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

Sunishtha S. Yadav, Shilpi Seth, Anwar J. Khan, Shailendra S. Maurya, Ankur Dhawan, Sidharth Pant, Mohan C. Pant, Devendra Parmar

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.

Keywords: CYP2C9, PMs, HNSCC, Radio-chemotherapy, Treatment response

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 antiangiogenants 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 (Schakl, 2003; 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)
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 & CYP2C9*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 chem- 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 Shahaji 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 (Qagen, CA) following the manufacturer’s 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 MgCl2, 25 mM KCl), 200 mM of each nucleotide, 200 nM of each of CYP2C9*2 or CYP2C9*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 Avall 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 α = 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 CYP2C9*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
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 polymorphism (Table 2). Likewise, the frequency of heterozygous and homozygous mutant genotypes CYP2C9*2 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 polymorphism, an increase in the frequency of heterozygous genotype (45.4%) of CYP2C9*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%). 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 (18.9%). 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 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 (42.4%)|
| 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%)|

### 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* (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%)              | 1.38 (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*: 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            | Cases  | OR (95% CI) | p-Value |
|                 | n = 205 (%)         | n = 275 (%) |             |         |
| CYP2C9*2        | Wild type           | 180 (87.9) | 169 (61.5) | 1 (ref.) | 431 (79.1) | 324 (68.2) | 1 (ref.) | 0.00 |
|                 | Variant              | 25 (12.1) | 106 (38.5) | 4.5 (2.78-7.32) | 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.) | 0.00 |
|                 | Variant              | 26 (12.8) | 98 (35.6) | 3.8 (2.35-6.2) | 86 (15.7) | 112 (23.5) | 1.65 (1.2-2.25) | 0.00 |
| Smokers         | Genotypes           | Controls | Cases  | OR (95% CI) | p-Value |
|                 | n = 203 (%)         | n = 424 (%) |             |         |
| CYP2C9*2        | Wild type           | 176 (86.5) | 261 (61.5) | 1 (ref.) | 435 (79.6) | 232 (71.3) | 1 (ref.) | 0.00 |
|                 | Variant              | 27 (13.5) | 163 (38.5) | 4.0 (2.6-6.4) | 112 (20.4) | 94 (28.7) | 1.6 (1.14-2.2) | 0.00 |
| CYP2C9*3        | Wild type           | 175 (86) | 293 (89) | 1 (ref.) | 463 (84.7) | 247 (75.7) | 1 (ref.) | 0.00 |
|                 | Variant              | 28 (14) | 131 (31) | 2.8 (1.78-4.4) | 84 (15.3) | 79 (24.3) | 1.8 (1.2-2.5) | 0.00 |
| Alcohol users   | Genotypes           | Controls | Cases  | OR (95% CI) | p-Value |
|                 | n = 107 (%)         | n = 200 (%) |             |         |
| CYP2C9*2        | Wild type           | 91 (85) | 114 (57.0) | 1 (ref.) | 520 (80.9) | 379 (68.9) | 1 (ref.) | 0.00 |
|                 | Variant              | 16 (15) | 86 (43.0) | 4.3 (2.35-7.8) | 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.) | 0.00 |
|                 | Variant              | 12 (11.1) | 56 (28.2) | 3.1 (1.5-6.04) | 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.

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)          | 0.000*  |
| CYP2C9*3      | 0.0000*           | 36 (35.6)     | 64 (64.4)          | 0.000*  |
| VarCYP2C9*2/3 | 96 (24.7)         | 36 (37.7)     | 60 (62.3)          | 0.000*  |
| HomCYP2C9*2/3 | 12 (3.1)          | 3 (25.0)      | 9 (75.0)           | 0.000*  |

Responders: Based on ≥50% reduction in tumor size, clinical response ≥50% & above by imaging, CTR/MRI, endoscopy techniques & symptomology (based on WHO criteria).
Non-responders: 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.

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 CYP1A1 and 2E1 and B[a]P is reported to be mutagenically activated by CYP2C9 and 2C19 along with other CYPs.
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. ITR Communication No.: 2716.

**References**

Adlitan, C., Gerard, N., Vasu, S., Balakrishnan, R., Shashindran, C.H., Krishnamoorthy, R., 2003. Allele and genotype frequency of CYP2C9 in Tamilnadu population. Eur. J. Clin. Pharmacol. 59, 707–709.

Ando, Y., Price, D.K., Dahut, W.L., Cox, M.C., Reed, E., Figg, W.D., 2002. Pharmacogenetic associations of CYP19 genotype with in vivo metabolisms and pharmacological effects of thalidomide. Cancer Biol. Ther. 1 (6), 660–673.

Bartsch, H., Nair, U., Risch, A., Rojas, M., Wilkman, H., Alexandrow, K., 2000. Genetic polymorphism of CYP19 genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiol. Biomark. Prev. 9, 3–28.

Bauer, E., Gao, Z., Ueng, Y.F., Bell, L.C., Zolda, D., Guengerich, F.P., 1995. Oxidation of benz[a]pyrene by recombinant human cytochrome P450 enzymes. Chem. Res. Toxicol. 8, 136–142.

Busch, T.M., Meijerman, I., Beijnen, J.H., Schellens, J.H., 2006. Genetic polymorphisms of drugs metabolising enzymes and drug transporters in the chemotherapeutic treatment of cancer. Clin. Pharmacokinet. 45, 253–258.

Brennan, P., Buffetta, P., 2004. Mechanistic considerations in the molecular epidemiology of head and neck cancer. IARC Sci. Publ. 157, 393–414.

Busse, D., Cosme, J., Beaune, P., Kroemer, I.K., Eichelbaum, M., 1995. Cytochromes of the P450 2C subfamily are the major enzymes involved in the O-demethylation of verapamil in humans. Naunyn Schmiedebergs Arch. Pharmacol. 353, 116–121.

Chan, A.T., Tranah, G.J., Giovannucci, E.L., Hunter, D.J., Fuchs, C.S., 2004. A prospective study of genetic polymorphisms in the cytochrome P-450 2C9 enzyme and the risk for distal colorectal adenoma. Clin. Gastroenterol. Hepatol. 2, 704–712.

Chang, T.K., Yu, I., Goldstein, J.A., Waxman, D.J., 1997. Identification of the polymorphically expressed CYP2C19 and the wild type CYP2C19/HE359 allele as low Km catalysts of cyclophosphamide and ifosfamide activation. Pharmacogenomics 71–221.

Dai, Z., Papp, A.C., Wang, D., Hampel, H., Sadee, W., 2008. Genotyping panel for assessing genetic and genotypic associations of CYP2C9 with in vivo metabolisms and pharmacological properties of cyclophosphamide and ifosfamide. Eur J. Clin. Pharmacol. 63, 1039–1045.

Degawa, M., Kojima, M., Yoshinari, K., Tada, M., Hashimoto, Y., 1994. DNA adduct formation of hepatocarcinogenic aromatic amines in rat liver: effect of cytochrome P450 inducers. Cancer Lett. 79 (1), 77–81.

Gautier, J.C., Leceour, S., Cosme, J., Perret, A., Urban, P., Beaune, P., Popom, D., 1996. Contribution of human cytochrome P450 to benz[a]pyrene and benz[a]pyrene-7,8-dihydriod metabolism, as predicted from heterologous expression in yeast. Pharmacogenetics 6 (6), 489–499.

Goldstein, J.A., De Morais, S.M., 1994. Biochemistry and molecular biology of the human CYP2C subfamily. Pharmacogenetics A, 285–299.

Griskovichius, L., Yasar, U., Sandberg, M., Hiderstrand, M., Elisson, E., Tybring, G., Hassan, D., M., 2003.Activation of cyclophosphamide: the role of polymorphic CYP2C enzymes. Eur. J. Clin. Pharmacol. 59, 103–109.

Grissivicoius, L., Yasar, U., Sandberg, M., Hiderstrand, M., Elisson, E., Tybring, G., Hassan, D., M., 2003. Activation of cyclophosphamide: the role of polymorphic CYP2C enzymes. Eur. J. Clin. Pharmacol. 59, 103–109.

Gonsalves, M., Koenig-Gregor, D., Jerg, M., Riechelmann, H., 2003. Genetic polymorphism in detoxification enzymes as susceptibility factor for head and neck cancer? Otolaryngology. Head Neck Surg. 128, 674–680.

Hamitouche, S., Poupoun, J., Dreano, Y., Amet, Y., Lucas, D., 2006. Ethanol oxidation into acetaldehyde by 16 recombinant human cytochrome P450 isoforms: role of CYP2C isoforms in human liver microsomes. Toxicol. Lett. 167, 221–230.

Hashibe, M., Brennan, P., Strange, R.C., et al., 2003. Meta- and pooled analyses of GSTM1, GSTTI, GSTPI, and CYP1A1 genotypes and risk of head and neck cancer. Cancer Epidemiol. Biomarkers Prev. 12, 1509–1517.

Hermida, J., Zarras, J., Alberca, I., Moncte, R., Lopez, M.L., Molina, E., Rocha, E., 2002. Differential effects of 2C9 and 2C9’ variants of cytochrome P-450 2C9 on sensitivity to acetomucosal. Am. Soc. Hematol. 99, 4237–4239.

Higashi, M.K., Veenstra, D.L., Kondo, L.M., Wirtkowsky, A.K., Srinuvarprachanah, S.L., Farin, F.M., Rettie, A.E., 2002. Association between CYP2C9 genetic variants and anticogulation-related outcomes during warfarin therapy. JAMA 287, 1690–1698.
