GSTM1 present genotypes were associated with 3.2 times and 2.2 times increased risk of stroke in diabetics, respectively. This risk was found as 2.5 times for GSTT1 present and 3.8 times GSTP1 313AG heterozygote genotypes in diabetics. The risk of smoker individuals having ischemic stroke was highest in the GSTM1 present genotype group (OR=3.150, *P*=0.013). The risk decreased to 1.9-fold in GSTM1 null, 2-fold in GSTT1 present, 2.8-fold in GSTT1 null and 2.5 fold in GSTP1 313AG heterozygote individuals. Similarly, GSTM1 present genotype was the highest risky genotype in obese people (OR=8.068, *P*=0.001). GSTM1 null (OR=3.445, *P*=0.003), GSTT1 present (OR=4.812, *P*=0.000), GSTT1 null (OR=3.961, *P*=0.014), GSTP1 313AA (OR=5.024, *P*=0.001) and GSTP1 313AG (OR=3.604, *P*=0.020) genotypes also represented a significant risk for stroke in obese individuals.

For NOS3 G894T polymorphism, 894GG wild type and 894GT heterozygote genotypes were observed as significant determinants of stroke in both hypertensive (OR=2.193, *P*=0.026; OR=4.535, *P*=0.000, respectively) and obese (OR=5.932, *P*=0.010; OR=3.358, *P*=0.003, respectively) people. The 894GG wild type was associated with 2.6 times and 4.2 times increased risk of stroke in diabetics and smokers, respectively. Except smokers, NOS3 -786TT wild type and -786TC heterozygote genotypes were found as significant determinants of stroke in hypertensive (OR=3.358, *P*=0.000; OR=3.600, *P*=0.000, respectively), diabetic (OR=2.226, *P*=0.015; OR=2.472, *P*=0.037, respectively) and obese (OR=5.613, *P*=0.000; OR=4.026, *P*=0.022, respectively) subjects. For NOS3 intron4 VNTR polymorphism “bb” genotype was associated with increased stroke risk in hypertensive (OR=4.142, *P*=0.000), diabetic (OR=3.034, *P*=0.000) and obese (OR=6.720, *P*=0.000) people. On the other hand, in smokers “ab” heterozygote genotype (OR=3.940, *P*=0.014) was found as significant determinant of stroke.

As a conclusion, we could not find any direct association between genotypes or alleles of studied genetic polymorphisms and ischemic stroke risk. However, various

genotypes were defined as significant risk factors for ischemic stroke in different subgroups such as hypertensive/normotensive, diabetic/non-diabetic, smoker/non-smoker and obese/non-obese.

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APPENDIX A

INFORMED CONSENT FOR PATIENTS

İnme-felç hastalığı için risk oluşturan faktörleri bulmak üzere yeni bir araştırma yapmaktayız. Araştırmanın ismi "Paraoksonaz 1'in gen ve aktivite polimorfizmlerinin iskemik inme riski ile ilişkisinin araştırılması" dır. Sizin de bu araştırmaya katılmanızı öneriyoruz. Bu araştırmaya katılıp katılmamakta serbestsiniz. Çalışmaya katılım gönüllülük esasına dayalıdır. Kararınızdan önce araştırma hakkında sizi bilgilendirmek istiyoruz. Bu bilgileri okuyup anladıktan sonra araştırmaya katılmak isterseniz formu imzalayınız. Araştırmaya davet edilmenizin nedeni sizde bu hastalığın bulunmasıdır. Size gerekli tetkikleri yaptıktan sonra bu hastalık için kabul görmüş klasik bir tedavi başlayacağız. Eğer araştırmaya katılmayı kabul ederseniz Prof.Dr. Okay Vural, Doç.Dr. Şeref Demirkaya ve Uz. Öğ.V. Semai BEK veya onların görevlendireceği bir hekim tarafından muayene edilecek ve bulgularınız kaydedilecektir. Bu çalışmayı yapabilmek için kolunuzdan 10 ml (2 tüp) kadar kan almamız gerekmektedir. Bu kandan çalışmada kullanılacak olan tetkikler çalışılacaktır. Bu çalışmaya katılmanız için sizden herhangi bir ücret istenmeyecektir. Çalışmaya katıldığınız için size ek bir ödeme de yapılmayacaktır. Kan alımı sizin hastalığınız klinik takibi sırasında alınacak kanlar alınır iken 2 tüp fazladan alınacaktır. Dolayısı ile size ek bir işlem yapılmayacaktır.

Yapılacak araştırmanın getireceği olası yararlar: Böyle bir analiz hastalığınıza sebep olan beyin damarlarınızın tıkanmasına yol açan veya damarınızın tıkanması için risk oluşturan faktörleri tespit edilmesinin öğrenilmesinde yararlı olacaktır. Şu

anda bu çalışmanın hemen size bir fayda olarak dönüp dönmeyeceğini bilmiyoruz. Ancak ilgili hastalığın temelinde yatan nedenlerin öğrenilmesinde ve gelecekte yeni tedavi yaklaşımlarının geliştirilmesi, bu hastalık geçirme riski olan hastaların önceden tespit edilmesi ve belki de hastalık geçirmeden önce önlem alınmasında fayda sağlayacaktır.

Bu çalışmaya katılmayı reddedebilirsiniz. Bu araştırmaya katılmak tamamen isteğe bağlıdır ve reddettiğiniz takdirde size uygulanan tedavide yada bundan sonra kliniğimizde size karşı davranışlarımızda herhangi bir değişiklik olmayacaktır. Yine çalışmanın herhangi bir aşamasında onayınızı çekmek hakkına da sahipsiniz.

Hastanın Beyanı

Sayın Prof Dr. Okay Vural, Doç.Dr. Şeref Demirkaya ve Uz.Öğ.V. Semai BEK tarafından Gülhane Askeri Tıp Akademisi Nöroloji Anabilim Dalı'nda tıbbi bir araştırma yapılacağı belirtilerek bu araştırma ile ilgili yukarıdaki bilgiler bana aktarıldı. Bu bilgilerden sonra böyle bir araştırmaya "katılımcı" olarak davet edildim. Eğer bu araştırmaya katılırsam hekim ile aramda kalması gereken bana ait bilgilerin gizliliğine bu araştırma sırasında da büyük özen ve saygı ile yaklaşılacağına inanıyorum. Araştırma sonuçlarının eğitim ve bilimsel amaçlarla kullanımı sırasında kişisel bilgilerimin ihtimamla korunacağı konusunda bana yeterli güven verildi.
Araştırma için yapılacak harcamalarla ilgili herhangi bir parasal sorumluluk altına girmiyorum. Bana da bir ödeme yapılmayacaktır.

İster doğrudan, ister dolaylı olsun araştırma uygulamasından kaynaklanan nedenlerle meydana gelebilecek herhangi bir sağlık sorunumun ortaya çıkması halinde, her türlü tıbbi müdahalenin sağlanacağı konusunda gerekli güvence verildi. (Bu tıbbi müdahalelerle ilgili olarak da parasal bir yük altına girmeyeceğim).
Bu araştırmaya katılmak zorunda değilim ve katılmayabilirim. Araştırmaya katılmam konusunda zorlayıcı bir davranışla karşılaşmış değilim. Eğer katılmayı reddedersem, bu durumun tıbbi bakımıma ve hekim ile olan ilişkime herhangi bir zarar getirmeyeceğini de biliyorum.

Bana yapılan tüm açıklamaları ayrıntılarıyla anlamış bulunmaktayım. Kendi başıma belli bir düşünme süresi sonunda adı geçen bu araştırma projesinde

“katılımcı” olarak yer alma kararını aldım. Bu konuda yapılan daveti büyük bir memnuniyet ve gönüllülük içerisinde kabul ediyorum.

Katılımcı

Adı, soyadı:

Adres:

Tel:

İmza:

Görüşme tanığı

Adı, soyadı:

Adres:

Tel:

İmza:

Katılımcı ile görüşen hekim

Adı soyadı, unvanı:

Adres:

Tel:

İmza:

APPENDIX B

INFORMED CONSENT FOR CONTROLS

Araştırma beyin damar tıkanması sonucu oluşan felç-inme hastalığına sebep olan veya katkıda bulunan durumların ortaya konmasına yönelik bir çalışmadır. İnme-felç için risk oluşturan birçok hastalık ve durumu şu an için biliyoruz. Bizim yapacağımız çalışma bunların dışında da bu hastalık için risk oluşturabilecek faktörlerin olup olmadığının araştırılmasıdır. Bu amaçla kanda yüksek yoğunluktaki yağ proteinine (HDL) bağlı olarak bulunan ve eksikliğinde damar sertliği ve sonuçta damar tıkanmasına sebep olabilen paraoksonaz 1 ve benzeri enzimlerin aktivitesi ve genetik durumu incelenecektir. Yapacağımız çalışma daha önce temelde aynı mekanizmaya dayanan kalp krizi için yapılmış ve anlamlı sonuçlar bulunmuştur. Bu işlem için sizden 2 tüp 10 ml kan alınacak ve çalışmalar buradan yapılacaktır. Kan alımı sizin hastalığınızın klinik takibi sırasında alınacak kanlar ile birlikte alınacak ve size ek bir işlem yapılmayacaktır. Sizden 2 tüp kan alımı dışında her hangi bir işlem veya bu çalışmayla ilişkili ek bir tedavi yapılmayacaktır. Araştırma sırasında oluşabilecek herhangi bir zararlı durumu yoktur. Sizden sadece kan alınacaktır. Araştırmaya gönüllü olarak katılmaktasınız ve araştırmaya katılmakta tamamen serbestsiniz. Çalışmada yer alacak gönüllü sayısı yaklaşık 150 hasta ve 150 sağlıklı kişi olacaktır.
Çalışmada yer aldığınız ve bilimsel gelişmelere katkılarınızdan dolayı teşekkür ediyoruz.

Açıklamaları Yapan Araştırmacının

Adı, Soyadı:

Görevi:

İmzası:

Açıklamayı başından sonuna kadar tanıklık eden kişinin

Adı, Soyadı:

Adresi:

İmzası:

Çalışmaya katılan gönüllünün

Adı, soyadı:

Adres:

İmzası:

APPENDIX C

ETHICAL COMMITTEE APPROVAL FORM

HİZMETE ÖZEL

T.C.
GENELKURMAY BAŞKANLIĞI
GÜLHANE ASKERİ TIP AKADEMİSİ KOMUTANLIĞI
A N K A R A

22 Şubat 2008

Y. ETİK KRL. : 1491 - 547 - 08
KONU : GATA Etik Kurulu

Doç. Dr. Şeref DEMİRKAYA

20 Eylül 2005 tarihli 43. Oturumda GATA Etik Kurulu'ndan onay almış olan **"Paraoksonaz 1'in Aktivite ve Gen Polimorfizmlerinin İskemik Strok Üzerindeki Etkisinin Araştırılması"** başlıklı çalışmanın adının **"HMG-Co Redüktaz, Lesitin Kolesterol Asetil Transferaz, GST Transferazlar, Lipoproteinler ve Sitokrom P450 Enzimlerinin Genetik Polimorfizmlerinin İskemik Strok Üzerindeki Etkisinin Araştırılması"** olarak değiştirilmesi ile ilgili protokol değişikliği başvurunuz ile ilgili, GATA Etik Kurulu'nun kararı EK'tedir.

Rica ederim.

Ali Uğur URAL
Prof. Tbp. Kd. Alb.
GATA Etik Kurulu Başkanı

EK :
1 Adet Etik Kurul Raporu

HİZMETE ÖZEL

T.C.
GENELKURMAY BAŞKANLIĞI
GÜLHANE ASKERİ TIP AKADEMİSİ KOMUTANLIĞI
ETİK KURUL TOPLANTI RAPORU

OTURUM NO : 103
OTURUM TARİHİ : 15 Şubat 2008
OTURUM BAŞKANI : Prof. Tbp. Kd. Alb. Ali Uğur URAL
OTURUM SEKRETERİ : Doç. Dr. Ecz. Kd. Alb. Adnan ATAÇ

GATA Etik Kurulu'nun 15 Şubat 2008 günü yapılan 103. oturumunda; GATA Nöroloji AD'dan Doç.Dr. Şeref Deirkaya'nın sorumlu araştırmacılığını yaptığı 20 Eylül 2005 tarihli 43. Oturumda GATA Etik Kurulu'ndan onay almış olan **"Paraoksonaz 1'in Aktivite ve Gen Polimorfizmlerinin İskemik Strok Üzerindeki Etkisinin Araştırılması"** başlıklı çalışmanın adının **"HMG-Co Redüktaz, Lesitin Kolesterol Asetil Transferaz, GST Transferazlar, Lipoproteinler ve Sitokrom P450 Enzimlerinin Genetik Polimorfizmlerinin İskemik Strok Üzerindeki Etkisinin Araştırılması"** olarak değiştirilmesi ile ilgili protokol değişikliği değerlendirildi.

Protokol değişikliğinin amaç, yöntem ve yaklaşım bakımından etik ilkelere UYGUN olduğuna karar verildi.

BAŞKAN
Ali Uğur URAL
Prof.Tbp.Kd.Alb.

ÜYE
Ali İhsan UZAR
Prof.Hv.Tbp.Kd.Alb.

ÜYE
Ayhan KUBAR
Prof.Tbp.Alb.

ÜYE
Adnan ATAÇ
Doç.Dr.Ecz.Kd.Alb.

ÜYE
Mükerrem SAFALI
Doç.Tbp.Kd.Alb.

ÜYE
K. Melih AKAY
Doç.Tbp.Kd. Alb.

ÜYE
Mustafa ÖZER
Doç.Tbp.Alb.

ÜYE
Ergun TOZKOPARAN
Doç.Tbp. Alb.

ÜYE
Nalan AKBAYRAK
Prof. Dr. Sağ. Yb.

ÜYE
Muharrem UÇAR
Yrd.Doç.J.Tbp.Yb.

APPENDIX D

BUFFERS AND 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 1L. 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 1L. Solution was autoclaved for sterilization and stored at 4°C.

TKME (Tris-KCl-$MgCl_2$-EDTA) Buffer, pH 7.6

10 mM 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), 1 mM 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 at 37°C), 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 Tango (digestion buffer of *Rsa*I, *Dra*I, *Dde*I, *Ban*II, *Pdi*I, *BsmA*I, *Dra*II) (Fermentas)

33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM Mg-acetate, 66 mM K-acetate, 0.1 mg/mL BSA. This buffer was supplied together with the restriction enzymes *Rsa*I, *Dra*I, *Dde*I, *Ban*II, *Pdi*I, *BsmA*I, *Dra*II. The restriction enzymes and buffers were stored at -20°C.

Buffer R (digestion buffer of *Hinf*I) (Fermentas)

10 mM Tris-HCl (pH 8.5 at 37°C), 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.

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 E

LIST OF STUDY POPULATION

Table E.1 List of study population composed of 245 stroke patients and 145 controls including demographic characteristics, lipid parameters, CYP2E1*5B, CYP2E1*6, CYP2E1*7B, FMO3 G472A, FMO3 A923G, NQO1*2, GSTP1 A313G, GSTM1, GSTT1, NOS3 G894T, NOS3 T-786C and NOS3 intron4 VNTR genotypes. P: Patient; C: Control; M: Male; F: Female; Y: Yes; N: No.

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
1	P	75	M	Y	Y	Y	N	3.094	1.281	1.846	0.615	c1c1	DD	GG	AA	AA	CC	AA	Null	Null	GG	TT	bb
2	P	57	F	Y	Y	N	N	5.460	1.528	3.385	1.308	c1c1	DD	GG	GG	AA	CT	GG	Present	Null	GT	TC	bb
3	P	41	M	N	N	Y	N	4.316	1.573	2.667	0.872	c1c1	DC	GG	GG	AA	CC	AA	Null	Null	GG	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
4	P	73	M	Y	N	N	N	3.718	0.719	1.897	1.462	c1c1	DD	GG	AA	AG	CT	AA	Null	Present	GT	TT	bb
5	P	53	M	Y	Y	N	N	10.426	2.596	7.590	1.513	c1c1	DD	GG	AA	AA	CC	AA	Null	Null	GT	TT	bb
6	P	66	M	Y	Y	Y	N	3.380	1.416	2.179	0.513	c1c1	DD	GG	AA	AA	CC	AG	Present	Present	GT	TT	bb
8	P	56	F	Y	Y	N	N	3.588	2.382	1.795	0.974	c1c2	DC	GG	GG	AA	CC	AA	Null	Present	GT	TC	bb
9	P	54	M	Y	N	Y	N	5.200	2.438	2.949	1.077	c1c1	DC	GT	GG	AA	CC	AA	Present	Null	GG	TT	bb
10	P	67	F	Y	N	N	N	5.434	1.191	3.564	1.256	c1c1	DD	GG	GA	AA	CT	AA	Present	Present	GG	TT	bb
11	P	76	M	Y	N	Y	N	4.030	0.764	2.333	1.282	c1c1	DD	GT	GA	AG	CC	AG	Present	Present	GG	TC	ab
12	P	78	F	Y	Y	N	N	3.692	1.539	2.026	0.923	c1c1	DD	GG	GA	AA	CT	AA	Null	Present	GG	TT	bb
13	P	75	F	Y	N	N	N	4.732	1.169	3.256	0.872	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TC	bb
14	P	74	F	Y	Y	N	N	4.342	0.697	2.744	1.231	c1c1	DD	GG	GA	AA	CT	AG	Null	Present	GT	TT	bb
15	P	68	F	Y	N	Y	N	3.640	2.270	1.923	0.641	c1c1	DD	GG	AA	AA	CC	AG	Null	Null	GG	TC	ab
16	P	72	F	N	N	N	N	6.656	2.843	4.231	1.026	c1c2	DC	GG	GG	AA	CT	AA	Present	Present	GT	TT	bb
19	P	73	F	Y	Y	N	N	5.382	2.270	3.205	1.077	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	bb
20	P	73	F	Y	N	N	Y	4.680	0.989	2.744	1.410	c1c1	DD	GG	GA	AA	CC	AG	Present	Present	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
21	P	67	F	Y	N	N	N	5.408	1.393	3.667	1.026	c1c1	DD	GG	GG	AA	CC	AA	Present	Null	GT	TC	bb
23	C	71	M	Y	N	N	N	3.848	1.337	2.103	1.077	c1c1	DD	GG	GA	AA	CT	AA	Present	Present	GT	TC	bb
24	P	61	M	Y	Y	N	N	4.394	1.371	2.769	0.949	c1c1	DD	TT	GG	AA	CC	AA	Null	Present	GT	TC	ab
25	P	40	M	N	N	Y	N	3.770	1.169	2.000	1.205	c1c1	DD	GT	AA	AA	CC	AG	Null	Present	GT	TC	ab
26	C	61	F	Y	N	N	N	3.380	0.933	1.179	1.744	c1c1	DD	GG	GA	AG	CC	AG	Present	Present	GG	TC	ab
27	P	60	F	Y	Y	N	Y	4.992	1.685	3.051	1.103	c1c1	DD	GG	GA	AA	CT	AA	Present	Present	GT	TT	ab
29	P	75	M	N	N	N	N	4.550	1.079	1.821	2.179	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GG	TT	bb
30	P	76	F	Y	N	N	N	3.224	1.517	1.282	1.179	c1c1	DD	GT	GA	AG	CC	AA	Present	Present	GT	TC	bb
31	C	76	M	Y	Y	N	N	3.432	1.045	1.897	1.000	c1c1	DD	GG	GG	AA	CT	AG	Present	Present	GT	TC	bb
32	C	51	M	N	N	Y	N	3.692	1.292	2.128	0.897	c1c1	DC	GG	AA	AA	CC	AA	Present	Present	GT	TC	bb
33	C	50	F	N	N	N	N	3.614	2.022	1.872	0.769	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GG	TT	bb
34	C	42	F	N	N	N	N	2.704	1.270	1.462	0.615	c1c1	DD	GG	AA	AA	TT	AA	Present	Present	GG	TT	bb
35	C	45	F	N	N	Y	N	3.666	0.596	1.872	1.462	c1c1	DD	GG	AA	AG	CC	AG	Null	Null	GT	TT	bb
36	P	70	M	Y	N	N	N	3.016	0.820	1.513	1.077	c1c1	DD	GG	AA	AG	CT	AG	Present	Null	TT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
37	P	76	M	N	N	Y	N	3.328	1.393	1.872	0.769	c1c1	DC	GG	GG	AA	TT	AA	Present	Present	GT	TT	bb
38	C	63	M	Y	Y	Y	N	3.042	1.124	1.538	0.949	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GG	TT	bb
40	C	63	F	N	N	N	N	5.200	2.483	3.128	0.872	c1c1	DD	GG	GA	AA	CT	AG	Null	Null	GG	TT	bb
42	C	75	M	Y	Y	N	N	4.732	1.719	2.564	1.308	c1c1	DD	GT	GA	AA	CC	AA	Present	Present	GG	TT	bb
43	C	58	F	Y	N	N	N	2.704	1.090	1.103	1.077	c1c1	DD	GG	GG	AA	CT	AA	Null	Present	GT	TC	bb
44	C	78	F	Y	N	N	N	3.614	1.067	1.821	1.256	c1c1	DC	GG	GG	AA	CC	AG	Null	Present	GG	TT	bb
46	C	74	M	N	N	N	N	4.342	0.921	2.564	1.308	c1c1	DC	GG	GA	AA	CT	AA	Null	Present	GG	TT	ab
47	P	71	M	Y	N	N	N	5.382	2.607	3.231	0.897	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TT	bb
48	C	32	M	N	N	Y	N	6.006	3.247	3.333	1.103	c1c1	DC	GG	AA	AA	CT	AA	Null	Present	GG	TT	ab
49	C	30	F	N	N	N	N	5.148	1.101	2.897	1.667	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GG	TT	ab
50	C	61	M	N	N	N	N	5.928	1.865	3.974	1.026	c1c1	DD	GG	GG	AA	CT	AA	Null	Null	GG	TC	ab
51	C	85	M	Y	N	N	N	3.094	1.798	0.615	1.615	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GG	TT	bb
52	C	65	F	N	Y	N	N	6.110	1.416	3.231	2.154	c1c1	DD	GG	GA	AA	CT	AG	Null	Present	GT	CC	ab
53	C	65	M	N	N	N	N	4.966	0.640	3.282	1.333	c1c1	DD	GG	GA	AA	CT	AG	Null	Null	GT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
54	C	58	M	N	Y	Y	N	5.954	1.202	4.333	1.000	c1c1	DD	GG	GG	AA	CT	AA	Null	Present	GT	TT	bb
55	C	61	F	N	N	N	Y	6.812	1.831	4.359	1.513	c1c1	DD	GG	AA	AG	CT	AA	Null	Null	GT	CC	ab
56	C	80	F	Y	N	N	N	6.058	2.270	2.128	0.615	c1c1	DD	GT	GG	AA	CT	AG	Present	Present	GG	TC	ab
57	C	80	F	N	N	N	N	3.354	0.708	1.179	1.821	c1c1	DD	GG	AA	AA	CT	AG	Null	Present	GG	CC	aa
58	C	67	F	Y	Y	N	N	3.926	1.427	2.179	1.051	c1c1	DD	GT	GG	AA	CT	AA	Null	Null	GG	TT	ab
59	C	76	M	N	N	N	N	6.006	2.315	3.667	1.205	c1c1	DD	GG	AA	AG	CT	AG	Present	Present	GT	TT	bb
60	C	66	M	Y	Y	N	N	4.108	1.427	2.513	0.897	c1c1	DD	GG	GG	AA	CT	GG	Null	Null	GG	TT	bb
61	P	61	M	N	Y	N	N	3.848	1.618	1.949	1.103	c1c1	DC	GG	GG	AA	CT	AG	Null	Present	GG	TT	ab
62	C	69	F	Y	N	N	N	3.926	1.764	1.897	1.179	c1c1	DD	GG	GG	AA	CT	AG	Null	Present	GG	TT	bb
63	C	66	M	N	Y	Y	N	6.968	3.921	4.103	0.974	c1c1	DD	GG	AA	AA	CT	AA	Null	Present	GT	TT	bb
64	C	60	F	Y	N	N	N	4.966	1.382	3.103	1.154	c1c1	DD	GG	GG	AA	CT	GG	Present	Present	GT	TC	ab
66	P	64	F	N	N	Y	Y	4.316	1.056	2.538	1.231	c1c1	DD	TT	GG	AA	CT	AA	Present	Null	GT	TT	bb
67	P	58	F	Y	Y	N	N	9.100	4.045	5.846	1.282	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TC	bb
68	P	74	F	Y	N	N	Y	6.266	1.730	4.154	1.231	c1c1	DD	GG	AA	AA	CT	AG	Present	Present	GT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
69	C	71	M	N	N	N	N	4.290	1.573	2.051	1.410	c1c1	DD	GG	AA	AG	CT	AA	Null	Present	GT	TC	bb
71	P	80	F	Y	N	N	Y	3.770	0.966	2.308	0.974	c1c1	DD	GG	AA	AG	CT	AA	Null	Present	GG	TT	bb
72	P	62	M	Y	Y	N	N	4.888	1.202	3.282	1.000	c1c1	DD	GG	AA	AA	CT	AA	Present	Present	GG	TC	bb
73	C	68	M	N	N	N	N	5.460	1.292	3.154	1.641	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TC	bb
74	C	65	F	N	N	N	N	5.200	1.764	3.154	1.179	c1c1	DC	GG	GG	AA	CT	GG	Null	Present	GT	TT	bb
76	C	72	F	Y	N	N	N	6.084	1.674	4.051	1.179	c1c1	DD	GG	AA	AG	CT	AG	Null	Present	TT	TT	bb
77	C	65	M	N	N	N	N	3.042	0.899	1.641	0.949	c1c1	DD	GG	GG	AA	CT	GG	Present	Present	GT	TT	bb
78	C	63	F	Y	Y	N	Y	5.018	3.685	1.692	1.103	c1c1	DC	GG	GG	AA	CC	AG	Present	Present	GG	TT	ab
79	C	70	F	N	N	N	N	4.862	1.483	3.026	1.103	c1c1	DD	GT	GA	AA	CT	AA	Null	Present	GG	TT	bb
80	C	70	F	Y	N	N	N	5.252	1.180	3.333	1.308	c1c1	DC	GG	AA	AA	CC	AA	Present	Present	GG	TT	bb
81	C	65	F	Y	N	N	N	5.954	1.764	3.769	1.308	c1c1	DD	GG	GG	AA	CC	AA	Present	Null	GT	TT	bb
82	P	63	M	Y	N	N	N	3.406	1.000	1.846	1.051	c1c1	CC	GG	GG	AA	CC	AG	Null	Present	GT	TT	bb
83	C	65	F	Y	N	N	N	4.524	1.270	2.179	1.692	c1c1	DD	GG	AA	AA	CC	AG	Present	Present	GT	TC	bb
84	C	78	F	Y	N	N	N	4.394	1.247	2.385	1.385	c1c1	DD	GG	GA	AG	CC	AA	Null	Present	TT	CC	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
85	P	68	M	Y	N	N	N	4.446	0.899	2.436	1.538	c1c1	DD	GG	AA	AA	CT	AA	Null	Present	GT	TC	ab
86	C	47	M	N	N	N	N	6.266	3.056	3.333	1.462	c1c1	DC	GG	AA	AA	CC	GG	Null	Present	GG	TT	bb
87	P	77	F	Y	N	N	N	5.070	1.067	3.513	1.000	c1c1	DD	GG	AA	AA	CC	GG	Null	Present	GG	TT	bb
88	C	77	F	Y	Y	N	N	4.056	0.978	2.641	0.923	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	TT	TC	bb
89	P	80	F	Y	N	N	N	5.226	1.056	3.077	1.590	c1c1	DD	GG	AA	AG	CC	AG	Null	Null	GT	TC	bb
90	C	71	M	N	N	N	N	4.368	1.472	2.308	1.333	c1c1	DC	GG	AA	AG	CT	AG	Null	Null	GT	TC	bb
91	P	55	M	Y	N	N	N	4.082	1.393	2.436	0.949	c1c1	DC	GG	AA	AA	CT	AG	Null	Null	GG	TT	bb
92	C	73	M	Y	N	N	N	3.640	2.472	1.949	0.513	c1c1	DD	GG	AA	AA	CC	AG	Present	Present	GT	TT	bb
93	C	61	M	N	N	Y	N	4.680	0.393	2.923	1.513	c1c1	DD	GG	AA	AG	CC	GG	Null	Null	GG	TC	ab
94	C	37	M	N	Y	N	N	4.784	1.348	2.821	1.282	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TT	bb
95	P	62	M	N	N	N	N	3.692	1.011	1.385	1.103	c1c1	DD	GG	AA	AG	CT	AG	Null	Null	GT	TT	bb
96	P	77	M	N	N	N	N	3.822	0.607	1.744	1.744	c1c1	DD	GG	AA	AA	CT	AA	Null	Present	GG	CC	bb
97	P	24	M	N	N	N	N	6.110	2.865	3.667	1.051	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GT	TC	ab
98	P	53	F	N	N	Y	N	2.496	2.157	0.590	0.897	c1c1	DD	GG	AA	AG	CC	AA	Null	Null	GG	TC	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
99	P	61	M	N	N	N	N	4.836	1.596	3.103	0.949	c1c1	DD	GG	AA	AG	CC	AA	Present	Present	GT	TT	bb
101	C	52	M	N	N	N	N	6.942	1.157	4.692	1.615	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TC	ab
102	P	78	M	Y	N	Y	N	5.226	2.056	3.077	1.128	c1c1	CC	GG	GG	AA	CC	AA	Present	Present	GT	TC	bb
103	C	65	M	N	N	N	Y	4.160	0.854	2.897	0.821	c1c1	DD	GG	AA	AA	CT	AG	Present	Present	GT	TC	ab
106	P	80	F	Y	N	N	Y	2.730	1.270	1.462	0.641	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GG	TT	bb
107	C	50	F	Y	N	N	N	3.198	0.854	2.154	0.615	c1c1	DD	GG	AA	AA	CT	AA	Present	Present	GG	TT	bb
108	C	87	F	Y	N	N	Y	2.756	1.022	1.385	0.872	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GT	TT	bb
109	C	67	M	N	N	N	N	4.368	1.146	2.103	0.154	c1c1	DD	GT	GA	AA	CT	AG	Present	Null	GT	TC	ab
111	C	38	M	N	N	Y	N	4.784	0.562	2.846	1.615	c1c1	DC	GG	GA	AA	CC	AG	Null	Null	TT	TT	bb
112	C	50	M	N	N	N	N	3.822	0.618	2.026	1.462	c1c1	DD	GG	GA	AA	CT	GG	Null	Null	GG	TT	ab
113	P	75	F	Y	N	N	N	2.860	0.652	1.333	1.179	c1c1	DD	GG	GA	AG	CC	AA	Present	Null	GT	TT	bb
114	P	26	M	N	N	N	N	4.212	1.067	2.051	1.615	c1c1	DD	GG	GA	AA	CC	AA	Present	Present	GG	TT	ab
115	P	55	M	Y	N	N	N	5.044	0.933	3.154	1.385	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TC	ab
116	P	26	M	N	N	N	N	4.056	0.719	2.923	0.744	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
117	P	73	F	Y	N	N	N	4.004	1.135	2.538	0.897	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TT	bb
119	C	80	F	N	Y	N	N	3.978	1.382	1.974	1.308	c1c1	DC	GG	GA	AA	CC	AA	Null	Null	GT	TC	bb
120	P	36	M	N	N	N	N	4.862	1.573	2.974	1.103	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
121	P	56	M	N	N	N	N	4.342	1.225	2.564	1.154	c1c1	CC	GG	GA	AA	CC	AG	Present	Null	GG	TC	ab
122	P	47	F	N	N	N	N	4.784	1.382	3.154	0.923	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GG	TT	bb
126	P	73	M	Y	Y	N	N	5.148	2.517	3.026	0.897	c1c1	DD	GT	GA	AA	CC	AA	Present	Present	GG	TT	bb
127	P	73	F	Y	N	N	N	4.004	1.135	2.538	0.897	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TT	bb
128	P	66	F	N	N	N	N	3.354	2.787	1.487	0.538	c1c1	DC	GG	GA	AG	CC	GG	Present	Present	GT	TC	ab
129	P	74	F	Y	N	N	N	5.980	1.427	3.949	1.308	c1c1	DC	GG	AA	AA	CC	AA	Present	Present	GG	TT	bb
130	C	44	M	N	N	Y	N	5.200	2.382	2.462	1.590	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	bb
131	C	51	F	N	N	N	N	4.368	0.573	2.821	1.231	c1c1	DD	GG	GA	AA	CT	AG	Present	Present	GG	TT	bb
132	C	67	M	Y	N	N	N	4.472	1.663	2.590	1.051	c1c1	DD	GG	GA	AG	CC	AG	Present	Present	GT	TC	bb
133	C	73	F	Y	Y	N	N	5.902	2.315	3.667	1.103	c1c1	DD	GG	GA	AA	CT	AG	Null	Present	GT	TT	bb
134	C	88	F	Y	N	N	N	4.004	0.798	2.564	1.026	c1c1	DD	GG	GA	AA	CC	AG	Present	Present	GG	TT	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
136	P	66	M	Y	Y	N	N	3.354	2.854	1.256	0.744	c1c2	DC	GG	GG	AA	CC	AG	Null	Present	GG	TT	ab
138	P	61	F	Y	Y	N	N	3.224	2.000	1.615	0.641	c1c1	DD	GT	GG	AA	CC	AA	Null	Present	GT	TC	bb
139	P	78	M	N	N	Y	N	3.380	1.236	1.795	1.026	c1c1	DD	GG	AA	AG	CC	AA	Present	Present	GG	TC	ab
140	C	59	M	N	N	N	N	4.082	1.236	2.308	1.154	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TC	bb
141	C	69	F	N	Y	N	N	6.266	1.337	4.026	1.538	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TC	bb
142	P	80	M	Y	N	N	N	7.904	0.831	6.128	1.282	c1c1	DD	GT	GG	AA	CC	AA	Null	Null	GT	TT	bb
143	P	76	F	Y	Y	N	N	4.108	1.236	2.513	0.974	c1c1	DD	GG	AA	AA	CC	AG	Present	Null	GT	TT	bb
144	P	79	F	Y	N	N	N	5.252	1.528	3.308	1.179	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TC	bb
146	C	51	F	N	N	N	N	2.860	1.011	1.385	0.974	c1c1	DD	GG	GG	AA	CT	AA	Null	Present	TT	TC	bb
147	P	21	M	N	N	N	N	3.874	0.933	2.564	0.821	c1c1	DC	GG	GG	AA	CC	AG	Present	Present	GT	TC	bb
148	P	76	M	N	N	N	Y	5.018	1.191	3.385	1.026	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TC	bb
150	C	35	M	N	N	Y	N	3.848	1.281	2.026	1.179	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GG	TT	bb
151	P	28	M	N	N	Y	N	3.926	1.640	2.000	1.128	c1c1	DD	GT	GG	AA	CT	AA	Present	Present	GT	TC	ab
152	P	20	M	N	N	Y	N	4.316	0.899	2.564	1.282	c1c1	DD	TT	GG	AA	CC	AA	Null	Present	GG	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
153	P	80	F	Y	N	N	N	5.018	2.820	2.205	1.462	c1c1	DD	GG	GA	AA	CC	AG	Present	Present	GG	TC	ab
154	P	64	F	Y	N	N	N	3.770	1.618	1.974	1.000	c1c1	DD	TT	GA	AA	CT	AA	Null	Present	GG	TT	bb
155	P	71	M	N	N	Y	N	4.940	0.910	3.385	1.077	c1c1	DD	GG	GG	AG	CT	GG	Present	Present	GT	TT	bb
156	P	67	F	Y	Y	N	Y	10.166	2.539	7.615	1.256	c1c1	DD	GG	GG	AA	CC	AG	Present	Present	GG	TT	bb
157	P	58	F	Y	Y	N	N	7.124	2.876	4.333	1.385	c1c1	DD	GG	GG	AA	TT	AG	Present	Present	GT	TC	ab
158	C	77	M	N	N	N	N	4.654	1.876	2.051	1.179	c1c1	DD	GT	AA	AA	CC	AA	Null	Present	GT	TC	bb
159	P	49	M	N	N	Y	N	5.876	2.292	3.564	1.179	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	ab
161	P	78	F	N	N	N	N	4.628	0.989	2.641	1.462	c1c1	DD	GG	AA	AA	CT	AG	Null	Present	GT	TC	ab
162	P	65	M	N	N	N	N	4.004	1.843	2.026	1.077	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TT	bb
163	P	75	M	Y	N	N	N	5.070	1.596	3.333	0.949	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TT	bb
164	P	79	M	N	N	Y	N	2.730	0.899	1.385	0.897	c1c1	DD	GG	AA	AA	TT	AG	Present	Present	TT	TC	ab
165	P	73	F	Y	Y	N	N	7.566	3.539	5.256	0.590	c1c1	DD	GG	GA	AA	CC	AG	Present	Present	TT	CC	bb
167	P	25	M	N	N	N	N	4.602	1.573	2.744	1.103	c1c1	DD	GG	GA	AA	CT	AG	Present	Present	GT	TC	bb
168	P	73	M	Y	N	N	N	5.070	0.888	3.051	1.538	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
169	P	74	M	Y	N	N	N	3.328	1.326	1.795	0.872	c1c1	DC	GG	GA	AA	CC	AA	Null	Present	GT	TT	bb
170	P	78	F	Y	Y	N	Y	4.706	1.551	2.795	1.128	c1c1	DD	GG	AA	AA	CT	AA	Present	Present	GG	TT	bb
172	P	56	M	Y	Y	N	N	4.602	1.764	2.692	1.051	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	bb
173	P	67	M	Y	N	N	N	4.290	1.730	2.282	1.103	c1c1	DC	GG	GG	AA	CC	AA	Null	Present	GT	TC	bb
174	P	74	M	Y	N	N	N	4.394	2.258	2.026	1.282	c1c1	DC	GG	AA	AA	CC	AA	Null	Present	GT	TT	bb
175	P	64	M	Y	Y	N	N	4.368	1.910	2.385	1.051	c1c2	DD	GG	AA	AG	TT	AA	Null	Present	GT	TT	ab
176	P	73	M	Y	Y	N	N	4.628	1.944	2.564	1.103	c1c1	DD	GG	GA	AA	TT	AG	Present	Present	GT	TT	bb
177	C	52	F	N	N	N	N	3.822	1.124	1.872	1.385	c1c1	DD	TT	GA	AG	CC	AA	Null	Present	GT	TT	bb
179	P	57	M	N	N	Y	N	4.862	1.674	2.974	1.051	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TT	bb
180	P	76	M	N	N	N	N	4.186	1.921	2.410	0.846	c1c1	DD	GG	GA	AG	CT	AA	Present	Present	GT	TT	ab
181	P	61	M	N	N	Y	N	4.862	1.393	2.974	1.179	c1c1	DD	GG	AA	AA	TT	AA	Null	Present	GT	TT	bb
184	C	77	F	Y	N	N	N	4.212	2.000	2.026	1.205	c1c1	DD	GG	AA	AA	CT	AG	Present	Present	GT	TT	bb
185	P	62	F	N	Y	N	N	4.082	1.629	2.333	0.949	c1c1	DD	GG	AA	AA	CC	AG	Present	Null	GT	TT	bb
186	P	73	M	N	N	Y	N	7.904	2.978	5.231	1.205	c1c1	DC	GG	GG	AA	CC	AA	Null	Present	GT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
187	P	63	M	N	N	Y	N	3.458	1.157	1.692	1.179	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GT	TC	bb
188	P	52	M	N	N	Y	N	4.108	1.101	2.769	0.795	c1c1	DD	GT	GA	AA	CT	AG	Present	Present	GT	TT	bb
189	C	79	M	N	N	N	N	5.252	0.865	3.846	0.949	c1c1	DD	GG	GA	AA	TT	AG	Null	Present	GT	TT	bb
190	P	61	F	Y	N	N	N	3.146	1.393	1.590	0.872	c1c1	DD	GG	GA	AA	TT	AA	Present	Present	GT	TC	bb
191	C	46	M	Y	N	Y	N	7.904	2.831	5.308	1.205	c1c1	DD	GT	GG	AA	CC	AG	Present	Present	GT	TT	ab
193	P	45	M	N	N	Y	N	3.926	3.742	1.308	0.846	c1c1	DD	GG	GG	AA	CT	AG	Null	Present	GG	TC	ab
194	P	64	M	N	N	Y	N	4.316	1.090	2.641	0.923	c1c1	DD	GT	AA	AA	CC	AA	Null	Present	GT	CC	ab
195	P	56	F	N	Y	N	N	7.020	3.191	4.333	1.128	c1c1	DD	GT	GG	AA	CC	AA	Null	Present	GG	TT	bb
196	P	67	M	Y	Y	N	N	6.916	2.843	4.385	1.128	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TC	bb
197	P	53	M	Y	N	N	N	7.384	3.393	4.513	1.231	c1c1	DD	GG	AA	AA	CC	AA	Present	Null	GT	TC	bb
198	C	35	M	N	N	N	N	4.082	1.966	2.256	0.872	c1c1	DD	GG	AA	AA	TT	AG	Null	Present	TT	TC	bb
200	C	36	M	N	N	N	N	3.146	0.775	1.923	0.821	c1c1	DD	GG	GA	AA	CT	AG	Present	Present	GT	TT	bb
201	C	38	M	N	N	Y	N	4.628	1.449	2.564	1.333	c1c1	DD	GT	GG	AA	CT	AG	Null	Present	GT	TC	bb
202	P	80	M	N	N	N	N	5.720	1.337	3.744	1.282	c1c1	DD	GT	GG	AA	TT	AA	Null	Null	GG	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
203	P	62	M	Y	Y	N	N	5.564	1.236	3.667	1.179	c1c1	DD	GT	GG	AA	CC	AG	Present	Null	GT	TC	bb
206	C	34	F	N	N	Y	N	4.004	0.978	2.692	0.821	c1c1	DD	GG	GA	AA	CT	AG	Null	Present	GT	TC	bb
207	C	41	F	N	N	N	N	3.900	1.461	2.154	1.026	c1c1	DD	GG	GA	AA	CT	AA	Present	Present	TT	TC	bb
209	P	80	M	N	N	N	N	5.278	1.685	3.154	1.282	c1c1	DD	GG	GA	AA	CC	AG	Null	Present	GT	TT	ab
210	P	61	M	N	N	Y	N	3.406	1.348	1.923	0.872	c1c1	DD	GG	GA	AA	CT	AA	Null	Present	GG	TC	ab
211	P	67	F	N	Y	N	N	4.940	1.517	3.205	0.974	c1c1	DC	GG	GG	AA	CC	AG	Present	Present	GG	TT	bb
213	C	48	F	N	N	N	Y	2.600	0.966	1.410	0.718	c1c1	DD	GG	AA	AA	CT	AG	Null	Present	GT	TC	bb
214	P	65	F	N	N	Y	N	5.772	1.180	3.821	1.333	c1c1	DD	GG	GG	AA	CC	AA	Null	Null	GT	TT	bb
215	C	35	F	N	N	N	N	3.510	1.663	1.923	0.769	c1c1	DD	GG	GG	AA	TT	AA	Null	Null	GT	TT	bb
216	P	79	F	Y	Y	N	N	4.264	1.685	2.641	0.795	c1c1	DD	GT	GA	AA	CC	AG	Null	Present	GT	TT	bb
217	P	61	M	Y	N	Y	N	4.706	1.202	3.051	1.051	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
218	P	36	F	N	N	Y	N	6.396	1.944	3.795	1.615	c1c1	DD	GT	AA	AA	CT	AG	Null	Present	GT	TC	aa
219	P	80	M	Y	Y	N	N	7.514	4.236	4.436	1.051	c1c2	DC	GG	GA	AG	CC	GG	Present	Present	GG	TT	ab
221	P	61	M	N	Y	Y	N	3.718	1.146	2.154	1.000	c1c1	DD	GG	GA	AA	CT	AG	Present	Present	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
222	P	69	M	Y	N	N	N	4.056	0.865	2.692	0.923	c1c1	DD	GG	GA	AG	TT	AA	Present	Present	GT	TC	ab
223	P	76	F	Y	Y	N	N	4.108	0.764	2.667	1.026	c1c1	DD	GG	GA	AA	CT	AA	Present	Present	GT	TC	bb
224	P	69	F	N	N	N	N	3.198	1.112	1.795	0.846	c1c1	DD	GG	GG	AA	CT	GG	Null	Null	GT	TT	ab
225	C	43	F	N	N	Y	N	5.772	1.843	3.513	1.333	c1c1	DD	GG	GA	AA	CC	AA	Present	Null	TT	TC	bb
226	P	77	M	Y	Y	Y	N	3.848	0.820	2.615	0.795	c1c1	DD	GG	GA	AA	CT	AA	Null	Present	GG	TT	bb
229	C	45	F	N	N	N	N	5.226	2.753	2.590	1.308	c1c1	DD	GG	AA	AG	CC	AA	Present	Null	GT	TT	bb
231	C	38	F	N	N	N	N	5.720	1.292	3.564	1.487	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GG	TC	aa
232	P	78	F	Y	N	N	N	4.888	0.697	3.179	1.333	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TC	bb
233	C	64	F	Y	N	N	Y	6.994	1.494	4.769	1.436	c1c1	DD	GG	GG	AA	CC	AG	Null	Null	GG	TC	ab
234	P	58	F	N	N	N	N	4.316	1.101	2.410	1.333	c1c1	DC	GG	GG	AA	CT	AG	Null	Present	GG	TT	bb
235	C	66	M	N	N	N	N	5.304	0.697	3.462	1.462	c1c1	DD	GG	AA	AG	CC	GG	Present	Present	GT	TT	bb
236	P	59	F	Y	Y	N	N	5.200	1.876	3.410	0.846	c1c1	DD	GT	AA	AA	CC	AA	Null	Present	GG	TT	bb
237	C	69	F	Y	N	N	N	4.862	1.169	2.000	1.205	c1c1	DD	GG	AA	AA	CT	AA	Present	Present	GG	TT	bb
239	P	53	M	N	N	Y	N	3.614	0.371	2.205	1.179	c1c1	DD	GG	GG	AA	CT	AG	Present	Null	GT	CC	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
240	C	42	M	N	N	Y	N	4.628	2.742	2.462	0.846	c1c1	DD	GG	AA	AA	CC	AG	Null	Null	GG	TT	bb
241	C	65	M	Y	N	N	N	3.848	1.292	1.949	1.256	c1c1	DD	GG	GG	AA	CC	GG	Null	Present	GT	TC	bb
242	C	87	M	Y	Y	N	N	2.704	1.236	0.974	1.128	c1c1	DD	GG	GG	AA	CT	GG	Present	Present	GT	TT	bb
244	C	75	F	N	N	Y	Y	4.784	0.910	2.897	1.410	c1c1	DD	GG	GA	AA	CC	GG	Null	Null	GT	TT	bb
245	P	54	M	N	N	N	N	4.420	0.663	3.103	0.949	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TT	bb
246	C	77	M	Y	N	N	N	3.614	0.674	2.436	0.821	c1c1	DD	GG	GA	AA	CT	GG	Null	Present	GG	TC	ab
247	C	69	F	N	N	N	N	4.628	1.292	2.718	1.256	c1c1	DD	GG	GA	AA	CC	AG	Present	Present	GT	TC	bb
248	P	75	F	N	Y	N	N	4.914	0.978	3.026	1.385	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GT	CC	bb
251	P	78	M	Y	Y	N	N	4.238	1.517	2.564	0.923	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TT	bb
252	P	40	F	N	N	Y	Y	4.992	1.056	3.256	1.179	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GG	TC	ab
253	P	48	M	Y	N	N	N	5.486	0.809	3.769	1.282	c1c2	DD	GG	GA	AA	CC	AG	Null	Present	GT	TT	bb
254	P	80	F	Y	Y	N	N	4.784	0.865	2.872	1.385	c1c1	DD	GG	GA	AG	CT	AG	Null	Present	GT	TC	bb
255	P	41	M	N	N	N	N	3.770	1.348	2.256	0.846	c1c1	DC	GT	GG	AA	CC	AG	Null	Present	GT	TC	bb
256	P	77	F	Y	Y	N	N	4.992	1.449	3.154	1.103	c1c1	DD	GG	AA	AA	CC	AG	Null	Present	GG	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
257	P	63	F	Y	Y	N	Y	3.926	0.775	2.564	0.949	c1c1	DD	GG	GG	AA	CC	AG	Null	Null	GT	TT	bb
258	P	55	M	Y	N	Y	N	7.202	1.831	5.308	0.949	c1c1	DD	GG	AA	AA	CT	GG	Present	Present	GG	TC	ab
259	P	79	M	Y	Y	N	N	3.120	0.764	1.564	1.154	c1c1	DD	GG	GG	AA	CT	AG	Present	Present	GT	TT	ab
260	P	67	F	Y	Y	N	N	5.122	1.427	2.923	1.487	c1c1	DD	GG	GG	AA	CT	AA	Null	Null	GT	TT	bb
261	P	63	M	Y	Y	N	N	3.770	1.764	2.128	0.795	c1c1	DD	GT	GG	AA	CC	GG	Present	Null	GT	TT	bb
262	P	75	F	Y	N	N	N	5.928	1.899	3.897	1.077	c1c1	DD	GG	AA	AA	CT	GG	Present	Present	GT	TT	bb
263	C	65	M	N	N	N	N	4.134	0.697	2.641	1.128	c1c1	DD	GG	GG	AA	CT	GG	Null	Present	GT	TC	ab
264	C	78	M	N	N	N	N	4.108	0.921	2.231	1.410	c1c1	DD	GT	GG	AA	CT	GG	Present	Present	GT	TT	bb
265	C	81	M	Y	N	N	N	2.808	0.607	1.231	1.256	c1c1	DD	GG	AA	AA	CC	AA	Null	Null	GG	TT	bb
266	C	56	M	N	Y	N	N	4.602	1.371	2.821	1.103	c1c1	DD	GT	GG	AA	CC	AA	Null	Null	GG	TT	bb
267	C	64	F	Y	Y	N	N	4.316	2.764	2.205	0.795	c1c1	DD	GG	AA	AA	CT	GG	Null	Present	GG	CC	aa
268	C	79	F	Y	N	N	N	4.706	1.236	2.821	1.256	c1c1	DD	GG	GG	AA	CT	GG	Null	Present	GT	TC	bb
269	C	67	F	N	N	N	N	7.748	1.146	5.846	1.282	c1c1	DD	GG	GG	AA	CT	GG	Null	Present	GT	TC	bb
270	C	76	F	Y	N	N	N	4.316	1.236	2.179	1.513	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
271	C	64	M	N	N	Y	N	3.380	1.022	2.205	0.667	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TT	bb
272	C	77	M	N	Y	N	N	8.788	1.775	6.231	1.615	c1c1	DD	GG	AA	AG	CC	AA	Null	Present	GG	TT	ab
273	C	75	M	N	N	N	N	4.966	1.674	2.795	1.333	c1c1	DD	GG	AA	AA	CT	GG	Present	Present	GT	TT	bb
274	P	54	F	N	N	N	N	5.226	3.382	2.923	0.692	c1c1	DD	GG	GG	AA	TT	AA	Present	Present	GG	TT	bb
275	P	61	F	Y	N	N	N	4.680	1.236	3.154	0.897	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
276	P	71	M	Y	N	N	N	3.250	0.798	1.872	0.974	c1c1	DD	GG	AA	AA	CT	AG	Present	Present	GT	TT	bb
277	C	68	F	Y	N	N	Y	4.836	1.910	2.487	1.410	c1c1	DC	GG	GG	AA	CC	AA	Null	Present	GT	TT	bb
278	P	74	F	Y	N	N	N	5.486	1.854	3.154	1.410	c1c1	DD	GG	GG	AA	CT	AG	Null	Present	GT	TT	bb
279	P	59	M	N	N	N	N	3.328	1.427	1.538	0.590	c1c1	DD	GG	GA	AG	CC	AA	Present	Present	GT	TC	ab
280	P	62	M	N	N	Y	N	4.004	0.787	2.641	0.949	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TT	bb
282	C	71	F	Y	N	N	N	3.874	1.090	2.308	1.026	c1c1	DD	GT	AA	AA	CC	AA	Present	Present	GG	TT	bb
283	C	52	F	N	N	N	N	4.004	0.798	2.564	1.026	c1c1	DD	GT	AA	AA	CC	AG	Null	Present	TT	TC	bb
284	C	78	M	Y	N	N	N	4.134	1.393	2.487	0.949	c1c1	DC	GG	AA	AA	CC	AG	Null	Null	GT	TC	ab
285	P	70	M	Y	N	N	N	5.148	0.854	3.436	1.256	c1c1	DD	GG	AA	AG	CC	GG	Null	Null	GT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
286	P	69	M	Y	N	N	N	4.420	0.933	2.846	1.077	c1c2	DC	GG	AA	AA	CT	AG	Present	Present	GG	TT	bb
287	C	80	M	Y	N	N	N	5.122	0.966	3.231	1.385	c1c1	DD	GG	GA	AA	CT	AA	Null	Present	GG	TT	bb
288	P	57	F	N	Y	N	N	5.460	1.730	3.462	1.128	c1c1	DD	GG	AA	AA	CT	AA	Null	Null	GG	TT	bb
289	P	58	F	Y	N	N	N	3.198	1.258	1.538	1.051	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	ab
292	P	80	M	Y	N	N	N	3.536	0.697	2.000	1.179	c1c2	DC	GG	GA	AG	CT	AA	Present	Present	GT	TC	bb
294	C	78	F	N	N	N	N	4.082	0.944	2.513	1.051	c1c1	DD	GG	GG	AA	CT	AA	Null	Present	GT	TC	bb
296	C	57	F	N	N	N	N	2.444	1.045	0.487	1.436	c1c1	DD	GG	AA	AA	CC	AG	Present	Present	GG	TT	bb
297	C	60	F	Y	N	N	N	5.356	2.225	2.872	1.385	c1c1	DD	GG	GG	AA	CC	AA	Present	Null	GT	TC	bb
298	C	63	F	Y	N	N	N	6.682	2.764	4.103	1.231	c1c1	DD	GG	AA	AA	CT	AA	Null	Present	GT	TT	bb
299	P	74	M	N	Y	N	N	3.380	0.730	1.667	1.333	c1c1	DC	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
300	P	50	M	Y	N	N	N	2.002	0.843	0.615	0.974	c1c1	DD	GG	GA	AA	CC	AA	Null	Null	GT	TC	bb
301	C	58	M	Y	N	N	N	3.900	2.247	2.051	0.769	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GG	TC	ab
302	C	54	M	Y	N	N	N	4.316	1.157	2.410	1.308	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GT	TT	bb
304	C	78	M	N	N	N	N	3.692	0.674	2.051	1.282	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GG	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
305	P	57	M	N	Y	N	N	5.018	1.427	3.564	0.744	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
306	C	75	M	Y	Y	N	N	4.810	0.798	2.949	1.436	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
307	C	77	M	Y	N	N	N	3.068	0.989	1.641	0.923	c1c1	DC	GG	AA	AA	CT	AA	Present	Present	GG	TC	ab
309	P	62	M	Y	N	N	N	5.070	2.618	3.051	0.744	c1c1	DD	GG	AA	AA	CT	AG	Null	Present	GG	TC	ab
311	P	77	F	Y	Y	N	N	3.016	2.101	1.564	0.462	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GG	TT	ab
312	P	54	M	Y	Y	N	N	4.134	1.337	2.846	0.615	c1c1	DC	GG	AA	AA	CC	AG	Present	Present	GT	TT	bb
316	P	71	F	Y	Y	N	N	4.238	1.933	2.333	0.974	c1c1	DD	GG	GA	AA	CC	AA	Present	Present	GT	TC	bb
318	P	80	F	Y	N	N	N	4.290	1.562	2.308	1.205	c1c1	DD	GG	GG	AA	CT	AG	Present	Present	GG	TT	bb
319	C	59	F	N	N	N	N	5.538	1.416	3.231	1.590	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GG	TT	bb
320	C	57	F	N	N	N	N	4.368	1.865	2.564	0.897	c1c1	DD	GG	GA	AA	CC	AG	Present	Present	GG	TT	bb
321	C	79	F	Y	Y	N	N	4.238	1.652	2.205	1.231	c1c1	DD	GG	GA	AA	CT	AG	Null	Null	GT	TT	bb
322	C	52	F	N	N	N	N	6.058	1.281	4.179	1.205	c1c1	DD	GT	GA	AA	CC	GG	Null	Present	GG	TT	bb
323	C	79	F	Y	N	N	N	1.534	1.112	0.590	0.410	c1c1	DD	GG	GG	AA	TT	AG	Null	Present	GT	TT	bb
324	P	69	M	Y	Y	N	N	5.382	2.506	2.795	1.359	c1c1	DD	GG	GA	AA	CT	AA	Null	Present	GT	TC	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
326	C	54	M	N	Y	N	N	7.332	2.472	4.897	1.205	c1c1	DD	GG	GA	AA	CT	AA	Null	Null	GT	TC	ab
327	P	54	F	Y	Y	N	N	5.148	1.202	2.590	1.385	c1c1	DD	GG	GG	AA	CT	AG	Null	Present	GG	TT	ab
328	P	70	F	Y	N	N	N	4.706	1.629	2.436	1.462	c1c1	DD	GG	GG	AA	CT	AA	Null	Present	GT	TT	bb
329	C	67	F	N	N	N	N	4.160	0.787	2.359	1.333	c1c1	DD	GG	GA	AG	CT	AG	Null	Null	GG	TT	bb
330	C	50	F	N	N	N	N	3.406	2.764	1.744	0.359	c1c1	DD	GT	GA	AA	CT	AA	Null	Present	GG	TT	ab
331	P	31	F	N	N	N	N	6.396	3.011	3.615	1.308	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GT	TC	ab
332	P	55	F	N	N	N	N	7.098	0.697	4.872	1.821	c1c1	DD	GG	GG	AA	CC	GG	Present	Null	GT	TC	bb
333	C	90	F	N	Y	N	N	5.564	1.640	3.410	1.333	c1c1	DD	TT	GA	AG	CC	AG	Null	Present	GT	TC	ab
334	P	71	M	Y	Y	N	N	5.616	2.000	3.744	0.872	c1c1	DD	GT	GA	AA	CC	AG	Present	Null	GT	TC	ab
336	C	77	F	Y	N	N	N	8.164	1.944	5.179	1.974	c1c1	DC	GG	GA	AA	CC	GG	Null	Present	GT	TC	bb
338	C	37	M	N	N	Y	N	2.574	0.798	0.359	1.821	c1c1	DD	GT	GA	AA	CC	AA	Null	Present	GG	TT	bb
339	P	77	M	Y	Y	N	N	4.576	2.169	2.359	1.154	c1c1	DD	GG	GG	AA	CC	AA	Null	Null	GG	TT	bb
340	C	57	M	Y	Y	N	N	4.524	0.551	2.641	1.564	c1c1	DD	GG	AA	AA	CC	AG	Null	Present	GT	TC	ab
341	C	68	M	N	N	N	N	3.536	0.584	1.846	1.385	c1c1	DD	GG	GG	AA	CT	AG	Null	Present	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
342	P	74	F	Y	Y	N	N	7.410	3.112	4.436	1.462	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	ab
343	P	47	M	Y	Y	N	N	4.550	1.449	2.436	1.385	c1c1	DD	GG	AA	GG	CC	GG	Null	Null	GG	TC	ab
344	P	43	M	N	N	Y	N	7.826	3.483	4.744	1.385	c1c1	DD	GG	GG	AA	CT	GG	Null	Null	GT	TC	ab
346	P	67	M	Y	Y	N	N	5.512	0.978	3.872	1.128	c1c1	DD	GG	GG	AA	CT	GG	Present	Present	GG	TC	ab
347	P	69	F	N	N	N	N	6.448	1.652	4.359	1.256	c1c1	DD	GG	GG	AA	CC	GG	Null	Present	GG	TT	ab
348	C	77	M	Y	Y	Y	N	5.226	0.910	3.256	1.487	c1c1	DC	GG	GG	AA	CT	AA	Null	Present	GT	TC	ab
349	C	60	M	Y	N	N	N	4.004	1.000	2.359	1.128	c1c1	DD	GG	GG	AA	CT	AA	Present	Null	GG	TT	bb
351	P	74	M	N	N	Y	N	4.004	1.079	2.718	0.744	c1c1	DD	GG	GG	AA	CC	AG	Present	Present	GG	TT	ab
353	P	71	M	Y	Y	Y	N	5.954	2.629	3.615	1.051	c1c2	DC	GG	GG	AA	CT	AG	Null	Present	GG	TT	bb
354	P	74	M	Y	N	N	N	3.640	1.371	2.051	0.923	c1c1	DD	GG	GG	AA	CC	AG	Present	Present	GT	TT	bb
355	P	76	F	Y	Y	N	N	4.420	1.955	2.205	1.256	c1c1	DD	GG	GG	AA	CC	AG	Null	Null	GT	CC	ab
356	C	44	M	N	N	N	Y	4.446	1.225	2.821	1.000	c1c1	DD	GT	GA	AA	CT	AA	Null	Null	GT	TT	bb
357	P	68	F	Y	Y	Y	N	3.042	1.225	1.410	1.026	c1c1	DD	GG	GA	AA	CT	GG	Present	Present	GG	TT	ab
359	C	52	F	Y	N	N	N	5.200	1.944	2.846	1.385	c1c1	DC	GG	GA	AA	CC	AA	Null	Null	TT	CC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
360	P	68	F	Y	N	N	N	5.330	1.034	3.615	1.179	c1c1	DD	GG	GA	AA	CT	GG	Null	Present	GT	TC	bb
361	P	74	F	Y	N	N	Y	3.900	0.787	2.487	1.000	c1c1	DD	GG	GA	AA	CT	AA	Null	Present	GT	TC	bb
363	C	58	F	N	N	N	N	4.082	1.674	2.308	0.949	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GG	TT	bb
364	P	57	M	Y	Y	Y	N	6.162	2.843	3.897	0.872	c1c1	DC	GG	GG	AA	CT	AA	Null	Present	GG	TT	bb
365	C	79	M	Y	Y	N	N	5.174	1.944	3.308	0.897	c1c1	DD	GG	GG	AA	CC	AG	Present	Present	GG	TT	bb
366	C	73	F	N	N	N	N	5.278	1.247	3.615	1.026	c1c1	DD	GT	GG	AA	CT	AA	Null	Null	GT	TC	bb
367	P	34	F	N	N	N	N	5.798	1.955	2.641	0.615	c1c1	DD	GG	GA	AG	CC	AG	Null	Present	GT	TT	bb
368	P	61	M	Y	N	N	N	4.576	1.483	2.923	0.923	c1c1	DD	GG	GA	AG	CT	AA	Null	Null	GT	TC	bb
369	P	62	M	N	N	N	N	4.706	2.112	2.692	0.974	c1c1	DD	GG	AA	AA	CC	AG	Null	Null	GG	TT	bb
371	P	62	M	Y	N	N	Y	5.694	1.517	3.795	1.128	c1c1	DD	GG	GG	AA	CT	AG	Null	Null	GT	TT	bb
372	P	63	F	Y	Y	N	N	6.396	1.843	4.513	0.949	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TT	bb
373	P	68	M	Y	N	Y	Y	4.862	1.056	3.590	0.718	c1c2	CC	GG	AA	AA	CT	AA	Present	Null	GT	TT	bb
374	P	71	F	N	N	N	N	5.954	1.101	4.436	0.923	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GT	TT	bb
376	P	70	M	Y	Y	Y	N	5.590	1.011	3.821	1.231	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes												
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
377	P	69	F	Y	N	N	N	5.356	0.517	3.821	1.231	c1c1	DD	GG	AA	AA	CC	AA	Null	Null	GT	TT	ab
378	P	71	M	Y	N	N	N	5.668	1.382	4.077	0.872	c1c1	DD	GT	AA	AG	CT	AA	Null	Present	GT	TT	bb
380	P	79	M	Y	Y	Y	Y	4.368	1.427	3.051	0.615	c1c1	DD	GT	GG	AA	CC	AA	Null	Present	GG	TT	bb
381	P	38	M	Y	N	Y	N	6.630	1.685	4.718	1.051	c1c1	DD	GT	AA	AA	CT	AG	Null	Null	GT	TC	bb
382	P	71	F	Y	N	N	Y	5.252	1.416	3.205	1.333	c1c1	DD	GG	AA	AG	CC	AA	Null	Present	GG	TT	bb
383	P	73	M	Y	N	Y	Y	6.370	1.281	4.538	1.154	c1c1	DC	GG	GG	AA	CC	AA	Null	Present	GG	TT	ab
385	P	63	M	N	N	Y	Y	4.472	0.775	2.590	1.462	c1c1	DD	GG	AA	AA	CC	AA	Present	Null	GT	TT	ab
386	P	30	M	N	N	N	Y	4.030	1.326	2.487	0.872	c1c1	DD	GG	AA	AA	CC	GG	Null	Present	TT	TT	bb
387	P	68	M	Y	N	Y	Y	4.550	1.124	3.026	0.949	c1c1	DD	GG	GG	AA	CC	AA	Null	Null	GT	TC	ab
388	P	61	F	Y	N	N	Y	4.940	1.292	2.564	1.641	c1c1	DD	GT	GG	AA	CC	AG	Present	Null	GT	TC	ab
389	P	75	M	N	N	N	N	3.640	0.685	2.256	1.026	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GG	TT	ab
390	P	42	M	N	N	N	N	5.980	0.876	4.282	1.205	c1c1	DD	GG	GG	AA	CT	AG	Null	Null	TT	TC	bb
391	P	59	F	Y	N	N	Y	6.812	1.820	4.051	1.846	c1c1	DD	GG	AA	AG	CC	AA	Present	Present	GT	TT	bb
393	P	73	M	Y	N	Y	Y	6.188	1.966	3.897	1.308	c1c1	DD	GG	AA	AA	CC	AA	Present	Present	GT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
394	P	78	M	N	N	N	Y	4.706	1.326	3.103	0.923	c1c1	DD	GG	GG	AA	CC	AA	Null	Null	GT	TT	bb
395	P	71	M	Y	Y	Y	Y	5.720	3.775	3.692	1.000	c1c1	DD	GT	GG	AA	CC	AG	Present	Present	GT	TT	bb
396	P	76	F	Y	N	Y	Y	5.122	2.067	3.333	0.769	c1c1	DD	GG	GG	AA	CC	AG	Present	Present	GT	TT	bb
397	P	74	F	Y	Y	N	Y	5.616	3.337	3.410	0.615	c1c1	DD	GT	AA	AA	CC	AG	Null	Present	GT	TC	ab
398	P	79	M	Y	Y	N	Y	5.070	1.079	3.769	0.744	c1c1	DD	GG	GG	AA	CC	AG	Null	Present	GT	TC	bb
399	P	59	M	Y	N	N	Y	4.394	1.281	2.795	0.949	c1c1	DD	GG	GG	AA	CT	GG	Present	Present	GT	TC	ab
400	P	70	F	Y	Y	N	Y	5.954	1.798	4.000	1.051	c1c1	DD	GG	AA	AA	CC	GG	Present	Present	GT	TC	aa
401	P	21	M	N	N	N	N	4.134	1.022	2.513	1.103	c1c1	DD	GG	AA	AA	CC	AG	Present	Present	GG	TT	bb
402	P	52	M	N	N	N	N	5.278	0.685	3.333	1.308	c1c2	DD	GG	GG	AA	CT	AA	Null	Present	GG	TT	bb
403	P	77	F	Y	Y	Y	Y	6.786	1.640	4.744	1.205	c1c1	DC	GG	GG	AA	CT	GG	Present	Present	GT	TC	ab
404	P	77	M	Y	N	Y	N	3.328	1.607	1.897	1.051	c1c2	DD	GG	AA	AG	CT	AG	Null	Present	GG	TT	ab
407	P	79	F	Y	N	N	N	4.550	0.809	3.051	1.077	c1c1	DD	GT	AA	AG	CC	AG	Null	Present	GG	TT	ab
408	P	55	M	Y	N	Y	Y	5.824	3.831	3.154	0.846	c1c2	DD	GG	GG	AA	CT	GG	Null	Present	GT	TT	bb
409	P	68	M	Y	N	Y	Y	6.344	1.180	4.641	1.077	c1c1	DD	GG	AA	AA	CC	AG	Null	Present	GT	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
411	P	79	F	Y	Y	N	Y	6.422	1.708	4.615	0.949	c1c1	DD	GG	GG	AA	CC	AG	Null	Null	GT	TT	bb
413	P	46	M	Y	Y	N	Y	5.018	1.157	3.154	1.256	c1c1	DD	GG	AA	AA	CC	AG	Null	Null	GT	TT	bb
414	P	73	M	Y	N	Y	Y	4.368	1.607	2.590	1.000	c1c1	DD	GG	GG	AA	TT	AG	Null	Null	GT	TT	bb
415	P	70	M	Y	Y	N	Y	6.604	2.180	4.513	1.000	c1c1	DD	GG	AA	AA	CC	AG	Null	Null	GT	TC	ab
416	P	73	F	Y	Y	N	N	3.354	1.079	2.179	0.641	c1c1	DD	GG	AA	AA	CC	AG	Null	Null	GG	TC	ab
417	P	79	F	N	N	Y	Y	6.630	1.034	3.974	2.103	c1c1	DD	GG	GG	AA	CT	AG	Present	Null	GG	TT	bb
420	P	79	M	Y	Y	N	Y	7.878	0.663	6.103	1.359	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TT	bb
421	P	66	M	Y	Y	Y	N	3.952	4.449	1.308	0.564	c1c1	DD	GG	GG	AA	CC	AG	Present	Null	GG	TC	ab
422	P	75	M	Y	N	Y	Y	6.474	2.775	4.000	1.128	c1c1	DD	GT	AA	AA	CC	AA	Null	Present	GG	TT	bb
425	P	64	M	Y	N	Y	Y	2.808	0.865	1.769	0.615	c1c1	DD	GG	GG	AA	CT	AG	Null	Null	GG	TT	bb
428	P	72	F	Y	N	Y	N	5.070	1.079	3.179	1.385	c1c1	DC	GG	AA	AA	CC	AA	Null	Present	GT	TC	bb
429	P	64	F	Y	Y	N	Y	4.550	0.921	3.103	0.974	c1c1	DD	GG	GG	AA	CT	AA	Null	Null	GG	TT	bb
431	P	71	M	Y	Y	Y	Y	4.316	1.157	3.026	0.692	c1c1	DD	GG	AA	AG	CC	AA	Present	Present	TT	TC	bb
432	P	64	F	Y	N	N	Y	5.954	1.258	4.179	1.128	c1c1	DD	GG	GG	AA	CT	AA	Present	Present	GG	TT	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes												
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
433	P	51	M	Y	N	Y	Y	4.524	1.921	2.923	0.667	c1c1	DD	GG	GA	AA	CT	AA	Null	Null	GT	TT	bb
434	P	65	M	Y	Y	Y	Y	5.382	3.753	2.487	1.103	c1c1	DD	GG	GG	AA	CC	AG	Null	Null	GG	TT	ab
435	P	60	F	N	N	Y	N	5.876	1.202	4.000	1.256	c1c1	DD	GG	AA	AG	CC	AA	Present	Null	GT	TT	bb
436	P	53	M	Y	N	Y	Y	5.330	1.820	3.308	1.128	c1c1	DD	GG	AA	AA	CT	AA	Present	Null	GG	TT	bb
437	P	63	F	Y	N	Y	Y	6.214	2.112	3.795	1.359	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TT	bb
438	P	77	F	Y	N	N	N	5.070	1.247	3.282	1.154	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TC	ab
440	P	71	F	Y	Y	N	Y	3.510	1.079	2.026	0.949	c1c1	DD	GG	GG	AA	CC	AA	Null	Null	GT	TT	bb
441	P	75	M	Y	N	Y	Y	4.966	1.112	2.923	0.923	c1c1	DD	GG	GG	AA	CT	AA	Null	Null	GT	TC	bb
442	P	65	M	Y	Y	Y	Y	5.408	2.101	3.333	1.128	c1c1	DD	GT	AA	AA	CT	AG	Null	Null	GT	TT	bb
443	P	72	F	Y	Y	N	N	4.264	1.584	2.385	1.103	c1c1	DD	GG	AA	AA	CC	AG	Present	Present	GT	TT	bb
444	P	70	F	Y	N	N	Y	5.538	0.955	3.385	1.641	c1c1	DD	GG	GA	AA	CC	AA	Present	Present	GT	TC	ab
445	P	57	M	N	N	N	Y	6.656	1.169	4.615	1.410	c1c1	DD	GG	GA	AA	CC	AG	Null	Null	TT	TC	bb
446	P	70	M	Y	Y	N	Y	3.770	1.281	2.359	0.769	c1c1	DD	GG	AA	AA	CC	AA	Null	Present	GT	TT	bb
447	P	67	F	Y	Y	N	N	4.862	1.573	3.359	0.718	c1c1	DD	GG	GA	AA	CC	AG	Null	Present	GT	TT	ab

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
450	P	76	F	Y	N	N	Y	5.980	1.955	4.026	0.974	c1c1	DC	GT	AA	AA	CC	AA	Present	Null	GT	TC	bb
453	P	32	M	N	N	Y	N	3.796	1.236	2.282	0.897	c1c1	DD	GG	AA	AA	CC	AA	Null	Null	GT	TC	ab
454	P	52	F	N	N	N	N	5.642	1.618	3.641	1.179	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TC	bb
455	P	80	M	Y	N	N	N	3.198	0.528	1.692	1.231	c1c1	DD	GG	GA	AA	CC	AG	Present	Null	GG	TT	bb
456	P	80	F	Y	N	N	Y	5.018	1.292	3.128	1.333	c1c1	DD	GT	GG	AA	CC	AA	Present	Present	GT	TT	ab
457	C	55	F	N	N	N	N	4.498	1.157	2.538	1.359	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TT	bb
458	C	52	F	N	N	N	N	4.420	1.371	2.692	1.077	c1c1	DD	GT	GG	AA	CC	AG	Present	Null	GG	TT	bb
459	C	51	M	N	N	N	N	3.952	1.180	2.359	1.026	c1c1	DD	GG	GA	AA	CC	AA	Null	Null	GG	TC	ab
460	P	61	F	Y	N	Y	N	4.472	1.191	2.513	1.308	c1c1	DD	GT	GG	AA	CC	AG	Null	Null	GG	TC	bb
461	C	66	M	N	N	N	N	5.044	0.854	3.282	1.308	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GT	TT	bb
462	C	66	F	N	N	N	N	6.318	1.528	4.154	1.385	c1c1	DD	GG	GA	AA	CC	GG	Null	Null	GT	TT	bb
463	C	54	M	N	N	N	N	5.460	1.876	3.256	1.359	c1c1	DC	GG	GA	AA	CC	AG	Present	Present	GT	TC	ab
483	C	33	M	N	N	Y	N	4.992	1.034	3.282	1.179	c1c2	DC	GG	GG	AA	CC	AG	Present	Present	GT	TT	bb
484	C	32	M	N	N	N	N	4.316	1.146	2.590	1.154	c1c1	DD	GG	GA	AA	CC	AA	Null	Null	GT	TC	bb

Table E.1 (continued).

		Demographic Characteristics						Lipid Parameters				Genotypes											
No	Patient-Control	Age	Gender	Hypertension	Diabetes	Smoking	Obesity	Total Cholesterol	Triglyceride	LDL-Cholesterol	HDL-Cholesterol	CYP2E1*5B	CYP2E1*6	CYP2E1*7B	FMO3 G472A	FMO3A923G	NQO1*2	GSTP1 A313G	GSTM1	GSTT1	NOS3 G894T	NOS3 T-786C	NOS3 intron4 VNTR
485	C	55	F	N	N	N	N	6.448	2.764	3.974	1.128	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TC	bb
488	P	66	M	Y	Y	N	N	5.148	1.202	2.974	1.410	c1c1	DD	GG	GA	AA	CC	GG	Present	Present	GT	TT	bb
489	P	56	F	N	N	N	N	4.940	1.169	3.282	1.051	c1c1	DD	GG	GA	AA	CC	AA	Null	Present	GT	TT	bb
490	P	46	M	N	Y	N	N	4.368	1.315	2.744	0.949	c1c1	DD	GG	GA	AA	CT	AA	Present	Present	GT	TC	bb
497	C	61	M	N	N	Y	N	6.188	1.494	4.103	1.282	c1c1	DD	GG	GG	AA	CC	AA	Present	Present	GT	TT	bb
500	P	72	M	N	N	N	N	6.838	0.719	4.077	2.333	c1c1	DD	GG	GA	AA	CC	GG	Present	Present	GT	TT	bb
506	C	74	M	N	N	N	N	6.448	2.011	4.205	1.231	c1c1	DD	GT	GG	AA	CC	GG	Null	Present	GT	TT	bb
507	C	52	M	N	N	N	N	5.642	2.011	3.308	0.923	c1c1	DD	GT	GA	AA	CT	AG	Null	Present	GT	TC	bb
508	C	53	M	N	N	N	N	6.474	2.202	3.641	1.744	c1c1	DD	GG	GG	AA	CC	AA	Null	Present	GG	TT	bb

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PUBLICATIONS

1. **Thesis**

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