RESEARCH ARTICLE

Frequencies of CYP1A2 Single Nucleotide Polymorphism in Indonesian and Its Effect on Blood Pressure

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Abstract

BACKGROUND: The association between caffeine with blood pressure (BP) still remains controversial. Caffeine is mainly metabolized by cytochrome-P450 (CYP)1A2 enzyme. Polymorphism of CYP1A2 is known to cause interindividual variation on enzymatic activity, thus affects caffeine metabolism and its effect on cardiovascular (CV) system.

METHODS: We conducted a cross-sectional study and recruited 121 Indonesian subjects aged 25-60 years with varying coffee-drinking habits. DNA was extracted from peripheral blood and genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Blood pressures were measured in the morning prior to the daily activity. Caffeine concentration in blood plasma was measured using high-performance liquid chromatography (HPLC). The differences between variables were analyzed using Mann-Whitney analysis and the correlations among variables were determined using multivariate logistic regression analysis.

RESULTS: This study showed that the frequencies of single nucleotide polymorphisms (SNPs) among Indonesian were 31.8%, 18.2%, 25.2% and 24.8% respectively for CYP1A2*1A, CYP1A2*1B, CYP1A2*1C and CYP1A2*1F alleles. The genotype analysis showed that the subject number of the wild type (*1A/*1A) and the variants were 9.92% and 90.08%. There were no significant differences in term of BP levels among CYP1A2 genotypes and coffee drinking habit groups.

CONCLUSION: The frequencies of CYP1A2 SNPs in Indonesian are different with frequencies in other populations. Since the association were not statistically significant, CYP1A2 polymorphisms as the predictor of elevated blood pressuare should be investigated further.

KEYWORDS: coffee, caffeine, blood pressure, single nucleotide polymorphism, CYP1A2

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Introduction

More than 80% of the world's population consumes caffeine (1,3,7-trimethylxanthine)-contained coffee, tea and soft drinks daily.(1) The effects of caffeine consumption on blood pressure (BP) and risk of cardiovascular diseases (CVD) have been widely reported. However, the results were inconsistent and still controversial. Although most cohort studies do not show significant associations between caffeine consumption and CVD, case-control studies tend to support significant associations.(1-3)

Caffeine is rapidly absorbed from gastrointestinal tract into the bloodstream. Approximately 95% of caffeine is metabolized by cytochrome P450 1A2 (CYP1A2).(4) The CYP1A2 enzyme is expressed primarily in liver and is responsible for oxidative metabolism of drugs such as theophylline, mexiletine, clozapine and phenacetin.(5,6) CYP1A2 genetic polymorphisms were known to cause interindividual variability on enzymatic activity. Up to 60-fold interindividual variation in CYP1A2 activity had ever been reported, including CYP1A2*1B, CYP1A2*1C and CYP1A2*1F (Figure 1). It is suggested that approximately 35 to 75% of the interindividual variability of CYP1A2 activity is due to genetic factors.(7,8)

Since interindividual variability of CYP1A2 gene is suggested to affect to BP elevation in substantial manner and frequency of CYP1A2 polymorphisms in Indonesia have not been characterized yet, therefore we aim to study the variability of CYP1A2 gene among Indonesian and its effect on BP.

Methods

Study Design

A cross-sectional study was conducted from May 23, 2012, until June 5, 2013. Initially 130 patients were enrolled; finally, 121 subjects aged 25-60 years with varying coffee-drinking habits participated in the study. All subjects had given full informed consent to participate. Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia (0566/H4.8.4.5.31/PP36-KOMETIK/2010).

Subjects

The subjects were enrolled from those who came to Prodia Clinical Laboratory in Denpasar and Jakarta, Indonesia, for having general medical check-up. All subjects were apparently in healthy conditions and willing to participate in the study. Subjects who were routinely consuming either vitamins or anti-inflammatory drugs, had kidney dysfunction (GFR <60 mL/min), diabetics (fasting glucose ≥126 mg/dL), had abnormal liver function (ALT/AST >2x reference range) and acute inflammation (hsCRP >10 mg/dL) were excluded from the study. Subjects with waist circumference (WC) of more than 90 cm (man) or 80 cm (woman) and current smokers were excluded from the study as well.

Demographic Data and Questionnaire

All tests were conducted in the morning prior to any physical activity. After the consent process, subjects were provided and completed a validated questionnaire. The questionnaire was used to assess subject's demographic data, past medical history, dietary intakes and coffee-drinking habits.

Anthropometric and Blood Pressure Measurement

Body weight (BW) was quantified in kilograms to the nearest 0.1 kg. Height and WC were measured in centimeters to the nearest 0.1 cm. WC was determined using a flexible non-elastic tape made by Roche (Roche, Basel, Switzerland). It was measured at the midway region between the lowest rib margin and the iliac crest, in standing position, abdomen relaxed, feet close together and weight equally divided over both legs.

Blood pressure was measured using calibrated sphygmomanometer and taken by trained healthcare professionals (nurses and physicians) who were instructed to follow standard procedures for BP measurement. An elevated BP reading was defined as a systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg.

Blood Analysis

Fasting blood samples were collected in the morning after the subjects completed questionnaire and anthropometric measurement. Serum was used for analysis of fasting blood glucose, creatinine, hsCRP, and liver function. Peripheral blood samples prepared with EDTA was used for genotype analysis and caffeine concentration measurement.

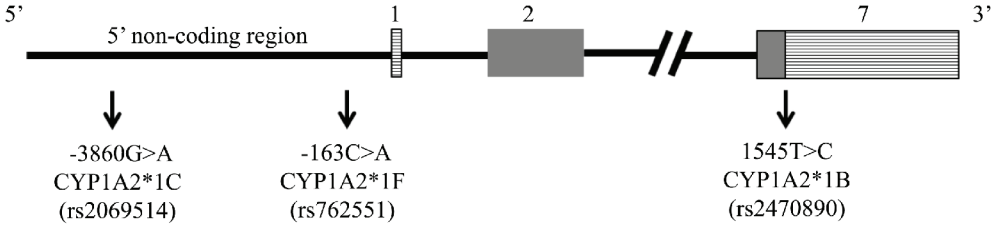


Figure 1. Approximate location of CYP1A2*1B, CYP1A2*1C and CYP1A2*1F SNPs. Figure is not scaled.

Genotype Analysis

Genomic DNA material was extracted from 5 mL of EDTA blood using High Pure PCR Template Preparation Kit (Roche Applied Science, Mannheim, Germany). DNA extracts were stored at -80°C prior to analysis.

Three polymorphisms were carried out; -1545T>C (CYP1A2*1B allele, rs2470890), -3860G>A (CYP1A2*1C allele, rs2069514) and -163C>A (CYP1A2*1F allele, rs762551). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was adopted from Sachse's and Tiwari's method (Table 1).(9,10)

Mastermix reagent contained namely: 2.5µL reaction buffer, 1.25mM $MgCl_2$, 0.2mM dNTPs (100mM dNTP Set, Roche Applied Science), 0.25µL of each primers (1st Base, Singapore), AmpliTag Gold DNA Polymerase (Roche Applied Science) for *1B, Kappa 2G Robust PCR Kit (Kappa Biosystem, Cape Town, SA) for *1C and *1F, DMSO (Applied Biosystems, Foster City, CA, USA), template and PCR grade water. Amplification was performed in Veriti Analyzer (Applied Biosystems).

According to RFLP method, the restriction enzymes were MluCI (New England Biolabs, Ipswich, MA, USA) for *1B, BslI (New England Biolabs) for *1C and PspOMI (New England Biolabs) for *1F. Analysis of restriction fragment was performed on 2% agarose gels (Promega, Madison, WI, USA). Electrophoresis was carried out and documented using GelDoc XR (Biorad, Hercules, CA, USA) (Figure 2).

Caffeine Concentration Measurement

Caffeine concentration measurement was performed by using high-performance liquid chromatography (HPLC). Briefly, 1 mL blood plasma was added with 200 µL of 20 µg/mL theophylline, 500 µL of 6M KOH, 5 mL of tert-Butyl methyl ether and 2 g of Na_2SO_4. The solution was homogenized with a rotary shaker and centrifuged. Resulted organic phase was collected and freeze-dried with nitrogen. Dried sample was then analyzed with HPLC. The organic solvents used for mobile phases was acetonitrile with flow rate of 1 mL/min. Photodiode array detector was used with the maximum wavelength of 273 nm. Commercially available caffeine (Sigma-Aldrich, St. Louis, MO, USA) was used to establish a standard curve.

Data Analysis

Data analysis was done using SPSS for Windows version 15 software (SPSS Inc., Chicago, IL, USA). Distributions of continuous variable were assessed for normality and equality of variance, subsequently using Kolmogorov-Smirnov and Leven's tests. The differences between variables were analyzed using Mann Whitney test. All tests were considered one-sided and significant at *p*-value of <0.05.

Results

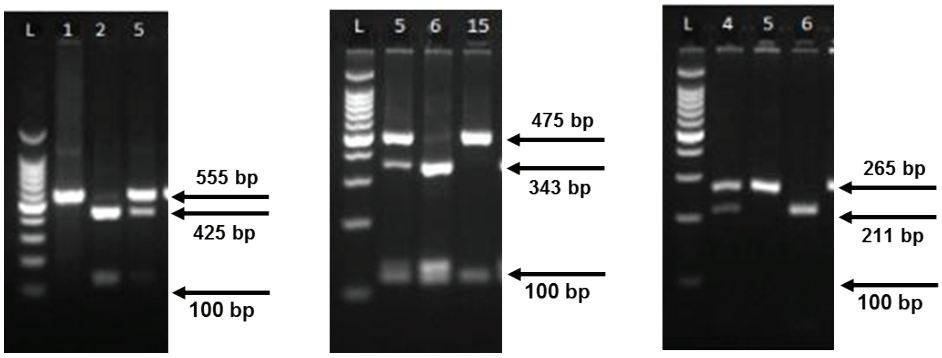
One hundred and twenty-one subjects were genotyped for CYP1A2 polymorphism. According to the data shown in Table 2, the frequencies of CYP1A2*1A, *1B, *1C, and *1F were calculated to be 31.8% (77/242), 18.2% (44/242), 25.2% (61/242) and 24.8% (60/242), respectively. The genotype analysis showed that the subject number of the wild type (*1A/*1A) and the variants were 9.92% and 90.08%. The complete frequencies of CY1A2 genotype were listed in Table 2.

Twenty-six of 121 subjects were excluded from the study due to various reasons and 95 subjects (Table 3) were still included in BP analysis. Table 4 presents the differences between CYP1A2 genotype, coffee drinking habits and BP levels. There were no significant differences in term of BP levels among CYP1A2 genotypes and coffee drinking habit groups.

Table 1. Primers and PCR conditions used for CYP1A2 genotyping.

SNP	Primer Sequence	PCR Program	Restriction Enzyme	Resulted Band (bp)	Reference
CYP1A2*1B	F: AGCCCTTGAGTGAGAAGATG R: GGTCTTGCTCTGTCACTCA	35x (30s 95°C – 30s 58 °C – 60s 72 °C)	*MluC* I	C: 555 T: 425-130	11
CYP1A2*1C	F: GCTACACATGATCGAGCTATAC R: CAGGTCTCTTCACTGTAAAGTTA	35x (30s 95 °C – 30s 52 °C – 60s 72 °C)	*Bsl* I	G: 343-132-93 A: 475-93	10
CYP1A2*1F	F: TGAGGCTCCTTTCCAGCTCTCA R: AGAAGCTCTGTGGCCGAGAAGG	35x (30s 95 °C – 30s 58 °C – 60s 72 °C)	*PspOM* I	A: 265 C: 211-54	10

s: seconds.



CYP1A2*1B
1 = C/C
2 = T/T
5 = C/T

CYP1A2*1C
5 = G/A
6 = G/G
15 = A/A

CYP1A2*1F
4 = A/C
5 = A/A
6 = C/C

Figure 2. CYP1A2*1B, CYP1A2*1C and CYP1A2*1F SNPs detection with PCR-RFLP. L: ladder.

Discussion

The frequencies of CYP1A2*1B (18.2%) and *1C (25.2%) in Indonesian are close to the reported frequencies in Japanese, whereas the frequency of *1F (24.8%) in Indonesian appears to be lower than that in Japanese. The frequencies of 19.2%, 23.6% and 62.8% for allele *1B, *1C, *1F in Japanese were reported by Soyama, *et al.*(5) Studies conducted by Nakajima, *et al.* and Chida, *et al.,* showed that the frequencies of *1C and *1F alleles were 23.0% and 61.3% among Japanese.(11,12) The frequencies of *1C allele among Chinese were 21-25%. (13-15) The *1A/*1A genotype was considered as wild type (extensive metabolizer) whereas the others as mutants (poor metabolizer).

Table 2 showed the frequencies of CYP1A2 genotype in Indonesian. This study was only genotyped 3 sorts of CYP1A2 polymorphisms. Up to now, there were more than 40 CYP1A2 alleles (haplotype) published in Human Cytochrome P450 Allele Nomenclature Committee homepage (http://www.cypalleles.ki.se).

CYP1A2 enzyme is responsible for 95% of caffeine metabolism. Consequently, there is up to 60-fold differences in enzymatic activity among individual. This interindividual variability is due to genetic (35-75%) and environmental factors.(7,8) This enzymatic activity is also induced by various xenobiotic compounds, such as 3-methylcholanthrene and other aromatic hydrocarbons. Smoking and heavy coffee drinking habits would have induced enzymatic activity, eventually BP rise.(16,17)

In this study, we found that there were no significant differences in term of BP levels and caffeine level between CYP1A2 genotypes and coffee drinking habit groups (Table 4). The BP of variant group was higher than wild type group among coffee drinkers (SBP: 109.31 ± 10.19 vs. 105.00 ± 5.77 mmHg; DBP: 73.63 ± 8.78 vs. 67.50 ± 5.00 mmHg, respectively). The caffeine level was higher in variant group compared with wild type group (0.667 ± 0.81 µg/mL vs. 0.375 ± 0.44 µg/mL). Both the BP and caffeine measurements were carried out in the morning after overnight fasting (10-12 hour).(18) This overnight fasting possibly caused the caffeine level, as well as BP, getting back to normal levels. Thus, the time lag between exposure/consumption and BP measurements is important to determine the association between coffee consumption and BP. The influence of coffee consumption (caffeine) on BP may vary, from zero to several mmHg. Longer coffee abstinence may also decrease BP.(2,19,20) Interestingly, caffeine was also found in the sample of non-coffee drinker

Table 2. CYP1A2 genotype in Indonesian population. (n total: 121 subjects).

SNP	n (%)
CYP1A2 *1A/*1A	12 (9.92)
CYP1A2 *1A/*1B	12 (9.92)
CYP1A2 *1A/*1C	20 (16.53)
CYP1A2 *1A/*1F	21 (17.36)
CYP1A2 *1B/*1B	2 (1.65)
CYP1A2 *1B/*1C	14 (11.57)
CYP1A2 *1B/*1F	14 (11.57)
CYP1A2 *1C/*1C	7 (5.79)
CYP1A2 *1C/*1F	13 (10.74)
CYP1A2 *1F/*1F	6 (4.96)

n: subject number.

Table 3. Subject characteristics.

Variable	Non-Coffee Drinker	Coffee Drinker	Total
n (%)	40 (42.1%)	55 (57.9%)	95 (100%)
Gender			
Male	11 (11.6%)	22 (23.1%)	33 (34.7%)
Female	29 (30.5%)	33 (34.7%)	62 (65.3%)
Average age (years)	37.62 ± 8.21	38.18 ± 7.52	37.95 ± 7.78
Average duration of coffee consumption (years)	0	9.16 ± 7.95	-
Family history			
Hypertension	19 (20.0%)	21 (22.1%)	40 (42.1%)
Diabetes mellitus	12 (12.6%)	12 (12.6%)	24 (25.3%)
Physical exercise	19 (20.0%)	22 (23.2%)	41 (43.2%)
Alcohol consumption	0	0	0
Tea consumption	21 (22.1%)	32 (33.7%)	53 (55.8%)

n: subject number.

group, suggesting that subjects have consumed any caffeine-contained meal or drink other than coffee, possibly tea and caffeinated soft drinks.

Although being reported harmful, coffee contains beneficial compounds as an antioxidant, including chlorogenic acid, flavonoids, melanoidins and various lipid-soluble compounds such as furans, pyrroles and maltol. Epidemiological studies revealed coffee is the main source of antioxidant.(21,22) The antioxidant compounds in coffee will be absorbed rapidly after ingestion and leads to increased circulating antioxidant levels. On the other hand, caffeine increases angiotensin II production by inhibiting adenosine A1 receptor.(23) Therefore, the balance between pro- and anti-oxidative compounds in coffee and inflammatory status may be important factors to affect BP.(24)

Coffee consumption has become part of modern lifestyle factor, together with smoking and alcohol consumption. Smoke was known to increase CYP1A2 activity.(2,25) In this study we were just able to control some confounders such as; age, alcohol consumption, smoking habits, weight circumference, medical history (acute inflammation, hypertension, diabetes, renal failure) and dietary factors (antioxidant/vitamin/energy drinks consumption) by design. However, the method of coffee preparation (boiled and filtered), the type of coffee (black coffee and instant coffee), varieties of coffee (arabica and robusta) and the exact amounts of coffee ingested were technically arduous to be adjusted in the study. Furthermore, hypertension is a multifactorial disorder and involves multiple environmental and genetic determinants. Many genetic analyses have been performed in different ethnic population, but the result is still inconsistent and specific cause of hypertension remains unclear. Further study need to be done, especially the experimental study to examine the acute effects of coffee consumption and its association with CYP1A2 polymorphism.

Conclusion

The frequencies of CYP1A2 SNPs in Indonesian are different with frequencies in other populations. Since the association were not statistically significant, CYP1A2 polymorphisms as the predictor of elevated blood pressuare should be investigated further.

Acknowledgement

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Table 4. CYP1A2 and blood pressure.

Variable	Non-Coffee Drinker			Coffee Drinker		
	Wild Type Mean ± SD	Variant Mean ± SD	*p*	Wild Type Mean ± SD	Variant Mean ± SD	*p*
n	4	36	-	4	51	-
Average age (years)	37.18 ± 8.38	37.67 ± 8.31	0.983	35.40 ± 3.20	38.41 ± 7.74	0.584
Caffeine (µg/mL)	0.21 ± 0.36	0.17 ± 0.23	0.778	0.375 ± 0.44	0.667 ± 0.81	0.542
SBP (mmHg)	107.50 ± 5.00	109.31 ± 13.53	0.948	105.00 ± 5.77	109.31 ± 10.19	0.370
DBP (mmHg)	75.00 ± 5.77	72.64 ± 7.88	0.557	67.50 ± 5.00	73.63 ± 8.78	0.175

n: Subject number; SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure.

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