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by Elly Usman

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Genetic Variation of the CYP2C9 Genetic of Minangkabau as a Predictor of Side Effect Providing Indications of Non-steroidal Anti-inflammatory Drugs

Yusticia Katar^{*}, Elly Usman[✉], Gestina Aliska[✉]

Department of Pharmacology, Faculty of Medicine, Andalas University, Padang, Indonesia

Abstract

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Correspondence: Yusticia Katar, Department of Pharmacology, Faculty of Medicine, Andalas University, Padang, Indonesia.
Email: yusticia.katar@gmail.com

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BACKGROUND: Non-steroidal Anti-inflammatory Drugs (NSAID) activity showed a varied response based on the genetic polymorphism of the CYP2C9 enzyme. Metabolism of some NSAIDs such as celecoxib, diclofenac, and ibuprofen is highly dependent on CYP2C9. This enzyme has several variants of the heterozygous and homozygous genotypes such as CYP2C9 *2/2 and CYP2C9 *17/3 which differ for each population. Genetic testing for specific variations of this allele will help determine whether the drug is a valid pharmacotherapeutic option for the patient or not.

AIM: This study aims to analyze the genetic variation of the CYP2C9 gene from the Minangkabau ethnic West Sumatra, Indonesia, which can later be used as a determinant of selecting the right NSAID with optimal effectiveness and to minimize side effects.

MATERIAL AND METHODS: Respondents of this study were users of NSAID drugs obtained from several hospitals in Padang City and were of Minang ethnicity. Blood samples taken from patients were stored in EDTA tubes and DNA was isolated using a genomic DNA isolation kit. DNAzol® Genomic DNA Kits (ThermoFischer Scientific). PCR primer and sequencing were designed using Primer Blast (NCBI) software. The synthesized primer was purified by HPLC. DNA fragment application was carried out using the PCR method. The amplicon DNA was purified and prepared as much as 500 ng for sequencing using Illumina's Next Generation Sequencing method. This analysis was performed with the help of SPSS software.

RESULTS: When analyzing the CYP2C9 gene on Primer F23, 44 people were found to be homozygous for the normal allele (AA genotype), and 11 people were heterozygous (GT genotype), 45 people were normal allele (TT genotype), five people were heterozygous (CG genotype), 49 people were normal allele (AA genotype), and five people were heterozygous (GA genotype).

CONCLUSION: This study concluded that relationship between CYP2C9 genetic variation and NSAID drug metabolism is found at the genotypic frequency rs229837 and rs1934969.

Introduction

Effectiveness, side effects or failure, and drug rejection reactions or pharmacogenetic risk factors are linearly related to the genetic variation possessed by the world's population [1]. CYP2C9 is a cytochrome P450 isoform that is responsible for the metabolism of most Non-steroidal Anti-inflammatory Drugs (NSAID) derivatives. Burian *et al.* (2002) reported that there is a relevance between genetic polymorphisms and increased toxicity of CYP2C9 substrates. CYP2C9 enzymes contribute greatly to the metabolism of a number of drugs, where polymorphisms in this gene express enzymes with different characters [2].

In vitro study conducted by Scordo *et al.* (2004) showed that conservative changes in amino acids in this gene can change the catalytic activity and sensitivity of the substrate [3]. Geographical and ethnic differences can also be associated with gene polymorphisms so that

they relatively affect the effectiveness of NSAID drugs in patients [4]. So that the significance of NSAIDs in the treatment of a number of diseases is also thought to have an effect on the Indonesian population, especially the Minang ethnic group.

Understanding the specificity of NSAIDs and their relationship to allele variations in the Indonesian population is an effective strategy. This study can optimize the effectiveness of the drug. Thus, studies related to the mapping of genetic variation in the CYP2C9 gene in the Indonesian population are urgent. Andalas University as a research and community service-based university is responsible for contributing to disease prevention. One of the first steps from a molecular perspective is to characterize and map variations in the CYP2C9 gene allele in an effort to develop drugs based on molecular metabolism specific to Indonesian populations. The aim of this study was to study the relationship between CYP2C9 genetic variation and NSAID drug metabolism.

Material and Methods

Population and sample

Respondents of this study were users of NSAID drugs obtained from several hospitals in Padang City and were of Minang ethnicity. Respondents stated their involvement in this study by providing informed consent. This study was approved by the Ethics Committee of the Faculty of Medicine, Andalas University. 5 mL blood sample was taken from the respondent who will be used to see the genetic variation.

Genomic DNA isolation and purification

Blood samples taken from patients were stored in EDTA tubes and DNA was isolated using a genomic DNA isolation kit, DNAzol® Genomic DNA Kits (ThermoFischer Scientific). The process of extracting genomic DNA from whole blood follows the protocol recommended by the provider. DNA is extracted by adding chloroform to homogenate to form aqueous, interphase, and organic layers. DNA was deposited from the interphase layer and the organic layer with the addition of 100% ethanol and Trizol reagent. Subsequently, the DNA pellets were washed with 0.1 M sodium citrate in 10% ethanol and 75% ethanol, respectively. The dried pellets were resuspended in 8 mM NaOH. DNA could be stored in a HEPES buffer pH 7–8 with 1 mM EDTA at –20°C.

Table 1: Primer F23

Index	Total
Patients with normal genome (AA)	44
Patients with a pathological gene (GT)	11
Patients with normal genome (TT)	45
Patients with a pathological gene (CG)	5
Patients with normal genome (AA)	49
Patients with a pathological gene (GA)	5

PCR and CYP2C9 gene sequencing

PCR primer and sequencing were designed using Primer Blast (NCBI) software. The synthesized primer was purified by HPLC. DNA fragment amplification was carried out using the PCR method. The PCR method was carried out using Gotaq™ PCR Core System kit (Promega) with a total volume of 50 µL for each reaction. The DNA sample was sampled for 35 cycles and after the DNA amplification process was completed, amplicons were stored at 4°C. The results of the amplification were separated by electrophoresis on 2% agarose gel which had been added by Gelred and DNA ladder. The amplicon DNA was purified and prepared as much as 500 ng for sequencing using Illumina's Next Generation Sequencing method.

Table 2: Genotype frequencies of investigated genes in the present study (n = 50)

SNPs	Genotype	Genotype frequency	n (%)
rs9332092	T/C	5	10
rs9332093	C/G	5	10
rs81804699	G/A	5	10
rs4918758	C/T	19	38
rs59890169/rs9332098	A/G	5	10
rs9332120	T/C	9	18
rs1799853	C/T	1	2
rs7900194	G/T	0	0
rs9332127	G/C	4	8
rs9332129	A/G	0	0
rs57505750	T/C	0	0
rs28371885	C/T	0	0
rs1057910	A/C	5	10
rs17847029	C/T	0	0
rs9332230	A/T	5	10
rs229837	C/T	18	38
rs1934969	A/T	23	46
rs1057911	A/T	5	10
rs9332242	C/G	1	2
rs9332244	A/G	0	0
rs9332245	T/A	5	10

Bioinformatic analysis of the structure of the CYP2C9 gene

The sequencing results were analyzed using the comprehensive amplicon sequencing workflow recommended by illumina. Genetic variations of the SLC22A1 gene were analyzed using Truseq Amplicon software. Alignment was performed using the Smith–Waterman banded algorithm. The calling variant was analyzed by somatic variant caller in germline or somatic mode. Ensembl and Refseq are used to annotate the output file. Base Space Variant Interpreter software is used to identify variants that have a significant biological function from human genome data.

Statistic analysis

Allele population frequencies and allele combinations were analyzed together with a 95% confidence limit. The differences in significance between ethnicities were tested by Fisher's test. The significant functional differences caused by CYP2C9 polymorphisms were calculated by analysis of variance after it was confirmed that the frequency distributions of all subgroups did not differ significantly from the normal distribution as calculated by the Kolmogorov–Smirnov test. This analysis was performed with the help of SPSS software (SPSS Inc., Chicago, USA).

Results

When analyzing the CYP2C9 gene on primer F23 (Table 1), 44 people were found to be homozygous for the normal allele (AA genotype), and 11 people were heterozygous (GT genotype), 45 people were normal allele (TT genotype), five people were heterozygous (CG genotype), 49 people were normal allele (AA

genotype), and five people were heterozygous (GA genotype) and genotype frequencies can be seen in Table 2.

Discussion

NSAID is drugs used worldwide to relieve pain as it has analgesic and anti-inflammatory effect. However, NSAID also gives side effect happened in patients. Polymorphism on gene encoding CYP2C9 enzyme, enzyme which metabolize NSAID, will increase the risk of bleeding because variants *2 and *3 decrease the activity of the enzyme. Thus, NSAID will be metabolized longer while the concentration of NSAID in plasma will be high, increasing the probability of side effect. Through determination of CYP2C9 polymorphism, NSAID dose adjustment could be done and hopefully will reduce the risk. To determine the polymorphism, one of the steps that needs to be done is genotyping. By knowing the CYP2C9 polymorphism, it is hoped that the incidence will increase the risk of side effects from NSAID therapy that is reduced [5].

The investigation of allelic distribution in human genes at a population level is practicing to determine numerous polymorphisms and to address the individual's SNPs associations to disease phenotypes [6], [7]. The allelic distribution based genetic structure inferences of drug-metabolizing enzymes are of utmost importance for the heterogeneous population holding complex demography [8]. Genetic variation in CYP2C9 is a recognized factor for ADRs as many of its drug substrates have a narrow therapeutic index. Numerous studies demonstrated the clinical significance of the CYP2C9*2 and *3 polymorphisms for most drug substrates mentioned above.

CYP2C9 polymorphism affects the metabolism of several NSAIDs [9]. The increased focus on safety in clinical trials represents a formidable hurdle regarding the availability of marketed drugs. The lengthy experimental process of ensuring the safety of a drug creates a need for faster, more efficient identification of drug toxicities. Profiling for individual genetic variability could be an essential screening process for potential adverse effects, especially within different ethnic populations. The identification of such variants should improve the management of patient care by, for example, identifying which patients should avoid a specific drug and which patients should be administered a modified dose. A suitable approach in implementing such a strategy could potentially reduce medical costs and improve the overall process and success of drug therapy. For example, polymorphisms in CYP2C9 gene, a gene involved in a variety of drug

metabolisms, should be considered during future drug development of novel NSAIDs [10].

Disorders that occur due to polymorphisms in CYP2C9 are changes in the metabolic rate of several drugs by this enzyme. Based on pharmacogenetic science, it is also stated that gene polymorphisms, especially genes encoding the CYP2C9 enzyme, have a significant role in increasing the risk of gastrointestinal bleeding. CYP2C9 is one of the CYP2C gene clusters on chromosome 10q24 [11]. CYP2C9 at most expressed in the liver with the second highest level of expression of the other CYP isoforms. CYP2C9 plays an important role in the process of drug metabolism, especially warfarin, and other drugs such as losartan, tolbutamide, phenytoin, and NSAIDs [12]. The decrease in metabolism will increase the concentration of drugs in the body which is at risk of increasing the side effects of the drug.

Conclusion

This study concluded that relationship between CYP2C9 genetic variation and NSAID drug metabolism is found at the genotypic frequency rs229837 and rs1934969.

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