Genetic Polymorphisms in CYP1A1 (m1), (m2), (m4) and CYP2A6 and Susceptibility to Hepatocellular Carcinoma in a Malaysian Study Population

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Abstract
The aim of this study is to investigate the relationship between Cytochrome P450 [CYP1A1 (m1), (m2), (m4) and CYP2A6] genetic polymorphisms and susceptibility to hepatocellular carcinoma (HCC) in a population-based case-control study. The frequency of the Cytochrome P450 polymorphisms was determined in 100 HCC patients and compared to frequencies determined in control population. DNA of peripheral white blood cells was isolated and the CYP1A1 and CYP2A6 genotypes were identified as restriction fragment length polymorphisms (RFLPs) by polymerase chain reaction (PCR) and restriction enzyme digests. Our results showed that there was a significant difference in the distribution of CYP1A1 (m2) and CYP2A6 genotypes among Malays, Chinese and Indians in Malaysia (p<0.05). CYP1A1 (m2) genetic polymorphism was found to have a significant relationship with HCC incidence ($\chi^2=31.87$, p<0.05) while no significant association between CYP1A1 (m1), (m4) and CYP2A6 genotypes with HCC risk was observed. Odds ratio (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression adjusting for known or suspected risk factors for HCC viz. hepatitis B status, family history of cancer, smoking status and alcohol intake. A significant increase in the risk of HCC was observed for individuals with CYP1A1 (m2) mutation (OR=3.69; CI=1.54-8.88). In conclusion, the distribution of CYP1A1 (m2) and CYP2A6 genotypes is different among ethnic groups in Malaysia and variant CYP1A1 (m2) genotype is associated with susceptibility to HCC in Malaysian population.

Keywords: CYP1A1, CYP2A6, polymorphisms, HCC risk.

CYP - Cytochrome, HCC - Hepatocellular Carcinoma, W - wild-type allele, D - deletion-type allele, C - conversion-type allele

Introduction
Hepatocellular carcinoma (HCC) is one of the most common cancer in Asia and Africa. Despite the remarkable advances in diagnostic and therapeutic techniques, the incidence of HCC remains on the increase [1]. There is a wide geographical and racial variation in the incidence of HCC which is high in China, South-East Asia and South African blacks [2] in figures ranging from less than 1/100,000 in parts of Northern Europe to over 100/100,000 in parts of China and Southern Africa [3]. The main etiological factor is chronic hepatitis B virus (HBV) infections, to which 50% to 80% of all cases are attributable. Other aetiological factors include chronic hepatitis C virus infections, exposure to aflatoxin, male gender and chronic liver disease of any type [4]. The male-to-female ratio of age-adjusted liver cancer incidence rates ranged from 0.7 (Ascucion, Paraguay) to 6.4 (Bas Rhin, France) but was mostly between 2 to 4. The gender difference may be due to discrepancies in the hepatitis B surface antigen (HbsAg) carrier rate, lifestyle, occupational exposure and hormone status between males and females [5]. Although the etiological factors associated are well recognized, the interactions between individual factors and the molecular mechanism by which they lead to cancer remain unclear [4].

The phase 1 cytochrome P450 enzymes catalyse the oxidative metabolism of diverse endogenous and exogenous chemicals from steroids to pollutants. During the oxidative process, electrophilic and carcinogenic intermediates can be created. P450 isoenzymes constitute a superfamily of enzymes and are divided into families based on their evolutionary relationship, which is determined by the degree of homology of the individual genes and thus amino acid sequence of the protein.

A number of genetic polymorphisms have now been established for several P450 enzyme systems, such as CYP1A1, CYP2A6, CYP2C9, CYP2D6 and CYP2E1. Considering the fact that bioactivation by P450 enzymes may play an important role in human drug toxicity and human cancer, these polymorphisms may result in large interindividual variations in the metabolism and toxicity of xenobiotics [6].

The gene product of CYP1A1 is aryl hydrocarbon hydroxylase which catalyses the first step of the
metabolism of polycyclic aromatic hydrocarbons (PAHs) to epoxides [7]. PAHs present in smoked food, tobacco smoke and ubiquitous in urban environment are believed to be responsible for an elevated risk in certain cancer [8]. Several polymorphisms of this gene has been reported [8] and it has been suggested that homozygotes variant allele of each mutation are correlated with an enhanced susceptibility to lung cancer especially in Japanese smokers [9].

In this study, we look into three types of CYP1A1 mutations which are m1, m2 and m4 mutations by referring to systematic nomenclature for mutations from Cascorbi et al. (1996) [10]. The CYP1A1 (m1) mutation is a point mutation at 3’ non-coding region (6235T → C) creating MspI cleavage site. This mutation has been associated with increased catalytic activity and in certain studies showed an increased risk for lung cancer [6]. The CYP1A1 (m2) mutation is the A → G transition at exon 7, codon 462 resulting in amino acid substitution of Val for Ile. This mutation results in a 2-fold increase in microsomal enzyme activity [11] and also associated with lung cancer in Japanese populations [12]. The CYP1A1 (m4) mutation is a point mutation at exon 7, codon 461 where the C → A transition occur resulting in the substitution of Asn for Thr.

CYP2A6 is an enzyme which plays a major role in the metabolic activation of several procarcinogens such as aflatoxin B1, N-nitosodimethylaniline and 1,3-butanediene [13]. CYP2A6 was found to activate a nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) which is well known as a potent lung carcinogen in rodents [14]. Among all 2A-genes, only CYP2A6 encodes an active protein [15]. The genetic polymorphism of CYP2A6 is represented by six allelic variants existing in addition to the wild-type allele CYP2A6*1. The variant allele CYP2A6*2 is a single base mutation (T/A) in exon 3 in this allele leads to one amino acid substitution (L160H) in the protein, which represents a catalytically inactive protein [15]. The next variant allele CYP2A6*3 is supposed to be generated through gene conversion between the CYP2A6 and the neighbouring CYP2A7 locus (16). CYP2A6*3 allele is supposed to encode a catalytically defective enzymes. Other polymorphic CYP2A6del allele defines a CYP2A locus completely lacking the CYP2A6 gene [15].

Few studies have correlated environmental factors and genetic susceptibility with the risk of hepatocellular carcinoma, especially in Asian population, which has one of the highest incidence of hepatocellular carcinoma in the world. Genetic polymorphism in CYPs P450 that contribute to chemical toxicity is likely to be a significant factor in determining susceptibility to diseases related to exposure to these compounds. An understanding of the extent of polymorphism in the genes coding these enzymes is likely to be useful in predicting those at high risk from certain types of exposure. In this study we evaluated the association between [CYP1A1 (m1), (m2), (m4) and CYP2A6] polymorphisms and the risk of hepatocellular carcinoma in a Malaysian study population.

Materials and Methods

Study subjects

The case group was comprised of 110 patients with primary hepatocellular carcinoma (HCC) (80 men, 30 women; aged mean = 55.7 ± 9.2, 31 to 76 years). The patients were consecutive cases at the Kuala Lumpur General Hospital and Universiti Kebangsaan Malaysia Hospital. All patients had been histologically diagnosed and 57% of them were hepatitis B surface antigen (HbsAg) carriers.

The control group was comprised of 317 subjects who were regular blood donors at Kuala Lumpur General Hospital Blood Bank. There were 239 men and 78 women (aged mean 33.8 ± 9.2, 18 to 69 years) and no specific age matching was carried out in the study. Control subjects had no clinical evidence of malignancy. At recruitment, each subject was personally interviewed to obtain information on demographic characteristics, habits of cigarette smoking, alcohol intake, dietary (fruits and vegetables) consumption frequency, hepatitis status as well as family history of cancer. All subjects gave verbal and written informed consent and were tested for hepatitis B surface antigen (HbsAg). Plasma HbsAg was assayed using an ELISA kit (Fujirebio Inc., Tokyo, Japan)

Blood samples

10 ml of blood was collected in EDTA tube and stored at 4°C before DNA extraction which was done within 1 week of sample collection. Blood samples were initially centrifuged to separate plasma from packed cells. Genomic DNA from leukocytes of anticoagulated blood was prepared by proteinase K (Roche Biochemicals, Germany) digestion and precipitated using a salting-out procedure with sodium chloride [17].

Identification of CYP1A1 polymorphisms

Identification of CYP1A1 polymorphisms was performed as described by Cascorbi et. al. [18]. Two types of oligonucleotide primers were used for the identification of CYP1A1 (m1) polymorphisms ; CYP1A1-M3F and CYP1A1-P80.

The sequence of the primers are:

CYP1A1-M3F : 5’- GGC TGA GCA ATC TGA CCC TA -3’
CYP1A1-P80 : 5’- TAG GAG TCT TGT CTC ATG CCT -3’

Polymerase chain reaction was carried out using 0.1 ug genomic DNA in 25 ul solution containing autoclaved ultrafiltered water, 10X PCR buffer (Promega Corp.,
Identification of CYP2A6 polymorphisms

Identification of CYP2A6 polymorphism was performed as described by Rautio et al. [19]. 0.1 ug of genomic DNA was added to 50 ul polymerase chain reaction mixture containing autoclaved ultrafiltered water, 10X LA-PCR buffer, 25 mM MgCl\(_2\), 2.5 mM dNTPs (Promega Corp., Madison, USA), 1 unit LA-Taq DNA (Promega Corp., Madison, USA) and 25 pmol of each oligonucleotide primer (Promega Corp., Madison, USA).

The sequence of the primers are:

- **CYP2A6-B4:** 5'-CAC CGA AGT GTT CCC TAT GCT -3'
- **CYP2A6-UTRASI:** 5'-TGT AAA ATG GCC ACC AAG-3' 

After initial denaturation (94°C, 3 min), thirty cycles of denaturation (94°C, 45 s) primer annealing (50°C, 1 min) and elongation (72°C, 1 min) followed by elongation (72°C, 5 min) were performed.

Two types of restriction enzyme were used for the identification of CYP2A6 polymorphisms; AccII and Eco81I. PCR products (5 ul) were digested (37°C, overnight) with 4 units AccII (Promega Corp., Madison, USA) in autoclaved ultrafiltered water and 10X M buffer. DNA fragments were resolved by electroforesis in 2% agarose gel containing ethidium bromide and photographed under u.v. light.

PCR products (5 ul) were also digested (37°C, overnight) with 4 units Eco81I (Promega Corp., Madison, USA) in autoclaved ultrafiltered water and 10X M buffer. DNA fragments were resolved by electroforesis in 2% agarose gel containing ethidium bromide and photographed under u.v. light.

Statistical analysis

To assess the relationship between CYP1A1 (m1), (m2), (m4) and CYP2A6 polymorphisms and HCC risk, the significant difference in the case-control distribution of cancer, smoking status and alcohol intake.

Results

Amplification of polymorphic CYP1A1 (m1), (m2), (m4)

DNA from subject with homozygous wild CYP1A1 (m1) genotype allowed amplification of undigested 899 bp DNA fragment (W/W alleles) while heterozygotes to 899 bp, 693 bp and 206 bp fragments (W/D alleles). DNA from subject with homozygous deletion mutation was digested to 693 bp and 206 bp fragments (D/D alleles).
Cytochrome P450 polymorphisms and HCC risk

Figure 1 shows the identification of the CYP1A1 (m1) mutation by MspI digestion of the PCR products in the presence of CYP1A1-M3F and CYP1A1-P80 primers. Lane 2 shows undigested 899 bp fragment indicating W/W alleles (wild genotype). Lane 3 shows incomplete digestion resulting in fragments of 899 bp, 693 bp and 206 bp indicating W/D alleles (heterozygote) and lane 4 shows complete digestion with MspI into fragments of 693 bp and 206 bp demonstrating the homozygous deletion mutation (D/D alleles). The study subjects carried the homozygous mutant gene (D/D alleles). Figure 2 shows the distribution of CYP1A1 (m1) mutation among ethnic groups in Malaysia. Comparison between major races in Malaysia showed no significant difference in the distribution of CYP1A1 (m1) mutation among Malays, Chinese and Indians ($\chi^2=1.74$; $p>0.05$).

For the identification of CYP1A1 (m2) mutation, DNA from subject with homozygous wild genotype was digested to 149 bp and 55 bp fragments (W/W genotype) while heterozygotes allowed digestion of 204 bp, 149 bp and 55 bp fragments (W/D genotype). DNA from subject with homozygous deletion mutation allowed amplification of an undigested 204bp DNA fragment (D/D genotype).

The control group was comprised of 102 Malays, 101 Chinese and 114 Indians. A total of 106 (33.4%) study subjects in control population carried the wild genotype (W/W alleles) of the CYP1A1 (m1), 53.9% were heterozygotes (W/D alleles) and another 12.6% of

![Figure 1: Identification of the CYP1A1 (m1) mutation by MspI digestion of the PCR products in the presence of CYP1A1-M3F and CYP1A1-P80 primers. Lane 2 shows undigested 899 bp fragment indicating W/W alleles (wild genotype). Lane 3 shows incomplete digestion resulting in fragments of 899 bp, 693 bp and 206 bp indicating W/D alleles (heterozygote) and lane 4 shows complete digestion with MspI into fragments of 693 bp and 206 bp demonstrating the homozygous deletion mutation (D/D alleles).](image1)

![Figure 2: Histogram showing the distribution of CYP1A1 (m1) alleles among ethnic groups in a Malaysian study population ($\chi^2=1.74$; $p>0.05$).](image2)

![Figure 3: Histogram showing the distribution of CYP1A1 (m1) alleles among male and female in a Malaysian study population ($\chi^2=0.28$; $p>0.05$).](image3)

![Figure 4: Identification of the CYP1A1 (m2) allele by BsrDI digestion of the PCR products in the presence of CYP1A1-M2F and CYP1A1-M2R primers. Lanes 1, 4 and 8 show complete digestion with BsrDI into fragments of 149 bp and 55 bp indicating W/W alleles (wild genotype). Lane 3 show incomplete digestion resulting in fragments of 204 bp, 149 bp and 55 bp indicating W/D alleles (heterozygote) and lanes 2, 5 and 6 show undigested 204 bp fragment demonstrating the homozygous deletion mutation (D/D alleles).](image4)
Figure 4 shows the identification of the CYP1A1 (m2) allele by BsrDI digestion of the PCR products in the presence of CYP1A1-M2F and CYP1A1-M2R primers. Lanes 1, 4 and 8 show complete digestion with BsrDI into fragments of 149 bp and 55 bp indicating W/W alleles (wild genotype). Lane 3 show incomplete digestion resulting in fragments of 204 bp, 149 bp and 55 bp indicating W/D alleles (heterozygote) and lanes 2, 5 and 6 show undigested 204 bp fragment demonstrating the homozygous deletion mutation (D/D alleles).

Analysis on CYP1A1 (m2) genetic polymorphism showed that there was a significant difference observed in the distribution of CYP1A1 (m2) genotype among Malays, Chinese and Indians in Malaysia ($\chi^2=11.69; p<0.05$) (Figure 5). From the results of the study it was shown that 40.1% of Malaysian study population carried wild genotype (W/W alleles), 29.3% were heterozygotes (W/D alleles) and 30.6% carried D/D mutant variant alleles.

For the identification of CYP1A1 (m4) mutation, DNA from subject with homozygous wild genotype was digested to 139 bp and 65 bp fragments (W/W genotype) while heterozygotes allowed digestion of 204 bp, 139 bp and 65 bp fragments (W/D genotype). DNA from subject with homozygous deletion mutation allowed amplification of an undigested 204 bp DNA fragment (D/D genotype).

Figure 7 shows the identification of the CYP1A1 (m4) allele by BsaI digestion of the PCR products in the presence of CYP1A1-M2F and CYP1A1-M2R primers. Lanes 1, 2, 3, 7, 8 and 9 show complete digestion with BsaI into fragments of 139 bp and 65 bp indicating W/W alleles (wild genotype). Lanes 4, 5, and 6 show incomplete digestion resulting in fragments of 204 bp, 139 bp and 65 bp indicating W/D alleles (heterozygote).

Figure 8 shows the distribution of CYP1A1 (m4) mutation among ethnic groups in Malaysia. Comparison between major races in Malaysia showed no significant difference in the distribution of CYP1A1 (m4) mutation among Malays, Chinese and Indians ($\chi^2=5.05; p>0.05$). From the results of the study it was shown that 97.8% of...
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Malaysian study population carried wild genotype (W/W alleles) of CYP1A1 (m4), and 2.2% were heterozygotes (W/D alleles). Meanwhile the D/D mutant variant alleles was not detected in this population.

Comparison between gender in Malaysian study population shows that there was no significant difference observed in the polymorphisms of CYP1A1 (m1), (m2), (m4) (Figures 3, 6, 9).

The odds ratio (ORs) and 95% confidence intervals (CIs) of CYP1A1 (m1), (m2), (m4) genotypes comparing hepatocellular carcinoma cases to control population after adjustment for hepatitis B status, family history of cancer, smoking status and alcohol intake are also shown (Tables 2, 3 and 4). Using wild genotype as referent, we found that the adjusted OR for the heterozygous mutant allele of CYP1A1 (m2) was significantly higher [OR = 3.69 (95% CI=1.54-8.88) (p<0.05)].

![Histogram showing the distribution of CYP1A1 (m4) alleles among male and female in a Malaysian study population (χ²=15.18; p>0.05)](image)

Figure 9: Histogram showing the distribution of CYP1A1 (m4) alleles among male and female in a Malaysian study population (χ²=15.18; p>0.05)

![Figures 10 and 11: Identification of the CYP2A6 allele by AccII and Eco81I digestion of the PCR products in the presence of CYP2A6-B4 and CYP2A6-UTRASI primers. Lanes 1, 2, 3, 4, 5, and 6 represent CYP2A6 allele[(W/W), (W/C), (W/D), (C/C), (C/D) and (D/D) respectively)](image)

Figures 10 and 11: Identification of the CYP2A6 allele by AccII and Eco81I digestion of the PCR products in the presence of CYP2A6-B4 and CYP2A6-UTRASI primers. The type of CYP2A6 alleles and its DNA fragments after AccII and Eco81I digestion is summarized in Table 1.

**Table 1: Type of CYP2A6 alleles and its DNA fragment size after digestion with endonuclease AccII and Eco81I**

<table>
<thead>
<tr>
<th>Restriction enzymes</th>
<th>Size of DNA fragments after digestion (bp)</th>
<th>W/W</th>
<th>W/C</th>
<th>W/D</th>
<th>C/C</th>
<th>C/D</th>
<th>D/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccII</td>
<td>1281</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>1021</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Eco81I</td>
<td>800</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>748</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>390</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

W, wild-type; C, conversion type; D, deletion type;

Analysis on CYP2A6 genetic polymorphism showed that there was a significant difference observed in the distribution of CYP2A6 genotype among Malays, Chinese and Indians in Malaysia (χ²=42.25; p<0.001) (Figure 12). From the results of the study it was shown that 25.6% of the study subjects in control population carried the wild genotype (W/W alleles) of the CYP2A6, 53% were heterozygotes (36.3%, 10.1% and 6.6% with W/C, W/D and C/D alleles respectively) and another 21.4% of the study subjects carried the homozygous mutant gene (20.5% and 0.9% with C/C and D/D alleles respectively). However comparison between gender in Malaysian study population shows that there was no significant difference observed in the polymorphisms of CYP2A6 (Figure 13).
Figure 12: Histogram showing the distribution of CYP2A6 alleles among ethnic groups in a Malaysian study population ($\chi^2=42.25; p<0.001$)

Figure 13: Histogram showing the distribution of CYP2A6 alleles among male and female in a Malaysian study population ($\chi^2=5.81; p>0.05$)

Table 2: CYP1A1 (m1) genotype and the risk of hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Percentage (n)</th>
<th>Adjusted OR$^a$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
</tr>
<tr>
<td>W/W</td>
<td>30.9 (34)</td>
</tr>
<tr>
<td>W/D</td>
<td>58.2 (64)</td>
</tr>
<tr>
<td>D/D</td>
<td>10.9 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>100 (110)</td>
</tr>
</tbody>
</table>

Insignificantly different among cases and controls ($\chi^2=6.22; p>0.05$)

$^a$OR adjusted for hepatitis B status, family history of cancer, smoking status and alcohol intake

Table 3: CYP1A1 (m2) genotype and the risk of hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Percentage (n)</th>
<th>Adjusted OR$^a$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
</tr>
<tr>
<td>W/W</td>
<td>30.0 (33)</td>
</tr>
<tr>
<td>W/D</td>
<td>58.2 (64)</td>
</tr>
<tr>
<td>D/D</td>
<td>11.8 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>100 (110)</td>
</tr>
</tbody>
</table>

Significantly different among cases and controls ($\chi^2=31.87; p<0.05$)

$^a$OR adjusted for hepatitis B status, family history of cancer, smoking status and alcohol intake

Table 4: CYP1A1 (m4) genotype and the risk of hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Percentage (n)</th>
<th>Adjusted OR$^a$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
</tr>
<tr>
<td>W/W</td>
<td>100 (110)</td>
</tr>
<tr>
<td>W/D</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D/D</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>100 (110)</td>
</tr>
</tbody>
</table>

Insignificantly different among cases and controls ($\chi^2=2.47; p>0.05$)

$^a$OR adjusted for hepatitis B status, family history of cancer, smoking status and alcohol intake
Table 5: CYP2A6 genotype and the risk of hepatocellular carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Percentage (n)</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>W/W</td>
<td>21.8 (24)</td>
<td>25.6 (81)</td>
</tr>
<tr>
<td>W/C</td>
<td>38.2 (42)</td>
<td>36.3 (115)</td>
</tr>
<tr>
<td>W/D</td>
<td>6.4 (7)</td>
<td>10.1 (32)</td>
</tr>
<tr>
<td>C/C</td>
<td>20.9 (23)</td>
<td>20.5 (65)</td>
</tr>
<tr>
<td>C/D</td>
<td>12.7 (14)</td>
<td>6.6 (21)</td>
</tr>
<tr>
<td>D/D</td>
<td>0 (0)</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>100 (110)</td>
<td>100 (317)</td>
</tr>
</tbody>
</table>

Insignificantly different among cases and controls (χ²=6.55; p>0.05)

*OR adjusted for hepatitis B status, family history of cancer, smoking status and alcohol intake

Table 5 shows the odds ratio (ORs) and 95% confidence intervals (CIs) of CYP2A6 genotypes comparing hepatocellular carcinoma cases to control population after adjustment for hepatitis B status, family history of cancer, smoking status and alcohol intake. Using wild genotype as referent, we found that the adjusted ORs for the heterozygous and homozygous mutant alleles of CYP2A6 was lower (not significant).

Discussion

CYP1A1 is a key enzyme in carcinogen metabolism. By virtue of its polymorphic regulation, CYP1A1 proved a promising genetic biomarker for susceptibility to certain malignancies, particularly lung cancer [20]. Previous study reported that CYP1A1 mutation varies among ethnic groups. Significant ethnic differences in the frequency of homozygous CYP1A1 MspI alleles (m1) was observed. The frequency of homozygous CYP1A1 MspI alleles (m1) was lower (7.3%) in Caucasians [21] compared to 33.2% in Japanese [22]. Studies by Quinones et al (2001) [23] showed that the frequencies of MspI site and Val alleles were 3 and 5 times higher in healthy Chilenean control group than in healthy French control group. Although in the Chileen healthy control group the frequency of the MspI allele was higher than in Caucasians, it was lower than in Asian. The CYP1A1 (m2) mutation is rare in Caucasians but occurs in about 20% of Japanese [24]. Comparison between major races in Malaysia shows significant difference in the distribution of CYP1A1 (m2) mutation with Chinese exhibit the highest percentage of homozygote mutation (43.6%) followed by Malays (30.0%) and Indians (26.4%).

The CYP1A1 (m1) and (m2) mutations were found to be overrepresented among lung cancer patients in Japan [22, 24] and also in a German study [21]. However only a trend was observed in Finland [25]. In our Malaysian study population, genotyping of CYP1A1 (m2) mutation shows that 30.6% of study subjects carried the homozygous mutant alleles and 29.3% were heterozygotes. Further analysis by comparing the frequency of CYP1A1 (m2) mutation in normal control population to HCC cases shows a significant difference among the two groups studied (χ²=31.87; p<0.05) suggesting that there was a relationship between CYP1A1 (m2) mutation and HCC risk.

Logistic regression was used to assess the interactions (or effect modifications) between CYP1A1 polymorphisms and other possible risk factors of HCC. The odds ratio (ORs) and 95% confidence intervals (CIs) were adjusted for hepatitis B status, family history of cancer, smoking status and alcohol intake. Using wild genotype as referent, we found that the adjusted OR for the heterozygous mutant allele of CYP1A1 (m2) was significantly higher [OR = 3.69 (95% CI=1.54-8.88) (p<0.05)]. This implied that HCC risk of an individual carrying m2 allele could be three times higher than that of an individual with the wild/wild genotype. However no significant association was found between CYP1A1 (m1), and (m4) mutations and HCC.

Very few studies in the past had reported the associations between CYP1A1 and the risk of HCC. The strongest evidence for the role of m2 in cancer was found in the Japanese [24]. Due to the relatively high prevalence of the m2-carrying allele (19.8%) in this population, an increase to 27.5% in lung cancer patients was of high statistical significance (ORs = 1.53; p=0.001). In Caucasians however, m2 represents a rare trait. The frequency of m2-carrying alleles rose by more than 2-fold among lung cancer patients compared to controls in Berlin (OR=2.16; p=0.03) [21]. Similarly, comparison of genotypes in Brazilian non-blacks resulted in an odds ratio of 2.40 (p=0.01). No impact of m2 was found for bladder cancer [26].

Analysis of CYP2A6 genetic polymorphisms shows that it was possible to distinguish the CYP2A6*1, the deletion-type mutant and the conversion-type mutant. There are six genotypes of CYP2A6 in Malaysian-studied population but very few possessed the deletion-type mutation. From the results of our study it was shown that
the Malays exhibited the highest percentage for homozygote deletion-type mutation (66.7%) followed by Chinese (33.3%) while no Indians possessed this genotype. Similar pattern was seen for homozygote conversion-type mutation in which Malays exhibited the highest frequency (45.5%) followed by Chinese (40.9%) and Indians (13.6%). Statistical analysis comparing the distribution of CYP2A6 genotypes among major races in Malaysia shows significant difference between Malays, Chinese and Indians. The results obtained from this study was similar to previous studies which reported a large inter ethnic variation of this polymorphism. Oscarson (2001) [27] stated that the frequency of the inactive alleles (deletion-type mutant) is low in European populations and very few metabolizers for the probe drug coumarin have been described in these populations. In contrast, a relatively high allele frequency of the CYP2A6 gene deletion has been found in Asians, resulting in a generally reduced activity in these populations.

CYP2A6 predominantly expressed in the liver and is responsible for the clearance of many drugs and environmental chemicals such as a naturally occurring plant compound coumarin, nicotine and precarcinogens such as aflatoxin B1 [27]. Previous studies showed that the frequency of CYP2A6 gene whole deletion was significantly lower in the lung cancer patients compared to the healthy populations [28]. In our studies, however no significant association was detected between CYP2A6 genetic polymorphism and HCC risk.

Comparison between gender in Malaysia shows that there was no significant difference observed in the polymorphisms of CYP1A1 (m1), (m2), (m4) and CYP2A6.

Conclusion

In conclusion, this study demonstrates a significant association between CYP1A1 (m2) genetic polymorphisms and HCC risk in Malaysian study population. In addition we found that there were significant differences in the polymorphisms of CYP1A1 (m2) and CYP2A6 genes among Malays, Chinese and Indians in this population.

References

Cytochrome P<sub>450</sub> polymorphisms and HCC risk


