RESEARCH ARTICLE

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CYP2D6, CYP2E1 Gene Polymorphisms and Gastrointestinal Cancer Risk in Rural Maharashtra: A Hospital Based Case-Control Study

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Abstract

Background: Cytochrome P450 (CYP) is a family phase I metabolizing enzymes important in xenobiotics metabolism. Genetic polymorphisms of CYPs have been comprehensively studied for their association with a range of diseases including cancer risk. In this study we assessed single nucleotide polymorphism (SNP) *CYP2D6* and *CYP2E1* genes and their role in gastrointestinal (GI) cancer susceptibility in the rural population of Maharashtra. **Methods:** Genotyping of *CYP2D6*4, CYP2E1*5B, CYP2E1*6, CYP2E1*7B* genes among 200 GI cancer cases and equal number of controls was studied by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The Odds ratio (OR) with 95% confidence interval and p-value were evaluated to get the level of association of polymorphisms with risk of GI cancer, where $p \leq 0.005$ was considered as statistically significant. **Results:** After the analysis of *CYP2D6* and *CYP2E1* gene polymorphisms, we noticed that *CYP2D6*4* (rs3892097) with heterozygous genotype (G/C) showed negative association with GI cancer risk (OR=0.43, 95% CI: 0.25-0.74; p=0.002) and *CYP2E1*6* (rs6413432) variant genotype showed positive association (OR=2.85, 95% CI: 1.40-5.81; p=0.003) showed positive association with GI cancer risk in studied population. **Conclusion:** The findings obtained from this study concluded that the polymorphic *CYP2D6* was negatively associated; however *CYP2E1*6* polymorphism was significantly associated with GI cancer risk in studied population.

Keywords: Gastrointestinal Cancer- *CYP2D6*- *CYP2E1*- Genetic polymorphism

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Introduction

Gastrointestinal (GI) cancer increasing alarmingly throughout the world encompasses principal malignancies of stomach, liver, esophagus, pancreas and colorectum. International agency for research on cancer (IARC) global cancer observatory reported 5121, 743 (25.63%) new cases and 2958, 922 (30.36%) deaths worldwide from the most common GI cancers in year 2022. Asia ranked highest in GI cancer incidence and mortality among all the cancers globally comprising 2951, 891(30.05%) new cases and 2053, 928 (37.60) deaths due to esophagus, stomach, liver, pancreas, gall bladder and colorectum [1]. The prominent risk factors including life style, diet, alcohol consumption and tobacco smoking are remarkably noted for development and advancement of GI cancer along with the infection with Helicobacter pylori [2-6]. Besides defined risk factors, genetic factors of an individual also confer the risk of GI cancer where genomic instability can cause uncontrolled cell growth and tumor formation lead to cancer development [7-8]. Genetic polymorphisms are important modifiers of cancer susceptibility where single nucleotide polymorphism (SNP) in different coding and non-coding regions of different genes can modulate cancer risk of an individual [9]. The Cytochrome P450 (CYP450) is a membrane associated heme containing phase I enzyme involved in metabolism of variety of dietary, environmental carcinogens and harmful xenobiotics in the human body [10]. *CYP450* genes are highly polymorphic in nature where the functional SNPs of CYP genes (*CYP1A1, CYP1B1, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1* and *CYP17*) can alter the expression of enzyme activity thereby increasing the cancer susceptibility [11-13].

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Cytochrome *P4502D6* and cytochrome*P4502 E1* are phase I enzymes encoded by *CYP2D6* and *CYP2E1* genes respectively and which are an important components of xenobiotics metabolism in human body. Studies have been carried out on functional association of polymorphisms in *CYP2D6* and *CYP2E1* genes with increased or decreased susceptibility to several cancers including esophagus, lung and colorectal cancer [14-20], and more recently breast [21], bladder [22] and head and neck cancer [23-24]. Indian studies also noted an association of *CYP2D6* polymorphism with breast cancer risk in South Indian population [25]. The role of *CYP2D6* polymorphism was also addressed in determining lung cancer susceptibility in North Indian population [26]. Similarly polymorphism in *CYP2E1* was illustrated in colorectal cancer risk in Kashmiri population [27], gastric cancer risk in West Bengal population [28] and head and neck cancer risk in North Indian population [29]. Other studies stated contradictory outcome with non significant correlation of *CYP2D6* and *CYP2E1* gene polymorphism with prostate [30-31], gastric [32] and colorectal cancer risk [33, 34]; however, the reason behind these inconsistent results is unambiguous. Therefore, we hypothesized that the environmental risk factors along with genetic polymorphism of *CYP2D6* and *CYP2E1* gene may modify the activity of metabolic enzymes which could alter the risk of GI cancer.

The incidence of gastrointestinal cancer is remarkably increasing in rural pockets of Maharashtra state of India where agriculture is the main component of life style. The heavy application of chemical fertilizers and non-degradable organophosphorous pesticides for decades led to intake of pesticides and increased occurrence of GI cancer cases in this area. The knowledge on the variability and expression of metabolizing enzymes in GI tract of the population is very poor. Therefore, it is necessary to understand the polymorphism of xenobiotic detoxification genes and their role in cancer susceptibility in the population of south-western Maharashtra. In view of literature when we reviewed the polymorphism of CYP450 gene polymorphism and its association with GI cancer risk, we noticed that no studies available in database stating the significance of *CYP2D6* and *CYP2E1* gene polymorphisms associated with GI cancer risk. We presumed that the polymorphisms of *CYP2D6* and *CYP2E1* genes may be contribute to the etiology of GI cancer in the studied population, therefore performed a hospital based case-control study to explore the association of *CYP2D6*4* (rs3892097), *CYP2E1*5B* (rs2031920), *CYP2E1*6* (rs6413432), *CYP2E1*7B* (rs6413420) polymorphisms with GI cancer risk in the population residing to rural areas of South-Western Maharashtra.

Materials and Methods

Selection of study subjects

This case control study was performed with two hundred clinically confirmed GI cancer cases and equal number of healthy, disease free, age and sex matched controls. The sample size was calculated by the formula n= [(p1xq1) + (p2 x p2)] X (Z1-α/2) +Z1-β)2/ (p1-p2)2; Where p1- presence of allele1, q1- absence of allele1, p2- presence of allele 2, q2- absence of allele 2, α- probability of detecting false results, β- power. All the cases ranged in age from 20-85 years (Mean ± SD) (59.0 ±13.32) enrolled immediately after diagnosis at Krishna Hospital and Medical Research Centre during the year 2018-2021. Written informed consent was obtained from all eligible cases and controls who agreed to participate after being given a detailed description of the study. The structured questionnaire was prepared to collect demographic and other clinical data. The study protocol (IEC-164/2017-2018) was approved by Institutional Ethics Committee of Krishna Institute of Medical Sciences 'Deemed to be University', Karad.

Blood Sample Collection and Genomic DNA Extraction and Purification

Five milliliter (mL) of whole blood from 200 patients was collected in sterile EDTA containing vacutainer after receiving informed consent. Genomic DNA extraction was carried out from the peripheral blood sample using HipurA®Blood genomic DNA miniprep purification kit. (Cat no. MB504-250PR) (HiMedia Laboratories) following the manufacturer's instructions. This pure genomic DNA was used for genotyping assays by polymerase chain reaction (PCR) and Restriction fragment Length Polymorphism (RFLP).

Genotyping assays of CYP2D6 and CYP2E genes

The genotyping of *CYP2D6*4*, *CYP2E1*5B*, *CYP2E1*6*, *CYP2E1*7B* genes was performed by PCR-RFLP. The PCR amplification were carried out separately in 20 micro liter (μL) reaction mixtures containing 1X PCR buffer 0.2 mM each dNTP, 10 picomole (pmol) of each primers (IDT technologies) , 1U Taq DNA polymerase (GeNei, Merck Bioscience) and 100 nanogram (ng) of purified genomic DNA. The primer sequence used to amplify the *CYP2D6* and CYP2E genes and the PCR conditions are shown in Table 1. After performing PCR programme for each reaction, the PCR products were analyzed by agarose gel electrophoresis in Tris-Acetate-EDTA (TAE) buffer. After confirmation of DNA amplification, each PCR product was digested with an appropriate restriction enzyme with specific conditions for genotyping. Ten micro liters of the PCR products digested at 37°C overnight with specific restriction enzymes in 20 μL reaction mixtures containing buffer supplied with each restriction enzyme (Table 1). After the overnight incubation, digestion products were separated on a 2-3% low EEO agarose (GeNei) gel at 100 V for 30 min stained with ethidium bromide and photographed with Gel Documentation System (BioRad).

Statistical Analysis

The association between the genotypes and risk of developing GI cancer were studied by Odds ratio (OR). Logistic regression model was used to calculate the OR and 95% confidence intervals (CI) with adjustment of variables to determine the GI cancer risk associated with genotypes. All p values were two-sided and differences were considered statistically significant for p ≤0.005.

All statistical analyses were performed with SPSS (IBM Version 11.0) software.

Results

Characteristics of selected demographic variables

The correlation of demographic variables of the study subjects including GI cancer cases and untreated controls was studied. The results of demographic characteristics showed that there was no statistically significant difference between age (p=0.307) of the cases and control group, where the Mean ± SD age in years was 59±13.33 for cases and 57.46±11.64 for controls. Similarly the data analysis of cases and control group did not show significant relationships between gender (p=0.133). However, it was observed that there is a significant relationship between tobacco chewing habit [OR: 4.03, CI: 2.65-6.10, p<0.0001, x^2=75.86] and alcohol consumption [OR: 4.45, CI: 2.15-9.22, p<0.0001, x^2=23.06] with the increased risk of GI cancer in the studied population.

Comparative analysis of genotypic polymorphism of CYP2D6 and CYP2E1 gene in gastrointestinal cancer cases and controls

CYP2D6 is an important toxicant metabolizing enzyme of CYP450 family. The frequency distribution of wild type rapid metabolizer (WRM), heterozygous rapid metabolizer (HRM) and variant poor metabolizers (VRM) was assessed to confirm the association with GI cancer risk. The results of analysis showed that the heterozygous rapid metabolizer with G/A genotype of *CYP2D6* was significantly lowered in cases as compared to controls. Thus, the frequency distribution of variant poor metabolizer of *CYP2D6* showed negative association with GI cancer risk in the studied population (OR 0.43; 95%CI, 0.25-0.74; p=0.002). The frequency of G/G, G/C and C/C genotypes of *CYP2E1*5B* in GI cancer cases was 78.50%, 13.50% and 8.0 % and that of controls were 81.50, 11.50% and 7.0 % in healthy controls which showed no difference. When we studied frequency distribution of *CYP2E1**6 (rs6413432), we observed that AA variant genotype increased significantly in GI cancer cases as compared to the controls (OR 2.85; 95% CI, 1.40 – 5.81; p=0.003) which showed the possible contribution of AA genotype in GI cancer development. The corresponding allele frequency of T7632A polymorphism of *CYP2E1*6* in studied population were 76 % for wild type *6A (T) allele and 24 % for mutated variant *6 (A) allele which also signified association with CI cancer risk in studied population (OR 1.98; 95%CI, 1.37-2.85; p=0.0002).

Investigation of *CYP2E1*7B* polymorphism yielded the genotype frequencies of 90 % for G/G genotype, 8.5 % for G/T and 1.50% for T/T genotype in GI cancer cases and 86.50 % for G/G, 11.50 % G/T and 2.0 % T/T genotypes in controls. The allele frequencies of *CYP2E1*7B* were 94.25 % and 5.75 % for G and T alleles respectively in GI cancer cases which showed no association with GI cancer risk in the studied population. The genotypic frequency distribution of *CYP2D6*4*, *CYP2E1*5B*, *CYP2E1*6* and *CYP2E1*7B* determined in GI cancer cases and healthy age and sex matched controls

Table 1. The List of Candidate *CYP2D6* and *CYP2E1* Genes Selected in the Present Study with Details of PCR and RFLP Procedures Including Primers and Restriction Enzymes and Expected Products of Selected Genes.

Gene Genotype	rs number	Amino acid/ nucleotide change	Primer Sequence Forward/Reverse	PCR Product size	Enzyme / Digestion conditions	Dominant (Wild type)	Heterozygous	Recessive (Variant)
*CYP2D6*4*	rs3892097	(G>A)	FP 5'-GCT TCG CCA ACC ACT CCG-3' RP 5'-AAA TCC TGC TCT TCC GAG GC-3'	334 bp	BstO1 37°C for 16h	230 bp, 105 bp	334 bp, 230 bp, 105 bp	334 bp
Intron-3 G1846A								
*CYP2E1*5B* 5' UTR G1293C	rs2031920	(G>C)	FP: 5'-ACC CCA ATG GGT GTC TGT C-3' RP: 5'-TCA TTC TGT CTT CTA ACT GGC AAT-3'	576 bp	PstI 37°C for 16h	294 bp, 284 bp	576 bp, 294 bp, 284 bp	576 bp
*CYP2E1*6* Intron -6 T7632A	rs6413432	(T>A)	FP: 5'-AGG CTC GTC AGT TCC TGA AA -3' RP: 5'-AAG GCA GGA GGA TGA CTT GA -3'	685 bp	DraI 37°C for 16h	376 bp, 309 bp	685 bp, 376 bp, 309 bp	685 bp
*CYP2E1*7B* G-71T	rs6413420	(G>T)	FP: 5'-CTG GAG TTC CCC GTT GTC TA-3' RP: 5'-GGG TGA AGG ACT TGG GAA TA -3'	547 bp	DdeI 37°C for 16h	301 bp, 246 bp	547 bp, 301 bp, 246 bp	547 bp

Table 2. The Distribution of Genotype and Allele Frequencies of *CYP2D6*4, CYP2E1*5B, CYP2E1*6* and *CYP2E1*7B* Gene Polymorphisms in Untreated Gastrointestinal Cancer Cases and Healthy Controls

Gene/SNP	Genotype/ Allele	Cases (n= 200) (%)	Control (n =200)(%)	OR (95% CI)	P value
*CYP2D6*4*	GG/GG	173 (86.50)	150 (75.0)	1 (Reference)	
Intron-3	GG/AA	24 (12.0)	48 (24.0)	0.43 (0.25-0.74)	0.002*
G1846A	AA/AA	3 (1.5)	2 (1.0)	1.30 (0.21-7.88)	0.775
rs3892097	G allele	370 (92.50)	348 (87.0)	1 (Reference)	
	A allele	30 (7.5)	52 (13.0)	0.54 (0.33-0.87)	0.011*
*CYP2E1*5B*	GG/GG	157 (78.5)	163 (81.5)	1 (Reference)	
5' UTR	GG/CC	27 (13.5)	23 (11.5)	1.21 (0.67-2.21)	0.516
G1293C	CC/CC	16 (8.0)	14 (7.0)	1.18 (0.56-2.51)	0.654
rs2031920	G allele	341 (85.25)	349 (87.25)	1 (Reference)	
	C allele	59 (14.75)	51 (12.75)	1.18 (0.79-1.77)	0.411
*CYP2E1*6*	TT/TT	133 (66.5)	157 (78.5)	1 (Reference)	
Intron -6	TT /AA	38 (19.0)	31 (15.5)	1.44 (0.85-2.45)	0.169
T7632A	AA/AA	29 (14.5)	12 (6.0)	2.85 (1.40-5.81)	0.003*
rs6413432	T allele	304 (76.0)	345 (86.25)	1 (Reference)	
	A allele	96 (24.0)	55 (13.75)	1.98 (1.37-2.85)	0.0002*
*CYP2E1*7B*	GG/GG	180 (90.0)	173 (86.5)	1 (Reference)	
G-71T	GG/TT	17 (8.5)	23 (11.5)	0.71 (0.36-1.37)	0.31
rs6413420	TT/TT	3 (1.5)	4 (2.0)	0.72 (0.15-3.26)	0.671
	G allele	377 (94.25)	369 (92.25)	1 (Reference)	
	T allele	23 (5.75)	31 (7.75)	0.72 (0.41-1.26)	0.261

SNP, Single nucleotide polymorphism; OR, Odds ratio; CI, Confidence Interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on χ^2

is summarized in Table 2.

When the polymorphism of variant genotypes of *CYP2D6*4*, *CYP2E1*5B*, *CYP2E1*6*, *CYP2E1*7B* and their association with GI cancer risk was studied in a recessive genotype model, we noted significant association of *CYP2DE1*4* (SNP: rs3892097) (OR=2.13; 95% CI: 1.27- 3.58; p=0.004). Similarly the recessive model for variant genotype of *CYP2E1**6 (rs6413432) showed negative association with risk of GI cancer (OR=0.54; 95% CI: 0.34-0.85); p=0.007) whereas the variant genotypes *CYP2E1*5b* and *CYP2E1*7b* showed no relationship with GI risk in the studied population (Table 3). The dominant model showed lack of involvement of *CYP2D6*4* (OR=0.63; 95% CI: 0.10-4.01); p=0.654) with GI risk. However, the significant negative association was noted in dominant model of *CYP2E1*6* (rs6413432) (OR=0.37; 95% CI: 0.18-0.76); p=0.006) with GI cancer risk in the studied population of South- Western Maharashtra (Table 4).

Discussion

Gastrointestinal Cancer is the most common malignancy increasing day by day in developing

Table 3. Association between Gastrointestinal Cancer Risk and the Single Nucleotide Polymorphism Variant of *CYP2D6*4, CYP2E1*5B, CYP2E1*6* and *CYP2E1*7B* Genes in the Recessive Model.

Genes (SNP)	Genotype	Cases (n= 200) (%)	Control (n =200)(%)	OR (95% CI)	P value
*CYP2D6*4* G1846A (rs3892097)	AA/AA + GG/AA	27 (13.5)	50 (25.0)	1 (Reference)	0.004*
	GG/GG	173 (86.50)	150 (75.0)	2.13 (1.27-3.58)	
*CYP2E1*5B* G1293C (rs2031920)	CC/CC + GG/CC	43 (21.5)	37 (18.5)	1 (Reference)	0.453
	GG/GG	157 (78.5)	163 (81.5)	0.82 (0.50-1.35)	
*CYP2E1*6* T7632A (rs6413432)	AA/AA+TT/AA	67 (33.5)	43 (21.5)	1 (Reference)	0.007*
	TT/TT	133 (66.5)	157 (78.5)	0.54 (0.34-0.85)	
*CYP2E1*7B* G-71T rs6413420	TT/TT + GG/TT	20 (10.0)	27 (13.5)	1 (Reference)	0.278
	GG/GG	180 (90.0)	173 (86.5)	1.40 (0.75-2.59)	

SNP, Single nucleotide polymorphism; OR, Odds ratio; CI, Confidence Interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on χ^2

Table 4. Association between Gastrointestinal Cancer Risk and the Single Nucleotide Polymorphism Variant of *CYP2D6*4, CYP2E1*5B, CYP2E1*6* and *CYP2E1*7B* Genes in the Dominant Model

Genes	Genotype	Cases (n= 200) (%)	Control (n =200)(%)	OR (95% CI)	P value
*CYP2D6*4 G1846A* (rs3892097)	AA/AA GG/AA +GG/GG	3 (1.5) 197 (98.5)	2 (1.0) 198 (99.0)	1 (References) 0.63 (0.10-4.01)	0.654
*CYP2E1*5B G1293C* (rs2031920)	CC/CC GG/CC +GG/GG	16 (8.0) 184 (92.00)	14 (7.0) 186 (93.0)	1 (References) 0.86 (0.41-1.82)	0.704
*CYP2E1*6 T7632A (rs6413432)*	AA/AA TT/AA + TT/TT	29 (14.5) 171 (85.5)	12 (6.0) 188 (94.0)	1 (References) 0.37 (0.18-0.76)	0.006*
*CYP2E1*7B G-71T* rs6413420	TT/TT GG/TT + GG/GG	3 (1.5) 197 (98.5)	4 (2.0) 196 (98.0)	1 (References) 1.34 (0.29-6.06)	0.703

SNP, Single nucleotide polymorphism; OR, Odds ratio; CI, Confidence Interval; Significance p< 0.05; *, Indicates significant Odds Ratio (p<0.05), p value determined based on χ^2

countries including China, Pakistan and India. Diet and life style factors along with genetics of an individual are the prominent etiological factors recognized in the process of carcinogenesis. Cytochrome P450 is a family of metabolic enzymes plays an important role in metabolism of carcinogenic metabolites including organophosphorous pesticides passively absorbed from the food. This study was planned to assess the polymorphism of *CYP2D6* and *CYP2E1* of *CYP450* gene family and discover its association with GI cancer risk in population of South-Western Maharashtra. In this hospital based case-control study we determined association of the polymorphism of *CYP450* genes including *CYP2D6* (*CYP2D6*4*) and *CYP2E1* (*CYP2E1*5B*, *CYP2E1*6*, and *CYP2E1*7B*) with GI cancer risk. The SNPs of *CYP2D6*4* (rs3892097), and *CYP2E1**5B (rs2031920), *CYP2E1*6* (rs6413432), *CYP2E1*7B* (rs6413420) were assessed for their association with GI cancer risk by logistic regression analysis of variant homozygous and heterozygous genotypes. These SNPs are comprehensively studied for their connection with range of diseases including cancer, however insufficient literature exist related to their association with GI cancer risk. The results obtained from this study indicated that the heterozygous G1846A genotype of *CYP2D6*4* polymorphism in the intron 3 region was negatively associated with GI cancer risk in the studied population. When the polymorphism of *CYP2E1* was studied, the results of *CYP2E1*6* (SNP: rs6413432) with homozygous variant genotype indicated significant association with GI cancer risk.

The functional polymorphism of CYP450 genes family including *CYP1A1, CYP1B1, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1* and *CYP17* are acknowledged for their involvement in cancer risk [10-13, 35]. *CYP2D6* and *CYP2E1* gene polymorphisms are documented for their role in risk of several cancers [18-24]. Similarly few Indian studies also denoted the significance of *CYP2D6* gene polymorphisms in breast [25] and Lung cancer [26] and *CYP2E1* polymorphism in colorectal and head and neck cancer [27-29]. However, other studies drawn contradictory opinions with no correlation of either *CYP2D6* or *CYP2E1* gene polymorphism with cancer risk among different ethnic groups [36-38]. The results on genotyping of *CYP2D6* (*CYP2D6*4*) showed negative association with GI cancer risk which were in agreement with other reports on protective role of *CYP2D6* in different cancers [37, 39-40]. The results of polymorphism of *CYP2E1* depicted significant association of homozygous variant 7632AA genotype of *CYP2E1*6* (T7632A) polymorphism with GI cancer risk in the studied population. These results are first of its own kind to reveal significant association of *CYP2E1* polymorphism (OR=2.85; 95% CI: 1.40 – 5.81; p=0.003) with GI cancer risk from the South-Western Maharashtra region of India. The findings obtained from this study on *CYP2E1* polymorphism consistent with other findings in gastric [28], breast [21], cervical cancer [40]. The findings are inconsistent in other studies which revealed no association of *CYP2E1* gene polymorphism with risk of colorectal [41], head and neck [42] and gastric cancer [43-44]. Thus, inaquitance of the earlier evidence on the polymorphisms of *CYP2D6* and *CYP2E1* and their association with GI cancer susceptibility influenced us to explore the role of rs3892097 SNPs of *CYP2D6* and rs2031920, rs6413432, 6413420 SNPs of *CYP2E1* in GI carcinogenesis in the rural population of Maharashtra.

In conclusion, the findings from current study revealed protective role of rs3892097 SNP of *CYP2D6**4 in GI cancer susceptibility. Further variation of rs6413432 SNP of *CYP2E1**6 has been recognized as a risk factor for the development of GI cancer in the studied population. To the best of our knowledge, this study is the first of its own kind to demonstrate the GI cancer susceptibility in association with *CYP2D6* and *CYP2E1* gene polymorphisms from the western peninsular region of India and established their role in cancer susceptibility.

Author Contribution Statement

Concept: KDD, RAG, AKG Design: KDD, AKG, RAG, Experimental Studies: MNP, ALM Clinical studies: RAG, AKG Data analysis: KDD, MNP, Statistical analysis: KDD Manuscript preparation: RAG, KDD, AKG, SRP. All authors read and approved the final manuscript.

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Approval

The study protocol was approved by protocol committee of Krishna Vishwa Vidyapeeth (Deemed to be University)

Declaration of Conflict of interest

The authors declare that they have no competing financial or any other conflict of interests that could have appeared to influence the work reported in this paper.

Ethics Committee Approval

The study protocol was approved by Institutional Ethics Committee of Krishna Vishwa Vidyapeeth 'Deemed to be University', Karad.

Abbreviations

GI: Gastrointestinal Cancer

DNA: Deoxyribose Nucleic acid

PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

SNP: Single Nucleotide Polymorphism

OR: Odds Ratio

CI: Confidence Interval

References

1. GLOBOCAN. Cancer Incidence and Mortality worldwide: International agency for research on cancer (IARC). Estimated number of new cases and death of cancer in 2022. IARC 2022. (Accessed: February 2024). Available from: https://gco.iarc.fr/today/home.
2. Thomson CA, LeWinn K, Newton TR, Alberts DS, Martinez ME. Nutrition and diet in the development of gastrointestinal cancer. Curr Oncol Rep. 2003;5(3):192-202. https://doi.org/10.1007/s11912-003-0110-y.
3. Sumathi B, Ramalingam S, Navaneethan U, Jayanthi V. Risk factors for gastric cancer in South India. Singapore Med J. 2009;50(2):147-51.
4. Moy KA, Fan Y, Wang R, Gao YT, Yu MC, Yuan JM. Alcohol and tobacco use in relation to gastric cancer: a prospective study of men in Shanghai, China. Cancer Epidemiol Biomarkers Prev. 2010;19(9):2287-97. https://doi.org/10.1158/1055-9965.EPI-10-0362.
5. Megraud F, Bessede E, Varon C. Helicobacter pylori infection and gastric carcinoma. Clin Microbiol Infect. 2015;21(11):984-90. https://doi.org/10.1016/j.cmi.2015.06.004.
6. Jain VS, Kawale D, Jain SM, Waghmare C, Pemmaraju G. Various addiction patterns, dietary habits, associated medical problems, and socioeconomic status in gastrointestinal malignancies: A prospective study in rural area of Maharashtra, India. J Cancer Res Ther. 2019;15(1):104-7. https://doi.org/10.4103/jcrt.JCRT_925_17.
7. Le D, Chen K, Husain S, Marathe A, Haq M. Molecular Genetics of Cancer. Int J Human Health Sci. 2018;2(4):199-208. https://doi.org/10.31344/ijhhs.v2i4.56.
8. Bapat B, Perera S. Genetic instability in cancer. Atlas of Genetics and Cytogenetics in Oncology and Haematology. 2007;11(2):155-64.
9. Al-Koofee Dhafer AF, Mubarak Shaden MH. Genetic Polymorphisms. The Recent Topics in Genetic Polymorphisms. Intech Open. 2019. https://doi.org/10.5772/intechopen.88063
10. Bag A, Jyala NS, Bag N. Cytochrome P450 1A1 genetic polymorphisms as cancer biomarkers. Indian J Cancer. 2015;52(4):479-89. https://doi.org/10.4103/0019-509X.178380.
11. Agundez JA. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab. 2004;5(3):211-24. https://doi.org/10.2174/1389200043335621.
12. Ghoshal U, Tripathi S, Kumar S, Mittal B, Chourasia D, Kumari N, et al. Genetic polymorphism of cytochrome P450 (CYP) 1A1, CYP1A2, and *CYP2E1* genes modulate susceptibility to gastric cancer in patients with Helicobacter pylori infection. Gastric Cancer. 2014;17(2):226-34. https://doi.org/10.1007/s10120-013-0269-3.
13. Elfaki I, Mir R, Almutairi FM, Duhier FMA. Cytochrome P450: Polymorphisms and Roles in Cancer, Diabetes and Atherosclerosis. Asian Pac J Cancer Prev. 2018;19(8):2057-70. https://doi.org/10.22034/APJCP.2018.19.8.2057.
14. Cai L, Zheng ZL, Zhang ZF. Cytochrome p450 2E1 polymorphisms and the risk of gastric cardia cancer. World J Gastroenterol. 2005;11(12):1867-71. https://doi.org/10.3748/wjg.v11.i12.1867.
15. Leng WD, Zeng XT, Chen YJ, Duan XL, Niu YM, Long RP, Luo ZX. Cytochrome P450 2E1 RsaI/PstI polymorphism and risk of esophageal cancer: A meta-analysis of 17 case-control studies. Exp Ther Med. 2012;4(5):938-48. https://doi.org/10.3892/etm.2012.687.
16. Shahriary GM, Galehdari H, Jalali A, Zanganeh F, Alavi SM, Aghanoori MR. *CYP2E1**5B, *CYP2E1**6, *CYP2E1**7B, *CYP2E1**2, and *CYP2E1**3 allele frequencies in iranian populations. Asian Pac J Cancer Prev. 2012;13(12):6505-10. https://doi.org/10.7314/apjcp.2012.13.12.6505.
17. Jiang O, Zhou R, Wu D, Liu Y, Wu W, Cheng N. *CYP2E1* polymorphisms and colorectal cancer risk: a HuGE systematic review and meta-analysis. Tumour Biol. 2013;34(2):1215-24. https://doi.org/10.1007/s13277-013-0664-8.
18. Zeng J, Li J, Bao M, Long Y, Li G, Luo Y. Association between *CYP2D6* polymorphisms and lung cancer risk: an up-date meta-analysis.Int J Clin Exp Med. 2017 Mar 1;10(3):4508-4517.
19. Shen ZT, Wu XH, Li B, Shen JS, Wang Z, Li J, Zhu XX. *CYP2E1* Rsa I/Pst I polymorphism and lung cancer susceptibility: A meta-analysis involving 10,947 subjects. J Cell Mol Med. 2018;22(7):3703. https://doi.org/10.1111/jcmm.13668.
20. Zhang H, Li H, Yu H. Analysis of the role of rs2031920 and rs3813867 polymorphisms within the cytochrome P450 2E1 gene in the risk of squamous cell carcinoma. Cancer Cell Int. 2018;18:67. https://doi.org/10.1186/s12935-018-0561-8.
21. Lu Y, Zhu X, Zhang C, Jiang K, Huang C, Qin X. Role of *CYP2E1* polymorphisms in breast cancer: a systematic review and meta-analysis. Cancer Cell Int. 2017;17:11. https://doi.org/10.1186/s12935-016-0371-9.
22. Yin X, Xiong W, Wang Y, Tang W, Xi W, Qian S, Guo Y. Association of *CYP2E1* gene polymorphisms with bladder cancer risk: A systematic review and meta-analysis. Medicine (Baltimore). 2018;97(39):e11910. https://doi.org/10.1097/MD.0000000000011910
23. Farhat F, Daulay ER. Chrestella J, Syari RP. The association of *CYP2E1* polymorphism and environmental factor in nasopharyngeal carcinoma patients. Maced J Med Sci. 2020;8(B):362-7. https://doi.org/10.3889/oamjms.2020.4639
24. Aday AD, Ozturk T, Teker BA, Aksoy F, Aydogan HY, Ozturk O, Isbir T. Association of *CYP2D6**4 gene polymorphism with early papillary thyroid carcinoma. Turk J Biochem.

2021;46(4):455-60. https://doi.org/10.1515/tjb-2020-0103.
25. Surekha D, Sailaja K, Rao DN, Padma T, Raghunadharao D, Vishnupriya S. *CYP2D6** 4 polymorphisms and breast cancer risk. Biol Med. 2010;2(4):49-55.
26. Sobti RC, Sharma S, Joshi A, Jindal SK, Janmeja A. CYP1A1 and *CYP2D6* polymorphism and risk of lung cancer in a North Indian population. Biomarkers. 2003;8(5):415-28. https://doi.org/10.1080/1354750031000161986O.
27. Sameer AS, Nissar S, Qadri Q, Alam S, Baba SM, Siddiqi MA. Role of *CYP2E1* genotypes in susceptibility to colorectal cancer in the Kashmiri population. Hum Genomics. 2011;5(6):530-7. https://doi.org/10.1186/1479-7364-5-6-530.
28. Ghosh S, Ghosh S, Bankura B, Shaha ML, Panda CK Chakraborty S, Das M. Polymorphisms of Cytochrome P450 2E1 Gene and Gastric Cancer Risk: A Case Control Study from West Bengal. J Clin Med Genom. 2017;5(1):1000148. https://doi.org/10.4172/2472-128X.1000148
29. Gupta S, Gupta OP, Srivastava S. Role of *CYP2E1* genetic polymorphism in the development of oral leukoplakia among tobacco users in North Indian population. Indian J Cancer. 2014;51(2):154-8. https://doi.org/10.4103/0019-509X.138266.
30. Murata M, Watanabe M, Yamanaka M , Kubota Y, Ito H, Nagao M, et al. Genetic polymorphisms in cytochrome P450 (CYP) 1A1, CYP1A2, *CYP2E1*, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. Cancer Lett. 2001;165(2):171–7. https://doi.org/10.1016/S0304-3835 (01)00398-6.
31. Ferreira PM, Medeiros R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveria J, Lopes C. Association between *CYP2E1* polymorphisms and susceptibility to prostate cancer. Eur J Cancer Prev. 2003;12(3):205–11. https://doi.org/10.1097/00008469-200306000-00007
32. Nishimoto IN, Hanaoka T, Sugimura H, Nagura K, Ihara M, Li XJ, et al. Cytochrome P450 2E1 polymorphism in gastric cancer in Brazil: case-control studies of Japanese Brazilians and non-Japanese Brazilians. Cancer Epidemiol Biomarkers Prev. 2000;9(7):675-80.
33. Butler WJ, Ryan P, Roberts-Thomson IC. Metabolic genotypes and risk for colorectal cancer. J Gastroenterol Hepatol. 2001;16:631-5. https://doi.org/10.1046/j1440-1746.2001.02501.x.
34. van der Logt EM, Bergevoet SM, Roelofs HM, Te Morsche RH, Dijk Yv, Wobbes T, et al. Role of epoxide hydrolase, NAD(P)H:quinone oxidoreductase, cytochrome P450 2E1 or alcohol dehydrogenase genotypes in susceptibility to colorectal cancer. Mutat Res. 2006;593(1-2):39-49. https://doi.org/10.1016/j.mrfmmm.2005.06.018.
35. Datkhile KD , Gudur RA , Gudur AK, Patil MN, Durgawale PP, Jagdale NJ, et al. Cytochrome P450 17 (CYP17) gene polymorphism (rs743572) and cervical cancer risk in women of rural Maharashtra. Gene Rep. 2021;24:101219. https://doi.org/10.1016/j.genrep.2021.101219.
36. Gutman G, Morad T, Peleg B. CYP1A1 and *CYP2D6* gene polymorphisms in Israeli Jewish women with cervical cancer. Int J Gynecol Cancer. 2009;19(8):1300-2. https://doi.org/10.1111/IGC.0b013e3181b9fa5d.
37. Zhou LP, Luan H, Dong XH, Jin GJ, Man DL, Shang H. Genetic variants of *CYP2D6* gene and cancer risk: a HuGE systematic review and meta-analysis. Asian Pac J Cancer Prev. 2012;13(7):3165-72. https://doi.org/10.7314/apjcp.2012.13.7.3165
38. Luo YP, Chen HC, Khan MA, Chen FZ, Wan XX, Tan B, et al. Genetic polymorphisms of metabolic enzymes-CYP1A1, *CYP2D6*, GSTM1, and GSTT1, and gastric carcinoma susceptibility. Tumour Biol. 2011;32(1):215–22. https://doi.org/10.1007/s13277-010-0115-8
39. Lemos MC, Carrilho F, Rodrigues F, Coutinho E, Gomes L, Carvalheiro M, Regateiro FJ. Genetic polymorphism of *CYP2D6* influences susceptibility to papillary thyroid cancer. Clin Endocrinol (Oxf). 2007;67(2):180-3. https://doi.org/10.1111/j.1365-2265.2007.02858.x.
40. Datkhile KD, Durgawale PP, Gudur RA, Gudur AK, Patil SR. *CYP2D6* and *CYP2E1* Gene Polymorphisms and their Association with Cervical Cancer Susceptibility: A Hospital Based Case-Control Study from South-Western Maharashtra. Asian Pac J Cancer Prev. 2022;23(8):2591-7. https://doi.org/10.31557/APJCP.2022.23.8.2591.
41. Gao CM, Takezaki T, Wu JZ, Chen MB, Liu YT, Ding JH, et al. *CYP2E1* Rsa I polymorphism impacts on risk of colorectal cancer association with smoking and alcohol drinking. World J Gastroenterol. 2007;13(43):5725-30. https://doi.org/10.3748/wjg.v13.i43.5725.
42. Balaji L, Singh KB, Bhaskar LV. Genetic polymorphisms of the *CYP2E1* gene do not contribute to oral cancer susceptibility in south Indians. Asian Pac J Cancer Prev. 2011;12(6):1523-7.
43. Malakar M, Devi KR, Phukan RK, Kaur T, Deka M, Puia L, et al. *CYP2E1* genetic polymorphism with dietary, tobacco, alcohol habits, H. pylori infection status and susceptibility to stomach cancer in Mizoram, India. Asian Pac J Cancer Prev. 2014;15(20): 8815-22. https://doi.org/10.7314/apjcp.2014.15.20.8815.
44. Zhang MX, Liu K, Wang FG, Wen XW, Song XL. Association between *CYP2E1* polymorphisms and risk of gastric cancer: An updated meta-analysis of 32 case-control studies. Mol Clin Oncol. 2016;4(6):1031-8. https://doi.org/10.3892/mco.2016.824.