Association of Functionally important polymorphisms in CYP2E1 Gene in North Indian Population

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Abstract: CYP2E1, enzymes belong to phase I group of drug-metabolizing enzymes, which are involved in the metabolism of various compounds and xenobiotics. In this study we have discuss only CYP2E1 gene. This gene, involved in metabolism of ethanol and tobacco-associated nitrosamines, may play a crucial role in the metabolism of various drugs or chemicals and carcinogenic, like vinyl chloride or tobacco associated nitrosamines. Several single nucleotide polymorphisms (SNPs) have been implicated in CYP2E1 gene which may have varied effects on the various processes such as gluconeogenesis, cirrhosis, and cancer. CYP2E1 gene has been shown to have large inter-individual and inter-ethnic variability in levels of expression and activity. This study was carried out to estimate (N =150) the allele and genotype frequencies of common variants in CYP2E1 gene in North Indian population. RFLP-PCR, allele-specific PCR, gradient PCR, and gene sequencing methods and restriction enzyme DraI and RsaI enzyme were used for the identification of gene polymorphisms. The frequency of RsaI wild type genotype (c1c1) was 99.5% while heterozygous genotype (c1c2) was found to be 2.5% in controls. In contrast, no homozygous mutant genotype (c2c2) was found. Genetic polymorphism exists in CYP2E1gene. A large proportion of individuals carried of CYP2E1 gene. The study of CYP2E1gene could further be utilized in case-control studies involving certain diseases like cancer, etc.

Key Words -CYP2E1 gene, gene polymorphism, genetic susceptibility, phase I enzymes, North Indian Populations.

I. INTRODUCTION

CYP2E1Gene:

CYP2E1 gene belong to phase I group of drug-metabolizing enzymes, which are involved in the metabolism of various compounds and xenobiotics.CYP2E1 gene which may have varied effects on the various processes such as gluconeogenesis, cirrhosis, and cancer. This is believed to be largely due to genetic polymorphism. A large proportion of individuals carried of CYP2E1 gene. Human CYP2E1 gene is located on the 10th chromosome, consists of 9 exones and 8 introns, contains a typical TATA-box and occupies 11413 b.p. of genomic DNA (Umeno et al., 1988). CYPE1 polymorphism was also identified in 5'-regulatory region with C→T replacement in position 1019 and RsaI restriction site loss (CYP2E1*5B) (Watanabe et al., 1990; Hayashi et al., 1991). Allele with RsaI site is defined as wild type and designated c1 and without this site — as variant or rare type and designated c2. Rare c2 allele frequency constitutes 24–30% for Asian populations (Watanabe et al., 1990), 2–3% for Caucasians (Kato et al., 1992), 0.3–7% for Afro-Americans (Kato et al., 1992) 15% for Mexican Americans (Wu et al., 1997) and 18% for Taiwanese (Hildeshiem et al., 1997). Variant c2 allele is expressed *in vitro* at higher rate compared to wild type, and homozygous c2/c2 genotype is associated with 10-times increase in CYP2E1 gene transcription (Watanabe et al., 1994;). Functional significance of CYP2E1*5B polymorphism may be due to its localization in presumed binding sites for hepatic transcription factor — HNF-1. In Caucasians, CYP2E1*5B polymorphism is closely related to point mutation in position –1259. No association between two polymorphisms was identified for other ethnic groups. Polymorphic gene in expression system differs from the normal gene by lower enzyme synthesis (63%) and catalytic activity (64%). CYP2E1*2 polymorphism was identified only in Chinese populations. Currently there is no convincing evidence on correlation between CYP2E1 gene polymorphism and catalytic activity of cytochrome P450 2E1.

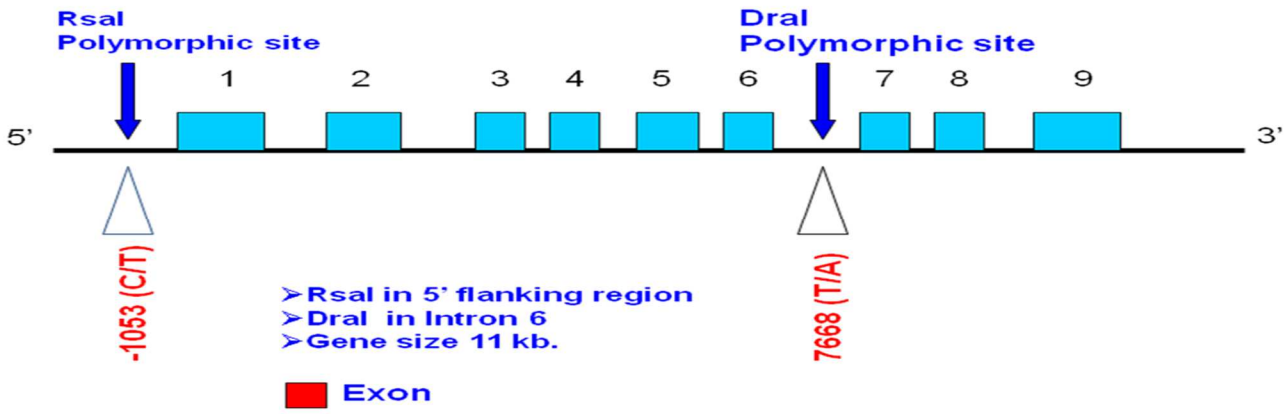


Fig- 1 Schematic Representation of Human CYP2E1 gene

II. MATERIALS AND METHODS

Blood samples were drawn from the healthy controls (n=150), Informed consent was taken before blood sampling.The blood collected from the controls was processed for isolation of DNA by using QIAamp DNA Blood Mini Kit.Genomic DNA isolated from blood of controls was used for identification of genetic polymorphisms in CYP2E1. Ethanol, Agrose powder, boric acid crystal and tris buffer, 10X buffer, $MgCl_2$, dNTPs, Forward Primer, Reverse Primer, Taq Polymerase for PCR amplifications ,EDTA, Genomic DNA isolation kit. Restriction Enzyme and its buffer,

Collection of blood for studying the polymorphisms in CYP2E1 genes, obtained from
controls individuals. Informed consent was taken from the individuals before sampling.

Primer Sequences used:

CYP2E1*5B: Forward Primer (FP):

-FP 5'-CCAGTCGAGTCTACATTGTCA-3

CYP2E1*5B: Reverse Primer (RP):

-RP 5'-TTCATTCTGTCTTCTAACTGG-3'

CYP2E1*6:

Forward Primer (FP):

-FP 5'-GTGTGCCGCCCTCCTCCTG -3 CYP2E1*6:

Reverse Primer (RP):

-RP 5'-TTTGGCTCCTTCACCCTTCACATT-3'

III. RESULTS

Standardization & Optimization of PCR conditions for detecting SNPs in CYP2E1 gene (CYP2E1*5B): Annealing temperature ranging between 55^0C to 64^0C. We have used 64^0C annealing temperature.

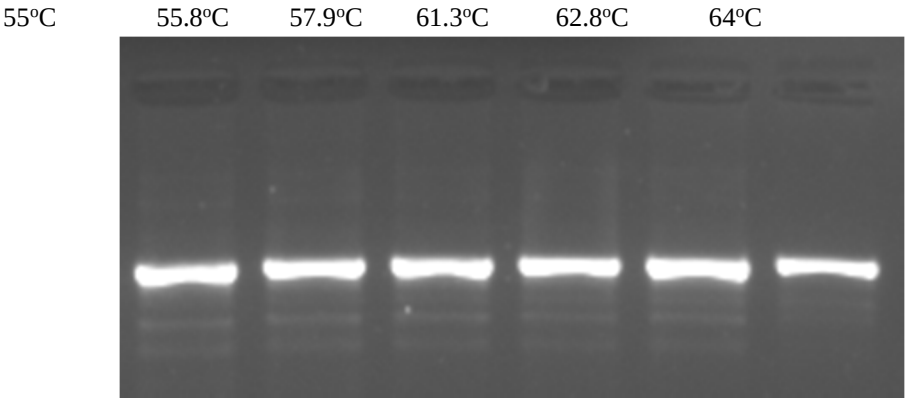


Fig-2 Standardization of PCR condition for detection of CYP2E1*5B Polymorphism 64^0C by temperature gradient PCR.

Standardization of PCR condition for detection of CYP2E1 polymorphisms by $MgCl_2$ gradient PCR: 1.8mM $MgCl_2$ concentration was used for subsequent PCR reactions.

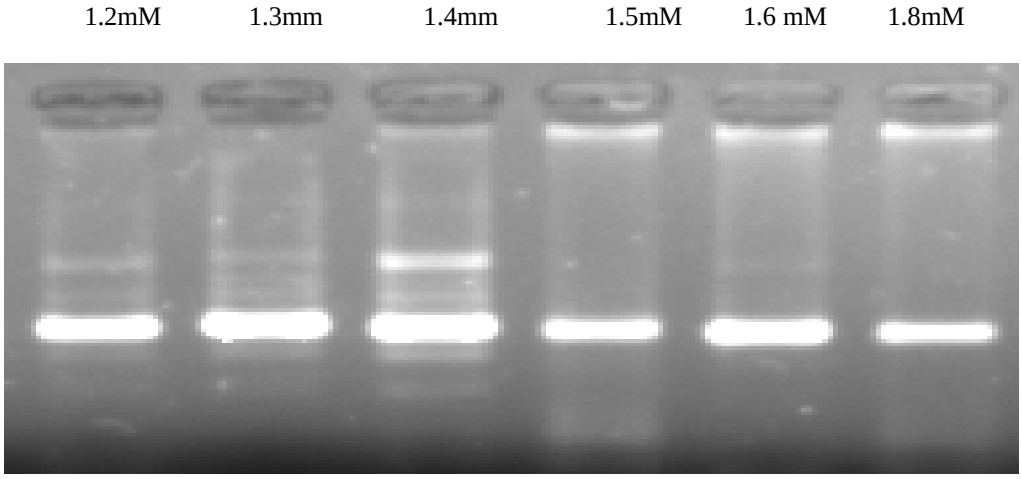
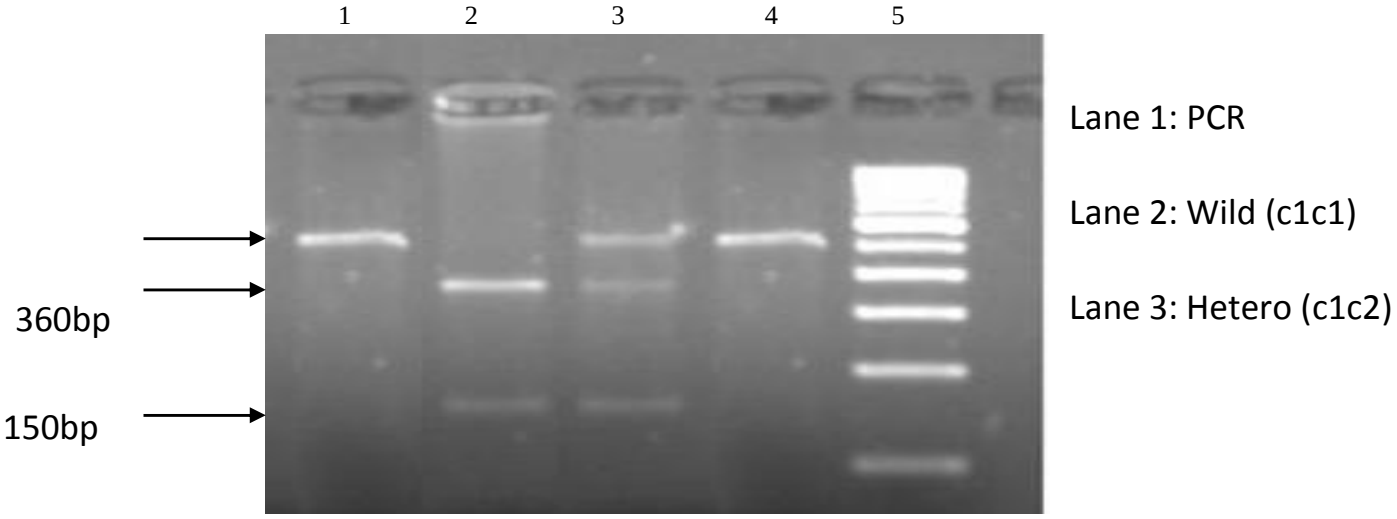


Fig-3 Standardization of PCR condition for detection of CYP2E1*5B polymorphism by MgCl2 gradient PCR.



CYP2E1*5B (RsaI digestion)

Fig.4: PCR-RFLP Method for detecting the CYP2E1 polymorphisms by RsaI digestion, CYP2E1*5B RsaI digestion

The genotype distribution of the CYP2E1 polymorphisms examined in controls is shown in table. The frequency of RsaI wild type genotype (c1c1) was 96% while heterozygous genotype (c1c2) was found to be 2.5% in controls. In contrast, no homozygous mutant genotype (c2c2) was found.

Genotype	Controls (%)
	(n=150)
CYP2E1*5B (RsaI)	
c1c1	99.5(99.5%)
c1c2	02.5(2.5%)
c2c2	00 (00)

Table1: Distribution of CYP2E1 genotypes among healthy controls.

IV. CONCLUSION

Finally we have found these data of CYP2E1 gene. Standarization was followed by genotyping of CYP2E1 genes in 150 healthy individuals. CYP2E1 gene firstly identified genotype frequency of CYP2E1 c1c1 was 99.5%, c1c2 2% and no homozygous mutant genotype c2c2 % .After that identified the allele frequency. The frequency of variant c2 allele of CYP2E1*5B was found to be 2.5% which is similar to that observed in the Indian studies (Soya et al., 2005; Mittal et al., 2005). The variant c2 allele has also been reported to be rare (2 – 3%) in the Caucasians (Kato et al., 1992; Carriere et al., 1996). However, it is markedly different from that observed for Chinese (25%) and Japanese (23%) populations which carry much higher frequency (23-25%) of the minor allele c2 (Yoshimura et al., 2003; Ran et al., 2007). The increase in risk for various cancers associated with the variant genotypes of CYP2E1 could be attributed to the increase in CYP2E1 gene transcription that may lead to an increased metabolic activation of the carcinogens and consequent initiation of malignancies. This results showed a presence of variant c2 allele of CYP2E1*5B in the studied healthy individuals.

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