Cytochrome P450 1A1 genetic polymorphisms as cancer biomarkers

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Abstract
Phase I metabolic enzyme CYP1A1 plays an important role in xenobiotics metabolism and has been extensively studied as a cancer risk biomarker. CYP1A1 is polymorphic and its four variants, e.g., CYP1A1*2A, CYP1A1*2C, CYP1A1*3 and CYP1A1*4 with trivial names m1, m2, m3 and m4 respectively, are most commonly studied for cancer link. Gene-gene interaction studies combining polymorphisms of this enzyme with those of phase II detoxifying enzymes, especially glutathione S-transferases (GSTs) revealed greater risk for cancer susceptibility. Variants of CYP1A1 have also been found to be associated with chemotherapeutic adverse effects. Results of these studies, however, remained largely contradictory mainly because of lack of statistical power due to involvement of small sample size. Strongly powered experimental designs involving gene-gene, gene-environment interactions are required in order to validate CYP1A1 as reliable cancer biomarker.

Key Words: Biomarker, cancer, combined genotype, CYP1A1, polymorphism, toxicity marker

Introduction
Gain or loss of gene functions may influence disease susceptibility or treatment outcome in an individual. Altered expression of xenobiotic metabolizing enzymes, which are responsible for detoxification of xenobiotics may have obvious effects on metabolism of environmental carcinogens or drugs in human body. Thus genetic polymorphisms of these enzymes may play significant role in cancer development and varied chemotherapeutic response. These enzymes are grouped into phase I and phase II enzymes, which complement one another in the detoxification process of xenobiotics.

Cytochrome P450 enzymes (CYPs) constitute important group of phase I enzymes. They are involved in metabolism of environmental chemicals, and their genetic variations have been found to be associated with risk of several forms of cancer.[1] CYPs are also responsible for metabolism of more than 90% of clinically prescribed drugs.[2]

CYP1A1 enzyme, previously known as aryl hydrocarbon hydroxylase, is a major phase I enzyme. Unlike majority of CYPs that are expressed mainly in human liver, CYP1A1 is expressed predominantly in extra hepatic tissues, e.g., lung, breast, ovarian follicles etc. Since CYP1A1 plays key role in the activation of pro-carcinogens, e.g., polycyclic aromatic hydrocarbons (PAHs) and aromatic amines, genetic polymorphisms of this enzyme have been extensively studied for susceptibility to chemically induced cancers. CYP1A1 converts these procarcinogens to their ultimate DNA binding forms. For example, benzo[a] pyrene, a weak carcinogenic PAH is converted to 7,8-epoxide with the action of CYP1A1, which is then hydrolyzed by microsomal enzyme epoxide hydroxylase (EPHX1) to form benzo[a]pyrene-7,8-dihydrodiol. This compound then undergoes another epoxidation by CYP1A1 to form benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, which is much more potent carcinogen and can form guanine adducts on DNA. If this DNA damage remains un-repaired, it may lead to carcinogenesis.

CYP1A1 is inducible by some carcinogenic substances. Induction of CYP1A1 is initiated by binding of specific ligand like PAHs to aryl hydrocarbon receptor (AHR), which is then translocated into the nucleus by AHR nuclear translocator (ARNT). AHR/ARNT heterodimer then binds to xenobiotic response element (XRE) of CYP1A1 gene leading to transcription.[3]

Other than activation of xenobiotics, CYP1A1 also plays a key role in estrogen metabolism. It catalyzes the hydroxylation of 17-β estradiol.[4] Hence, CYP1A1 polymorphisms may be related to gynecological cancer risk also.

Genetic polymorphisms
It has been found that 40% of human CYP450 forms that are involved in xenobiotic metabolism are polymorphic.[5] The CYP1A1 gene is located at 15q24.1 and has seven exons and six introns. A total of 11 variant alleles have been identified for CYP1A1, CYP1A1*1 to CYP1A1*11. Different nomenclature systems have been developed for CYP1A1 alleles,[6] which have often created confusion regarding identity of a particular CYP1A1 allele. In the present article nomenclature system recommended in http://www.imm.ki.se/CYPalleles has been followed. Other than 11 alleles, some additional single nucleotide polymorphisms (SNPs) have also been detected and cited in this homepage, for which haplotypes have not been determined. Among these variants, four alleles, CYP1A1*2A (3698 T >C), CYP1A1*2C (2454 A >G), CYP1A1*3 (3204 T >C) and CYP1A1*4 (2452C >A), with trivial names m1, m2, m3 and m4 respectively, have been studied mostly for the association with cancer. CYP1A1*1 is the wild type allele. CYP1A1*2C and CYP1A1*4 are lying only two bases apart, both on exon seven. The m2 polymorphism leads to an amino acid substitution of Val for Ile (I462V) in the heme-binding region; m4 causes substitution of Asn for Thr (T461N) in the same region of the enzyme. While m2 mutation leads to an enhanced enzymatic activity in comparison to its wild type allele, effect of m4 is not clearly understood.[7]

Both m1 and m3 do not cause any amino acid substitution as m1 is on 3’ non-coding region and m3 is on intron
seven. However, *m1* allele may lead to an elevated enzyme activity.[7] The *m1* mutation is also known as *MspI* polymorphism as it gains a *MspI* restriction site.

Distribution of *CYP1A1* polymorphisms in different populations, Caucasians, African- Americans and Asians has been described in an exhaustive review by Masson et al.[8] They found in pool analysis that the *m1* variant allele is most prevalent in Asian populations (13%) and it was present in much lower frequency in Caucasians (1%). And in African- Americans the frequency was intermediate between that of Asians and Caucasians (6%). Likewise, *m2* homozygous variant allele was found to be common among Asians (5%) and very rare in Caucasians (0.7%) and absent in African Americans.[8,9] The *I462V* polymorphism when linked with *MspI* polymorphism is designated as *CYP1A1* *2B*, a common genotype in Caucasians.[6] The *m1* and *m2* allele have been found to be in linkage disequilibrium (LD) in many other populations also.[10,11] *CYP1A1* *I462V* variant is prevalent in African- Americans. Asian studies rarely observed the presence of *m4* variant[7,11,12] while it is more common among whites.[13]

In the present review we summarize the studies on *CYP1A1* genetic polymorphisms as cancer susceptibility biomarkers. Here we also include the studies exploring CYP1A1 as marker for chemotherapeutic outcome. Studies were identified through electronic databases primarily from MEDLINE using keywords “CYP1A1 polymorphisms and cancer”.

**Association with cancer susceptibility**

**Head and neck cancer**

Association of *CYP1A1* polymorphisms with risk of head and neck cancer has been extensively studied. The main etiological factor of head and neck cancer includes excessive intake of tobacco either by smoking or by chewing. Smoking related laryngeal and hypopharyngeal squamous cell carcinoma (SCC) risk was evaluated by Tai et al.[14] in a Chinese population. Significant relation between *m1* and *m2* polymorphisms and increased cancer risk was observed. Further, combined effect of smoking and *m1* polymorphism was also found in this study. A positive association between *m1* polymorphism and laryngeal SCC was also noted in Caucasians of Portugal.[15]

*CYP1A1* *I462V* has been found to be linked with oral cancer.[16,17] However, in many studies no relation was established between this polymorphism and oral cancer risk.[18,19] *m1* was not found to be linked with oral cancer risk.[16,20] In a meta- analysis and pooled analysis Varela-Lema et al.[21] found a positive association of *2A* as well as *2C* polymorphism with oral and pharyngeal cancer in ever smokers. Gajecka et al.[22] found *CYP1A1* *I*462V variant to be associated with laryngeal SCC. In India oral cancer is one of the leading cancers in men,[23] Sreelekha et al.[24] found a positive association of *m2* polymorphism with oral cancer in Indians while findings of Chatterjee et al.[25] did not reveal any association of *m2* with leukoplakia. The *MspI* genotype was not found to be associated with oral pre- cancer or cancer in Indians by Anantharaman et al.[26] In a recent meta- analysis Zhuo et al.[27] found *MspI* polymorphism as a risk factor in Caucasians, not for Asians.

**Lung cancer**

Lung cancer is the leading cause of cancer death in men and second major lethal cancer in women.[9] This disease has been found to be conclusively associated with tobacco smoking. Tobacco smoke comprises of nearly 60 carcinogenic compounds including PAH(s). It was estimated that 85% lung cancer in men and 45% cases in women were caused due to tobacco smoking.[28] However, other environmental carcinogens, like smoky coal combustion etc., with carcinogenic constituents also can contribute to the development of lung cancer significantly.[29]

It is known today that not all but only a fragment of smoker population (<20%) develops lung cancer,[7] which indicates involvement of genetic variants in lung cancer development. A number of studies have been carried out on the association between *CYP1A1* variants with lung cancer risk in last few decades although results were contradictory.[11] While varying gene frequencies in different ethnicities might be one of the plausible explanations for these conflicting results, the main factor responsible is a lack of adequate statistical power as these are low penetrance alleles and require large sample size from a specific ethnicity.

Several studies indicate association of *m1* and *m2* alleles with lung cancer risk in Chinese population. In a case- control study Song et al.[7] assessed the role of *CYP1A1 MspI*, *CYP1A1 I462V* and *CYP1A1 T461N* in the development of lung cancer in Chinese. They noted significantly higher risk of lung cancer for *MspI* and *H462V* variant alleles, even when individuals were heterozygous for these variants. However, this elevated risk was restricted to squamous cell carcinoma (SCC) only, not for adenocarcinoma (AC) or other histological types of lung cancer. Similarly, smokers with at least one variant allele of *m1* and *m2* had twice the risk than with homozygous wild type alleles. They also found gene- smoking interaction for both of these variants when they stratified their data by pack- years; the risk of variant alleles was higher for heaviest smokers. Yang et al.[30] tested *CYP1A1 I462V* mutation as a risk factor for lung cancer in Chinese women. They observed that a combined *m2* genotype (*Ile/Val and Val/Val*) was significantly associated with lung cancer risk. A study by Wang et al.[31] however, did not reveal any heightened risk of *m1* polymorphism for lung cancer in Chinese population. In a meta- analysis Shi et al.[32] concluded that *CYP1A1* variants were associated with lung cancer in Chinese. Early Japanese studies documented *m1* and *m2* as risk factors for developing lung cancer.[6] Other studies on Asians include a Korean study documenting a positive association between *CYP1A1* variant and lung cancer risk[33] while in South Indians individuals with *MspI* homozygous alleles were found to be at significantly higher risk for lung cancer.[34]

A study on North Indian population documented positive association of both *MspI* and *H462V* variants with lung cancer susceptibility.[11] Both the Indian studies found higher risk for respective variants in heavy smokers.

In a pooled analysis, including 1950 cases and 2617 controls Marchand et al.[35] found association between inducible form of *CYP1A1 I462V*, and lung cancer. The interaction was
stronger for SCC than AC. And the effect was particularly higher in never smokers and women. Other case-control studies demonstrated $H62V$ polymorphism as a susceptibility factor for non-small cell lung carcinoma (NSCLC) in Caucasians.$^{36,37}$ Larsen et al.$^{36}$ recruited large number of individuals (1050 cases and 581 controls), and found the women carriers with Val alleles of $m2$ polymorphism as more prone to develop NSCLC. CYP1A1*4 was found to be associated with lung cancer in Mexicans.$^{38}$ The role of MspI variant in lung cancer formation has been found to be conflicting in Caucasians. In a pooled analysis of 2451 cases and 3358 controls Vineis et al.$^{39}$ found MspI homozygous variant was significantly associated with SCC as well as AC(s) among Caucasians, and there was a stronger association in men than in women. However, no significant association could be detected for the Asians. In a pooled analysis Taioli et al.$^{40}$ detected that MspI variant allele (CYP1A1*2 A and *2B) genotype was a risk factor in Caucasian never smokers. In studies from Portugal’s midland$^{10}$ and Brazilian$^{41}$ populations CYP1A1*2 A, however, could not be detected as risk factor. In a case-control study Cote et al.$^{42}$ interestingly found that the Caucasians with Ile/Val genotypes of $m2$ polymorphism was at decreased risk for lung cancer than those with homozygous wild type alleles (Ile/Ile). However, such protective role could not be replicated in African-Americans in the same study.

CYP1A1 gene is inducible by tobacco carcinogen hence a large number of experiments have been carried out to study the association of this gene with lung cancer risk in smokers. However, parallel studies in non-smoking populations would reveal a true disease-genotype association as any susceptibility for lung cancer in these cases will be generated in non-inducing environment. According to Song et al.$^{7}$ non-smokers with $m1$ variants had elevated risk than those homozygous for wild type alleles. Ng et al.$^{43}$ conducted their experiment in life-time non-smoking Chinese women, and found elevated risk of lung cancer for both homozygous $m1$ and $m2$ genotypes. Furthermore, lung cancer risk associated with both polymorphisms was higher in women with lower exposure to environmental tobacco smoke. Yang et al.$^{30}$ also found such association was more pronounced among non-smokers than among smokers. Hung et al.$^{9}$ conducted a pooled analysis of case-control experiments carried out in non-smoker Caucasians. They found that hetero- and homozygous variant alleles of $H62V$ had higher risk for lung cancer, especially for lung adenocarcinoma. In this analysis in non-smoker Caucasians the authors ruled out any significant influence of the MspI polymorphism in lung cancer. Although low level association of MspI polymorphism with lung cancer could occur due to its link with $H62V$ polymorphisms ($*2B$) in Caucasians.$^{19}$

Gastrointestinal tract cancer

CYP1A1 has been studied for its link with gastrointestinal tract cancer also. Mostly, CYP1A1 MspI polymorphism has not been found to be related with esophageal cancer.$^{44,45}$ The results were not changed for patients exposed to smoke or occupational exposure.$^{46}$ Moreover, in the patients with alcohol habits, the non-variant genotype (TT) showed a higher (not-significant) risk for esophageal cancer in Indians. Interestingly in a case-control study in Chinese population, Wang et al.$^{47}$ observed a protective effect of MspI polymorphism in esophageal carcinoma. Unlike MspI the Val allele of $H62V$ polymorphism have often been linked to this cancer$^{44,48,49}$ with few exceptions.$^{50}$

In another case-control study on Indian gastric cancer patients, Malik et al.$^{51}$ did not find CYP1A1 MspI polymorphism as a susceptibility marker for gastric cancer. Similarly, MspI was not found be linked with gastric cancer in other studies also,$^{52,53}$ though sample size in these studies was too small to bring a conclusive result. Notably Agudo et al.$^{54}$ observed inverse association for $m1$ genotype in gastric cancer and increased risk for relatively uncommon SNP $m4$ in a nested case-control study developed in 10 European countries. Roth et al.$^{55}$ observed a reduced risk of CYP1A1*2 A hetero- or homozygous variant alleles for gastric cardia cancer in Asian population. CYP1A1*2 A was found to be more pronounced among Lebanese gastric cancer patients by Darazy et al.$^{56}$

An over representation of the CYP1A1*2C variant was documented in a large case-control study employing 490 colorectal cancer patients and 593 controls$^{57}$ and in a Brazilian study.$^{58}$ MspI polymorphism was not linked to the development of colorectal cancer in the Scottish,$^{59}$ while higher prevalence of $*2A$ variant allele (T/C) was noted among such type of cancer patients without any smoking habits.$^{60}$ Little et al.$^{59}$ detected an inverse association of CYP1A1*4 polymorphism with colorectal cancer. According to Pande et al.$^{61}$ TC genotype of CYP1A1 MspI was associated with earlier onset of colorectal cancer among individuals with Lynch syndrome. However, the authors suggested that this SNP might not be the source of this age shift. Rather any other SNP at different locus in LD with the CYP1A1 SNPs could lead to this shift. A composite genotype of CYP1A1*2 A or CYP1A1*2C and GSTT1 polymorphisms was found to be associated with a decreased risk of colorectal cancer in a study involving 685 cases and 778 controls.$^{62}$ Hou et al.$^{63}$ found another gene combination of CYP1A1 H62V and NQO1 ser (I87) linked with colorectal adenoma particularly among smokers.

Biliary tract cancers are although uncommon, they can be fatal sometimes. Park et al.$^{64}$ in their study on a Chinese population found that CYP1A1 was significantly associated with the cancer of gall bladder and ampulla of Vater. CYP1A1 H62V was found to be significantly associated with increased risk of both adeno- and non-adenocarcinoma of gall bladder for Hungarian women.$^{65}$ Although only 37 cases were involved in this study. Tsuchiya et al.$^{66}$ found that Val allele of m2 mutation was associated with an increased risk of gall bladder cancer (GBC) in Japanese women. Pandey et al.$^{67}$ also found CC genotype of MspI polymorphism to be significantly associated with GBC; a gene-environment interaction was detected in men taking tobacco.

Interaction between CYP1A1 polymorphisms and cigarette smoking is thought to promote hepatocellular carcinoma (HCC) and results of the association
studies on this carcinoma remain largely inconclusive. In a meta-analysis of eight studies (1,752 cases and 2,279 controls) for $I462V$ polymorphism and eight studies (933 cases and 1,449 controls) for $MspI$ polymorphism Yu et al.\textsuperscript{(68)} found that neither $I462V$ nor $MspI$ was associated with HCC. Although borderline significant associations with HCC risk were detected for both the genotypes among cigarette smokers.

**Cancer of urinary system**

Tumours of the kidney accounts for 2% of all cancers and its incidence rate is two-fold higher in men than in women.\textsuperscript{(69)} The commonest type of renal tumour is renal cell carcinoma (RCC). Main etiological factor is cigarette smoking. In a case-only analysis Smits et al.\textsuperscript{(70)} found $CTPIA1$ polymorphism as RCC risk factor in relation to smoking in 245 renal cancer patients. They found increased incidences (statistically non-significant) of RCC in smokers with $CTPIA1*2C$ genotype.

Bladder cancer is strongly associated with cigarette smoking and environmental carcinogens. Therefore, polymorphisms of carcinogen metabolizing enzymes have been extensively studied for bladder cancer risk. While the variant genotype $CTPIA1*2C$ polymorphism was found to be higher in bladder cancer patients than in normal patients\textsuperscript{(71)} in a Turkish population wild type (Ile/Ile) was found to be a risk factor for bladder cancer.\textsuperscript{(72)} No significant association with this cancer was found for $*2A$ genotypes in a North Indian population.\textsuperscript{(73)}

**Male reproductive system**

$CTPIA1$ polymorphisms have been widely studied for prostate cancer, the commonest cancer of men. Results of these studies are largely inconclusive. Vijayalaksmi et al.\textsuperscript{(74)} found that $T/C$ genotype of $m1$ mutation was significantly associated with development of prostate cancer while $A/G$ genotype of $m2$ polymorphism was found to be associated with reduced prostate cancer risk. However, no association was found between $m1$ and prostate cancer in many studies.\textsuperscript{(75-78)} Interestingly, Chang et al.\textsuperscript{(79)} demonstrated that for $m1$, $m2$ and $m4$ polymorphisms, $TAC$ haplotype was significantly associated with prostate cancer risk, and $CAC$ was associated with a decreased risk.

Testicular cancers are comparatively uncommon. Although aetiology of this cancer is not known fully, an unbalanced level of estrogen and androgens in utero is often thought to lead to develop this cancer.\textsuperscript{(80)} Since $CTPIA1$ is involved in hormonal metabolism, its polymorphisms have been studied for testicular cancer risk. In a study including large number of cancer patients ($n = 652$) and 199 controls of Norwegian Caucasian origin, both $CTPIA1*2A$ and $*2C$ polymorphic alleles were found to be associated with significantly reduced risk of testicular cancer.\textsuperscript{(80)}

**Gynecologic cancer**

Among gynecologic cancers, ovarian, endometrial and cervical cancers are most commonly studied for link with $CTPIA1$ polymorphisms. Since this gene has a role in the hydroxylation of estrogens to catechol intermediates that cause oxidative DNA damage, lipid peroxidation, and leads to DNA adducts\textsuperscript{(81)} a possible effect of $CTPIA1$ mutations on gynecologic cancers is conceivable. An association between tobacco smoking and $CTPIA1$ $MspI$ polymorphism on the risk of ovarian cancer was detected by Goodman et al.\textsuperscript{(82)} An elevated risk for ovarian cancer was demonstrated for women with Ile/Ile genotype and who consumed more than medium level of caffeine.\textsuperscript{(82)} In a recent meta-analysis Huang et al.\textsuperscript{(83)} demonstrated that $CTPIA1$ $I462V$ was linked to ovarian cancer and the risk was significantly increased for Caucasians and Asians. Although no significant association was found for $MspI$ with this cancer.

Esinler et al.\textsuperscript{(84)} found an association between Val allele of $I462V$ mutation and endometrial cancer. However, no relationship was found for $m1$, $m2$ mutations with endometrial as well as ovarian cancer in several investigations.\textsuperscript{(85-88)} Although this gene has role in oxidative metabolism of estradiol and activation of tobacco-smoke constituents,\textsuperscript{(86)} endometrial cancer is probably the only cancer type whose risk is decreased in smokers.\textsuperscript{(89)} Doherty et al.\textsuperscript{(90)} observed that presence of at least one $CTPIA1$ $m1$ or $m2$ variant allele was associated with a decreased risk of endometrial cancer. Also, a combination of $TC + CC$ genotype of $m1$ was in decreased frequency in endometrial cancer patients and the $TA$ haplotype of $CTPIA1$ $m1$ or $m2$ was increased in the patients.\textsuperscript{(91)} In a meta-analysis on $m1$, $m2$ and $m4$ Sergentanis et al.\textsuperscript{(92)} found no significant association for these three polymorphisms and endometrial cancer risk in Caucasian women.

Although the relationship between host genetic factors and human papillomavirus associated cancer is a debatable issue, a positive association has been documented with $CTPIA1*2A$, $CTPIA1*2C$, $CTPIA1*3$ polymorphisms and cervical cancer risk.\textsuperscript{(93-95)} Moreover, women with smoking habits and homozygous genotype for $MspI$ variant allele had a 19.4 fold higher risk for cervical cancer.\textsuperscript{(94)} However, no relation with $CTPIA1$ genetic polymorphisms and cervical cancer risk was detected by other studies.\textsuperscript{(96,97)} In a meta-analysis Sergentanis et al.\textsuperscript{(98)} found that homozygous mutant of $MspI$ and both heterozygous as well as homozygous mutants of $I462V$ polymorphisms are associated with increased risk of cervical cancer.

**Breast cancer**

Breast malignancy is the most prevalent cancer among women. While genetic factors like $BRCA1$ and $BRCA2$, and reproduction history constitute 30% of breast cancer causes,\textsuperscript{(99)} a low penetrance gene like $CTPIA1$ has been found to be responsible for the development of breast cancer. Results of the studies for breast cancer and $CTPIA1$ polymorphisms have remained largely inconclusive. In a recent meta-analysis of 10,520 cases and 14,567 controls Yao et al.\textsuperscript{(100)} concluded that there was no significant association between $CTPIA1*2A$ and breast cancer risk. However, in a recent case-control study on Indian women Syamala et al.\textsuperscript{(101)} detected a familial breast cancer risk for $CTPIA1*2A$ hetero- and homozygous variant genotypes. In yet another study involving 1140 Chinese patients of cohort design, Long et al.\textsuperscript{(102)} concluded that $CTPIA1*2A$ might be considered as a genetic marker for breast cancer.
prognosis in Chinese women. Earlier studies in Caucasians did not reveal any link between CYP1A1*2C polymorphism and breast cancer risk.\textsuperscript{[6]} Finnish Caucasians also did not show any significant risk of CYP1A1*2C for breast cancer development.\textsuperscript{[103]} However, in a recent meta-analysis of 11909 breast cancer patients and 16,179 controls, Sergentanis et al.\textsuperscript{[104]} found that Caucasians homozygous for variant alleles of H62V polymorphism, not the carriers, were at elevated breast cancer risk; the same polymorphism was not detected to be associated with such risk in Chinese subjects. An Indian study\textsuperscript{[105]} found that homozygous genotype (G/G) of m2 polymorphism was significantly associated with breast cancer. The m4 polymorphism was found to be a risk factor for breast cancer among French-Canadians by Krajinovic et al.\textsuperscript{[99]}

Interestingly many studies have demonstrated a reduced risk for breast cancer in individuals with MspI variant alleles\textsuperscript{[106-108]} and so with H62V polymorphism.\textsuperscript{[106,108,109]} This reduced risk for *2A and *2C variant alleles were found to be prevalent particularly among lean women with long-term endogenous estrogen exposure (>30% yrs of menstruation duration). In a haplotype analysis of two loci, CYP1A1 MspI and CYP1A1 H62V, Shin et al.\textsuperscript{[110]} demonstrated that CA haplotype was associated with the lowest risk of breast cancer.

**Lymphomas**

Acute lymphoblastic leukemia (ALL) accounts for approximately 74% of the leukemia cases among children.\textsuperscript{[111]} It is hypothesized that polymorphisms of xenobiotic metabolizing enzymes may have influence on susceptibility to childhood ALL. Swinney et al.\textsuperscript{[111]} studied association of CYP1A1 to ALL susceptibility in three ethnic groups, e.g., Caucasian, Hispanic and African-American children. Overall, an increased risk of ALL was documented for CYP1A1*2C and *2B homozygous variant alleles. There was a significant association of these two polymorphisms and also CYP1A1*2 A polymorphisms for Hispanics. Significant association of CYP1A1 m1 and m2 homozygous polymorphisms with paediatric ALL risk was found for Indian children.\textsuperscript{[112]} In a recent meta-analysis, Vijayakrishna and Houlston\textsuperscript{[113]} conclude that a significant association exists between CYP1A1*2A and childhood ALL. For adults CYP1A1*2 A homozygous polymorphism was detected as significant risk factor for susceptibility to ALL in a Mexican population.\textsuperscript{[114]} Further, Val allele of H62V mutation was found to be associated with chronic myeloid leukemia.\textsuperscript{[115]} CYP1A1*2A was detected for multiple myeloma in Koreans.\textsuperscript{[116]}

**Other cancers**

Sporadic studies on other types of cancer and CYP1A1 association are found in the literature. A variant allele of CYP1A1*4 mutation (C/A or A/A) was found to be a risk factor for papillary thyroid cancer.\textsuperscript{[117]}

**Combined genotype and cancer association**

Genetic association study is considered as a powerful approach for identification of low penetrance disease-susceptibility alleles.\textsuperscript{[118]} It has been often found that the association studies have mainly followed candidate gene approach,\textsuperscript{[118]} investigating one or a few SNP (s) leading to functional changes of a gene. However, carcinogenesis is a multi-step process involving cross-talk between a numbers of genes. It is expected that studying gene- gene interaction may better evaluate the contribution of low penetrance genes in cancer development. A higher phase I enzyme activity in combination with a lower phase II enzyme activity can be expected to generate several fold higher risk for cancer than an individual phase I/II enzyme. For xenobiotic-derived cancer CYP1A1, the phase I enzyme, has been most commonly studied in combination with glutathione-S-transferase (GSTs) phase II detoxifying enzymes. Both GSTM1 and GSTT1 are involved in the metabolism of tobacco smoke constituents and a deletion polymorphism for GSTM1 or GSTT1 has been found to be linked with the risk of lung cancer.\textsuperscript{[119]} Wang et al.\textsuperscript{[31]} in their experiment on genetic association for lung cancer in a Chinese population did not find any statistically significant association either for CYP1A1 MspI or for GSTM1 polymorphism separately, however, a strong association was observed for combination-genotype of MspI homozygous variant and GSTM1 homozygous null. A combination of variant MspI allele, and GSTM1 or GSTT1 null allele were recognized as a risk factor for lung cancer in other studies also.\textsuperscript{[34,43,120]} In a pooled analysis on non-smoker lung cancer patients Raimondi et al.\textsuperscript{[121]} concluded that a combination of CYP1A1 wild type, GSTM1 null and GSTT1 non-null genotypes might have a protective effect from lung cancer. Yang et al.\textsuperscript{[33]} identified a genotype combination of GSTP1, myeloperoxidase gene (MPO) and CYP1A1 variants as a risk factor for lung cancer development. A joint effect of GSTM1 null genotype and CYP1A1 MspI variant on head and neck cancer was also found.\textsuperscript{[17,122]} A high risk for esophageal cancer was detected for individuals with CYP1A1 Val/Val and GSTM1 deletion genotype.\textsuperscript{[48]} The transformation of PAH into its ultimate carcinogenic form requires oxidation by CYP1A1 and hydrolysis by EPHX. Agudo et al.\textsuperscript{[84]} found a synergistic interaction of these two genes in gastric cancer patient, although not significant. For colorectal cancer, a combination of N-acetyltransferase NAT 2 rapid and CYP1A1*2 A heterozygous genotypes was identified as a susceptibility marker.\textsuperscript{[60]} Hou et al.\textsuperscript{[63]} studied gene- gene interaction between CYP1A1 m2 and Ser187 polymorphism of NQO1 gene in colorectal cancer, which influences activation of carcinogenic compounds in tobacco smoke. While Val462 or Ser187 was only weakly associated with colorectal adenoma, a combination of these genotypes showed increased risk for cancer, particularly among recent and heavy smokers. Estrogen metabolizing genes CYP1A1 and GST were found to interact in breast cancer cases also.\textsuperscript{[123,124]} Genes involved in the disposition of estrogen include catechol-o-methyltransferase (COMT), progesterone receptor (PGR), sulfotransferases (SULT1A1 and SULT1E1), CYP1A1, CYP1A2, CYP1B1, and CYP3A4. Rebbeck et al.\textsuperscript{[125]} found that synergistic interactions existed between CYP1A1*2C and SULT1A1*2 for breast cancer risk. Sulfotransferases cause sulfation of estrogen and catecholestrogens to more hydrophilic forms that leads to
excretion of these compounds. A combination of CYP1A1, CYP1A2, and CYP1B1 genotypes led to a reduced risk for endometrial cancer. \[^{[90]}\] GSTM1 null and CYP1A1 m1 in combination were found to have higher risk for prostate cancer. \[^{[126, 127]}\] Recipients of solid organ transplant were found to have high risk to develop non-melanoma skin cancer (NMSC). \[^{[128]}\] Polymorphisms in detoxifying enzyme may alter this risk. In their case- control study Lira et al. \[^{[128]}\] found that a combination of risk genotypes, i.e., GSTM1 null homozygous and carrying CYP1A1 Val462 allele had higher chance of developing NMSC. In acute lymphoblastic leukemia (ALL) polymorphisms of individual genes were not found to be risk factors, however, combination of GSTM1 null with CYP1A1 heterozygous mutant were found in individuals with high chance to develop ALL. \[^{[129]}\] Again, a combination of GSTT1 null genotype and CYP1A1*2B and CYP1A1*4 alleles was found to increase the risk of acute myeloid leukemia. \[^{[130]}\] Table 1 shows a brief comparison between the CYP1A1 and cancer- link either alone or in combination with other genes. These findings suggest that experimental designs including polymorphisms of more than one enzyme involved in metabolic pathway of a particular xenobiotic would produce better results.

**Association with therapeutic outcome**

An individual may have good response to cancer chemotherapeutic agent while at the same time, another person generates resistance and even adverse effects to the same drug. Genetic constitution of an individual is largely responsible for this difference; genetic polymorphisms of drug metabolizing enzymes are known to be important source of variability in drug responses. In humans important group of drug metabolizing enzymes include CYP(s) with CYP3A4, CYP2C9 and CYP2D6. A current trend of research includes CYP1A1 polymorphisms in cancer drug toxicity studies.

According to Krazinovic et al. \[^{[131]}\] approximately 20-40% of the childhood acute lymphoblastic leukemia (ALL) patients develop resistance to current therapeutic protocols. CYP1A1*2A and NQO1*2 variants were found to be associated with a worse therapeutic outcome in children with ALL. \[^{[131]}\] Therapy- related acute myeloid leukemia/ myelodysplastic syndrome (t-AML/t-MDS) might occur as a result of impaired ability to detoxify chemotherapeutic drugs. \[^{[132]}\] According to Bolufer et al. \[^{[132]}\] a polymorphism profile consisting of CYP1A1*2 A, GSTT1 null and NQO1*2 is a strong modifier of t-AML/t-MDS risk. They also demonstrated that the absence of all three polymorphisms decreased the risk of this therapy related disease. Platinum drugs represent a unique and important class of anticancer compounds and are frequently used in the treatment of various types of cancer. Heubner et al. \[^{[133]}\] observed a statistically significant association between the 462 Val allele and platinum resistance in ovarian cancer patients. Erlotinib, the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, is approved for second and third- line treatment of NSCLC. \[^{[134]}\] However, treatment with this drug has often been associated with life threatening adverse effects. In humans, erlotinib is extensively metabolized predominantly by CYP3A4, and to a lesser extent by CYP1A2 and the inducible form CYP1A1. \[^{[134]}\] Li et al. \[^{[134]}\] demonstrated that CYP1A1 along with CYP3A4 contributed to the formation of erlotinib- glutathione adducts. Also, the increased expression of CYP1A1 in the lungs of smokers may increase the risk of erlotinib associated interstitial lung disease.

For cancer chemotherapy, where cytotoxic agents are administrated at doses close to their maximum tolerable dose and therapeutic windows are relatively narrow, minor differences in individual drug handling may lead to severe toxicities. \[^{[135]}\] Hence, identification of predictive genetic markers for chemotherapeutic toxicity can make it possible to identify patients unlikely to respond to drug or at risk of severe drug- toxicity. Then an individualized plan for treatment with appropriate drug regimen can be prepared to achieve maximum treatment success with least amount of toxicity.

**Discussion**

Despite the advent of new high- throughput techniques in the field of biomarker studies only few genetic polymorphisms possess accountability as cancer risk marker. For example GSTM1 null and NAT2 slow acetylator genotypes have been associated with increased overall risk of bladder cancer. \[^{[136]}\] And a few gene- drug combinations are recommended by US Food and Drug Administration (FDA) for pharmacogenetic testing. \[^{[137]}\] In general, only very few of the candidate gene studies depicting positive association between genetic polymorphism and cancer susceptibility could be replicated in the subsequent studies. The probable causes of this failure are discussed in several reviews. \[^{[137, 138]}\] Briefly most important reason is the small sample size recruited in these studies. It is known that high penetrance genes render high risk for cancer but only <5% of all cancers can be explained in this way. \[^{[139]}\] Hence low penetrance common alleles with minor allele frequency (MAF)>5% have been chosen in genetic association studies. However, these alleles contribute only modestly to the risk for cancers. A large sample size is required in order to get statistically significant power (ability to detect a true association) in such cases. \[^{[140]}\] Published case- control studies typically have been conducted on a small sample size with fair possibility to generate false positive association for such low penetrance alleles. CYP1A1 studies are also no exception. Other than study design, publication bias for positive findings also influences the validation of a gene- disease association. Spitz and Bondy \[^{[138]}\] further pointed out that selection of SNPs for association study is a challenging job. In complex diseases like cancer many genes of different cross-talking pathways can be involved. Some uncharacterized genes having plausible role can make the selection more complex. Further, an allele common in one population can be present in very low frequency in other populations with least relevance for a disease. For example, m3 variant of CYP1A1 gene...
is prevalent in African-Americans but not in other populations, which emphasizes the need of searching a universal marker applicable for all ethnic groups.

Holmes et al.,[137] recommended some strategies to be followed in present day pharmacogenetic research. These included the need of high quality updated systematic reviews and meta-analyses in order to obtain conclusive data. Also, a careful look at precisely measurable intermediary risk markers like DNA adducts, cytogenetic damages and mutations, rather than cancer as an end point, would lead to a more comprehensive understanding of the genetic basis for cancer development.[6] While susceptibility biomarkers contribute to the identification of high-risk subgroups in the population, intermediate biomarkers measure early non-persistent biological events that take place after exposure and before the cancer development.[141] Intermediate (effect) markers include chromosomal aberrations, changes in DNA adducts, protein expression etc., and another category of biomarker, the exposure biomarkers, could be used to understand environmental influence on cancers, e.g., urinary adducts formed by aflatoxin, a strong human carcinogen.[141]

**Conclusion**

This is need of the hour to identify genetically susceptible individuals so that preventive measurements could be undertaken in order to reduce cancer load in the population. Further, identification of toxicity markers can reduce chemotherapy related death cases. Variations in metabolic enzymes have long been hypothesized as risk factors for the genetic susceptibility of cancer development, or chemotherapeutic drug toxicity and drug resistance. CYP1A1 for its key role in xenobiotic metabolism, have been extensively studied as a cancer marker though large discrepancies prevail in the results of these studies. More thorough studies recruiting large sample size, gene-gene, gene-environment interactions are required to understand the potential of CYP1A1 as candidate gene for cancer susceptibility or drug toxicity biomarker.

**Acknowledgement**

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<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Ref</th>
<th>Population/country</th>
<th>Cancer type</th>
<th>Case/ control</th>
<th>CYP1A1 genotype</th>
<th>Combined genotype</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
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<td>Oral</td>
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<td>*2A, GSTT1+</td>
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<td>Lung</td>
<td>91/138</td>
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<td>2.27 (1.1-4.52)</td>
<td>3.85 (1.43-10.33)</td>
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<td>South India</td>
<td>Lung</td>
<td>146/146</td>
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<td>3.18 (0.95-10.6)</td>
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<td>Lung</td>
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</tbody>
</table>

**Table 1: Association of CYP1A1 polymorphisms with cancer risk either alone or in combination with other genes**

AML=Acute myeloid leukemia; NMSC=Non melanoma skin cancer; ‡Not defined; *Number of variant alleles; †Non significant; SCC=Squamous cell carcinoma

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