INTRODUCTION
Cytochrome P450s (CYPs) are an important superfamily of phase I xenobiotic metabolizing enzymes that are involved in the biotransformation of majority of the drugs and chemicals including chemicals present in the environment. CYP dependent biotransformation often determines to a large extent whether a chemical will be detoxified or activated to a reactive species to produce toxicity. A potential relationship between CYP activation in target tissues and diseases such as cancer has been shown. Genetic polymorphism has also been reported for several of the xenobiotic metabolizing CYPs and the susceptible genotypes are reported to be at increased risk to the adverse effects/toxicity of drugs and chemicals. Genetic variations in the CYPs are often an important resource for studying various complex diseases and have been identified as biomarker of susceptibility. Much of the variability in the CYPs is in the form of stable substitution of a single base termed as the single nucleotide polymorphisms (SNP). SNPs create an extraordinary degree of variability among genomes of different individuals. SNPs in general serve as markers with those occurring in the coding region (cSNP) having the potential to alter protein structure and function and, therefore, to be the causative gene alteration influencing disease susceptibility, drug efficacy, and toxicity of xenobiotics. There has been an increased interest in identifying the role of SNPs in the human genome in modifying the effect of exposures to environmental health hazards, which might render some individuals or groups in the population more or less susceptible to develop disease after exposure. Since SNPs may vary among populations it is essential to discover and/or validate them in the population of interest for disease association or pharmacogenetic study. Since the complex diseases are not caused by a mutation in
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single gene but by interaction of multiple genes, so called susceptibility genes, as well as environmental factors, it is important to identify genes that influences individual’s susceptibility to environmental factors. Genetic polymorphism in genes encoding drug-metabolizing enzymes, drug transporters and receptors contribute, at least in part, to the inter-individual variability in drug response. In recent years there has been an interest to develop biomarkers that predict the susceptibility and exposure of the toxic agents. SNP in CYPs has been used as biomarker of susceptibility to predict the toxicity of environmental chemicals including carcinogens as well as adverse drug effects.

Cytochrome P450 2E1 (CYP2E1) is a major component of microsomal ethanol oxidation system, involved in the activation of many procarcinogens and several drugs to highly reactive metabolites. It also metabolizes various endogenous substrates, such as ethanol, acetone, and acetal, as well as exogenous substrates including benzene, carbon tetrachloride, ethylene glycol and nitrosamines. Genetic polymorphism has been reported for CYP2E1 gene which has a clinical importance. The molecular genetic basis for the different phenotypes has been well characterized for this gene in Caucasians and Oriental population. However, not much information is available about the polymorphism in CYP2E1 gene in Indian population. The Indian population is unique due to its population structure and is subdivided into a multitude of large endogamous ethnic groups which are genetically less heterogeneous than conglomerate populations, enabling greater success in genomic and epidemiological studies on them. Thus the vast and diverse Indian genetic pool provides a unique opportunity to discover functional significance of the genetic variations. It is likely, that the SNPs found in the genes involved in the toxification-detoxication pathways may be conserved and these could be exploited to find the risk factors for the environment induced diseases. As the CYP2E1 enzyme is responsible for the metabolism of ethanol, and its activity increases up to 20-fold following continuous alcohol consumption, the polymorphisms in CYP2E1 may consequently modulate the risk of developing alcoholic liver diseases involving alcoholic liver cirrhosis.

Alcoholic liver cirrhosis, a chronic liver disease, results from damage to liver cells from chronic ethanol intake where damaged and dead liver cells regenerate in an abnormal pattern primarily forming nodules that are surrounded by fibrous tissue. Alcoholic liver cirrhosis is most common in the western world and its occurrence is increasingly seen in countries such as India and Japan which traditionally had a low
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prevalence of the disease. The development of alcoholic cirrhosis depends upon the amount and regularity of alcohol intake and usually occurs after 10 to 15 years of heavy drinking. Though, alcohol consumption is one of the main factors responsible for alcoholic liver cirrhosis, only 20% of habitual drinkers develop cirrhosis. Genetic polymorphisms (SNPs) in alcohol metabolizing enzymes such as CYP2E1 and detoxification enzymes for example glutathione-S-transferases (GSTs) and manganese superoxide dismutase (MnSOD), may play a major role in determining individual’s susceptibility to cirrhosis and thus may act as useful markers to predict the susceptibility for cirrhosis in individuals and populations at large. Though the pathogenesis of alcoholic liver cirrhosis involves oxidative stress, the disease seems to be multifactorial involving gene-environment interaction. It would be therefore interesting to focus on polymorphisms in the genes that are involved in ethanol metabolism particularly CYP2E1 and detoxification of reactive oxygen species generated during metabolism of ethanol such as GSTs and MnSOD.

As compared to genotyping, phenotyping studies have shown to provide more clinically relevant information because it is a reflection of the combined effects of genetic, environmental and endogenous factors on CYP activity. In recent years, there has been great interest in developing assays that can be used as biomarkers of the extent and persistence of effects caused by exposure to toxic agents. As blood cells are easily available these cells can be used as surrogate for the status of CYP in the target tissues and changes in CYP levels in blood cells could be used to predict xenobiotic induced alterations in the target tissues. CYP2E1 protein activity and mRNA expression is also known to increase several fold in the liver of experimental animals following alcohol administration suggesting that CYP2E1 is regulated at various level such as pretranscriptional, pretranslational, translational, and posttranslational. Studies have also shown that freshly isolated peripheral blood lymphocytes of human and laboratory animal express measurable levels of CYP2E1 mRNA and protein. Expression of CYP2E1 mRNA and protein in the peripheral blood lymphocytes is known to be influenced by the same factors that affect the concentration of hepatic enzymes including exposure to xenobiotics and certain physiological states particularly those associated with higher plasma cholesterol. Since expression of CYP2E1 is altered by many factors affecting chemical metabolism and toxicity, monitoring of CYP2E1 levels in individuals, who are
regular alcohol users could help in identifying individuals who are at risk to develop alcohol induced toxicity.

The candidate in the present dissertation has therefore carried out studies with the following objectives:

➢ To identify and validate Single Nucleotide Polymorphisms (SNPs) in cytochrome P450 2E1 (CYP2E1) gene in Indian population, stratified on linguistic and geographical basis.

➢ Using case-control studies identify functional significance of SNPs in CYP2E1, a phase I genes and glutathione S-transferases (GSTs), and manganese superoxide dismutase (MnSOD), phase II genes by studying their association with alcoholic liver cirrhosis in North Indian population.

➢ To explore the importance of possible genotypic combinations in the above mentioned genes involved in the pathogenesis of alcoholic liver cirrhosis.

➢ To develop peripheral blood lymphocyte CYP2E1 expression as a possible biomarker of alcoholic liver cirrhosis by investigating CYP2E1 mRNA and protein expression in blood lymphocytes isolated from alcoholic cirrhotic patients.