Applied & Translational Genomics 3 (2014) 8–13

Contents lists available at ScienceDirect

Applied & Translational Genomics

journal homepage: www.elsevier.com/locate/atg

Association of polymorphism in cytochrome P450 2C9 with susceptibility to head and neck cancer and treatment outcome ☆

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ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 23 July 2013
Accepted 25 July 2013

Keywords:
CYP2C9
PMs
HNSCC
Radio-chemotherapy
Treatment response

ABSTRACT

The present case–control study involving 750 cases and equal number of healthy controls investigates the association of polymorphism in cytochrome P450 2C9 (*CYP2C9*) with head and neck squamous cell carcinoma (HNSCC) and response in patients receiving chemotherapy or combination of radio-chemotherapy. The frequency of heterozygous or homozygous genotypes of *CYP2C9*2* & *CYP2C9*3*, which leads to the poor metabolizer (PM) genotype was significantly higher in HNSCC cases when compared to the healthy controls resulting in significantly increased risk in the cases. Tobacco use in the form of tobacco smoking or tobacco chewing was found to increase the risk several fold in cases when compared to the non-tobacco users. Likewise, alcohol intake in cases with variant genotypes of *CYP2C9*2* or *CYP2C9*3* also significantly increased the HNSCC risk in cases when compared to non-alcohol users. Further, majority of the cases carrying variant alleles of both *CYP2C9*2* or *CYP2C9*3* were found to respond poorly to the chemotherapy or combination of radio-chemotherapy. The data suggests a significant association of the *CYP2C9* polymorphism with HNSCC and treatment outcome.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) accounts for approximately 5% of all cancers worldwide. In India they account for one-quarter to one-third of male cancers and one-tenth of cancers in females. HNSCC is often associated with heavy tobacco and alcohol use. People who use both tobacco and alcohol are at greater risk for developing these cancers than people who use either tobacco or alcohol alone (Hunter et al., 2005; Brennan and Boffetta, 2004). Polymorphisms in genes such as cytochrome P450s (CYPs) & glutathione-S-transferases (GSTs), involved in the metabolism and detoxification of alcohol and constituents of tobacco are shown to influence an individual's susceptibility to cancer (Bartsch et al., 2000; Gronau et al., 2003). Studies including meta- and pooled analysis have shown that polymorphisms in phase I (*CYP1A1*, *CYP2E1*) & phase II (*GST*) enzymes are associated with tobacco induced HNSCC (Hashibe et al., 2003; Singh et al., 2008; Shah et al., 2008).

CYP2C9 is one of the major drugs metabolizing CYP in human liver and contributes to the metabolism of a number of clinically important drugs such as anticoagulants and antihypertensives (Hermida et al., 2002). *CYP2C9* is also known to be involved in the metabolism of some of the anti-neoplastic drugs such as cyclophosphamide, etoposide, tamoxifen and ifosfamide (Schaik, 2005; Bosch et al., 2006). A 3-fold lower intrinsic clearance for cyclophosphamide was observed with recombinant *CYP2C9*2* and *CYP2C9*3* protein when compared to *CYP2C9*1* protein in a yeast expression system (Griskevicius et al., 2003). The MDR modulator, verapamil, has been reported to be metabolized by *CYP2C9*, which may have an impact on the concomitant use of chemotherapeutic drugs and verapamil (Busse et al., 1995). *CYP2C9* enzyme has been shown to play a key role in the metabolism of non-steroidal anti-inflammatory drugs (NSAIDs) that are frequently used in cancer patients suggesting that the doses of NSAIDs, should be carefully individualized in these patients (Goldstein and De Morais, 1994).

In addition, *CYP2C9* enzyme also metabolizes several carcinogenic and mutagenic substrates including heterocyclic aromatic amines and polycyclic aromatic hydrocarbons, PAHs (Shou et al., 1996; Nakamura et al., 1999). It has also been shown that some of the reactions catalyzed by *CYP2C9* lead to detoxification of carcinogens (Bauer et al., 1995; Chan et al., 2004). Genetic polymorphism has also been reported for *CYP2C9*. *CYP2C9*2* and *CYP2C9*3* genotypes account for "poor metabolizer" (PM)

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http://dx.doi.org/10.1016/j.atg.2013.07.002

phenotype resulting in the slow metabolism of drugs and other substrates metabolized by *CYP2C9* (Miners and Birkett, 1998; Higashi et al., 2002). Significant differences are known to exist in the distribution of the variant alleles of *CYP2C9* in different population. In general, the polymorphism in *CYP2C9* is more frequent in Caucasians when compared to Orientals (Chinese & Japanese population) (Wang et al., 1995; Takahashi et al., 2003; Musbah et al., 2007). An increased frequency of *CYP2C9*2* allele in the patients with lung cancer has been reported (London et al., 1996). Variant alleles of *CYP2C9* were reported to increase the risk of distal colorectal adenoma (Chan et al., 2004; Tranah et al., 2005).

Previous study from our laboratory has shown that PMs of *CYP2C19* are involved in modulating the susceptibility to HNSCC and exhibit poor response to chemotherapy (Yadav et al., 2008). The present study, therefore, now attempted to investigate the association of *CYP2C9* genotypes which leads to poor metabolizer (PM) status with HNSCC risk and outcome of treatment in the patients receiving chemotherapy and combination of radio-chemotherapy. Attempts were also made to investigate the interaction of *CYP2C9*2* & *CYP2C19*3* genotypes with tobacco and alcohol in modifying the HNSCC risk.

2. Materials and methods

2.1. Study subjects

A case–control study was conducted at King George's Medical University (KGMU), Lucknow, India. 750 males suffering from HNSCC and visiting the OPD facility of Radiotherapy Department of KGMU and equal number of controls were included in the study. The cases had squamous cell carcinoma of the oral cavity or pharynx or larynx which was confirmed by cytological, imaging and histopathological examinations and were advised a combination treatment of chemo- and radiotherapy. All the subjects included in the study belonged to the same ethnic group (Indo-European community) of North India. Controls were frequency-matched to cases by year of birth in 5-year classes. Based on medical check-up, controls were not found to suffer from any chronic disease.

The protocol of the study was approved by the human ethics committee of Chhatrapati Shahuji Maharaj Medical University, Lucknow, where the patients were registered and it conforms to the provisions of the declaration of Helsinki. Informed consent was obtained from the study subjects for inclusion in the study and before the collection of blood samples and it was also ensured that the subject anonymity was maintained. All study subjects completed a questionnaire covering medical, residential and occupational history. Information pertaining to dietary habits, family history of disease, smoking, tobacco chewing and alcohol drinking was also included in the questionnaire filled by the subjects. Subjects having regular smoking habits and smoking index (cigarettes/day × 365 days) of 730 or more were classified as smokers (Quinones et al., 2001). Likewise, smokeless tobacco dose was estimated as 'chewing year' (i.e. CY = frequency of tobacco chewed or kept/day × duration of year). Those who had CY of 365 or more were considered as tobacco chewers (Sikdar et al., 2003). Similarly, cumulative exposure to alcohol drinking was derived by multiplying the total yearly consumption of alcohol (in L/year) by the duration of habitual alcohol drinking (in years). Those who had cumulative exposure to alcohol about 90 L were considered as regular alcohol users in our study (Hung et al., 1997).

2.2. DNA isolation and CYP2C9 genotyping

500 μL blood samples collected in citrate containing tubes from the study subjects were processed for the isolation of genomic DNA whole blood using QIAamp DNA mini kit (Qiagen, CA) following the manufacturers' protocol. For identifying the polymorphism in *CYP2C9* (*CYP2C9*2* and *CYP2C9*3*), the reaction mixture in 50 μL contained 1× buffer (10 mM Tris–Hcl pH 8.3, 1.5–3.0 mM of $MgCl_2$, 25 mM KCl), 200 mM of each nucleotide, 200 nM of each of *CYP2C9*2* or 2C9*3 primers (Wang et al., 1995), 1.5 unit of Taq polymerase (MBI Fermentas, Germany), 100 ng of genomic DNA and sterile milliQ water and was processed for PCR. Amplified PCR products were digested with AvaII or NsiI (MBI Fermentas, Germany) for identifying *CYP2C9*2* and *CYP2C9*3* respectively (Wang et al., 1995). The products were resolved by 3% agarose gel containing ethidium bromide as described earlier (Wang et al., 1995).

For quality control, randomly 10% of the samples were selected and re-genotyped to confirm the authenticity of the results obtained earlier and they were found to be in 100% concordance.

2.3. Statistical analysis

We determined whether genotype or allele frequencies of *CYP2C9* polymorphism amongst the cases and controls were in Hardy–Weinberg equilibrium (HWE) using the standard chi square tests. The association between genetic polymorphisms and risk of HNSCC was estimated by calculating crude odds ratio (OR). A p-value of <0.05 was considered statistically significant. The statistical analysis was performed with the SPSS software package (version 11.0 for Windows; SPSS Chicago, IL). The power of the present study was found to be >80% as analyzed by power genetic association analysis software (http://dceg.cancer.gov/bb/tools/pga) at the level of significance $\alpha = 0.05$ with sample size of 750 in HNSCC and 750 in controls.

2.4. Treatment and treatment response

Patients were subjected to 3 cycles of neo-adjuvant chemotherapy (NACT), before radiotherapy or concurrent chemotherapy with radiotherapy (CT–RT). Each cycle of NACT consisted of cisplatin (50 mg/day) from days 1 to 3 and 5-fluorouracil (1 g/day) from days 1 to 3. Each cycle was administered once in 3 weeks. CT–RT included administration of 50 mg of cisplatin once every week for 7 weeks along with 70 Gy of radiation (which could be 200 cGy or 2 Gy/fraction depending on tumor size), daily for 7 weeks.

For assessing the treatment response, patients are asked to return for follow-up for five years. During follow-up, monitoring of the patients is done by thorough serial inspection of the head and neck region — looking for disease recurrence as well as second primary tumors. On the basis of WHO criteria the treatment outcome is divided into the following three categories:

i. Complete response (CR): No detectable tumor
ii. Partial response (PR): More than or equal to 75% decrease in tumor in its largest dimension
iii. No response (NR): Less than or equal to 50% decrease in tumor in its largest dimension

Those exhibiting CR & PR are categorized as responders while patients exhibiting NR are classified as non-responders (Yadav et al., 2008).

3. Results

The distribution of demographic variables and putative risk factors of HNSCC are summarized in Table 1. In general, cigarette smoking, tobacco chewing and daily alcohol use were found to be prevalent in the patients when compared to the controls (Table 1). Table 2 summarizes the genotypic frequencies of *CYP2C9*2* & *CYP2C9*3* in the controls and cases respectively. The distribution of CYP2C9*2 (chi square: 34.93 at 1 d.f.) and CYP2C9*3 (chi square: 32.49 at 1 d.f.) genotypes in the controls showed deviation from Hardy–Weinberg equilibrium (HWE) while that of CYP2C19*2 (chi square: 3.22 at 1 d.f.) was in HWE, though at a borderline significance. The frequency of heterozygous genotypes (CT) of *CYP2C9*2* polymorphism was found to be higher

Table 1
Distribution of demographic variables and putative risk factors of HNSCC cases.

Characteristics	Controls n (%)	Cases n (%)
Subjects	750	750
Age (mean ± S.D.)	50 ± 11	57 ± 9.2
Non-tobacco chewers	545 (72.6)	475 (63.3)
Smokers	164 (30)	394 (83.0)
Alcohol users	55 (10.0)	109 (23.0)
Tobacco chewers	205 (27.4)	275 (36.7)
Non-smokers	547 (72.9)	326 (43.4)
Tobacco chewers	170 (31)	251 (77.0)
Alcohol users	71 (13.0)	75 (23.0)
Smokers	203 (27.1)	424 (56.6)
Non-alcohol users	643 (85.7)	550 (73.3)
Tobacco chewers	129 (20.0)	259 (47.0)
Smokers	116 (18.0)	281 (51.0)
Alcohol users	107 (14.3)	200 (26.7)

in the cases (26.6%) when compared to the controls (14%). This increase in frequency resulted in an increased OR (2.22; 95% CI 1.51–3.26) in cases which was found to be statistically significant. Likewise, the frequency of homozygous genotype (TT) was slightly higher in cases (7.7%) when compared to the controls (4.6%) that resulted in an increase in risk (OR: 1.75) which, however, was not found to be statistically significant. Adjustment of the data for age, cigarette smoking, and tobacco chewing and alcohol consumption revealed that the risk continued to be significantly increased (Adj. OR: 2.72; 95% CI: 1.78–4.14) in the cases with heterozygous genotype (CT) of *CYP2C9*2* polymorphism (Table 2). Likewise, the frequency of heterozygous and homozygous mutant genotypes *CYP2C9*3* was found to be increased in the cases when compared to the controls that increased the HNSCC risk which was statistically significant. Adjustment of the data for age, cigarette smoking, tobacco chewing and alcohol consumption revealed that the risk continued to be significantly increased in the cases with heterozygous genotype (AC) of *CYP2C9*3* polymorphism (Table 2).

As with *CYP2C9*2* polymorphism, an increase in the frequency of heterozygous genotype (45.4%) of *CYP2C19*2* was observed in cases when compared to the controls (34.3%). The increase in frequency was associated with an increase in the OR (1.60; 95% CI 1.17–2.16) which was found to be statistically significant. An increase in the frequency of homozygous mutant genotype (18%) was also observed in the cases when compared to the controls (8.3%) and that resulted in a statistically significant increase in OR (2.43; 95% CI 1.17–2.16) in the cases (Table 2). Adjustment of the data for age, cigarette smoking, tobacco chewing and alcohol consumption revealed that the risk continued to be significantly increased in the cases for both, heterozygous (adjusted OR: 2.05; 95% CI: 1.45–2.91; p-value: 0.000) or homozygous mutant genotype (adjusted OR: 3.25; 95% CI: 1.93–5.49; p-value: 0.000) when compared to the controls (Table 2).

The effect of interaction of the risk modifiers such as tobacco chewing, cigarette smoking and alcohol consumption with the *CYP2C9* genotypes in the controls and cases is summarized in Table 3. The number of individuals with variant genotypes (homozygous & heterozygous) of *CYP2C9*2* was significantly increased in cases (38.5%), who were regular tobacco chewers as compared to the controls (12.5%) with similar habit of tobacco chewing. The increase in the frequency resulted in several fold statistically significant increase in the OR (4.3; 95% CI: 2.2–8.6) amongst the tobacco chewing cases. As observed with *CYP2C9*2*, the frequency of individuals who were regular tobacco chewers with variant genotypes of *CYP2C9*3* was also increased significantly in cases (35.6%) when compared to the controls (14.6%). The increase in the frequency resulted in 3–4 fold statistically significant increase in the risk (OR: 3.2, 95% CI: 1.7–6.1) (Table 3).

Cigarette smoking also increased the risk to HNSCC in the cases with *CYP2C9* polymorphism when compared to the smokers in the controls (Table 3). The frequency of individuals who were regular smokers and carried variant genotypes of *CYP2C9*2* (40.9%) was significantly increased in the cases as compared to the controls (13.7%). The OR associated with cigarette smoking increased several fold in the patients with the variant genotypes of *CYP2C9*2* (4.4; 95% CI: 2.3–8.4) which was found to be statistically significant (p-value: 0.000). As observed with *CYP2C9*2* variants, the number of cases with variant genotypes of *CYP2C9*3* was also increased amongst smokers (30.3%) as compared to the controls (14.7%). Further, this increase was associated with an increased risk (OR: 2.5, 95% CI: 1.3–4.8), which was found to be statistically significant (Table 3).

Our data further showed that frequency of the individuals with variant genotypes of *CYP2C9*2* and who were regular alcohol users significantly increased in the cases (57%) when compared to the controls (16.9%). This increase in frequency was associated with almost 4-fold increase in the risk (OR: 3.7, 95% CI: 2.0–7.0) to HNSCC in the cases (Table 3). As observed with *CYP2C9*2* variants, the number of cases with variant (homozygous & heterozygous) genotypes of *CYP2C9*3* also increased amongst alcohol users (28.2%) as compared to the controls (8.4%). A similar increase in risk was also observed in cases amongst the alcohol users with variant genotypes of *CYP2C9*3* when compared to the alcohol users in the controls. Moreover, this increase in the risk (OR: 4.3; 95% CI: 1.9–9.8) was also found to be statistically significant (Table 3).

A follow-up study was also carried out in 390 patients to investigate the effect of treatment on the patients with different genotypes of *CYP2C9* (Table 4). Amongst the patients with wild type genotype of *CYP2C9* (*CYP2C9*1*), 73% responded to the treatment of chemo- and radiotherapy (Responders) while 27% showed almost negligible response (non-responders). Amongst the patients with variant genotypes of *CYP2C9*2*, only 37% could be categorized as responders while 63% were found to be non-responders. Likewise, amongst the PMs with *CYP2C9*3* genotypes, only 34.2% responded to the treatment while 65.8% could be categorized as non-responders (Table 4). Interestingly, amongst 7 cases who carried compound heterozygous genotype of *CYP2C9*2/*3* (cases with both the heterozygous i.e. *CYP2C9*1/*2* &

Table 2
Distribution of CYP2C9*2 & CYP2C9*3 genotypes amongst HNSCC cases and healthy controls.

Genotype frequency	Control n = 750 (%)	Patients n = 750 (%)	Crude OR (95% CI)	p-Value	Adjusted OR[a] (95% CI)	p-value
*CYP2C9*2*						
CC	611 (81.4)	493 (65.7)	1 (ref.)		1 (ref.)	
CT	105 (14)	200 (26.6)	2.36 (2.8–3.1)	**0.00**	2.5 (1.88–3.32)	**0.00**
TT	34 (4.6)	57 (7.7)	2.1 (1.33–3.22)	**0.00**	2.3 (1.4–3.75)	**0.00**
*CYP2C9*3*						
AA	638 (85)	540 (72)	1 (ref.)		1 (ref.)	
AC	90 (12)	173 (23)	2.27 (1.72–3.0)	**0.00**	2.6 (1.92–3.52)	**0.00**
CC	22 (3)	37 (5)	1.98 (1.15–3.4)	**0.01**	2.3 (1.3–4.1)	**0.00**

OR: Odds ratio; CI: confidence interval; ref.: reference category, Adjusted OR[a]: adjusted in multivariate logistic regression models including age, smoking status, daily consumption of alcohol, tobacco chewing. Values in bold are statistically significant at the 0.05 levels.

Table 3
Interaction between CYP2C9 genotypes and tobacco chewing, smoking and alcohol consumption and risk to HNSCC.

Tobacco chewers					Non-tobacco chewers			
Genotypes	Controls n = 205 (%)	Cases n = 275 (%)	OR (95% CI)	p-Value	Controls n = 545 (%)	Cases n = 475 (%)	OR (95% CI) p-value	p-Value
*CYP2C9*2*								
Wild type	180 (87.9)	169 (61.5)	1 (ref.)		431 (79.1)	324 (68.2)	1 (ref.)	
Variant	25 (12.1)	106 (38.5)	4.5 (2.78–7.32)	**0.00**	114 (20.9)	151 (30.8)	1.76 (1.3–2.3)	**0.00**
*CYP2C9*3*								
Wild type	179 (87.2)	177 (64.4)	1 (ref.)		459 (84.3)	363 (80.1)	1 (ref.)	
Variant	26 (12.8)	98 (35.6)	3.8 (2.35–6.2)	**0.00**	86 (15.7)	112 (23.5)	1.65 (1.2–2.25)	**0.00**
Smokers					Non-smokers			
Genotypes	Controls n = 203 (%)	Cases n = 424 (%)	OR (95%CI)	p-Value	Controls n = 547 (%)	Cases n = 326 (%)	OR (95% CI)	p-Value
*CYP2C9*2*								
Wild type	176 (86.5)	261 (61.5)	1 (ref.)		435 (79.6)	232 (71.3)	1 (ref.)	
Variant	27 (13.5)	163 (38.5)	4.0 (2.6–6.4)	**0.00**	112 (20.4)	94 (28.7)	1.6 (1.14–2.2)	**0.00**
*CYP2C9*3*								
Wild type	175 (86)	293 (69)	1 (ref.)		463 (84.7)	247 (75.7)	1 (ref.)	
Variant	28 (14)	131 (31)	2.8 (1.78–4.4)	**0.00**	84 (15.3)	79 (24.3)	1.8 (1.2–2.5)	**0.00**
Alcohol users					Non-alcohol			
Genotypes	Controls n = 107 (%)	Cases n = 200 (%)	OR (95% CI)	p-Value	Controls n = 267 (%)	Cases n = 148 (%)	OR (95% CI)	p-Value
*CYP2C9*2*								
Wild type	91 (85)	114 (57.0)	1 (ref.)		520 (80.9)	379 (68.9)	1 (ref.)	
Variant	16 (15)	86 (43.0)	4.3 (2.35–7.8)	**0.00**	123 (19.1)	171 (31.1)	1.9 (1.46–2.2)	**0.00**
*CYP2C9*3*								
Wild type	95 (88.9)	144 (71.8)	1 (ref.)		543 (84.5)	396 (72)	1 (ref.)	
Variant	12 (11.1)	56 (28.2)	3.1 (1.5–6.04)	**0.00**	100 (15.5)	154 (28)	2.1 (1.6–2.8)	**0.00**

OR: odds ratio; CI: confidence interval; ref.: reference category; variant — heterozygous and homozygous mutant genotype. Values in bold are statistically significant at the 0.05 levels.

*CYP2C9*1/*3*) of *CYP2C9*, 5 (71.4%) did not respond to the treatment while only 2 (28.6%) were found to be responders (Table 4).

4. Discussion

The data of the present study has shown that functionally important polymorphism of *CYP2C9* exists in North Indian population. The frequency of the variant genotypes of *CYP2C9* (*CYP2C9*1/*2* & *CYP2C9*3*) was found to be higher (14% & 3% respectively) than that reported in South Indian (7% and 1%) population (Adithan et al., 2003; Rosemary et al., 2005). This could be partly attributed to the population structure of India comprising a mixture of endogamous ethnic groups (Rosemary et al., 2005). The frequency of the *CYP2C9*2* genotypes in our control population was higher than that observed in other Asian population (Chinese & Japanese) but was comparable to the Caucasians (Wang et al., 1995; Takahashi et al., 2003; Musbah et al., 2007). It was observed that the *CYP2C9*3* polymorphism was more common in North Indian population. The frequency of the variant genotype *CYP2C9*3* was relatively higher (3%) when compared to the Chinese population (0.01%) but relatively lesser than found in the Caucasians (7%) (Wang et al., 1995; Takahashi et al., 2003; Musbah et al., 2007). As observed with other studies (Higashi et al., 2002; Kramer et al., 2008;), the distribution of CYP2C9*2 and CYP2C9*3 genotypes in the controls showed deviation from Hardy–Weinberg equilibrium (HWE) which may be possibly due to recent population admixture. As reported earlier (Kudzi et al., 2009), CYP2C9*2 and CYP2C9*3 genotypes do not exhibit linkage disequilibrium (LD) in our study.

A relatively higher prevalence of cases with variant genotypes of CYP2C9*2 or *3 have clearly indicated that individuals inheriting PM genotypes of CYP2C9 are at increased risk to develop HNSCC. Though the association of CYP2C9 polymorphism has not been relatively well characterized with HNSCC, an increased frequency of CYP2C9*2 allele has been reported in the cases suffering from lung cancer (Ozawa et al, 1999; London et al., 1996). Using reconstituted system, microsomes prepared from *Saccharomyces cerevisiae* expressing recombinant human CYPs, CYP2C9 was found to catalyze both activation and inactivation reactions of benzo[a]pyrene, B[a]P (Bauer et al., 1995; Shou et al., 1994; Gautier et al., 1996). Similarly, amongst human CYPs expressed in vaccinia virus, CYP2C9 gave the highest activity for the metabolism of BP-7,8 diol to the diol epoxides, thus identifying the role of CYP2C9 in the metabolism and activation of B[a]P (Bauer et al., 1995; Shou et al., 1994; Gautier et al., 1996). Further, higher affinity for CYP2C9 has been shown for B[a]P when compared to PAH-metabolizing CYPs such as CYP1A and 2E1 and B[a]P is reported to be mutagenically activated by CYP2C9 and 2C19 along with other CYPs

Table 4
Treatment responses in patients of HNSCC with CYP2C9 genotypes.

Genotypes	Cases n = 390 (%)	Responders (%)	Non-responders (%)	p-Value
*CYP2C9*1*	148 (37.8)	108 (73.0)	40 (27.0)	Ref
*CYP2C9*2*	142 (36.5)	54 (38.0)	88 (62.0)	0.0000*
*CYP2C9*3*	0.0000*	36 (35.6)	64 (64.4)	0.0000*
*VarCYP2C9*2/*3*	96 (24.7)	36 (37.7)	60 (62.3)	0.0000*
*HomCYP2C9*2/*3*	12 (3.0)	3 (25.0)	9 (75.0)	0.0000*

Responder: Based on 50% reduction in tumor size, clinical response 50% & above by imaging, CTMRI, endoscopy techniques & symptomology (based on WHO criteria).
Non-responder: Less than 50% clinical response.
Var: Cases carrying both homozygous and heterozygous variants of both CYP2C9*2 & CYP2C9*3. *$p < 0.05$ is considered statistically significant.
Hom: Cases carrying only homozygous variants of both CYP2C9*2 & CYP2C9*3.

(Yilmaz et al., 2001; Yamazaki et al., 2002). It has also been shown that CYP2C9 is involved in the detoxification of PAHs derived from tobacco smoke (Sugimoto et al., 2004; Bauer et al., 1995; Tranah et al., 2005; Chan et al., 2004). Consistent with these reports, a high correlation has also been observed between total DNA adducts and CYP2C isoenzymes in larynx (Degawa et al., 1994). Thus, the association of CYP2C9 polymorphism with HNSCC risk could be attributed to the possible involvement of CYP2C isoenzymes in the metabolic activation as well as detoxification of PAHs and other tobacco derived products (Degawa et al., 1994).

Our study has further indicated that the risk to HNSCC was increased in cases who were tobacco or alcohol users, suggesting that interaction of *CYP2C9* genotypes with tobacco or alcohol increases the susceptibility to HNSCC. The increased risk observed in cases using tobacco with PM genotypes of *CYP2C9* could thus be explained by higher affinity of *CYP2C9* for B[a]P (Yilmaz et al., 2001). *CYP2C9* variant alleles have also been associated with altered metabolism of alkylating agents that are well established mutagens (Chang et al., 1997). It has also been shown that the risk of colorectal cancer or gastric cancer was found to be increased in cases who had a history of smoking and carried PM genotypes of CYP2C9 (Sugimoto et al., 2005; Tranah et al., 2005). An association has also been reported between high levels of bronchial bulky DNA adduct with CYP2C9 genotypes in lung cancer cases with increased formation of bronchial bulky DNA adduct in cases with CYP2C9*2 alleles compared to those with CYP2C9*3 (Ozawa et al, 1999). Likewise, an increase in risk in cases of drinking alcohol with *CYP2C9* PM genotypes has suggested that alcohol possibly interacts with *CYP2C9* genotypes in increasing the risk to HNSCC. Epidemiological studies have also reported that alcohol acts synergistically with tobacco to promote carcinogenesis in HNSCC. Though not much data is available on interaction of *CYP2C9* genotypes & alcohol, ethanol is known to inhibit *CYP2C9* activity (Hamitouche et al., 2006). The increase in risk in alcohol users could be explained by delayed detoxification of tobacco derived products in cases with PM genotypes of CYP2C9.

Our study has further shown that chemotherapeutic response is modified in patients with PM genotypes of CYP2C9. The poor response rate in the patients with PM genotypes and treated with radio & chemotherapy regimen could probably be a result of decreased availability of metabolites in PMs. Pharmacogenetic studies have shown that polymorphism in genes involved in drug metabolism and transport influences interindividual variability in drug efficacy and adverse effects in cancer treatment (Schaik., 2005; Bosch et al., 2006; Dai et al., 2008). Cases carrying variant alleles of thiopurine methyltransferase (*TPMT*) or dihydropyrimidine dehydrogenase (*DPYD*) or UDP-glucuronosyltransferase 1A1 (*UGT1A1*) were found to be predisposed to the toxicity/adverse effects associated with the drugs metabolized by these enzymes (Weinshilboum, 2001; Van et al., 2002). It has been reported that the dose and therapeutic response for several drugs in the cases vary according to the genotypic status of *CYP2C9* (Kirchheiner et al., 2004; Sanderson et al., 2005). CYP2C subfamily (including CYP2C9 and CYP2C19) is involved in the metabolism of commonly used anticancer drugs such as ifosfamide, cyclophosphamide, thalidomide and could have an impact on the efficacy & toxicity of chemotherapeutic agents & other drugs used in standard oncology (Chang et al., 1997; Ando et al., 2002). In vitro studies have demonstrated that wild type CYP2C9 was more efficient than the mutant allele CYP2C9*3 in cyclophosphamide 4-hydroxylation and ifosfamide 4-hydroxylation (Chang et al., 1997). Kinetic studies have revealed a 3-fold lower intrinsic clearance (Vmax/Km) for cyclophosphamide in a yeast expression system with recombinant CYP2C9*2 and CYP2C9*3 protein when compared to CYP2C9*1 protein (Griskevicius et al., 2003). A recent study from our laboratory has also shown the involvement of PMs of *CYP2C19* in modulating the chemotherapeutic outcome in HNSCC cases (Yadav et al., 2008).

In conclusion, the results of the present study have demonstrated that *CYP2C9* variants modulate the susceptibility to HNSCC. A several fold increase in the risk to HNSCC in the cases with variant genotypes (PMs) of *CYP2C9* and who were tobacco or alcohol users have indicated that *CYP2C9* genotypes interact with environmental risk factors in modifying the susceptibility to HNSCC. Furthermore, it was also demonstrated that PMs of *CYP2C9* modify the treatment outcome in cases receiving chemotherapy or a combination of radio- and chemotherapy.

Conflicts of interest

The authors report no conflicts of interest.

Acknowledgments

The authors are grateful to the Director, Indian Institute of Toxicology Research, Lucknow for his keen interest and support in carrying out the study. The financial support of SIP-08 of Council of Scientific & Industrial Research (CSIR), N. Delhi in carrying out the above studies is gratefully acknowledged. The technical assistance of Mr. B. S. Pandey is also gratefully acknowledged. IITR Communication No.: 2716.

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