The Implication of the Polymorphisms of COX-1, UGT1A6, and CYP2C9 among Cardiovascular Disease Patients Treated with Aspirin

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ABSTRACT - Purpose. Enzymes potentially responsible for the pharmacokinetic variations of aspirin include cyclooxygenase-1 (COX-1), UDP-glucuronosyltransferase (UGT1A6) and P450 (CYP) (CYP2C9). We therefore aimed to determine the types and frequencies of variants of *COX-1 (A-842G), UGT1A6 (UGT1A6*2*; *A541G* and *UGT1A6*3*; *A522C*) and *CYP2C9* (*CYP2C9*3*; *A1075C*) in the three major ethnic groups in Malaysia. In addition, the role of these polymorphisms on aspirin-induced gastritis among the patients was investigated. **Methods**: A total of 165 patients with cardiovascular disease who were treated with 75-150 mg daily dose of aspirin and 300 healthy volunteers were recruited. DNA was extracted from the blood samples and genotyped for *COX-1 (A-842G), UGT1A6 (UGT1A6*2* and *UGT1A6*3*) and *CYP2C9* (*CYP2C9*3*; *A1075C*) using allele specific polymerase chain reaction (AS-PCR). **Results**: Variants *UGT1A6*2,*3* and *CYP2C9*3* were detected in relatively high percentage of 22.83%, 30.0% and 6.50%, respectively; while *COX-1 (A-842G)* was absent. The genotype frequencies for *UGT1A6*2 and *3* were significantly different between Indians and Malays or Chinese. The level of bilirubin among patients with different genotypes of *UGT1A6* was significantly different (p-value < 0.05). In addition, *CYP2C9*3* was found to be associated with gastritis with an odd ratio of 6.8 (95 % Cl OR: 1.39 – 33.19; $P = 0.033$). **Conclusion**: Screening of patients with defective genetic variants of *UGT1A6* and *CYP2C9*3* helps in identifying patients at risk of aspirin induced gastritis. However, a randomised clinical study of bigger sample size would be needed before it is translated to clinical use.

Keywords: *COX-1*, *UGT1A6*, *CYP2C9*, Aspirin

What This Study Adds: Genotyping Screening for *UGT1A6*2, UGT1A6*3* and *CYP2C9*3* helps to identify patients at risk of aspirin induced gastritis.

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INTRODUCTION

Aspirin with a dose of 75-150 mg daily has been found to reduce risk of vascular events by approximately 32% in patients with cardiovascular disease (CVD) [1]. However, a substantial number of patients do not respond optimally to aspirin treatment [2]. The inter-individual variability of patients' responses is due to variation either in pharmacokinetic (PK) or pharmocodynamic (PD) properties of aspirin [3]. Those polymorphic enzymes potentially affecting the PK-PD of aspirin include cyclooxygenase-1 (COX-1), UDP-glucuronosyltransferase 1A6 (UGT1A6) and CYP2C9 enzymes.

COX-1 is directly and irreversibly inhibited by aspirin resulting in reduction or inhibition of the formation of precursors of prostaglandins and thromboxanes. Genetic variants of *COX-1* gene were suggested to modulate arachidonic acid-induced platelet aggregation and serum thromboxane B2 (TXB2) levels in patients treated with aspirin. The Polymorphic UDP-glucuronosyltransferase (UGT1A6) and CYP2C9*3

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enzymes are reported to be associated with altered enzyme function which affects aspirin metabolism and efficacy. . The amino acid changes for two variants of *UGT1A6 (UGT1A6*2*; *A541G* and *UGT1A6*3*; *A522C*) result in a 30%-50% reduced enzyme activity compared to enzyme encoded by the wild-type allele [4]. Both variants of *UGT1A6*2* and **3* are in complete linkage disequilibrium [5]. The variant alleles for *CYP2C9*3* also produce enzymes bearing some 5%-30% of the activity of the wild-type enzyme [6]. All these polymorphic enzymes might modulate the therapeutic effect of aspirin in treatment or prevention of CVD. Carriers of variant alleles are more prone to develop acute gastrointestinal problems when they receive aspirin as compared to non-carriers [7,8].

Several biochemical tests are routinely screened among the patients in order to assess the risk of early stage CVD. Altered Lipid profiles which includes HDL, LDL, trigylcerides and total cholesterol are common risk factors for CVD. The higher the cholesterol level, the higher the risk of CVD disease. However, HDL is "good cholesterol" which protects one against the disease by removing cholesterol and excess fat from blood vessel walls and transports them to the liver to be removed from the body. Besides, there is an apparent protective effect of bilirubin which is in similar magnitude as HDL. A study shows that a 50% decrease in total bilirubin was associated with a 47% increased risk of having more severe coronary artery disease (p=0.02) [9]. Since, UDP-glucuronosyltransferase (UGT1A6) is highly expressed in liver and plays a major role in the metabolisms of bilirubin, their genetic polymorphisms and bilirubin levels are potentially important risk factors of CVD.

The aims of this study were to investigate the relationship between the clinical parameters and genetic polymorphisms of *COX-1*, *UGT1A6* and *CYP2C9* in cardiovascular patients. This allows the assessment of the impact of genetic polymorphisms of *COX-1*, *UGT1A6* and *CYP2C9* on gastritis or other gastrointestinal symptoms in cardiovascular patients treated with aspirin.

METHODS

Subjects

The protocol of the study was approved by the local Research and Ethics Committee. The study comprised of 165 patients and 300 healthy volunteers aged 18 to 65 years old. All patients were diagnosed with cardiovascular disease and treated with aspirin (acetylsalicylic acid, 75-150 mg daily dose); while the healthy volunteers were unrelated individuals not receiving any treatment. All the participants were healthy mentally and physically, understand the study protocol and willing to sign the consent form.

Data Collection

Medical records for all patients were reviewed. The clinical data include medical history, biochemical test result (i.e. liver function test, renal function test, full blood count, lipid profile and blood glucose), dosage regimen, INR measurement, concurrent drugs intake and adverse effects experienced by patients were recorded. Sign and symptoms of gastritis such as gastric pain, vomiting and loss of appetite were reviewed and diagnosed by the medical doctors in charged.

Genotyping

Five milliliters of blood samples were drawn into tubes which contained tri-sodium citrate (4%).The blood samples were used for DNA extraction and genotyping for variants of *UGT1A6*, *COX-1* and *CYP2C9*. The DNA was extracted using lysis method that has been optimized previously [10]. The extracted DNA was dissolved in 1x TE buffer and kept at -20 ºC freezer until use. Allele specific PCR (AS-PCR) method was developed for detection of each variant. This technique is based on single-nucleotide variations which introduced destabilizing mismatch at the 3' end of the allele-specific primers [11].

A nested PCR was developed to amplify *COX-1*(*A-842G)* variants. Amplicon sizes for the first step PCR and allele specific *COX-1*(*A-842G)* variant are 369 bp and 150 bp, respectively. The optimal primer concentrations used was 0.2 μM for common forward (5'-GGTGATCTTGGACCTATTCC-3') and reverse (5'-CTTGGACAAGGTACTTATCTT-3') primers; 0.3 μM for variant (5'-GCACCTACTACATGCTGTG -3') and wild-type (5'- GCACCTACTACATGCTGTA-3') primers. The thermal cycling condition comprised of 30 cycles of 1st step and 15 cycles for the 2nd step PCR reaction. DNA denaturing was set at temperature of 94ºC for 30 seconds, while annealing and extension steps were set at 50ºC for 30 seconds and 72ºC for

30 seconds, respectively. Fragments of PCR product are shown in Figure 1(a) (2nd step).

One set of forward and reverse primers flanking 150 bp of Beta-2-microglobulin (*B2M*) gene was used as the internal control. The sets of primers used for detection of *UGT1A6*2* (*A541G)* variant were forward primer, 5'-CGATCATTCCTAACTGCTCCTC-3', 0.2 µM; reverse primer, 5'- TGGGCTTCTGCTGAATGC -3', 0.3 µM); and wild-type (forward primer, 5'-CGATCATTCCTAACTGCTCCTC-3', 0.2 µM; Reverse primer, 5'- TGGGCTTCTGCTGAATGT -3', 0.3 µM). For determination of *UGT1A6*3* (A522C), the primers used were forward primer, 5'-CGATCATTCCTAACTGCTCCTC-3', 0.2 µM; reverse primer, 5'- GGAGCATACATTCAGCAGC -3'; 0.3 µM and wild-type forward primer, 5'-CGATCATTCCTAACTGCTCCTC-3', 0.2 µM; reverse primer, 5'- GGAGCATACATTCAGCAGA -3' 0.3 µM. The fragment size of amplicons for variant detection of *UGT1A6*2* (*A541G)* and *UGT1A6*3* (A522C) are 255 bp and 301 bp, respectively. The thermal cycling conditions for *UGT1A6* variants are the same as COX-1 except for the annealing temperature. DNA was denatured at temperature of 94ºC for 30 seconds, while annealing temperature for *UGT1A6*2* and **3* are 54ºC and 60ºC, respectively. The extension steps were set at 72ºC for 30 seconds. The fragments of PCR products for both polymorphisms are shown in Figure 1(b) *UGT1A6*2* and Figure 1 (c) *UGT1A6*3.*

For *CYP2C9*3*, the amplified fragments for internal control and allele specific are 250 bp and 470 bp, respectively (Figure 1 (d)). The fragments were amplified using specific primers (variants type forward primer, 5'- CGAGGTCCAGAGATACC-3', 0.3 µM; reverse primer, 5'-CCTCAACGTGTCAAGATTCAGT-3', 0.2 µM); and wild-type forward primer, 5'-CGAGGTCCAGAGATACA-3', 0.3 µM; reverse primer, 5'-CCTCAACGTGTCAAGATTCAGT-3', 0.2 µM). The thermal cycling conditions were the same as for amplification of variants of *COX-1* and *UGT1A6*, but the optimum annealing temperature was 55ºC. For confirmation of the results, PCR products for each variants were sequenced using direct sequencing approach. The sequencing results are illustrated in Figure 1.

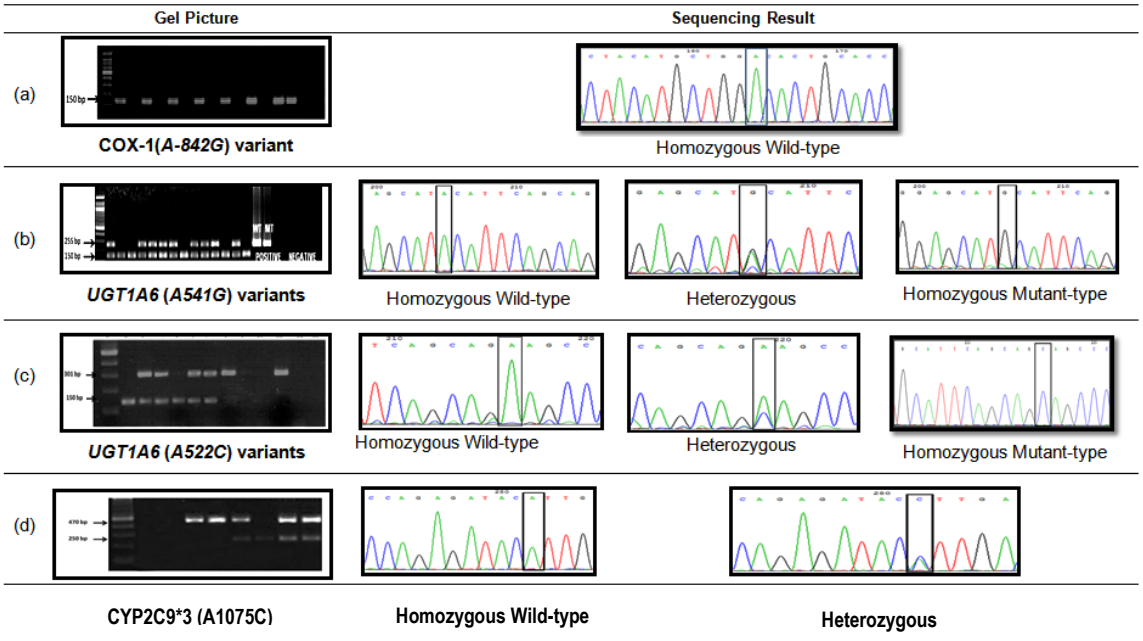


Figure 1. Nucleotide Sequence of the PCR Products of *COX-1(A-842G), UGT1A6 (A541G* and *A522C*) and *CYP2C9*3 (A1075C)* Allele

Data Analysis

The genotypes and allelic frequencies of *COX-1*, *UGT1A6* and *CYP2C9*3* were analyzed. Statistical analysis was carried out using Statistical Package for Social Science (SPSS), version 20. The observed and expected genotype frequencies were compared. Chi-square test was used to evaluate the fitness of the genotypic distribution to Hardy-Weinberg equilibrium. Numerical variables such as age, BMI, bilirubin and albumin were analyzed using Independent t-test, Mann- Whitney or Kruskal-Wallis test when appropriate. Categorical variants such as ethnicity and gender were analyzed using Chi-square test. For *UGT1A6* gene, the patterns of linkage disequilibrium (LD) for both SNPs (A541G and A522C) were analyzed and visualized, pair-wise of both SNPs were computed using Haploview (SNPs Tools) (http://www.bioinformatics.org/snp-tools-excel) [12]. The relative risk of aspirin induced gastritis due to genetic variant was tested using odd ratio (OR) (http://www.medcalc.org/calc/odds_ratio.php).

RESULTS

Three hundred healthy volunteers, 100 each of Malays, Chinese and Indians were recruited. A total number of 165 patients with cardiovascular disease (CVD) who met the inclusion and exclusion criteria of the study were also recruited. Most of the CVD patients were male (84.52%) and the remaining were female (15.48%). About 86.67% of the total patients were overweight and most of them were smokers (59.39%); 75.15% of the patients have dyslipidaemia (Table 1).

Table 1. Demographic and Clinical Data of the CVD Patients (Categorical variables)

Variable		n (165)	Gender		% (100)	*p*
			Male	Female		
Ethnicity	Malays	69	62 (89.9 %)	7 (10.1 %)	41.82	<0.001*
	Chinese	73	58 (79.5 %)	15 (20.5 %)	44.24	
	Indian	23	20 (87.0 %)	3 (13.0 %)	13.94	
BMI	Normal (≤ 24.99)	22	22 (100 %)	0 (0 .0 %)	13.33	<0.001*
	Overweight (≥ 25.00)	143	118 (82.5 %)	25 (17.5 %)	86.67	
Smoker	Yes	98	96 (98 %)	2 (2 %)	59.39	0.016*
	No	67	44 (65.7 %)	23 (34.3 %)	40.61	
Diabetes mellitus[b]	Yes	89	72 (80.9 %)	17 (19.1 %)	53.94	0.312
	No	76	68 (89.5 %)	8 (10.5 %)	46.06	
Dyslipidaemia[b]	Yes	124	108 (87.1 %)	16 (12.9 %)	75.15	<0.001*
	No	41	32 (78.0 %)	9 (22.0 %)	24.85	

[a]Chi-square test, [b] Risk Factor, n: Sample Size, %: Percentage, BMI: Body Mass Index

Biochemical results for triglycerides, TC, HDL, LDL, PT and INR of the patients were significantly different between the three ethnic groups (Kruskal-Wallis test; p-value < 0.05). The variables which were significantly different were further analyzed using Mann-Whiney tests.

A total of 300 healthy volunteers participated in this study were successfully screened for *COX-1 (A-842G)*, *UGT1A6 (A541G* and *A522C)*, and *CYP2C9*3 (A1075C)* polymorphisms. For *COX-1 (A-842G)*, no variants were detected in healthy volunteers. Out of the 300 healthy volunteers, 245 and 55 were carriers of wild-type and heterozygous genotypes for *CYP2C9*3*, respectively. No homozygous *CYP2C9*3* genotype was detected. The genotypes were in Hardy-Weinberg Equilibrium (HWE). The genotype frequencies were significantly different between Indians and Malays as well as between Indians and Chinese; the Indians carry 14% of *CYP2C9*3* allele as compared to 2.5% and 3% in Malay and Chinese respectively (Table 2). The genotype frequencies for both *UGT1A6*2* and **3* among the healthy volunteers

Table 2. Allele Frequencies of CYP2C9*3 (A1075C), UGT1A6 (*2 &*3) among the Healthy Volunteers in Three Ethnic Groups

Race	*CYP2C9*3 (A1075C)* Allele Frequency (%) (95% CI: Lower limit, Upper limit)				*UGT1A6*2* (*A541G*) Allele Frequency (%) (95% CI: Lower limit, Upper limit)				*UGT1A6*3* (*A522C*) Allele Frequency (%) (95% CI: Lower limit, Upper limit)			
	Wild-type (A)		Variant-type (C)		Wild-type (A)		Variant-type (G)		Wild-type (A)		Variant-type (C)	
	n	Observed	n	Observed	n	Observed	n	Observed	n	Observed	n	Observed
Malays	19 5	97.50 (92.26-99.22)	5	2.50 (0.78-7.74)	169	84.50 (76.15-90.30)	31	15.50 (9.70-23.85)	155	77.50 (68.39-84.58)	45	22.50 (15.42-31.61)
Chinese	19 4	97.00 (91.55-98.97)	6	3.00 (1.03-8.45)	171	85.50 (77.29-91.09)	29	14.50 (8.91-22.71)	163	81.50 (72.78-87.89)	37	18.50 (12.11-27.22)
Indian	17 2	86.00 (77.86-91.47)	28	14.00 (8.53-22.14)	123	61.50 (51.71-70.44)	77	38.50 (29.56-48.29)	102	51.00 (41.35-60.58)	98	49.00 (39.42-58.65)

n: Sample size

were in Hardy-Weinberg Equilibrium (HWE). The genotype frequencies for both *UGT1A6* variants were significantly different between Indian and Malay, also Indian and Chinese ethnicity with p-value less than 0.001. The frequencies of *UGT1A6*2* and **3* were higher in Indian population with 38.50% and 49.00%, respectively as compared to the Malay (15.50% and 22.50%, respectively) and Chinese (14.50% and 18.50%, respectively) populations (Table 2). *UGT1A6*1* (wild type allele) occurs at frequencies of 0.52 and 0.61 for CVD patients and healthy volunteers of Malay ethnicity. While for the Chinese, the frequencies of the *UGT1A6*1* (wild type allele) for CVD patients and healthy volunteers were 0.56 and 0.64, respectively. The frequency of the polymorphism of *UGT1A6*1* (wild type allele) for the Indians CVD patients was 0.26 whereas the frequency for Indians healthy volunteers was 0.25. *UGT1A6*2/*2 (A541G)* and *UGT1A6*3/*3 (A522C)* was not detected in any ethnic groups. The allelic variants of *UGT1A6*2* and **3* were observed at 20.30% and 28.18%, respectively in patients with CVD; 22.83% and 30%, respectively in healthy volunteers. The genotype frequencies of *UGT1A6* were similar and consistent in both patients and healthy volunteers. The patients were classified according to different *UGT1A6*2* and **3* genotypes and the patients' bilirubin levels show significant differences when compared according to genotypes with p-value less than 0.05 (Table 3).

Table 3. Concentration of Bilirubin with Respect to *UGT1A6 (*2 & *3)* and *CYP2C9*3* Genotypes

Genotype		**Bilirubin**[c] (umol/L) (n = 164)	***p***
*UGT1A6*2*	AA (Homozygous wild-type) Median (CI: 95 %)	10 (9 – 11)	0.045*[a]
	AG (Heterozygous) Median (CI: 95 %)	12 (9 – 13)	
	GG (Homozygous variant) Median (CI: 95 %)	13 (8 – 21)	
*UGT1A6*3*	AA (Homozygous wild-type) Median (CI: 95 %)	10.0 (9.0 – 11.0)	0.013*[a]
	AC (Heterozygous) Median (CI: 95 %)	11.0 (9.0 – 13.0)	
	CC (Homozygous variant) Median (CI: 95 %)	13.5 (10.0 – 19.0)	
*CYP2C9*3*	AA (Homozygous wild-type) Median (CI: 95 %)	10 (9 – 11)	0.643[b]
	AC (Heterozygous) Median (CI: 95 %)	13 (8 – 15)	

[a] Kruskal-Wallis test, [b] Mann-Whitney test, [c] Laboratory data, N: Sample Size, Cl: Confident Interval * Statistical significance (*P*-value < 0.05), AA: Homozygous wild-type, AG: Heterozygous, GG: Homozygous variant, AC: Heterozygous, CC: Homozygous variant.

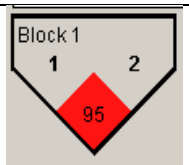
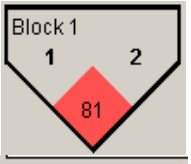
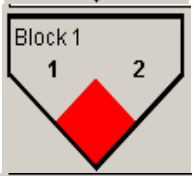
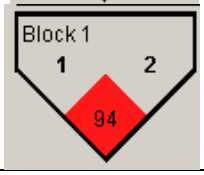
The association between genotype and gastritis event among patients who took aspirin as their anticoagulant drug were calculated using chi-square test. The odds ratio (OR) was used to examine the risk of each genotype in association with the development of gastritis event. Referring to the odds ratios in Table 4, individual who were heterozygous CYP2C9* 3 genotype shows 6.8 times more likely to have gastritis when compared to the individual with homozygous wild-type.

Table 4. Odd Ratios of Gastritis in Aspirin Treated Patients with Different Genotypes of *UGT1A6 (*2 &*3)* and *CYP2C9* 3*.

Gene	Genotype	Gastritis		OR (CI: 95 %)	*P*
		With Event N%	Without Event N%		
*UGT1A6*2*	AA (Homozygous Wild-Type)	7.69	92.31	2.8 (1.35-5.57)	0.776
	AG (Heterozygous)	5.41	94.60		
	GG (Homozygous Variant)	0.00	100.00		
*UGT1A6*3*	AA (Homozygous Wild-Type)	4.84	95.16	2.3 (1.19-4.61)	0.360
	AC (Heterozygous)	9.43	90.57		
	CC (Homozygous Variant)	0.00	100.00		
*CYP2C9*3* (*A1075C*)	AA (Homozygous Wild-Type)	4.67	95.33	6.8 (1.39-33.19)	0.033*
	AC (Heterozygous)	25.00	75.00		

[a] Chi-square test , OR: Odd Ratios, * Statistical significance (*P*-value < 0.05).

Table 5. Analysis of linkage disequilibrium of *UGT1A6*2* and *UGT1A6*3* in Malaysian Population

Haplotype	Ethnic	N	D'	r^2	LD Plot
A541G – A522C	Malays	100	0.95	0.572	
	Chinese	100	0.81	0.501	
	Indian	100	1.00	0.646	
	Total	300	0.94	0.614	

n: Sample size

Haplotypes was analysed using haploview which was downloaded from http://www.broadinstitute.org/haploview. This software was used to observe the linkage between two *UGT1A6* variants (*2 and *3). This analysis was done for the three major ethnic groups in Malaysian population which are the Malays, Chinese and Indians. A hundred samples from each ethnic group were used to study the degree of linkage between the three ethnic groups. As shown in Table 5, Indians have a strong degree of linkage disequilibrium with D' value of 1. Both variants were found in different percentage of linkage disequilibrium for different ethnic groups, which are 95%, 81% and 100% in Malay, Chinese and Indian ethnic groups, respectively (Table 5).

DISCUSSION

Most of the smokers were males and this is in line with the high number of male smokers (2.61 million) in Malaysia compared to 120,000 female smokers [13,14]. The latest projections indicate that approximately 3.1 million smokers were between 25 and 64 years in Malaysia. The Malays constitute the largest number of smokers in Malaysia, with 60.9% [15]. This situation might provide one of the reasons why the Malays tends to have higher incidences of CVD at earlier age compared to the Chinese.

Among 165 patients with CVD in this study, 86.67% were overweight and 75.15% have dyslipidemia. Singh *et al*. [16] have suggested that obesity and dyslipidemia are related and play major roles in the development of CVD. In addition, this study also showed that there was a significant difference in the lipid profile between the Malays and two other ethnic groups, which was in agreement with the results of Khoo *et al*. [17]. Further, there was also a significant association between body weight and ethnicity (p = 0.007), where the BMI of the Malays were higher. This finding is supported by Malaysia's National Health and Morbidity Survey 2 (1996 – 1997) which found that obesity was significantly associated with ethnicity. This observation may be explained by differences in dietary habits or lifestyle, or by genetic factors between the three ethnic groups.

PT and INR values of the Malay patients were found to be significantly different when compared with the values observed in the Indian and Chinese (p values of 0.016 and 0.014, respectively). The differences of PT and INR may be affected by daily diet. According to Rombout *et al*. [18], an increase in the amount of food rich in vitamin K can lower the PT and INR of an individual. Green and leafy vegetables such as broccoli, lettuce and spinach are part of the routine diet of the Chinese ethnic group in Malaysia and therefore it is not surprising for the Chinese to have lower PT and INR values [19].

Halushka *et al*.[20] have found that heterozygocity of *COX-1* (*A-842G)* gene significantly shows greater inhibition in the formation of prostaglandin H_2 by acetylsalicylic acid compared with common homozygous wild-type allele. Unfortunately this variant is not detected in this study, even though it was detected at a high percentage among the Caucasians (19% heterozygous and 1% homozygous) [20,5].

Nagar *et al.*, (2004) reported that the frequencies of *UGT1A6*2* and *UGT1A6*3* were 5.7% and 5.2% in Caucasian and, 10% and 5% in African-American [21]. The percentage frequency of *UGT1A6*2* and *UGT1A6*3* for the Chinese population in China were 22% and 24.7%, respectively [22]. Similarly, in the Japanese, *UGT1A6*2* and *UGT1A6*3* were present in 21.8% and 2.26%, respectively in the population, [23]. Limenta *et al*.[24] reported that *UGT1A6* variants were found in 19% of the Thailand population. The percentage of *UGT1A6* variants reported in this study was somewhat consistent with findings among the Chinese in China and Japanese population. Both variants of *UGT1A6* were reported to be in complete linkage-disequilibrium of >98% [5]; while in this study, the linkage was found to be 94%.

The significant association between the concentration of the patients' bilirubin and *UGT1A6*2* and **3* was in accordance with a previous finding by Peters *et al*.[25]. From the existing evidence, the concentration of bilirubin in patients with CVD was associated with the enzyme activity of *UGT1A6*. As shown in our results, higher concentration of bilirubin was found in patients who possess variants allele of *UGT1A6* with slow metabolizing capacity. In other studies by Djousse *et al*. [26] and Lin *et al*. [27], high levels of bilirubin was associated with decreased risks of coronary heart disease (CHD) and cardiovascular disease (CVD). Similarly in the Framingham

Offspring Study, higher serum bilirubin concentrations were associated with decreased risk of CVD, CHD, and myocardial infarction (MI) [28]. The association between the genetic polymorphisms of *UGT1A6* and the concentration of bilirubin are interesting finding and a case-control study with longer follow-up time might be useful to confirm the relationship.

Homozygous genotype of *CYP2C9*3* was reported to present at low frequency of only 1% in the Malay and Indian population [29], but no homozygous *CYP2C9*3* was found in this study. There were also no subjects with homozygous *CYP2C9*3* in the 115 Han Chinese as well as 218 Japanese and 98 Taiwanese [30-32]. The genotype frequency of *CYP2C9*3* in this study is in agreement with the reported rare frequency in most population worldwide.

Similar with findings from Van Oijen *et al*.[5], no significant association between the occurrence gastric events and polymorphisms of *UGT1A6* gene in aspirin treated patients were detected. However, there was a significant association between the slow metabolizer (SM) of heterozygous *CYP2C9*3* with the gastritis events among the CVD patients treated with aspirin. There were 25% occurrences of gastritis in patients with heterozygous *CYP2C9*3* while only 4.67% of gastric event among patients with homozygous wild type. Martin *et al.*, [33] revealed that 30% of NSAID treated subjects experienced ulceration event in non-wild type *CYP2C9* genotypes. The calculated odd ratios (OR) in this study indicated that individuals who were taking aspirin and with heterozygous *CYP2C9*3* genotype have 6.8 times higher risk to get gastritis as compared to the individual with homozygous wild-type (*p*-value 0.033).

CONCLUSION

In conclusion, we have successfully developed allele specific PCR (AS-PCR) methods for detection of *COX-1* (*A-842G*), *UGT1A6*2* and *3 and *CYP2C9* (*A1075C*) alleles. Besides, we also have successfully genotyped all the 165 patients and 300 healthy volunteers.

There was an association between the bilirubin concentration of CVD patients and variants of *UGT1A6*2* and *3 gene. In addition, this study also found an association of *CYP2C9*3* variant allele (slow metabolizer of aspirin) with the occurrence of gastritis event among CVD patients treated with aspirin.

This study provides support to recommend genotyping of CVD patients for *UGT1A6**2 and *3 and *CYP2C9*3*. However, further studies are needed to confirm the association between the genetic polymorphisms of *UGT1A6* with the concentration of bilirubin. A case-control study with longer follow-up period might be useful to provide answers to the relationship. In addition, the potential of bilirubin as therapeutic target or tool in monitoring the disease of CVD patients, a clear cut–off concentration or range of normal bilirubin concentration need to be established. Case controlled studies using bigger sample size is required.

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