Prevalence of CYP2C19 681G>A and 636G>A Gene Polymorphisms in Javanese Farmers Exposed to Chlorpyrifos

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ABSTRACT

Objective. The objective of our study was to determine the genotype frequencies of CYP2C19*2 and *3 gene polymorphisms among Javanese farmers exposed to chlorpyrifos (CPF) in Central Java, Indonesia.

Methods. This cross-sectional study was conducted from July to October 2020 in Central Java, Indonesia, involving 151 vegetable farmers aged 18-65 years who used CPF for at least one year. CYP2C19*2 and *3 gene polymorphisms were analyzed using PCR-RFLP. Direct calculations were applied to calculate allele and genotype frequencies. The difference in genotype frequencies among the sex and cumulative exposure level (CEL) group was performed using the Chi-square test. In contrast, the proportion difference of allele frequencies was analyzed using Z-test.

Results. The frequency of CYP2C19 genotypes *1/*1, *1/*3, *1/*2, and *2/*2 were 64 %, 7 %, 23 % and 6 %. We observed no significant difference in the genotype distribution according to sex group and CEL group.

Conclusion. In summary, the prevalence of toxicologically relevant CYP2C19 polymorphisms was determined in the Javanese agricultural population. The CYP2C19 genotype may be helpful as an essential biomarker of genetic susceptibility towards CPF exposure. Nevertheless, further studies to confirm the role of CYP2C19 in this context are still needed.

Key Words: CYP2C19, farmer, pesticide, susceptibility biomarker

INTRODUCTION

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Indonesia is an agricultural country, and farmers generally use insecticides to control pests. The most widely used insecticide is chlorpyrifos (CPF) and is most often used in vegetable farming and other crops like shallots, chilies, cocoa, soybeans, rice, and peanuts.[1] Farmers' behavior in using insecticides is generally poor with higher concentrations and more frequent applications. Moreover, using multiple pesticides was a common practice. Still, proper personal protective equipment (PPE) use was rare otherwise.[2] Farmer exposed to CPF may develop certain risks of health problems, including but not limited to neurological symptoms and hormonal alteration.[3,4] A recent report from a study on farmers in Central Java, Indonesia, revealed that 68.3% of participants

had cholinesterase levels below the reference value indicating exposure to acetylcholinesterase inhibitor pesticides, and neuropathic symptoms were found in 59.2% of participants.[5]

Previous studies have elucidated the critical role of CYP450 (CYP) in the human bio metabolism of xenobiotic substances. Human CYP is known to have several essential effects regarding its role as a catalyst in phase 1 xenobiotic metabolism and clearance.[6,7] However, it is not expressed at the same level for all individuals, thus impacting different individual responses to xenobiotics. Chlorpyrifos, an organophosphate (OP) insecticide, is metabolically activated to its oxon form through bioactivation (desulfuration) reaction triggered by CYP2B6 and CYP3A4, and also can undergo a detoxification (dearylation) reaction, which is predominantly catalyzed by CYP2C19, resulting in inactive metabolites 3,5,6-Trichloro-2-pyridinol (TCPy).[8,9] The balance between the activation and detoxification in CPF metabolism is affected by age, the co-existence of other xenobiotics, paraoxonase-1, CYP enzymes such as CYP2B6, and CYP2C19, then determine their toxic effects.[10,11]

Since the CYP2C19 gene is highly polymorphic and the *2 and *3 alleles are the most common variations in the CYP2C19 gene, such variations may alter the metabolic properties of the CYP2C19 enzyme (i.e., increased or reduced function) in the metabolism of many drugs and chemicals including CPF.[10,12] Concerning the vital role of CYP2C19 in CPF metabolism, individuals with lower CYP2C19 expression due to the genetic variations may have a greater susceptibility to the effects of CPF exposure.[13,14]

Therefore, the objective of our study was to determine the genotype frequencies of CYP2C19*2 and *3 gene polymorphisms among Javanese farmers exposed to CPF in Central Java, Indonesia. We hope our results will provide valuable and relevant information about the biomarker of susceptibility of CPF exposure and subsequently will be helpful in further exposure management, particularly in agricultural populations with extensive use of CPF.

MATERIALS AND METHODS

All methods were performed following the relevant guidelines and regulations. The study protocol was approved by the Ethical Committee of the Faculty of Medicine Universitas Indonesia on March 23, 2020 (No. KET-339/UN2.F1/ETIK/PPM.00.02/2020). Written informed consent was obtained from all study participants.

Study population

This cross-sectional study was held from July to October 2020. A consecutive sampling method was applied. We included 151 vegetable farmers from 2 villages, Pancot village, Tawangmangu District, and Adipuro village, Kaliangkrik District, known as the most prominent garlic and vegetable farming areas in Central Java, Indonesia, the study participants. The eligibility criteria were vegetable farmers who

were actively using CPF for a minimum of 1 year before the study, aged between 18-65 years. They gave written consent to participate in the study. A small remuneration was given to the participants for their participation in this study.

Characteristics of the study population and cumulative exposure level

A structured interviewer-administered questionnaire was used to identify the individual and occupational characteristics of the study participants. We estimated the intensity level and cumulative exposure level (CEL) of CPF using a validated quantitative method in the Agricultural Health Study from Dosemeci.[15] The intensity level was estimated as the function of activity related to the application (i.e., mixing, application method, repairing, washing, or both the equipment), use of PPE, gloves replacement, personal hygiene practices, and spill treatment when further combined with the lifetime years of pesticide use and the number of days spraying per year to estimate CEL. The participants were then classified as high and low exposure groups based on the median CEL value as described previously.[2]

CYP2C19 Genotyping

Genotyping assay was performed by Prodia Clinical Lab, Jakarta, Indonesia. Venous blood samples were collected from each participant using 3mL EDTA anticoagulant tubes and then transported to the laboratory and stored at -20°C before analysis. *CYP2C19*2* (681G>A – rs4244285) and *3* (636G>A – rs4986893) were analyzed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) as described previously.[16,17] During the RFLP phase, a specific restriction enzyme/ endonuclease was added to the DNA fragment and identified with electrophoresis gel. SmaI restriction enzyme was used in CYP2C19*2 analysis while primers *forward* 5'–CAGAGCTTGGCATATTGTATC–3' and 5'–GTAAACACACAACTAGTCAATG – 3' as primers *reverse* were used in the PCR amplification process. In addition, BamHI restriction enzyme was used in CYP2C19*3 analysis, and the amplification process were done using primers *forward* 5'-AAATTGTTTCCAATCATTTAGCT-3' and *reverse* 5'-ACTTCAGGGCTTGGTCAATA-3'. The RFLP genotyping was visualized in Figure 1.

Statistical Analysis

The analysis was performed using SPSS 20 for Windows. The characteristics of the study population were described using mean (SD) or median (minimum-maximum). We directly calculated the allele and genotype frequencies. The CYP2C19 gene polymorphisms were classified as follows: *1/*1; *1/*2; *1/*3; and *2/*2. The difference in genotype frequencies among the sex and CEL group was performed using the Chi-square test, while the proportion difference of allele frequencies was analyzed using Z-test. All p values were two-sided, with $p < 0.05$ was considered significant.
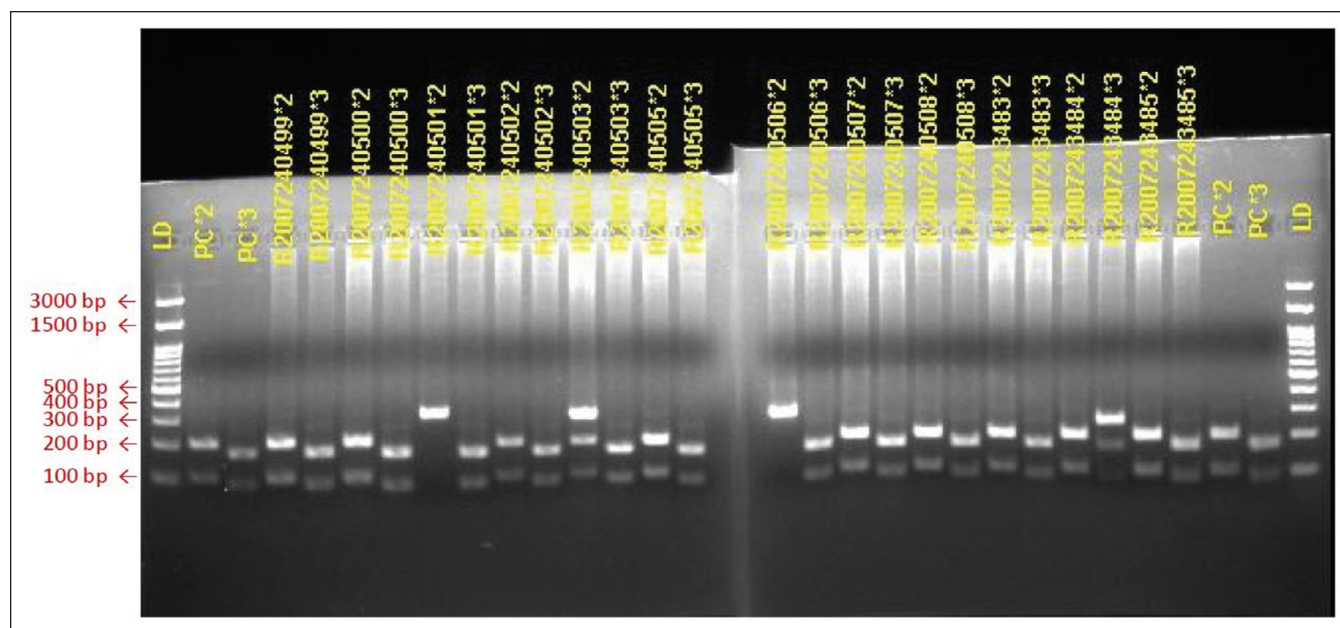
Figure 1. Visualization of CYP2C19 genotyping with PCR-RFLP.

RESULTS

Our study focused on determining the most common variant of the CYP2C19 allele associated with CPF exposure. We provide data regarding the distribution of the most common and clinically significant allelic and genotypic frequency of CYP2C19*2 (681G>A) and *3 (636G>A) in Javanese agricultural populations.

The mean age of our study population was 50 (SD 9.4) years and the median of lifetime years of pesticide use was 25 (1–45) years. Among them, 91% were male, and 47% were classified as a high CEL group. The observed frequency of the CYP2C19 genotype was in Hardy-Weinberg equilibrium. The frequency of CYP2C19 genotypes *1/*1, *1/*3, *1/*2, and *2/*2 were 64 %, 7 %, 23 % and 6 %, respectively as shown in Table 1.

The *2/*3 haplotypes were not found in our population. We observed no significant difference in the genotype distribution according to sex group (Table 2) and CEL group (Table 3), indicating that the members of both groups shared a similar genetic susceptibility to CPF exposure.

As shown in Table 4, our findings indicated that reduced function alleles were present in 21% of our study population. The CYP2C19*2 minor allele frequency (MAF) was 17.5%, while MAF for *3 allele was 3.6%.

DISCUSSION

The distribution of CYP2C19 gene polymorphisms has been previously studied in various populations. The frequency of the *wildtype* CYP2C19 allele in the Asian population is 60.5%. The most common allele was *2 with

Table 1. Distribution of CYPC19 genotype in study population

Genotype	Frequency (n=151)	Expected Phenotype
AA (*2/*2)	9 (6,0)	Poor Metabolizer (PM)
GA (*1/*2)	35 (23,2)	Intermediate Metabolizer (IM)
GA (*1/*3)	11 (7,3)	Intermediate Metabolizer (IM)
GG (*1*1)	96 (63,6)	Extensive Metabolizer (EM)

Table 2. Distribution of CYPC19 genotype according to the sex group

Genotype	Sex group#		p^{cs}
	Female (n=14)	Male (n=137)	
AA (*2/*2)	0 (0)	9 (6,6)	0.559
GA (*1/*2)	5 (35,7)	30 (21,9)	
GA (*1/*3)	1 (7,1)	10 (7,3)	
GG (*1*1)	8 (57,1)	99 (64,2)	

n (% within sex group)
cs – Chi-square for testing of male and female group

Table 3. Distribution of CYPC19 genotype according to CEL

Genotype	Cumulative exposure level#		p^{cs}
	High (n=71)	Low (n=80)	
AA (*2/*2)	3 (4,2)	6 (7,5)	0.580
GA (*1/*2)	14 (19,7)	21 (26,2)	
GA (*1/*3)	6 (8,5)	5 (6,2)	
GG (*1*1)	48 (67,6)	48 (60,0)	

n (% within CEL group)
cs – Chi-square for testing of high and low CEL group

Table 4. The minor allele frequency of CYP2C19*2 and *3 gene polymorphisms and the genotype frequencies of CYP2C19*2 and *3 in different population

Population	Sample size	MAF CYP2C19 (%)		Genotype CYP2C19 (%)			
		681G>A	636G>A	*1/*1	*1/*2	*1/*3	*2/*2
Present study	151	17.5	3.6	63.6	23.2	7.3	6.0
Egyptian[18]	120	3.8#	0.0#	93.3#	5.8#	0.0#	0.8#
Han Chinese[19]	100	25.5	2.0	42.0#	41.0#	3.0#	3.0
Thai[20]	1205	25.6#	2.5	48.2#	37.9#	3.5#	5.3
Kinh Vietnamese[21]	100	20.5	2.5	58.0	32.0	4.0	3.0

# p<0.05 by Z-test indicates a significant difference of MAF or genotype frequencies compared to the present study

an allele frequency of more than 70%, followed by *17, and *3.[14] The minor allele frequency (MAF) of CYP2C19*2 and *3 in our study was similar to that of the Han Chinese and Kinh Vietnamese population, but particularly lower *2 MAF than Thai. CYP2C19*3 is considered an Asian mutation, so, understandably, no *3 allele was found among the Egyptians.[22] Our findings indicate that the prevalence of poor metabolizers in this study was significantly higher than that of Egyptians and higher than those of Han-Chinese, Thai, and Kihn-Vietnamese Kinh, although not significantly different.

Individual responses to xenobiotics may be influenced by genetic polymorphisms in genes encoding enzymes that play a role in phase 1 and phase 2 metabolism, particularly enzymes catalysing the oxidation and conjugation reactions. This condition explained the activity of the bioactivation of xenobiotics to form products that are more reactive than the parent compound but can also promote the detoxification process and facilitate the subsequent excretion process.[12] Four phenotypes have been identified for CYP2C19 according to their enzyme activity, i.e., extensive metabolizer (EM), intermediate metabolizer (IM), poor metabolizer (PM), and ultra-rapid metabolizer (UM).[12,23]

The CYP2C19*2 and *3 gene polymorphisms were present in 1 out of 3 among our study population. We found that 30% of the studied population could be categorized as intermediate metabolizers since their genotype contained one reduced function allele (*1/*2 or *1/*3). In contrast, the other 6% with two reduced function alleles (*2/*2) can be classified as poor metabolizers. Since the CYP2C19 has a significant role in the detoxification process of CPF to form the inactive metabolite TCPy, individuals with genetic variations of CYP2C19*2 and *3 was thought to have lower enzymatic activity resulting in decreased CPF detoxification capacity.[10,12] CYP2C19*2 is the result of a G>A transition at position 681 in exon 5 which causes a splicing defect in pre-RNA that affects the proline amino acid at position 227. In contrast, CYP2C19*3 is a G>A transition at position 636 in exon 4, a vital point mutation due to replacing the tryptophan codon TGG with TGA. Both variants produce premature stop codons and, of course, non-functional proteins.[23]

With a better understanding of the CPF metabolic pathways, the human population's significant risks resulting from CPF exposure can be estimated. The metabolism of CPF, mainly the detoxification process, is quite complex, with many enzymes involved. Thus, with CYP2C19 as the main enzymes involved in this process, the CYP3A4 and 1A2 also play a role in the CPF detoxification reactions.[10,24] Individuals with low levels of CYP2C19 can be expected to produce fewer dearylation products. Therefore, intermediate or poor metabolizer individuals with lower CYP2C19 expression may be more susceptible to the effects of CPF exposure,[13] as the CPF parent compound is more readily available for bioactivation.

Our study has limitations as we did not examine other CYP isoforms that are important in CPF detoxification, i.e., CYP3A4 and CYP1A2, as potential genetic risk factors, so it is not clear how these CYP variants will interact with each other.

CONCLUSION

In summary, our results provide a basic profile, the prevalence of toxicologically relevant CYP2C19 polymorphisms in the Javanese agricultural population. These results provide information on most polymorphisms in genes encoding essential enzymes involved in CPF metabolism that may lead to the health impacts of occupational exposure to CPF among farmers' populations. Because the prevalence of CYP2C19*2 and *3 was considered high in this farming population, a periodic medical examination needs to be implemented to maintain farmers' health and early detection of the potential health problems. The CYP2C19 genotype may be helpful as an essential biomarker of genetic susceptibility towards CPF exposure. Nevertheless, further studies to confirm the role of CYP2C19 in this context are still needed.

Statement of Authorship
All authors participated in the data collection and analysis and approved the final version submitted.

Author Disclosure

Funding Source

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